Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

Colecalciferol
Cholecalciferol
Vitamin D3

EC Number: 200-673-2
CAS Number: 67-97-0

CLH-O-0000001412-86-144/F

Adopted
9 December 2016

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON COLECALCIFEROL; CHOLECALCIFEROL, VITAMIN D3

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: colecalciferol; cholecalciferol; vitamin D3
EC number: 200-673-2
CAS number: 67-97-0
Dossier submitter: Sweden

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
24.02.2016	Switzerland	DSM Nutritional Products AG	Company-Manufacturer	1
Comment received				
DSM Nutritional Products AG (referred as DSM thereafter) with its headquarter in Kaiseraugst, Switzerland manufactures vitamin D3 (also known as cholecalciferol) and vitamin D3 containing formulations at its sites in the European Union and in Switzerland. DSM sells vitamin D3 into the food, the feed, and the pharma market. Thus, DSM complies with the CLP Regulation requirements regarding packaging and labelling of its products and other regulations regarding worker safety. Vitamin D3 is an essential nutrient for human being. Insufficient exposure is known to produce serious health problems such as rickets or osteoporosis. Vitamin D3 is available for human being either by synthesis in the skin from UV-light exposure or through the diet. Intake of vitamin D3 in the modern developed Western-style of living resulted in too low exposure to vitamin D3. In fact infants are supplemented with vitamin D3 orally during their 1st year of life. Likewise, elderly are usually recommended to take vitamin D3 supplements to overcome serious health implications. Additionally, vitamin D3 is used for animal nutrition to support their production. DSM is aware of the current intention to further expand the existing classification and labelling of vitamin D3 based on a biocide registration process. The Rapporteur Member State Sweden submitted a respective CLH report which is available from the ECHA webpage (referred as CLH report (2016)). In this CLH report the Swedish authority provides argumentation on their rationale for proposing vitamin D3 as a germ cell mutagen (category 2) and carcinogen (category 2). DSM would like to express its serious concerns in that respect, since vitamin D3 is a biological compound present in the bodies of animals and humans to support the homeostasis of calcium, in particular for the bones formation and maintenance. Indeed, vitamin D3 is synthesized in the skin thanks to the light of sun. From a biomonitoring survey performed in African tribes having high sun exposures, it can be estimated that				

this production from sun exposure is equivalent to oral vitamin D3 doses of 100-250 µg/day. Insufficient exposure to the sun, motivates its supplementation in the diet of both people and animals. There is currently high concern in the countries of northern latitudes as well in countries in which part of the population has to be highly covered due to cultural practices, and the supplementation of vitamin D3 is promoted to ensure a healthy population.
Classification and labelling of vitamin D3 as a mutagen (category 2) and carcinogen (category 2) may have regulatory implications within the EU and potentially health issues for both human being and livestock: "The Scientific Committee is of the opinion that substances which are both genotoxic and carcinogenic should not be approved for deliberate addition to foods or for use earlier in the food chain, if they leave residues with are both genotoxic and carcinogenc in food." (EFSA 2005). Considering this opinion, the use of vitamin D3 in animal production and for people supply could be limited.
Based on an in depth analysis of the currently available data consisting of the CLH report, DSM proprietary data, and published literature, DSM does not consider vitamin D3 as a mutagen or carcinogen fulfilling the requirements laid down in the Guidance on the Application of the CLP criteria (CLP 2015).

ECHA note - The following attachments were submitted with the comment above:

- *D3 CLH DSM Position paper 22_02_2016_FINAL_submitted.pdf*
- *DSM 2015.pdf*
- *DSM 2016.pdf*

Dossier Submitter's Response

Classification is based on the intrinsic properties of a substance meaning that also effects that may occur following exposures above endogenous levels (resulting from production in skin or from intake via food and food supplements) are considered. We understand the concern for any downstream consequences a decision on classification may bring. However, in our view, classification must be based on the conclusions made from a scientific assessment without taking any downstream consequences into account. These should rather be handled in separate processes (e.g. possibility to derogate from criteria in different legislations).

Comments on D3 CLH DSM Position paper:

Mutagenicity:

Ames test: the basis for the proposal Muta. 2 is principally based on the in vivo comet assay. Therefore, even if the reproducible increase of revertants observed in the Ames test (which was the only one considered to be of sufficient reliability) would be disregarded, the concern still remains.
In vivo comet assay: effects were due to liver damage such as hepatocyte necrosis and mitochondrial damage in the test animals, at least hepatocyte necrosis would have been observed in the histopathological investigation. The lack of liver toxicity is indeed what raises a strong concern for genotoxicity despite the concomitant toxicity manifested as effects on bodyweight and biochemical parameters.

Carcinogenicity:

Proliferative lesions (hotspots) 8/10, hyperplastic nodules (5/10) and pheocytochroma (1/10) were observed also in the mid dose group.
In our view, there is no data clearly demonstrating that pheocytochromas arise solely in rats due to a disturbed calcium homeostasis. Therefore, it is not considered safe to exclude that tumors arising already after 26 weeks do indicate a carcinogenic potential of cholecalciferol that m be relevant for humans.

CLH proposal versus other competent bodies: it should be noted that the purposes of the different reviews differ. For risk assesmsment, it is possible to take uncertainties into consideration and a reference value can be set at a "safe level" where no effects have

been noted. Classification is based on intrinsic properties and must be valid for a range of exposure levels, also those above the physiological range.

Epidemiological data:

Undoubtedly, as cholecalciferol is an essential vitamin, exposure is beneficial for humans at certain levels. However, classification is based on the intrinsic potential for carcinogenicity, i.e. it takes into account also effects that may occur at exposure levels outside of the physiological range as a result from other uses of the substance. Therefore, if the substance also causes adverse effects, classification may be required despite beneficial properties at lowere exposure levels.

RAC's response

The comments will be taking into account for the opinion making process and are very appreciated. However, RAC works from a scientific point of view and the opinion will be on the background of the intrinsic properties of the substance. The RAC rapporteurs are fully aware of the benefits of therapeutic uses of the substance, but the assessment and possible labeling must cover all uses of the substance and not include a risk assessment. The endpoints for mutagenicity and carcinogenicity are cases where we are aware of the data underlying is debatable and will be so in plenary. All argumentations are specified in the opinion.

Date	Country	Organisation	Type of Organisation	Comment number
07.03.2016	United Kingdom	European Medicines Agency	EU Agency	2

Comment received

General comment on the use VitD3 in medicines and consistency of labelling

Vitamin D3 is used in veterinary medicinal products, including for food producing animals, to correct vitamin D3 deficiency. In relation to its use in food producing animals, vitamin D3 has a "No MRL required" classification (in Regulation No. 37/2010) indicating that numerical MRLs were not considered necessary for the protection of human health. Vitamin D3 is also widely used in human medicines for prevention and treatment of vitamin D3 deficiency.

We are concerned that, from the public's point of view, classification of colecalciferol as Muta. 2 and Carc. 2 may be confusing as such a classification could be considered inconsistent with existing therapeutic uses of the substance. We therefore encourage ECHA to ensure that the relevant documents adequately address this.

Committee for Medicinal Products for Human Use - Safety Working Party Discussion

- **CLH Report from Swedish Chemicals Agency for Cholecalciferol (Vitamin D3)**

Two toxicological issues have been raised by the CLH report, i.e. on (germline) genotoxicity, and on (human relevant) carcinogenicity.

In the sections below we first discuss the Genotoxicity, and then the Carcinogenicity of cholecalciferol.

- **Safety Working Party (SWP) comments to proposed classification as "category 2 germ cell mutagen"**

Background

The assessment of the genotoxic potential of cholecalciferol to support the proposed classification as a category 2 germ cell mutagen according to Regulation 1272/2008 *("Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans"*) is based on an *in vitro* 3-test battery and one *in vivo* study.

In vitro tests:

- Ames test (TA98, 100, 1535, 1537, E.coli WP2 uvrA)
- Mammalian cell gene mutation assay (Mouse Lymphoma L5178 TK assay)
- Mammalian cell chromosome aberration assay (Chinese Hamster V79 cells)

In vivo test:

- Combined bone marrow micronucleus (MN) test and liver/duodenum Comet assay in Wistar rats

The mammalian cell gene mutation assay and chromosome aberration test as well as the rat bone marrow MN study and the comet assay in rat duodenum showed all negative results with cholecalciferol. In contrast, the Ames test and the rat liver comet assay were considered positive.

The criteria for a 'category 2 germ cell mutagen' according to Regulation 1272/2008 read:

The classification in Category 2 is based on 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.

The latter criteria (last bullet) are considered fulfilled since cholecalciferol was positive in the comet assay ("other *in vivo* somatic genotoxicity test") supported by positive results from an Ames test ("*in vitro* mutagenicity assay").

SWP assessment of genotoxic potential of cholecalciferol (& derivatives)

Ames test

According to the CLH Report positive mutagenic effects with cholecalciferol in the Ames test (study IIIA 6.6.1/01) were identified for two of the five tester strains, namely TA 100 and TA1535. However, the evaluation of the findings with TA 1535 as positive is debatable. There were three runs without S9 and two runs with S9 using TA1535. A weak increase at the highest concentrations (3750 and 5000 µg/pl) was seen in one experiment without S9 but this was not confirmed in the two other experiments. With S9 a borderline effect was seen in TA 1535 at the highest concentration in one experiment but the second experiment was completely negative. The overall evaluation of the results with TA 1535 is therefore considered as negative.

Both experiments without S9 in TA 100 were negative up to 5000 µg/pl whereas a 2-3-fold increase in number of revertants were observed in both experiments with S9 at the highest concentration. These weak effects in TA 100 can be considered as a borderline positive finding.

In another Ames study conducted by the NTP (http://ntp.niehs.nih.gov/) cholecalciferol was tested at concentrations from 33 up to 10000 µg/plate using the S. typhimurium strains TA1535, TA1537, TA97, TA98

and TA100 in the absence and presence of rat or hamster liver S9 (Mortelmans et al., 1986). The study was completely negative and thus does not confirm the borderline effects seen in the new CLH Report study. The NTP study was considered as a non-key study of low reliability in the CLH Report because of deficiencies in reporting. However, according to our evaluation Ames study protocols used by NTP are fully acceptable and the study outcome was reported in sufficient detail so that the negative NTP results are considered as reliable and valid findings.

Further (unpublished) results from GLP-compliant Ames studies with cholecalciferol metabolites and analogues have been submitted for marketing authorization to EU regulatory authorities including 1-hydroxy-cholecalciferol, 24,25-dihydroxycholcalciferole, or calcipotriol (a synthetic calcitriol derivative). All Ames studies were negative.

Overall, the data from several studies in the Ames test provide sufficient evidence to conclude that cholecalciferol and closely related derivatives do not induce relevant mutagenic effects in the bacterial gene mutation assay.

Mammalian cell gene mutation assays

The lack of significant mutagenic activity of cholecalciferol (& derivatives) in bacterial assays (Ames test) is further confirmed by clearly negative findings in a number of mammalian cell gene mutation assays. Negative results in the mouse lymphoma TK gene mutation assay are reported for cholecalciferol (CLH Report), 1-hydroxy-cholecalciferol and calcipotriol (both from regulatory submissions).

Tests for chromosomal aberrations in vitro and in vivo

All in vitro and in vivo studies with cholecalciferol and two derivatives (24,25-dihydroxycholcalciferole and calcipotriol) for testing chromosomal aberrations/ micronuclei were consistently negative suggesting that these compounds are non-clastogenic.

In vivo Comet assay

After treatment of male Wistar rats with cholecalciferol at 0, 3.75, 7.5 and 15 mg/kg bw/day for three days p.o. the comet analysis in cells from duodenum showed no increased DNA migration. However, in the liver an increase in % tail intensity and tail moment was observed at the two highest dose levels. The increases were statistically significant when compared to the concurrent vehicle control group and was also exceeding the historical control range.

The positive rat liver comet assay result is unexpected given that all other genotoxicity assays with cholecalciferol and derivatives did not provide evidence for a relevant genotoxic potential (see above). The comet findings are therefore most likely due to a secondary effect either related to the pharmacology (e.g., Ca-activated nucleases that may have caused DNA strand-breaks) or toxicity (comet effects occurred at doses above the MTD). More research would be needed to explore the underlying mechanism of increased DNA migration in rat liver cells. A direct DNA damaging through a DNA-reactive liver-specific metabolite (as seems to be suggested by the Swedish dossier submitter) is considered unlikely as cholecalciferol (& -metabolites) are human endogenous hormones present chronically at significant systemic physiological levels.

Randomized clinical trial to test the effect of vitamin D3 on a marker of oxidative DNA damage in humans (Fedirko et al. 2010)

A pilot, randomized, double-blind, placebo-controlled clinical trial was conducted to test the effect vitamin D3 supplementation at 20 µg/day for 6 months on a marker of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine, in the normal colorectal mucosa. The results suggest that vitamin D3 may decrease oxidative DNA damage and provide support for further investigation of vitamin D3 as chemopreventive agent against colorectal neoplasms.

SWP's conclusion on (germ cell) Genotoxicity

Based on the weight-of-evidence assessment of the available genotoxicity data it is concluded that cholecalciferol has no relevant mutagenic and/or genotoxic potential. A classification of cholecalciferol as a "substance which causes concern for humans owing to the possibility that it may induce heritable mutations in the germ cells of humans" (category 2 germ cell mutagen) is not supported.

- **SWP comments to proposed classification as "possible human carcinogen"**

Carcinogenicity

In the framework of the International Conference (now Council) on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use an international discussion is ongoing on the need for conducting life-time carcinogenicity studies in rats. Part of the work is related to a retrospective analysis of a large number of rat carcinogenicity studies on human pharmaceuticals. Data are gathered from PhRMA (192 compounds), FDA (44 compounds), and JPMA (63 compounds). A paper has been written by members (and a former member) of the SWP to analyse the relationship between the pharmacological class of compounds and the specific pattern of tumours that are observed in the rat carcinogenicity studies of all these compounds.

The paper has been submitted for publication and has been accepted for publication.

Four Vitamin D analogues are included in this dataset and all were found to induce pheochromocytomas in the adrenal gland, and no other tumours have been reported.

The authors put this into perspective of data from general literature. Tischler and DeLellis (1988) described the proliferative lesions in adrenal medulla. The group observed the effect of VitD3 stimulating chromaffin cell proliferation in this organ. Also Ikezaki et al (1999) reported medullary hyperplasia and pheochromocytoma of the adrenals in rats after 57 weeks treatment with 24R,25-dihydroxyvitamin D3. Using PCNA staining the indices for intact adrenal medulla, medullary hyperplasia and pheochromocytoma in the treated group were all higher than that for the adrenal medulla in the control group. However, no change in serum calcium had been observed with that dose, suggesting that rats may be too sensitive for results to be relevant to human risk assessment. Tischler et al (1999) have described already an increase in BrdU labelling in the adrenal medulla of rats treated with Vit D3 since after treatment of 4 weeks , and also after week 26 proliferative lesions were found.

The human relevance of this effect on the adrenal medulla is likely to be low as pheochromocytoma is reported only once in a population of 700 patients with hypercalcaemia (Roe and Bar, 1985). However, based on the mechanism of action – i.e. nuclear receptor mediated cascades - adverse cell proliferative responses cannot be totally excluded.

Williams et al (2014) also discussed the human relevance of pheochromocytomas. Pheochromocytomas might be caused by hypoxemia, stimulating catecholamine secretion from the adrenal medulla. Chronic endocrine hyperactivity may lead to compensatory hyperplasia and neoplasia, as discussed above. Agents include lactose, sugar alcohols, and Ca2+. Pheochromocytomas through this type of interference are most likely not relevant for humans. (Greim et al, 2009)

SWP conclusion on Carcinogenicity:

VitD3 and its analogues are likely to induce adrenal pheochromocytomas in rats after long-term administration. These tumours are most likely associated with the effects of cholecalciferol and its analogues

on Ca2+ metabolism. In this case of well documented secondary pheochromocytomas, the experimental conditions in animals have little relevance for humans. Therefore such findings are not considered to be relevant for classification.

References:

- Greim H, Hartwig A, Reuter U, Richter-Reichhelm HB, Thielmann HW (2009). Chemically induced pheochromocytomas in rats: Mechanisms and Relevancefor risk Assessment. Crit. Rev. Toxicol. 39: 695-718.
- Ikezaki S, Nishikawa A, Furukawa F, Tanakamaru Z, Nakamura H, Mori H, Hirose M. (1999) Influences of long-term administration of 24R,25-dihydroxyvitamin D3, a vitamin D3 derivative, in rats. J. Toxicol. Sciences 24: 133-139
- Roe FJC, Bar A (1985) Enzootic and epizootic adrenal medullary proliferative disease of rats; influence of dietary factors which affect calcium absorption. Human Toxicol, 4: 27-52.
- Tischler AS, DeLellis RA (1988) The rat adrenal medulla. II Proliferative lesions. J.Am.Coll.Toxicol. 7, 23-44
- Tischler AS, DeLellis RA, Nunnemacher G, Wolfe HJ (1988) Acute stimulation of chromaffin cell proliferation in the adult rat adrenal medulla. Lab Invest. 1988 Jun;58(6):733-5.
- Tischler AS, Powers JF, Pignatello M, Tsokas P, Downing JC, McClain RM (1999) Vitamin D3-induced proliferative lesions in the rat adrenal medulla. Toxicological Sciences 51: 9-18
- Williams GM, Iatrapoulos MJ, Enzmann HJ, Descl UF. (2014) Carcinogencity of Chemicals. Assessment and human extrapolation. In: Hayes' Principles and Methods of Toxicology. 6th Edition. Hayes W, Kruger CL, Eds). Taylor & Francis, CRC Press. Pp.1251-1304.

ECHA note: The comment above was originally submitted as separate attachment.

Dossier Submitter's Response
Please note our response to comment 1. *Regading comments on the in-vivo assay:* the only tissue investigated besides the liver was the duodenum. Since this organ is exposed to the substance prior to the hepatic metbolism takes place (see section on toxicokinetics) we propose that this may explain why a mutagenic response was observed in the liver but not in the duodenum.
RAC's response
Noted and very appriciated. Please note our response to comments no. 1.

Date	Country	Organisation	Type of Organisation	Comment number
03.03.2016	Germany		MemberState	3
Comment received				
Hazardous to the aquatic environment Table 7, page 7: It is stated that the hazard class "Hazardous to the aquatic environment" is not assessed in the current CLH dossier. However, as Colecalciferol is a biocidal active substance, according to article 36 (2) CLP Regulation all hazard classes are subject to a harmonized classification and labelling. The current existing Annex VI entry does not cover environmental hazards. As this harmonized classification was probably introduced before the CLP Regulation came into force, the basis for this non-classification is not clear (based on available data, due to lack of data or not known); some more information about the history of this classification would be appreciated. Colecalciferol is currently under review as a new biocidal active substance according to Regulation EU No 528/2012 and therefore ecotoxicological data for classification purposes is available. At least the new criteria for the long-term aquatic hazard assessment introduced with (EU) No 286/2011 (2. ATP) should be checked and the result documented in the current CLH dossier.				

Dossier Submitter's Response
We certainly agree that a decision on classification covering all hazard is preferable, however, the currently available resources for CLP restrict our contribution to hazard classes for human health since these are crucial for the decision on approval under the BPR and/or for concluding on any user restrictions for the approval of products containing the active substance.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
03.03.2016	United States		Individual	4
Comment received				
As the principal investigator on the paper Tischler A.S., Powers J.F., Pignatello M., Tsokas P., Downing J.C. and McClain R.M. (1999) Vitamin D3-Induced Proliferative Lesions in the Rat Adrenal Medulla. Toxicological Sciences 51, 9-18, I strongly suggest that the paper offers no evidence that vitamin D3 is a carcinogen. A large volume of literature demonstrates that rats as a species are uniquely susceptible to induction of pheochromocytomas by dietary factors, drugs and other agents that do not cause tumors in humans or mice. Most of the implicated agents are not mutagenic and are believed to act indirectly. The purpose of the above study was to test the hypothesis that pheochromocytomas in rats can be caused by perturbations of calcium homeostasis. The hypothesis was based on previous studies in which rat pheochromocytomas were associated with several non-toxic food additives. Our study was done with very high doses of vitamin D3 in order to more markedly perturb calcium balance. The rats with pheochromocytomas received the highest doses of vitamin D3. Consequently, all had severe kidney disease, which has itself been associated with pheochromocytomas in some rat studies (Nyska, A., Haseman, J.K., Hailey, J.R., Smetana, S., and Maronpot, R.R. (1999). The association between severe nephropathy and pheochromocytoma in the male F344 rat – the National Toxicology Program experience. Toxicol. Pathol. 27, 456-462). Further, almost all of the adrenal medullary lesions in the study were hyperplastic foci, not pheochromocytomas, and none of the lesions was malignant.				
Dossier Submitter's Response				
Thank you for further clarifying the aim of the study. Since classification is based on the intrinsic potential for carcinogenicity, the use of doses above the physiological range and a plausible Mode of Action (MoA) exerted via calcium perturbation do not necessarily invalidate findings. However, conclusive evidence for a MoA without relevance for humans could justify a decision not to classify. Since cholecalciferol was positive in an in vivo comet assay, we do not find it safe to exclude a genotoxic mode of action. This is discussed in more detail in section 10.9.1 of the CLH report. As also discussed in the CLH report, we are fully aware of the existence of a normal background of pheochromocytomas in aging male rats. In this study, tumours occurred already after 26 weeks exposure and adrenal chromaffin cell proliferation was observed in both males and females in a separate 90 day study. The frequencies differed from the concurrent controls.				
RAC's response				
Noted. Please note our response to comment no. 1. With regards to the assumed association between CPN and pheochromocytomas, Nyska et al. (1999) found a link between severe CPN and a higher tumour incidence only in control				

animals. This association was not found for substance-related pheochromocytomas. 6 out of 125 chemicals produced increased incidences of pheochromocytomas without showing an increase in CPN severity. 28 out of 125 substances produced higher severity CPN (> 0.5 in mean than controls), only 3 of them showed an increase in pheochromocytomas.

With regards to colecalciferol, there is no evidence on spontaneous CPN in the available long-term studies on colecalciferol and 24,25-dihydroxcholecalciferol. Thus there is no link to a role of spontaneous CPN.

In general, an association between CPN (irrespective of its cause) and pheochromocytomas appears biologically plausible, as severe CPN can result in disturbed calcium/phosphorus homeostasis and secondary hyperparathyroidism. This does not mean that substance-related calcium dysbalance-mediated pheochromocytomas in rats are meaningless for humans.

Date	Country	Organisation	Type of Organisation	Comment number
03.03.2016	Israel		Individual	5
Comment received				

As the principal investigator on the papers:
Nyska, A., Haseman JK, Hailey JR, Smetana S, Maronpot RR. The association between severe nephropathy and pheochromocytoma in the male F344 rat - the National Toxicology Program experience, Toxicol. Pathol., 27, 456, 1999
Ozaki, K., Hailey JR, Maronpot RR, **Nyska A**. Association of adrenal pheochromocytoma and lung pathology in inhalation studies with particulate compounds in the male F344 rat - The National Toxicology Program experience, Toxicol. Pathol., 30, 263, 2001.
I strongly believe that my investigations are suggesting that pheochromocytoma in rats are caused by dietary factors, drugs and other agents that do not cause tumors in humans or mice. Most of the implicated agents are not mutagenic and are believed to act indirectly.
In my two retrospective evaluation of the National Toxicology Program (NTP) database, I demonstrated the potential involvement of indirect mechanisms in the adrenal medullary proliferative response in the male F344 rat.
In the first retrospective study, the potential chromafin proliferative effect induced by chronic renal disease, was tested (Nyska et al, 1998). Chronic progressive nephropathy (CPN) is a commonly occurring spontaneous disease in aging F344/N rats. Severity of CPN is greater in males than in females. Chronic renal failure is known to be associated with the inability to secrete phosphate, resulting in hyperphosphatemia and reduced production of the active metabolite of vitamin D, due to decreased numbers of nephrons, and hypocalcemia, due to decreased calcium intestinal absorption. In severe cases of CPN in rats, associated with disturbed calcium/phosphorous homeostasis, chronic stimulation of the chromaffin cells to proliferate may result, which may eventually lead to hyperplasia and neoplasia.
The retrospective analysis indicated that there was no definitive cause-and-effect relationship between pheochromocytoma incidence and severity of CPN, and a suggestion was made that more work is required to better understand this correlation and what impact, if any, CPN may have on the interpretation of experimental results (Nyska et al, 1998)..
In the second retrospective study, the potential effect of space-occupying, toxic-related pulmonary disease, on the incidence of pheochromocytoma, was tested (Ozaki et al, 2002). The NTP performed several 2-year inhalation studies in F344 rats to evaluate the effects of particulate compounds. The results demonstrated variably extensive pulmonary

inflammatory lesions and/or lung tumors and significantly increased incidences of adrenal medullary hyperplasias and pheochromocytomas induced by several compounds in males and females. Retrospective evaluation of 9 of these studies in male F344 rats revealed significant ($p < 0.01$) associations of pheochromocytoma with the severity of inflammation and fibrosis in the cases of nickel oxide, cobalt sulfate, indium phosphide, talc, and nickel subsulfide. Studies of gallium arsenide, vanadium pentoxide, molybdenum trioxide, and nickel sulfate hexahydrate revealed an increased incidence and/or severity of nonneoplastic lung lesions, but no increased incidence of pheochromocytoma. However, pheochromocvtoma, was significantly correlated ($p < 0.01$) with the severity of pulmonary fibrosis and inflammation per se in the gallium arsenide and molybdenum trioxide studies. In the studies of vanadium pentoxide and nickel sulfate hexahydrate, no relationship between nonneoplastic lung lesions and pheochromocytoma was manifested. Retrospective investigation therefore supported possible roles of pulmonary fibrosis and inflammation in the induction of pheochromocytoma in some, but not all, studies of F344 male rats. In those studies in which no association between the severity of lung pathology and pheochromocytoma was noted, hypoxemia may not have been sufficiently severe and/or prolonged to promote the proliferation of adrenal medullary cells. The existence of a hypoxemic threshold, defined as a factor of severity of alveolar space occupation multiplied by the duration of reduced normal oxygenation, has been suggested (Ozaki et al, 2002). *ECHA note: The comment above was originally submitted as non-confidential attachment.*
Dossier Submitter's Response
Thank you for this information. As stated above, we do not consider the available data to convincingly prove that the pheocytochromas and hotspots observed at a higher frequency in treated animals and already after 26 weeks are caused by a mechanism that lacks human relevance.
RAC's response
Noted. Please note our response to comment no. 1. The observation regarding the role of lung fibrosis and inflammation and the hypoxia may be less relevant for the colecalciferol.

Date	Country	Organisation	Type of Organisation	Comment number
02.03.2016	France		MemberState	6
Comment received				
We agree with the classification proposal				
Dossier Submitter's Response				
Thank you for informing us about your position.				
RAC's response				
Noted.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
07.03.2016	United Kingdom	Exponent International (for BASF/BCS TF)	Company-Manufacturer	7
Comment received				
Exponent submits the publication: Bikle DB, (2014). Vitamin D and cancer: the promise not yet fulfilled. Endocrine 2014; 46:29–38.				

This publication provides evidence that cholecalciferol shows cellular actions which are expected to be protective against cancer. Cholecalciferol should not be classified for carcinogenicity. ECHA note - The following attachment was submitted with the comment above: *Bikle 2014 vitamin D cancer mechn.pdf*
Dossier Submitter's Response
In similarity with other vitamins, cholecalciferol may cause both beneficial and adverse effects depending on the dose. Safe levels where only beneficial effects are expected have been concluded under different legislations. However, the substance may also cause adverse effects which is why it is used as a biocide for rodent control. Since classification is based on the intrinsic hazard of the substance, all harmful effects, also those occurring at non-physiological exposure levels must be considered.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
07.03.2016	Germany		Individual	8
Comment received				
The submitter of the CLH report does not discuss the overall database of the colecalciferol toxicity and the historical contrl data in rats for phechromocytoma. A CLH report should take into account all available information on the classification. It is therefore suggested to revise the dossier also taking into account all available information.				
Dossier Submitter's Response				
The lack of relevant historical control data is discussed in section 10.9.1 of the CLH report. *"[…] 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."* The CLH report aims at presenting all information reasonably available and considered to be of relevance for the endpoint. This includes several repeated dose toxicity studies in animals as well as human data summarized in different reviews. To our understanding, the purpose of the public consultation is to ensure that any robust data that may have been overlooked by mistake should be brought to attention. Since no such data has been submitted, we do not see the need to withdraw and revise the CLH proposal.				
RAC's response				
Noted.				

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON COLECALCIFEROL; CHOLECALCIFEROL, VITAMIN D3

Date	Country	Organisation	Type of Organisation	Comment number
03.03.2016	Germany		MemberState	9
Comment received				
In a 26 week study with male rats phaeochromocytoma were observed in 1/10 animals at the medium and 1/9 animals at the high dose. This type of tumour is relatively common in aging male rats. However, no relevant historical control data for a 26 week study is available. Furthermore, no phaeochromocytoma were observed in the control group and at the low dose. In addition proliverative lesions including hot spots and hyperplastic nodules were observed. So, it cannot be excluded that further tumours may develop with time. It has been suggested that hypercalcemia could cause phaeochromocytoma. We would ask for more details that disturbance in calcium homeostasis results in phaeochromocytoma. However, since cholecalciferol was positive in an in vivo comet assay a genotoxic mode of action cannot be excluded. Overall, the proposed classification Carc. 2, H351 seems to be plausible.				
Dossier Submitter's Response				
Thank you. Unfortunately we have no further information to add to the discussion in section 10.9.1 of the CLH report.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
03.03.2016	United States		Individual	10
Comment received				
Please see general comment above, pertaining to Section 10.9 ECHA note: This comment refers to Comment #4 of this table.				
Dossier Submitter's Response				
Please see our response to the general comment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
02.03.2016	United Kingdom	Exponent International (on behalf of the BASF-BCS Task Force)	Company-Manufacturer	11
Comment received				
The Applicant disagrees with the proposed classification of cholecalciferol as a Carcinogen, Category 2. Under Regulation 528/2012, Cholecalciferol is intended for use as a rodenticide therefore typical rodent carcinogenicity studies would not be conducted. Moreover, considerable epidemiological evidence is available. The regulatory strategy recognised that for cholecalciferol, the epidemiological data in human is more relevant than data generated in animals. The CLH classification proposal basis is confined to the results of one published investigative experiment in rats with cholecalciferol and another but with a cholecalciferol metabolite. The proposal ignores the relevant epidemiological data available on this				

essential vitamin. Incorporation of the epidemiological and experimental data into a weight of evidence approach leads to the conclusion that no classification for carcinogenicity is appropriate.
Namely:
• Cholecalciferol is an essential vitamin (D3) in humans for its role in calcium homeostasis and skeletal health, and is primarily synthesized in the body.
• Tolerable upper intake levels have been defined by the European Food Safety Agency (2012) at 100µg/day (approximately 1.25 µg/kg/day).
• The published investigative experiment in Crl:CD rats used Cholecalciferol levels 100, 200 or 400 fold above the EFSA upper intake levels.
o The experiment design was not run according to regulatory standards i.e. not OECD test guideline compliant, lasted only 26 weeks, and compliance with GLP was not claimed.
o The mid and high doses provoked a decrease in bodyweight gain of 25-82%, marked hypercalcaemia and adrenal chromaffin cell proliferation but with only a single incidence of pheochromocytoma in each group (1/10 and 1/9 respectively).
• A single pheochromocytoma (1/20) was observed in the 1-year experiment with a single dose level of the metabolite (24R,25 (OH)2VitD3) concurrent with evidence of calcium dysregulation.
• The proliferative lesions and the benign pheochromocytoma are secondary to the known primary mode of action i.e. calcium dysregulation (hypercalcaemia). The occurrence of pheochromocytomas under toxic conditions in animal experiments is to be assessed as a secondary effect (Greim et al 2009).
Therefore these two studies are considered insufficient evidence of a primary carcinogenic effect and do not support classification for carcinogenicity under CLP.
• Epidemiological data have been recently and intensively reviewed e.g. the EFSA Panel on Dietetic Products, Nutrition and Allergies (2012) which stated 'no studies reported an association between vitamin D intake and increased risk for adverse long-term health outcomes.' Similar conclusions are reached by UK Scientific Advisory Panel on Nutrition (SACN, draft 2015). None of these reviews conclude an increased cancer risk.
• There is increasing information about the possible role of Vitamin D3 in cancer prevention in humans:
o intervention and observational epidemiology studies indicate protective or no associations with overall and site-specific cancer risks (i.e., not an increased hazard)
o these extensive epidemiological data on cholecalciferol cover numerous studies, involving hundreds of thousands of human subjects in total
o numerous mechanistic studies in vitro show anti-proliferative and pro-differentiation effects thought to be protective against cancer
o Cholecalciferol remains under study in numerous clinical trials for various health benefits including cancer protection.
Since cholecalciferol (vitamin D3) is beneficial to health at physiological exposure levels, and appears possibly protective of cancer at physiological dose levels, the animal tumours (of secondary origin, seen at excessive doses irrelevant to human, without statistical significance, in studies inadequate for carcinogenicity assessment) are not reason for classification. The extensive epidemiology data (of high relevance to human), together with mechanistic data supporting a protective effect, are considered sufficiently meaningful that classification for carcinogenicity is not appropriate. These issues are explained in clear detail in Exponent document 1408446.uk0-7619, which is attached.

ECHA note - The following attachment was submitted with the comment above:
1408446 UK0 - 7619 Carcinogenicity WoE evaluation_v4.docx

Dossier Submitter's Response
Undoubtedly, as cholecalciferol is an essential vitamin, exposure is beneficial for humans at certain levels. However, classification is based on the intrinsic potential for carcinogenicity, i.e. it takes into account also effects that may occur at exposure levels outside of the physiological range that may result from other uses of the substance. To a reasonable extent, the epidemiological data available has been included and discussed in the CLH report. However, since this data is poorly described and since it considers exposures comparable to the physiological range (rather than a range from no/little toxicity to the MTD as is requested in guidelines to assess carcinogenic potential), this data is not considered sufficiently robust to overrule the findings observed already after 26 weeks in rats. *Regarding conclusions made in other regulatory reviews:* it should be noted that these reviews were made for a different purpose and they do not dicuss the need for classification. For risk assessment purposes, it is possible to take uncertainties into consideration and a reference value can be set at a "safe level" where no effects have been noted. However, classification is based on intrinsic properties and must be valid for a range of exposure levels, also those above the physiological range. *Regarding deficiencies in the study by Tischler:* we agree that the study has several short-comings when used to assess carcinogenicity. However, despite a low number of animals and a short exposure time, proliferative lesions and/or pheochromocytoma were yet observed in treated animals and we do not find it safe to disregard these in the context of classification. In our view, even if pheochromocytoma would be a secondary effect, it would still be an effect of exposure to cholecalciferol thus we consider the major issue to be whether or not there is evidence to exclude relevance for humans. Please also note our response to comment 4.
RAC's response
Noted. Please see our response to comment no. 1.

Date	Country	Organisation	Type of Organisation	Comment number
29.02.2016	Netherlands		MemberState	12
Comment received				
• Human studies do not suggest an association between exposure to vitamin D3 and cancer at doses comparable with or slightly above the supplement range. No information is available for higher doses. • In the 26 week rat study proliferative adrenal lesions were observed in all dose groups (dose dependent) and hyperplastic nodules and hotspots in the adrenals were observed in the mid and high dose groups (5/10 and 7/9). It is noted that the high dose group in the 26 week study exceeds the MTD, as is indicated by a ≥ 10% reduction in body weight gain. • The only increase in tumours observed is a low number of pheochromocytomas in the 26 week rat study (1 in mid dose and 1 in high dose group, none in controls) and the 57 week rat study (1 versus 0 in controls). Again, it is noted that the high dose group in the 26 week study exceeds the MTD. In addition, there is a high background frequency of adrenal phaeochromocytomas in male rats. An increase in low number of tumours at a young age was observed which was not statistically significant. However, due to the observed mitogenic stimulation of the adrenal and presence of hyperplastic nodules in combination with other carcinogenicity studies with sugar and sugar alcohols showing that alteration of the calcium homeostasis results, as observed for Vitamin D3, in an increase of pheochromacytomas in the rat (RIVM report 601516), the low increase is considered			

some evidence of a substance induced increase in carcinogenicity. Although, the relevance of this mechanism to humans is doubtful seen the high spontaneous background at old age it has not been shown that it is not relevant. The mechanisms for induction of pheochromacytomas in rats via alteration of the calcium homeostasis can also be expected in humans (Greim, 2009). Therefore, Cat 2 is considered correct.

Dossier Submitter's Response

Thank you for sharing your line of reasoning.

RAC's response

Noted (and see above the response to comment 4 regarding the CPN).
Please note the small group sizes (1 tumour in 9 and 10 animals, respectively) in the Tischler study. The BW gain was lower (30-35%) in the high dose group (no data on food consumption found), without any evidence on systemic toxicity (clinical signs or organ toxicity) was seen in the animals. Thus, the data at the high dose are considered as relevant, in particular as the cell proliferation/hot spots/hyperplasia started at the lowest dose tested where BW gain was normal or slightly reduced.

Date	Country	Organisation	Type of Organisation	Comment number
24.02.2016	Switzerland	DSM Nutritional Products AG	Company-Manufacturer	13

Comment received

Reference to section 10.9, page 32
The proposal for considering vitamin D3 as a carcinogen (category 2) is based on two published studies (Ikezaki et al., 1999, Tischler et al., 1999) where vitamin D3 or a downstream metabolite (24R,25-dihydroxy vitamin D3) were tested in male rats. A low incidence of adrenal medulla tumors (pheochromocytoma) was noted in the treated groups at high dose levels only.
The dose levels used in the Tischler study with vitamin D3, produced severe systemic toxicity as evident by mortality, no or only very limited body weight gain, and hypercalcemia. The applied dose levels clearly exceeded the MTD and are therefore of limited relevance for classification and labelling (CLP 2015).
Pheochromocytoma have a high background incidence in rats and the low incidence seen in this study is certainly within the normal biological variation rather than a biologically relevant increase in tumor incidence.
In the Ikezaki study, a different test substance was used. The test substance is 24R,25-dihydroxy vitamin D3, CAS 126356-63-6 a down-stream metabolite of vitamin D3. Oral dosing of this substances produces a qualitative different plasma metabolite profile as compared to the metabolite profile after dosing vitamin D3. Vitamin D3 metabolites like 25-hydroxy vitamin D3 or 1,25-dihydroxy vitamin D3 cannot be produced upon oral administration of 24,25-dihydroxy vitamin D3 (Shepard & DeLuca 1980, DeLuca 1986). We therefore believe that the use of data for this down-stream metabolite is not justified to evaluate the hazard of vitamin D3. Further the respective study does not comply to any testing guideline, is non-GLP, and thus has a low reliability.
Pheochromocytoma induction in rats due to disturbed calcium homeostasis is a species specific mode of action for rats. This mode of action has no relevance for human as already discussed by other competent bodies like the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1997).
It has been demonstrated that vitamin D3 is non-carcinogenic in human being. Human data provide no evidence of increased tumor incidences in relation to high doses of 100 µg/d vitamin D3 supplementation even in susceptible population (Marshall et al., 2012). 100 µg/d is the agreed Upper Level of vitamin D3 in adults (EFSA, 2012). A recent meta-

analysis indicates that cancer occurrence in vitamin D3 supplemented groups is identical to the non-vitamin D3 supplemented control groups (Bjelakovic et al., 2014). Likewise, human being is exposed ever since to vitamin D3 due to vitamin D3 production in skin. The produced amount is equivalent to high oral doses (> 100 µg/d).
We therefore conclude that the available data for vitamin D3 do not justify classification as a carcinogen category 2 according to CLP requirements (CLP 2015). For further details see attached DSM document.

ECHA note - The following attachment was submitted with the comment above:

- *D3 CLH DSM Position paper 22_02_2016_FINAL_submitted.pdf*
- *DSM 2015.pdf*
- *DSM 2016.pdf*

Dossier Submitter's Response

The proposal for classification carcinogenicity category 2 is mainly based on the findings in the 26 week study which is considered to be supported by proliferative changes observed in the 90 day study and findings in the 57-week study.
With respect to other arguments made, please note our response to previous comments.

RAC's response

Noted. Please see our response for comment no. 1. In addition the points raised in this comment were reflected in the assessment and comparison with the classification criteria of the Opinion Document.

Date	Country	Organisation	Type of Organisation	Comment number
20.02.2016	United States		Individual	14

Comment received

I am an expert on the role of vitamin D and health. In my role as a university faculty member I educate students on the role of vitamin D and health. I am also called upon to provide expert advice on vitamin D and health to consumers, companies, government agencies, and other groups. My research interest is in the nutritional uses of vitamin D to support normal whole body calcium homeostasis and bone health. I also conduct research on the potential cancer chemoprotective effects of dietary vitamin D. Regulatory decisions on either the utility or potential risks of vitamin D have an impact on my communications with students, lay people, and granting agencies.

To me, the issue with the report is that the Tischler study shows proliferative adrenal gland lesions in rats using levels that are at least 500X higher than those that are in a commercial rodent chow diet. The paper clearly shows that these long-term effects (after 26 wks) are accompanied by classical symptoms of vitamin D toxicity (hypercalcemia, hypercalciuria, soft tissue calcification). If the doses are translatable directly to humans, the human equivalent dose for this effect would be >300,000 IU per day. As a result, my position is (1) the observations in this one paper about increased adrenal lesions should be verified before they are given credence in the form of regulatory guidelines) and, (2) the doses examined are irrelevant to human health because those levels can't be reached by consumers with available products. As a result, the findings have very little regulatory meaning. It is also critical to recognize that by placing vitamin D3 in classification level 2, the ECHA could scare the public unnecessarily and lead them to avoid the use of health protective doses of vitamin D as a supplement (600-5000 IU/d) or a medical intervention (e.g. the controlled dosing strategies that include monthly loading doses of up to 100,000 IU).

Dossier Submitter's Response
Undoubtedly, as cholecalciferol is an essential vitamin, it is beneficial for humans at certain levels. However, classification is based on the intrinsic potential for carcinogenicity, i.e. it takes into account also effects that may occur at exposure levels outside of the physiological range that may result from certain uses of the substance. Therefore, if a substance also causes adverse effects, classification may be required even if it also has beneficial properties. We understand the concern that the classification proposed may scare the general public however it must be assumed that the general public understands the difference between recommended doses and excessive doses. Likewise, the general public could be alarmed by the existing classification STOT-RE 1 and labelling GHS08 "danger".
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
09.03.2016	Germany	BASF SE, Nutrition & Health	Company-Manufacturer	15
Comment received				
We disagree with the proposed classification of Cholecalciferol as a Carcinogen, Category 2. The CLH classification proposal is based on the results of one published investigative experiment in rats with Cholecalciferol and another with a Cholecalciferol metabolite (Tischler et al., 1999 and Ikezaki et al., 1999). We consider these two studies not to be adequate for classification of Cholecalciferol as a carcinogen Cat 2. In the 26-week study of Tischler et al., 10 male rats/dose received by gavage daily doses of Cholecalciferol at 0.125, 0.25, or 0.5 mg/kg bw/day which is equivalent to 100, 200 and 400-fold the human upper limit of intake of 100 µg/day (EFSA 2012). The study was not intended as a study for carcinogenicity but was designed as a mechanistic study to investigate the effect of Cholecalciferol on adrenal proliferative changes and progression. Particularly, the number of animals was small (only 10 male rats per group instead of 50 animals/sex/group as required by the OECD Guidelines for carcinogenicity). There was no histology examination in any other organs except the duodenum and the overall tumour incidence in these animals was not measured. Results showed marked decreased body weight gain (by more than 80 and 25% at the high and mid dose), markedly increased of blood calcium and phosphorus levels and urinary/calcium creatinine ratio mainly at the two highest doses levels. So, the doses were by far exceeding the MTD limit of 10% and higher than a dose that would be tested in a rat carcinogenicity study. Histopathology examination revealed dose-related changes in the two highest dose groups in the two organs examined, i.e. kidney (nephrocalcinosis) and adrenals (increased incidence of hyperplastic nodules and one pheochromocytoma in each of the high and the middle dose groups). There was no pheochromocytoma at the lowest dose level which did not exceed the MTD. Tischler et al. (1999) specifically discussed that the pheochromocytoma associated with Cholecalciferol in rats do not represent a risk to man. The authors suggested that the rat adrenal medulla to be unusually susceptible to perturbation of calcium homeostasis and considered the proliferative effect of Cholecalciferol on the rat adrenal medulla to be secondary. In addition, neither Cholecalciferol nor the active metabolite 1,25-(OH)2-D3 induced chromaffin cell proliferation in vitro. Isobe (2012), using infusion of a calcium salt, then demonstrated adrenal medullary cell proliferation is stimulated by calcium. This supports the pheochromocytoma to occur by a secondary process, making the relevance				

to classification questionable.
In the review article of Greim et al. (2009), it was noted that pheochromocytomas occur with relatively higher frequency in male rats, especially when conditions such as hypoxia, uncoupling of oxidative phosphorylation, disturbance in calcium homeostasis, and disturbance of the hypothalamic endocrine axis are involved. The underlying biochemical mechanisms suggest that other substances that interfere with these biochemical endpoints also produce pheochromocytomas. To date, there is no indication that the substances inducing pheochromocytomas in animal experiments also induce corresponding tumours in humans. In the analysis of Greim et al. which included the Tischler et al (1999) study such secondary pheochromocytomas are considered not to be relevant for classification and human risk assessment. This view is also in line with the more recent expert review by Edler et al (2014) on carcinogenicity risk assessment in which the human relevance of specific rodent tumour types and the modes of action by which they can be induced was discussed.
In the second study (Ikezaki, 1999), 20 male rats/dose were administered 24R,25-dihydroxy Cholecalciferol (24R,25(OH)2D3), which is a derivative of Cholecalciferol, at a single concentration of 5 ppm in the diet (ca. 0.23 mg/kg bw/day) over a period of 57 weeks. 24R,25(OH)2D3 can be formed in conditions of Cholecalciferol excess as an alternative (detoxification) pathway. Similarly to the Tischler study, the experiment was performed with the objective to assess the influence of 24R,25(OH)2D3 on calcium homeostasis and the occurrence of adrenal proliferative lesions. This investigative experiment was not intended as a study for carcinogenicity and suffers from several limitations from a regulatory point of view (number of animals too low, only one dose level tested, no background tumour data, extent of histopathological examinations). In addition, the relevance of findings for a metabolite of cholecalciferol studied at excessive doses is doubtful for the substance itself.
The treatment had no effects on body weight, on food consumption and on serum calcium, although urinary calcium was well increased above the control levels since the 3rd week of administration. Femur and adrenal medulla weight were increased and associated with histopathology findings, e.g. thickening of cortical bones in the femurs, increased PCNA labelling indices, medullary hyperplasia (6/20 rats) and a single benign pheochromocytoma (1/20 rats) in the adrenals.
The CLH-report notes that the benign pheochromocytoma occurred in the absence of hypercalcaemia (serum calcium was only investigated at study termination). However, hypercalcuria was shown, and disturbance of calcium levels was also implied from thickening of cortical bone. It has been shown, that pheochromocytoma were attributed to disturbed calcium homeostasis in the case of the sugar alcohols also in the absence of hypercalcaemia (Lynch et al., 1996). Similarly, to the results in the Tischler study, the adrenal proliferative changes observed can be attributed to a secondary mechanism (disturbance of calcium regulation) to which the rat is particularly sensitive and this mechanism should not be the basis for classification of Cholecalciferol as a carcinogen Cat 2.
In the CLP Guidance (Vers. 4.1, June 2015, page 375 above), it is noted that
" There are several reasons why a tumour observed in animals may be judged to be not relevant for humans or may be judged to be of lower concern. In most of these cases the tumour arises via a mode of action which does not occur in humans (see this Section part k). In some cases the tumour may arise in a tissue known to be overly susceptible in the species tested to development of certain tumours and consequently may be judged to be less relevant for humans."
Furthermore, in the CLP Guidance, pheochromocytomas in male rats exposed to particulates through inhalation secondary to hypoxemia (see page 381, reference Ozaki et al., 2002) are mentioned as an example of a mechanisms of tumour formation considered not relevant to humans. In analogy, the calcium dysregulation induced by high doses of

Cholecalciferol may be seen as a secondary mechanism where adrenal proliferative changes are induced by an indirect, chemically-unspecific effect in an oversensitive species.
Finally, Cholecalciferol does not pose a concern with respect to genotoxicity as the overall biological significance of the weak effects observed in the Comet Assay at excessive dose levels above the MTD is considered to be biologically irrelevant. Therefore, we do not see evidence that Cholecalciferol has a carcinogenic potential caused by a mutagenic activity in vivo.
In conclusion, we consider the findings of the two mechanistic studies, one of which was conducted with a derivative of Cholecalciferol, not relevant with respect to carcinogenicity classification for the following reason:
• The investigative non-guideline, non-GLP experiment in male rats used Cholecalciferol levels 100, 200 or 400 fold above the EFSA upper intake levels.
• The mid and high doses induced a decrease in bodyweight gain of 25-82%, marked hypercalcaemia and adrenal chromaffin cell proliferation but with only a single incidence of pheochromocytoma in each group (1/10 and 1/9 respectively).
• A single pheochromocytoma (1/20) was observed in the 1-year experiment with a single dose level of the derivative 24R,25 (OH)2VitD3 with evidence of calcium dysregulation.
• The proliferative lesions and the benign pheochromocytoma are considered secondary to the known primary mode of action i.e. calcium dysregulation (hypercalcaemia). The occurrence of pheochromocytomas under toxic conditions in animal experiments is to be assessed as a secondary effect (Greim et al. 2009).
Therefore these two studies should not be used for classification of Cholecalciferol as a carcinogen Cat. 2. By contrast, human data shows that Cholecalciferol is not known to be carcinogenic:
• Cholecalciferol is an essential vitamin (D3) in humans for its role in calcium homeostasis and skeletal health and Tolerable upper intake levels have been defined by the European Food Safety Agency (2012) at 100µg/day.
• Epidemiological data have been recently and intensively reviewed e.g. the EFSA Panel on Dietetic Products, Nutrition and Allergies (2012) which stated 'no studies reported an association between vitamin D intake and increased risk for adverse long-term health outcomes.' Similar conclusions are reached by UK Scientific Advisory Panel on Nutrition (SACN, draft 2015). None of these reviews concluded an increased cancer risk.
Therefore and in view of all available data from vivo animal studies and human data, we do not see a concern for carcinogenicity and classification of Cholecalciferol as a carcinogen is not justified.

References:
Greim et al. (2009). Chemically induced pheochromocytomas in rats: mechanisms and relevance for human risk assessment. Critical Reviews in Toxicology, 2009; 39, 8, 695 - 718

Edler et al. (2014). Selection of appropriate tumour data sets for Benchmark Dose Modelling (BMD) and derivation of a Margin of Exposure (MoE) for substances that are genotoxic and carcinogenic: Considerations of biological relevance of tumour type, data quality and uncertainty assessment. Fd. Chem. Toxicol. 70, 264 - 289
EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (2012); Scientific Opinion on the Tolerable Upper Intake Level of vitamin D. EFSA Journal 2012, 10, 7, 2813. doi:10.2903/j.efsa.2012.2813. Available online: www.efsa.europa.eu/efsajournal
Ikezaki et al. (1999). Influences of Long-term Administration of 24,25-Dihydroxyvitamin D3, a Vitamin D3 Derivative, in Rats. Journal of Toxicological Sciences 24, 2, 133-139
Isobe et al. (2012). Stimulation of Adrenal Chromaffin Cell Proliferation by Hypercalcemia Induced by Intravenous Infusion of Calcium Gluconate in Rats. J Toxicol Pathol 2012, 25,

281 - 285
Lynch et al. (1996). Low digestible carbohydrates (polyols and lactose): significance of adrenal medullary proliferative lesions in the rat. Reg. Toxicol. Pharmacol, 23, 256 - 297
Ozaki et al (2002). Association of Adrenal Pheochromocytoma and Lung Pathology in Inhalation Studies with Particulate Compounds in the Male F344 Rat- The National Toxicology Program Experience. Toxicol. Pathology, 30, 263–270
UK Scientific Advisory Committee on Nutrition (SACN). Draft Vitamin D and Health Report (2015). Available at:
https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/447402/Draft_SACN_Vitamin_D_and_Health_Report.pdf

Dossier Submitter's Response

We certainly agree that the 26-week study has limitations when used to assess the intrinsic carcinogenic potential of cholecalciferol. Nevertheless, pheochromocytomas could be observed already after 26 weeks despite using a low number of animals. If a higher number of animals had been used (50/sex/dose is the recommended size in OECD 451), the tumor frequency would probably have been higher. In our view, the key issue is whether or not this effect and the plausible MoA (calcium perturbation) are relevant for humans. Since we do not consider existing data to convincingly prove that this would not occur in humans, we do not find it safe to disregard these findings.
With respect to other arguments made, please note our response to previous comments.

RAC's response

Noted and very appreciated. Please note our response to comment no.1.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
24.02.2016	Switzerland	DSM Nutritional Products AG	Company-Manufacturer	16

Comment received

Reference to section 10.8; page 21
The proposal for considering vitamin D3 as a germ cell mutagen (category 2) is based on a recent Ames Test and a recent in vivo Comet assay in the liver; both studies performed with vitamin D3. These studies were considered positive in the CLH report.
Ames Test: The CLH report gives consideration that the most recent Ames Test overrules the other evidence from in vitro mutagenicity testing. This other evidence consists of a negative mutation test in vitro in mammalian cells and two earlier but publically available Ames tests with vitamin D3.
When testing natural substances in standard toxicological testing care needs to be taken into account for susceptibility of the isolated material towards oxygen and light being not protected with e.g. antioxidants. Such artificial conditions may lead to degradation of the material and consequently results from toxicological studies which are irrelevant for the real-life situation. For example it was recently published that improper handling of the carotenoid zeaxanthin produced artificial oxidation products being responsible for a positive response in the Ames Test. Using appropriate protection, a negative Ames Test was obtained (Edwards 2016).
Vitamin D can be artificially oxidized to epoxide and cyclic peroxide structures once being unprotected from light and oxygen in solvents (Min & Boff 2002). It is known that peroxides and epoxides are reactive and such artificially produced deterioration products could have caused the equivocal positive responses in one out of three Ames Tests.
DSM investigated the degradation of vitamin D3 in different solvents without protection against light and oxygen. Within 1 hour incubation oxidation products were not detected

by analytical means in ethanol, methanol, and acetone. Whereas in DMSO, already after 1h, oxidative products were seen. One of them was identified as 7,8-epoxy vitamin D3 (DSM 2015).
Based on these results a further Ames test was performed using tester strains TA100 and TA1535 (the two ones with the equivocal positive results as cited in the CLH report) under GLP and OECD guideline thereby using protection against oxygen and light as much as possible. The results of this new study (DSM 2016) confirm the negative responses seen previously in the earlier published Ames Tests and put the results of this single weakly positive study into questions.
In addition, Ames Test data on 25-hydroxy vitamin D3, the metabolite of vitamin D3 formed in the liver, also showed no indication of an increased revertant frequency (DSM 2013).
Overall, the weight of evidence using the currently available reports of three negative Ames Tests and one negative in vitro mutation test in mammalian cells, the negative Ames Test of 25-hydroxy vitamin D3, as well as the possibility of artefacts due to improper handling indicate that vitamin D3 poses no mutagenic properties in vitro.
In vivo Comet assay: Considering the overall weight of evidence on the mutagenic activity of vitamin D3 in vitro discussed above, it is difficult to understand why the DNA-damage observed in the liver should be the result of a direct DNA-interacting activity of vitamin D3 or of vitamin D3 metabolites. Therefore, the present in vivo Comet assay study should be looked at in more details.
Literature data show liver damage in laboratory animals shortly after vitamin D3 intoxication consisting of hepatocyte necrosis and mitochondrial damage (partly using non-standard techniques such as electron microscopy, Gascon-Barre & Cote 1978, Kocher et al., 2010, Chavan et al., 2011). It is possible that such effects may not be seen in standard histopathological examination in such short-term studies as discussed by Speit et al. (2015).
Furthermore, the animals showed all signs of a severe hypervitaminosis D evident by hypercalcemia and hyperphosphatemia resulting in severe body weight loss. Thus, the dose levels used in the study seem to be clearly above the Maximum Tolerated Dose (MTD) and are close to dose levels causing death after single application. This severe intoxication could have resulted in the observed strand breaks.
Overall, the increased DNA-migration seen in the in vivo Comet assay in the liver after administration of doses being close to the LD50 are likely a secondary response to the hypercalcemia and/or liver damage.

Therefore, vitamin D3 should not be considered a germ cell mutagen (category 2) because it does not match the criteria laid down in CLP (2015). For further details see attached DSM document.

ECHA note - The following attachment was submitted with the comment above:

- *D3 CLH DSM Position paper 22_02_2016_FINAL_submitted.pdf*
- *DSM 2015.pdf*
- *DSM 2016.pdf*

Dossier Submitter's Response

Comments on attachments:

Ames test: the basis for the proposal Muta. 2 is principally based on the in vivo comet assay. Therefore, even if the reproducible increase of revertants observed in the single Ames test considered to be of sufficient reliability would be disregarded, the concern still remains.
In vivo comet assay: if, as suggested in the attachment, the liver mutagenicity is due to liver damage such as hepatocyte necrosis and mitochondrial damage in the test animals,

at least hepatocyte necrosis would have been observed in the histopathological investigations. The lack of liver toxicity is indeed what raises a strong concern for genotoxicity despite the concomitant toxicity manifested as effects on bodyweight and biochemical parameters.
RAC's response
Noted and very appreciated. Please note our response to comment no. 1.

Date	Country	Organisation	Type of Organisation	Comment number
09.03.2016	Germany	BASF SE, Nutrition & Health	Company-Manufacturer	17

Comment received

We disagree with the suggested classification for Germ Cell Mutagenicity Category 2. Cholecalciferol was tested in a complete set of GLP and guideline conforming in vitro and in vivo mutagenicity tests. The proposal of the Dossier Submitter is based on weakly positive responses in a single Ames test (Hargitaj, 2013a) and borderline findings in an in vivo Comet Assay using high, toxic dosages above the MTD (Beevers, 2014).
In the Ames test, Cholecalciferol was clearly negative in strains TA98, TA1537 and E.coli WP2uvrA (Hargitai, 2013a). It induced a weak positive response in TA100 with metabolic activation at high dose levels in the presence of precipitate (2.6-fold increase over control) and in TA1535 without metabolic activation at high dose levels and again in the presence of precipitate (3.3-fold increase over control). Precipitation indicates the limit of solubility has been reached and may impact the interpretation of results. In addition, one of the two increases was reported with metabolic activation, the other only observed without metabolic activation. Overall the effects are interpreted as weak effects with regard to the criteria for a positive response in the report (factor 2 in TA 100 and factor 3 in TA 1535). This assessment is in line with further studies showing negative results in several bacterial strains including TA 100 and TA 1535, even when tested at higher dose levels than dose tested in the more recent Ames test (Anonymous, 1977; Mortelmans et al., 1986; see table).
Summary table of Ames tests (see attachment)

In the in vivo Comet Assay, rats were dosed with Cholecalciferol at 3.75, 7.5 and 15 mg/kg/day by oral gavage over 3 consecutive days. When tested under these conditions Cholecalciferol was clearly negative at 3.75 mg/kg/day, but induced slight statistically significant increases in Tail Intensity (TI) in the liver of rats treated at 7.5 and 15 mg/kg/day (1.9 and 1.8- fold increase, respectively). However, the changes observed at the mid and high dose were slight (<2-fold increase), not dose-related and not associated with a concurrent significant increase in Tail Moment (TM) although this parameter is the product of the TI and the comet length. The values of TI and TM remained within the range of laboratory's historical control data.
Knowing that excessive toxicity can play a key role in the interpretation and the reliability of the results of the Comet Assay, it should be emphasized that in the Comet Assay dose levels were used which clearly exceeded the MTD based on severe body weight losses over a short period of time of 3 days and which were close to lethal dose levels observed in the pre-study. Toxicity was indicated by severe alterations in clinical chemistry (increases in Ca/P, urea and liver enzymes) which reflect renal and muscular failure and decreases in liver glycogen vacuolation. DNA migration (within the range of historical control data) was only seen at dose levels which exceeded the MTD, no DNA migration was observed at dose levels that induced some tolerable systemic toxicity. It is therefore concluded that the available Comet Assay should not be the basis of classification. The absence of a concern for mutagenicity is further supported by clearly negative results in

mammalian cell lines in a Mouse Lymphoma Assay, a Chromosome Aberration test (Hargitaj, 2013b and c) and the in vivo Micronucleus test (Beevers, 2014).
In summary, we conclude that the criteria for germ cell mutagenicity as defined in Regulation No. 1272/2008 are not met for Cholecalciferol for the following reasons:

The effects in the Ames test were

• only slight and found at dose levels where precipitation was observed
• inconsistent with regard to the effect of metabolic activation
• not in line with negative results from published studies using higher dose levels
• not confirmed by higher tier tests in mammalian cells (Mouse Lymphoma Assay)
The effects in the in vivo Comet Assay were found to be
• only slight (<2 increase in Tail Intensity)
• not associated with an increase in Tail Moment
• effects within historical control data
• were only found in presence of overt signs of excessive toxicity
• not dose related at mid and high dose
According to the CLP-criteria the classification in Category 2 is based on the:
• 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.
The Dossier Submitter concluded in section 10.8.2 of the CLH proposal that
" 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."
We consider this conclusion to be inappropriate as in the Annex I of the CLP, section 3.5.2.3.9 it is stated that
" the classification of individual substances shall be based on the total weight of evidence available, using expert judgement. In those instances where a single well-conducted test is used for classification, it shall provide clear and unambiguously positive results."
Moreover, the criteria of the current OECD Test Guideline 489 (2014) for a clearly positive result are not fulfilled as the statistically significantly increased values for tail intensity at the low and mid dose (mean values 9.65 and 9.18) are still within the historical negative control data of the laboratory for studies started at July 2010 and November 2011 (observed range 0.01 – 12.64). Consequently, the results of the Comet Assay cannot be assessed to be clear and unambiguously positive as required by the CLP guidance. In addition, there are no known structure activity relationships of Cholecalciferol to known germ cell mutagens.
Therefore and in view of all available data from in vitro and in vivo studies, we do not see a concern for genotoxicity and classification of Cholecalciferol as a mutagen is not justified.

References:
Anonymous (1977) Mutagenicity Evaluation of FDA 75-81 Vitamin D3 (Cholecalciferol)
Beevers (2014) Cholecalciferol: Combined bone marrow micronucleus test and Comet Assay in the liver and duodenum in treated rats. Unpublished and confidential report
Hargitai (2013a) Cholecalciferol: Bacterial Reverse Mutation Assay. Unpublished and confidential report
Hargitai (2013b) Cholecalciferol: In vitro Mammalian Cell Gene Mutation Test (Mouse Lymphoma Assay). Unpublished and confidential report

Hargitai (2013c) Cholecalciferol: in Vitro Mammalian Chromosome Aberration Test Unpublished and confidential report
Mortelmans et al. (1986) Salmonella Mutagenicity Tests: II. Results from the testing of 270 chemicals. Environ. Mutagen, 8, Suppl. 7, 1-119
OECD TG 489 (2014). OECD guideline for the testing of chemicals. In vivo mammalian alkaline comet assay.

ECHA note - The following attachment was submitted with the comment above: *Summary table of Ames tests.docx*

Dossier Submitter's Response

The objections raised regarding the interpretation of results from the Ames test and the Comet assay are already considered in the CLH report. As stated in the CLH report, the mean per cent tail intensity was above the historical control data for the vehicle and laboratory in question. The mean values should be compared to mean values of the HCD, not to a range. In the HCD referred to, the mean value of the range (0.01-12.64) is 2.22 thus the results are clearly above this value.
Comment on the attachment (***Summary table of Ames tests):*** Strains TA 100 and TA 1535 were exposed to eight different concentrations. According to the study report *"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 and without metabolic activation and in Confirmatory Mutation Test in all examined bacterial strains at 5000 µg/plate with metabolic activation."*
Precipitation may have masked additional revertants but it is noted that a dose-dependent increase of revertants was observed also at concentrations where no precipitation was observed.

RAC's response

Noted and very appreciated. The relevant information on the strengths and weaknesses of the available studies will be reflected in the Opinion Document. Please note our response to comment no. 1.

Date	Country	Organisation	Type of Organisation	Comment number
07.03.2016	United Kingdom	Exponent International (for BASF/BCS TF)	Company-Manufacturer	18

Comment received

Exponent submits as confidential data a QSAR report (Carling, 2012) demonstrating cholecalciferol shows no structural alerts for mutagenicity. Cholecalciferol should not be classified for carcinogenicity.

ECHA note - The following attachment was submitted with the comment above: *EWC 0028 Cholecalciferol DEREK report.*pdf

Dossier Submitter's Response

A QSAR analysis is based on structural similarity with substances included in the database. While this can be useful if no information on genotoxicity is available, the lack of genotoxic structures in the database matching structures within the test compound is not considered to overrule the existing in vivo test data available for the active substance.

RAC's response

Noted.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON COLECALCIFEROL; CHOLECALCIFEROL, VITAMIN D3

Date	Country	Organisation	Type of Organisation	Comment number
07.03.2016	Germany		Individual	19
Comment received				
The proposed classification is not in line with the legal requirements. The classification is justified with a positive comet assay in vivo. Since the assay in this case is to be considered as genotoxicity assay, the CLP regulation clearly highlights that such finding need to be supported by positive results in vitro. Since this is not the case and secondly, the positive findings of the comet assay were seen at levels of clear liver and general toxicity. The findings therefore need to be interpreted as secondary to toxicity and not a direct genotoxic effect.				
Dossier Submitter's Response				
Please note our response to previous comments on mutagenicity.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
03.03.2016	Germany		MemberState	20
Comment received				
We agree with the proposed classification Muta 2, H341 based on a positive in vivo comet assay which is supported by positive results from in vitro test with bacterial cells (Ames test).				
Dossier Submitter's Response				
Thank you for informing us about your position.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
02.03.2016	United Kingdom	Exponent International (on behalf of the BASF-BCS Task Force)	Company-Manufacturer	21
Comment received				
The Applicant disagrees with the proposed classification of cholecalciferol as a Mutagen, Category 2. Experimental data available from in vitro and in vivo GLP studies, epidemiological reports and public scientific literature show that Cholecalciferol poses no concerns with respect to genotoxicity. Notwithstanding this weight of evidence, the CLH report considers that borderline results in the in vitro Ames and in vivo comet assay might suggest some concern for genotoxicity and therefore proposes to classify Cholecalciferol as a Mutagen Category 2. The Applicant considers that the weak positive response observed in the in vitro Ames test at high doses and in presence of precipitate is not relevant based on clear negative responses reported in in vitro higher tier mammalian test and two other Ames tests from the public literature.				

The Applicant disagrees with CLH report's interpretation of the comet test data which is discordant from the Study Director's conclusion. The Applicant considers that the weak DNA migration observed in the liver of highly intoxicated animals is irrelevant with respect to genotoxicity for the following reasons:
• The effects are slight (<2-fold increase),
• The effects are neither dose-related nor associated with a concurrent significant increase in Tail Moment although this parameter is the product of the Tail Intensity and the comet length,
• The effects remained within the range of laboratory's historical control data,
• The effects are only observed in the presence of excessive systemic toxicity in the liver of highly intoxicated animals undergoing cholecalciferol toxicosis with severe hypercalcaemia.

This position is further supported by negative QSAR analysis for mutagenicity structural alerts, extensive literature which suggests a protective role of Cholecalciferol against cellular processes leading to cancer.

The Applicant considers that Cholecalciferol does not meet any criteria for Mutagenicity classification as defined in the Regulation (EC) No 1272/2008 taking into account the extensive animal data and human experience.

Cholecalciferol is an essential vitamin in humans for its role in calcium homeostasis and skeletal health. Cholecalciferol has a long history of beneficial use in fortified foodstuffs, dietary supplements and human medicinal products. Tolerable upper intake levels have been defined by the European Food Safety Authority (EFSA, 2012) and national supplementation and fortification policies have been adopted across Europe to prevent Vitamin D deficiency disorders, e.g. impaired bone mineralization and bone-softening diseases (EFSA, 2012 and Spiro, 2014). Classification of Cholecalciferol as a mutagen is not justified and is likely to impair community health by discouraging adequate intake of Vitamin D3.
These issues are explained in clear detail in Exponent document 1408446.uk0-5431, which is attached.

ECHA note - The following attachment was submitted with the comment above:
1408446.uk0 - 5431 Cholecalciferol Genotoxicity_v4.docx

Dossier Submitter's Response

Please note our response to previous comments on genotoxicity.
The basis for the proposal Muta. 2 is principally the results from the in vivo comet assay. Therefore, even if the results of the Ames test considered to be of sufficient reliability would be disregarded, the concern still remains.
Moreover, classification is based on the intrinsic hazard, i.e. it takes into account also effects that may occur at exposure levels outside of the physiological range. Therefore, classification may be required even if cholecalciferol may have beneficial properties such as those indicated in the study by Fedirko (2014).

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
29.02.2016	Netherlands		MemberState	22
Comment received				
• The exact substances used for the mutagenicity assays are not clear ('not stated' and 'D0074' as stated in tables 10.8.A and 10.8.C) • One out of 3 Ames tests showed a weak positive response. In test strain TA100 +S9, a response of >2x was observed at doses ≥ 2500 µg/plate in the initial test. This was however not confirmed in the confirmatory test (only a response >2x at the slightly cytotoxic dose of 5000 µg/plate, although with clear dose response). In test strain TA1535, a positive response (>3x) was only observed at ≥ 3750 µg/plate in the complementary test. In the initial test responses were <2x, and in the confirmatory test no effect was observed without S9 mix. • A combined in vivo micronucleus / comet assay with doses up to > MTD was provided. Despite the high doses used, the results of the micronucleus study were negative. No cytotoxicity effect was seen in bone marrow, however, systemic toxicity (severe body weight loss and alterations of clinical chemistry parameters) was observed and therefore it may be expected that the bone marrow was reached. The results of the micronucleus study can therefore be seen as valid. • The comet assay was positive in liver (at dose levels inducing severe systemic toxicity), but negative in duodenum. We agree that the absence of inflammation and necrosis in the liver indicate that the increased DNA migration is not a secondary effect of excessive liver toxicity. Nevertheless, we question whether results from dose levels inducing excessive toxicity should be used for classification purposes. • In conclusion, considering the fact that only 1:3 Ames tests was weakly positive and a mouse lymphoma test (TG 476) was negative, and the fact that data from a Comet assay, positive only at doses exceeding the MTD, can only be used as evidence for genotoxicity, we do not agree with classification for mutagenicity for Colecalciferol.				
Dossier Submitter's Response				
Thank you for informing us about your position. The batch denoted D0074 is claimed to comply with the requirements of the European Pharmacopoeia. For the other studies in table 10.8.A, the batch(es) used and their purity is not stated in the original report. This is one of the reasons why the study was given low reliability. *(It is unclear on what basis a purity of 97% has been reported by the applicant under BPR).*				
RAC's response				
Noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
24.02.2016	Switzerland	DSM Nutritional Products AG	Company-Manufacturer	23
Comment received				
Reference to section 10.10, page 48 DSM agrees to the conclusion of non-classification provided in the CLH report with respect to this endpoint. There is even more human data on pregnancy outcomes upon supplementation with vitamin D3 which supports the non-classification: • In a double-blind trial, pregnant Iranian women were safely supplemented with 100'000 IU/month (2500 µg vitamin D3/month, Sabet et al., 2012). • Likewise, no adverse effects were seen in three randomized controlled trials in pregnant				

women with treatment up to 4000 IU/day (100 µg vitamin D3/day, Dawodu et al., 2013, Hollis et al., 2011, and Wagner et al., 2013). • Supplementation of breast feeding mothers with up to 6400 IU/day (160 µg vitamin D3/day) showed no indication of adverse effects in the mothers as well as their infants (Wagner et al., 2006) ECHA note - The following attachment was submitted with the comment above: • *D3 CLH DSM Position paper 22_02_2016_FINAL_submitted.pdf* • *DSM 2015.pdf* • *DSM 2016.pdf*
Dossier Submitter's Response
Thank you for informing us about your position. Since classification is based on intrinsic hazard, data from trials investigating only a certain dose range is of limited use for this purpose. Consequently, this data can only be considered in combination with more robust data.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
02.03.2016	United Kingdom	Exponent International (on behalf of the BASF-BCS Task Force)	Company-Manufacturer	24
Comment received				
The Applicant supports "no classification" for reproductive toxicity. Epidemiological evidence is of greater relevance than the inconsistent results of some dubious animal studies. There is no clear evidence of any effect of cholecalciferol on pregnancy, and adequate levels of cholecalciferol are clearly essential for healthy development of the infant.				
Dossier Submitter's Response				
Please note our response to the previous comment.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
03.03.2016	Germany		MemberState	25
Comment received				
We agree with the removal of the asterisk from Acute Tox. 2, H330 and tightening the classification for the oral and dermal route from Acute Tox. 3* to Acute Tox. 2.				
Dossier Submitter's Response				
Thank you for informing us about your position.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
03.03.2016	Germany		MemberState	26
Comment received				
We agree with the proposed classification STOT RE1, H372 due to hypercalcemia with tissue mineralisation. However, the derivation of the proposed SCL is not plausible. For setting of specific concentration limits (SCL) the effective dose is used. The effective dose is the lowest dose inducing significant/severe target organ toxicity. At 0.06 mg/kg bw/d no severe toxicity was observed (minimal kidney changes). We propose to use a dose of 0.3 mg/kg bw/d (minimal to moderate kidney changes with higher incidence and tissue mineralisation in several organs) for derivation of the SCL.				
Dossier Submitter's Response				
Thank you for informing us about your position.				
RAC's response				
In addition to the kidney lesions, the mineralisations in the aorta and heart as such are considered as a seriously adverse effect. Here, your suggestion that it may be appropriate to use 0.3 mg/kg bw as a starting point for SCL calculation will be discussed in plenum.				

Date	Country	Organisation	Type of Organisation	Comment number
29.02.2016	Netherlands		MemberState	27
Comment received				
• The proposed classification is based on progressive hypercalcemia with tissue mineralisation in several organs and proliferative adrenal pathology, with symptoms already visible at 0.06 mg/kg bw/day, we agree with classification as STOT RE1, without specifying a target organ. We also agree with the proposed SCLs of 0.6% for STOT-RE1 and 0.06% for STOT-RE2. However, rounding down to the nearest preferred values as stated in the CLP guidance is suggested resulting in 0.5% and 0.05%.				
Dossier Submitter's Response				
Thank you for informing us about your position.				
RAC's response				
Noted. This will be discussed in plenum.				

NON-CONFIDENTIAL ATTACHMENTS:

1. *Summary table of Ames tests.doc.* Submitted on 09/03/2016 by BASF SE, Nutrition & Health. [Please refer to comment No 17]
2. *1408446 UK0 - 7619 Carcinogenicity WoE evaluation_v4.docx.* Submitted on 02/03/2016 by Exponent International (on behalf of the BASF-BCS Task Force). [Please refer to comment No 11]
3. *1408446.uk0 - 5431 Cholecalciferol Genotoxicity_v4.docx.* Submitted on 02/03/2016 by Exponent International (on behalf of the BASF-BCS Task Force). [Please refer to comment No 21]
4. *D3 CLH DSM Position paper 22_02_2016_FINAL_submitted.pdf.* Submitted on 24/02/2016 by DSM Nutritional Products AG. [Please refer to comments No 1, 13, 16, 23]

CONFIDENTIAL ATTACHMENTS:

1. *Bikle 2014 vitamin D cancer mechn.doc.* Submitted on 07/03/2016 by Exponent International (for BASF/BCS TF). [Please refer to comment No 7]
2. *EWC 0028 Cholecalciferol DEREK report.pdf* Submitted on 07/03/2016 by Exponent International (for BASF/BCS TF). [Please refer to comment No 18]
3. *ECHA Vit D Gentox and Carcino SWP comments.docx.* Submitted on 07/03/2016. [Please refer to comment No 2]
4. *DSM 2015.pdf.* Submitted on 24/02/2016 by DSM Nutritional Products AG. [Please refer to comments No 1, 13, 16, 23]
5. *DSM 2016.pdf.* Submitted on 24/02/2016 by DSM Nutritional Products AG. [Please refer to comments No 1, 13, 16, 23]