Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

divanadium pentaoxide; vanadium pentoxide

EC Number: 215-239-8
CAS Number: 1314-62-1

CLH-O-0000006927-60-01/F

Adopted

10 December 2020

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON DIVANADIUM PENTAOXIDE; VANADIUM PENTOXIDE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during 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 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 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. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Substance name: divanadium pentaoxide; vanadium pentoxide
EC number: 215-239-8
CAS number: 1314-62-1
Dossier submitter: France

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2019	Germany	Vanadium Consortium / Vanitec	Industry or trade association	1
Comment received				
The Vanadium industry, represented by Vanitec and the Vanadium Consortium, takes this opportunity to submit scientific comments on the Proposal for Harmonised Classification and Labelling of divanadium pentaoxide. Our comments are put forward in detail endpoint-by-endpoint in the public attachment" Vanadium Consortium-Vanitec_Comments on CLH proposal of V2O5_2019-11-22.pdf". We propose an alternative science-based, justifiable classification since we disagree with the unbalanced analysis, methodology, and findings in the CLH proposal ECHA note – An attachment was submitted with the comment above. Refer to public attachment Vanadium Consortium-Vanitec_Comments on CLH proposal of V2O5_2019-11-22.pdf				
Dossier Submitter's Response				
Thanks but comments addressing management issues are not under the scope of this consultation. Comments related to specific endpoints are adressed in the comments below (number 6, 10, 11 and 14)				
RAC's response				
Thank you for your comments. Comments related to management issues are not under the scope of the RAC evaluation.				

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2019	Spain	Frit Consortium	Industry or trade association	2

Comment received

The Frit Consortium is the organization that manages the REACH and CLP obligations of companies that manufacture or import frits into the EU. The Consortium currently includes 34 members from different European countries, mainly Spain, Italy and Germany. The members of the Frit Consortium are the largest producers in Europe for frits.
Frits are chemical substances, result of a mixture of inorganic chemical substances (typically consisting of metal oxides and salts) produced by rapidly quenching a molten, complex combination of materials, confining the chemical substances thus manufactured as nonmigratory components of glassy solid flakes or granules. Divanadium pentaoxide and other vanadium compounds are used as important raw materials for some specific types of frits and cannot be substituted. During the manufacturing process, raw materials are transformed via melting and the resulting substances contain vanadium ions confined into the vitreous structure of the frit. These substances are exclusively manufactured at industrial sites by trained workers and their main end uses are ceramics, glass and metals.

The Frit Consortium fully supports the scientific and technical position of the Vanadium REACH Consortium in relation to the current Public Consultation to modify the existing entry for divanadium pentaoxide in Annex VI of the CLP Regulation.

Impact of the CLH proposal in our sector:
The proposed harmonised classification and labelling for divanadium pentaoxide would have a negative impact in our sector mainly due to its use as raw material, but also due to potential stigmatisation of vanadium-containing frits.
The following main consequences have been identified:
- Changes in the manufacturing and handling practices
- Market impact due to stigmatisation of all substances and mixtures containing divanadium pentaoxide
- Stigmatisation of articles containing vanadium substances, even if they are bound in a matrix and thus not biologically available
- Changes to labelling, packaging and eSDS
- Additional investment and/or equipment costs
- Additional RMMs required
- Possibility for a future authorisation or restriction under REACH affecting vanadium-containing frits
- Possible precedent setting for classification outside the EU
- Impact on other related legislation (e.g. waste, occupational health and safety legislation, etc)
- Impact for other raw materials where divanadium pentaoxide may be present as an impurity

All the above would result on an increase of operational costs and loss of competitiveness face to our competitors in the rest of the world and may trigger delocalisation.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Contribution V2O5 CLH Public Consultation_FC.pdf
Dossier Submitter's Response
Thanks but comments addressing management issues are not under the scope of this consultation
RAC's response
Thank you for your comments. Comments related to management issues are not under the scope of the RAC evaluation. See also responses to Vanadium REACH Consortium comments.

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2019	Spain	Inorganic Pigments Consortium	Industry or trade association	3
Comment received				
The Inorganic Pigments Consortium is the organization that manages the REACH and CLP obligations of companies that manufacture or import inorganic pigments into the EU. The Consortium currently includes 25 members from different European countries, mainly Spain, Italy, Germany and UK. The members of the IP Consortium are the largest producers in Europe for inorganic pigments. Complex inorganic pigments are chemical substances manufactured by means of an industrial process, which involves a chemical reaction. In this process, a mixture of raw materials (typically consisting of metal oxides and salts) undergoes a calcination reaction at high temperatures forming a specific crystalline matrix. Divanadium pentaoxide is a key raw material for the manufacture of some specific complex inorganic pigments to obtain certain colour ranges and cannot be substituted. During the pigment manufacturing process, raw materials are transformed via calcination and the resulting inorganic pigments contain vanadium ions bound to the crystalline structure. These substances are exclusively manufactured at industrial sites by trained workers and their main end uses are ceramics, metals, plastics and paints or coatings. The Inorganic Pigments Consortium fully supports the scientific and technical position of the Vanadium REACH Consortium in relation to the current Public Consultation to modify the existing entry for divanadium pentaoxide in Annex VI of the CLP Regulation. Impact of the CLH proposal in our sector : The proposed harmonised classification and labelling for divanadium pentaoxide would have a negative impact in our sector mainly due to its use as raw material, but also due to potential stigmatisation of vanadium-containing pigments, even if scientific information show that vanadium ions are not released from the crystalline structure of pigments and therefore not bioavailable. The following main consequences have been identified: - Changes in the manufacturing and handling practices - Market impact due to stigmatisation of all substances and mixtures containing vanadium compounds - Stigmatisation of articles containing vanadium-containing pigments, even if their constituents are bound in a matrix and thus not biologically available. - Changes to labelling, packaging and eSDS - Additional investment and/or equipment costs				

- Additional RMMs required
- Possibility for a future authorisation or restriction under REACH affecting vanadium-containing pigments
- Possible precedent setting for classification outside the EU
- Impact on other related legislation (e.g. waste, occupational health and safety legislation, etc)
- Impact for other raw materials where divanadium pentaoxide may be present as an impurity

All the above would result on an increase of operational costs and loss of competitiveness face to our competitors in the rest of the world and may trigger delocalisation.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Contribution V2O5 CLH Public Consultation_IPC.pdf

Dossier Submitter's Response

Thanks but comments addressing management issues are not under the scope of this consultation

RAC's response

Thank you for your comments. Comments related to management issues are not under the scope of the RAC evaluation. See also responses to Vanadium REACH Consortium comments.

Date	Country	Organisation	Type of Organisation	Comment number
21.11.2019	United States		Individual	4

Comment received

Throughout the CLH report insufficient consideration is give to the complex nature of vanadium chemistry so that information has been included in the assessment that is not relevant to divanadium pentaoxide.

See attachment: CLH-V2O5 chemistry comments by David White-2019-11-21.pdf

ECHA note – An attachment was submitted with the comment above. Refer to public attachment CLH-V2O5 chemistry comments by David White-2019-11-21.pdf

Dossier Submitter's Response

Read-across with sodium and ammonium metavanadate is only used for classification as toxic for reproduction (fertility and development) and causing effects on or via lactation. Read-across approach is based on chemical structure, physico-chemical and toxicological (including endpoint on toxicokinetics and effects on fertility, development and lactation) properties.

We acknowledge your comments on the complex nature of vanadium chemistry. That's why we only use the read-across on substances with the same oxidative degree of 5.

Regarding your comment 1b: we agree that V2O5 reacts with water when administered in solution. After direct exposure, V2O5 will react similarly in the organism (as you note in "biological fluid") when administered orally. Thus, using V2O5 in aqueous solution can be used for classification purpose.

In fine, the read-across is particularly supported by similar toxicity on reproduction, development and effects *via* lactation. The studies are performed either by oral route or parental route (never by inhalation for which specific effect of V2O5 dust is expected).

For fertility and developmental toxicity, there are some data on V2O5. However, since they generally suffer from methodological limitations, data on sodium and ammonium metavanadates are used as a weight of evidence for the classification proposal. All substances induce consistent effects on male reproductive organ, supporting the read-across. Consistent effects (malformations, decreased weight and lethality) are also observed regarding developmental toxicity.

For lactation, there are no study with V2O5. Neurotoxic effects are reported with sodium metavanadate after exposure during lactation. Neurotoxic effect are reported in adults exposed to V2O5, which supports the read-across.

Concerning occupational exposure studies, they are judged not adequate for reaching conclusion on classification proposal.

RAC's response

Thank you for the comments regarding vanadium chemistry. As explained by dossier submitter, read-across was used only for reproductive endpoints and only using data on other pentavalent vanadium species. Bioelution data was available to support the read-across. RAC considers the use of read across in this case appropriate.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2019	Germany		MemberState	5

Comment received

The French MSCA proposes to add classification as Carc. 1B (H350) to the Annex VI entry.
The proposal is based on two reliable 2-year carcinogenicity tests with mice and rats exposed to V2O5 via inhalation. Increased incidences of tumours (alveolar, bronchiolar neoplasms: adenoma and carcinoma) without excessive toxicity at test doses in male and female B6C3F1 mice were shown. Some evidence of carcinogenic activity in male F344/N rats was observed (NTP, 2002). In addition to these results, V2O5 exposure significantly promoted lung tumours (solid adenomas) in A/J and BALB/cJ mice (Rondini et al., 2010). No human data with positive association between cancer and exposure to V2O5 is available.

In a weight of evidence approach and considering additional important factors to assess the overall level of concern (tumour type and background incidence, progression of lesions to malignancy, reduced tumour latency, responses in male and female mice, route of exposure, no excessive toxicity) V2O5 is presumed to have carcinogenic potential for humans. We agree that classification as Carc. 1B (H350) would be justified.

However, there are also arguments for a classification in category Carc. 2. Dose dependent effects (alveolar/ bronchiolar carcinoma or adenoma) were only observed in one species (B6C3F1 mice) with the lung as the only site of response. Moreover, in historical controls (NTP historical controls report) the overall incidence of alveolar/

bronchiolar carcinoma or adenoma in lung is high (about 32 %). These arguments should be included in the discussion regarding the classification of V2O5 in Carc. 1B or Carc. 2.
Dossier Submitter's Response
Thank you for your support that Carc. Cat. 1B. would be justified for V2O5. Concerning your comments about historical controls in the NTP study in F344/N rats,two different NTP historical controls were included: historical control with NTP-2000 diet and with NIH-07 diet, with some differences in ranges of alveolar/bronchiolar adenoma/carcinoma. They are both concurrent in time (December 2000 for NTP-2000 and December 1999 for NIH-07). Since diet is a significant factor that can affect the background incidence of neoplasms at a variety of sites, it is more relevant to consider the historical control using the same diet as given to animals exposed to V2O5: namely NTP-2000 diet: 26/609; 4.5 ± 3.9%; range 0-14%. Regarding males, even if the increase of the incidence of alveolari/bronchiolar adenoma or carcinoma is not statistically significant compared to concurrent control, it exceeds the NTP historical control at 0.5 and 2 mg/m^3. Incidence of lung tumours in the control group is within historical control but somewhat at the upper end of the range. This may explain that the difference is not statistically significant between the groups. However, since the incidences at 0.5 and 2 mg/m3 are outside the historical control ranges, this should be considered as related to V2O5 exposure. At 1 mg/m3, the incidence is at the very upper end of the historical control range (adjusted rate = 14%). In male rats fed the NTP-2000 diet, only 2 of 609 control rats developed alveolar/ bronchiolar carcinomas, and there was never more than one of this neoplasm in a study. In contrast, there are 3 males with carcinoma at 0.5 and 2 mg/m3 of V2O5. In addition, analysis of genetic alterations (K-ras) suggest that the mechanism of lung carcinogenesis following vanadium pentoxide exposure was different than that of spontaneously induced lung neoplasms. This is in favour of an association between lung tumours and exposure to V2O5. In addition, mouse alveolar/bronchiolar adenomas and carcinomas are similar to human adenocarcinomas in histomorphology and molecular characteristics including activation of the *K-ras* gene[1] [2]

[1] 25. Meuwissen R, Berns A. Mouse models for human lung cancer. Genes & Development. 2005;19:643–664.
[2] Nikitin AY, Alcaraz A, Anver MR, Bronson RT, Cardiff RD, Dixon D, Fraire AE, Gabrielson EW, Gunning WT, Haines DC, Kaufman MH, Linnoila RL, Maronpot RR, Rabson AS, Reddick RL, Rehm S, Rozengurt N, Schuller HM, Shmidt EN, Travis WD, Ward JM, Jacks T. Classification of proliferative pulmonary lesions of the mouse recommendations of the mouse models of human cancers consortium. Cancer Res. 2004;64:2307–2316.

TABLE 13
Incidences of Neoplasms and Nonneoplastic Lesions of the Respiratory System in Rats in the 2-Year Inhalation Study of Vanadium Pentoxide

	Chamber Control	0.5 mg/m³	1 mg/m³	2 mg/m³
Male				
Lung[a]	50	49	48	50
Alveolar Epithelium, Hyperplasia[b]	7 (2.3)[c]	24** (2.0)	34** (2.0)	49** (3.3)
Bronchiole Epithelium, Hyperplasia	3 (2.3)	17** (2.2)	31** (1.8)	49** (3.3)
Alveolar Epithelium, Metaplasia, Squamous	1 (1.0)	0	0	21** (3.6)
Bronchiole Epithelium, Metaplasia, Squamous	0	0	0	7** (3.7)
Inflammation, Chronic Active	5 (1.6)	8 (1.8)	24** (1.3)	42** (2.4)
Interstitial, Fibrosis	7 (1.4)	7 (2.0)	16* (1.6)	38** (2.1)
Alveolus, Infiltration Cellular, Histiocyte	22 (1.3)	40** (2.0)	45** (2.3)	50** (3.3)
Alveolus, Pigmentation	1 (2.0)	0	2 (1.5)	28** (2.1)
Alveolar/bronchiolar Adenoma, Multiple	0	2	0	0
Alveolar/bronchiolar Adenoma (includes multiple)[d]	4	8	5	6
Alveolar/bronchiolar Carcinoma, Multiple	0	1	0	0
Alveolar/bronchiolar Carcinoma (includes multiple)[e]	0	3	1	3
Alveolar/bronchiolar Adenoma or Carcinoma[f]				
Overall rate[g]	4/50 (8%)	10/49 (20%)	6/48 (13%)	9/50 (18%)
Adjusted rate[h]	10.0%	23.3%	14.0%	21.3%
Terminal rate[i]	4/20 (20%)	8/29 (28%)	4/26 (15%)	6/27 (22%)
First incidence (days)	729 (T)	608	694	558
Poly-3 test[j]	P=0.232	P=0.092	P=0.416	P=0.134

According to NTP conclusion, there was clear evidence of carcinogenicity in mice (both sexes), some evidence in male rats and equivocal evidence of carcinogenic activity in female rats.

In addition, according to CLP criteria:
"Sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. **An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence.** A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites;"

As the NTP protocol is recognised as a gold standard for carcinogenicity testing, this NTP study performed with V2O5 must be considered as a well-conducted study. Since, a clear causal relationship between lung tumours and exposure to V2O5 is reported in both sexes of mice, criteria for Carc. 1B is fulfilled. In addition, even if the evidence is less strong than in mice, similar effect is reported in rats (in particular in males) which supports the findings observed in mice.

RAC's response

Thank you for your comments. RAC agrees that there are aspects which can be considered to decrease the concern for humans. Discussion on these has been included in RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2019	Germany	Vanadium Consortium / Vanitec	Industry or trade association	6

Comment received

Carcinogenicity:
In the view of the Registrant, the NTP (2002) inhalation study in rats and mice does not provide sufficient data to draw definitive conclusions regarding thresholds and/or mechanisms of action or mode of action. The relevance of the lung tumours in mice is severely confounded by the chronic and persistent inflammation, presumed to have occurred over the whole duration of the two-year exposure, and the lack of a dose-response relationship.
Divanadium pentaoxide reacts strongly acidic, so that this is likely to contribute to the chronic inflammation. Further, V2O5 is not expressing its carcinogenic activity via genotoxicity since tumours did not develop at other organ sites following 2-years of inhalation even though divanadium pentaoxide circulated systemically. Based on the absence of genotoxicity, divanadium pentaoxide is thought to exert genotoxicity by indirect mechanisms (secondary genotoxicity, i.e. chronic inflammation and oxidative stress).
The tumour promoting activity in mice, although strain-dependent (Rondini et al., 2010) may be considered part of that mode of action of V2O5. It is further noted that there is a high degree of discordance between rats and mice in the development of chemically induced lung tumours, as shown in a statistical analysis of 58 compounds tested in NTP two-year inhalation studies (Smith & Anderson, 2017). From this data, it has also been concluded that bronchioalveolar lung tumours induced only in mice by non-genotoxic chemicals are of low relevance for human lung cancer risk (Smith et al., 2018). Further, the genomic responses of mice to inflammation are of questionable comparability to those of humans (Seok et al., 2013).
Finally, the available carcinogenicity data relate only to inhalation exposure of rats and mice to divanadium pentaoxide, which affected only the respiratory tract, and with tumour formation only in mice lungs which is considered a substance-specific local effect. The NTP (2002) study does not report any treatment related lesions in other tissues whatsoever, despite measured vanadium levels in blood indicating systemic exposure.
In conclusion, the evidence of carcinogenicity in mice of both sexes (NTP, 2002) is not considered sufficient for classification of divanadium pentaoxide as a Category 1B substance. Instead, a classification in Category 2 – H351i appears to be most appropriate and adequately conservative. Additionally, any classification should be route-specific, i.e. restricted to inhalation.

Please refer for a detailed argumentation to the attachment "Vanadium Consortium-Vanitec_Comments on CLH proposal of V2O5_2019-11-22.pdf".

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Vanadium Consortium-Vanitec_Comments on CLH proposal of V2O5_2019-11-22.pdf

Dossier Submitter's Response

You consider that the NTP (2002) inhalation studies in rats and mice do not provide sufficient data to draw definitive conclusions regarding thresholds (for risk-assessment) and/or mechanisms of action or mode of action. But in the CLP process (only hazard-based),

determination of a threshold effect and/or mechanisms of action or either mode of action are not needed for carcinogenicity labelling proposal.

Secondly, you consider that *"V2O5 is not expressing its carcinogenic activity via genotoxicity since tumours did not develop at other organ sites following 2-years of inhalation even though divanadium pentaoxide circulated systemically. Based on the absence of genotoxicity, divanadium pentaoxide is thought to exert genotoxicity by indirect mechanisms (secondary genotoxicity, i.e. chronic inflammation and oxidative stress)*." However, according to CLP criteria, carcinogenicity labelling applies either for a genotoxic carcinogenic substance as well as for non-genotoxic carcinogenic substance. Both are considered.
Then, you discuss the difference of response observed in mice compared to rat, considering that there is a high degree of discordance between rats and mice in the development of chemically induced lung tumours (Smith & Anderson, 2017; Smith et al., 2018). This statement is mainly based on the analysis of the results of NTP studies conducted on 58 compounds, tested via inhalation: 11 chemicals inducing lung tumors in mice and not in rats, and being negative in the Ames test. Although there is a difference of response between B6C3F1 mice and F344 rats, this does not mean that B6C3F1 is too sensitive compared to humans. In contrast, rats are also generally considered more sensitive than mice to lung tumours induced by particles and to oxidative damage (based on IARC classifications). A more comprehensive comparaison would need further data including on other strains. Lastly, B6C3F1 mice are not considered as among the most sensitive strains whereas A/J and SWR mice are (Meuwissen and Berns, 2005[3]).

The other argument you brought is that: " *the available carcinogenicity data relate only to inhalation exposure of rats and mice to divanadium pentaoxide, which affected only the respiratory tract, and with tumour formation only in mice lungs*". According to CLP process, a single tumor site does not preclude a classification labelling for carcinogenicity.

Lastly, mouse /bronchiolar adenomas and carcinomas, which are common spontaneous and chemical induced lung tumors in mice, are similar to human adenocarcinomas in histomorphology and molecular characteristics including activation of the *K-ras* gene (Meuwissen and Berns, 2005[4] and Nikitin et al., 2004)[5] which are genetic alterations common for human pulmonary neoplasms (Tuveson DA, Jacks T, 1999 ; You et al., 1998; Herzog et al., 1996).[6]

Overall:
-According to NTP conclusion, there is clear evidence in mice (both sexes), some evidence in male rats and equivocal evidence of carcinogenic activity in female rats.

-According to CLP criteria:

[3] Meuwissen R, Berns A. Mouse models for human lung cancer. Genes & Development. 2005;19:643‑664.

[4] Meuwissen R, Berns A. Mouse models for human lung cancer. Genes & Development. 2005;19:643‑664.

[5] Nikitin AY, Alcaraz A, Anver MR, Bronson RT, Cardiff RD, Dixon D, Fraire AE, Gabrielson EW, Gunning WT, Haines DC, Kaufman MH, Linnoila RL, Maronpot RR, Rabson AS, Reddick RL, Rehm S, Rozengurt N, Schuller HM, Shmidt EN, Travis WD, Ward JM, Jacks T. Classification of proliferative pulmonary lesions of the mouse recommendations of the mouse models of human cancers consortium. Cancer Res. 2004;64:2307‑2316.

[6] Tuveson DA, Jacks T. Modeling human lung cancer in mice: similarities and shortcomings. Oncogene 1999;18:5318–24.
You M, Candrian U, Maronpot RR, Stoner GD, Anderson MW. Activation of the Ki-ras protooncogene in spontaneously occurring and chemically induced lung tumors of the strain A mouse. Proc Natl Acad Sci USA 1989;86:3070–4.
Herzog CR, Soloff EV, McDoniels AL, et al. Homozygous codeletion and differential decreased expression of p15INK4b, p16INK4a-_ and p16INK4a-_ in mouse lung tumor cells. Oncogene 1996;13:1885–91.

"Sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. **An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence.** A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites;"

As the NTP protocol is recognised as a gold standard for carcinogenicity testing, this NTP study performed with V2O5 must be considered as a well-conducted study. Since, a clear causal relationship between lung tumours and exposure to V2O5 is reported in both sexes of mice, criteria for Carc. 1B is fulfilled. In addition, even if the evidence is less strong than in mice, similar effect is reported in rats (in particular in males) which supports the findings observed in mice.

RAC's response

Thank you for your comments. RAC agrees that NTP study in rats remained inconclusive for carcinogenicity. However, in mice, increased incidence of lung tumours was seen at all doses in both males and females. According to current classification criteria, an increased incidence of tumours in both sexes of a single species in a well-conducted study can provide sufficient evidence for classification to cat 1B. Aspects, which may be considered to decrease the concern for humans, including the high background incidence in male mice, lack of direct genotoxicity, general toxicity/lung inflammation, and dose selection resulting in flat dose-response, have been taken into account and discussed in RAC opinion. Careful evaluation of the data did not, however, provide enough evidence to decrease the concern.

As discussed in RAC opinion, RAC considers NTP study sufficient and do not consider that there are limitations which would compromise the validity of the cancer findings in mice.

According to CLP regulation the route of exposure should be stated if it is conclusively proven that no other routes of exposure cause the hazard. In practise, this means negative data via oral route. Although it is acknowledged that the main concern in the case of vanadium pentoxide is related to the lung cancers in inhalation exposure, it is not possible conclusively to exclude e.g. GI-tract carcinogenicity after oral exposure and therefore, route of exposure cannot to be limited to inhalation.

Date	Country	Organisation	Type of Organisation	Comment number
21.11.2019	United Kingdom		Individual	7

Comment received

see Public Attachment: Comments on CLH carcinogenicity-Len Levy-2019-11-20.pdf

see pages 50-62

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Comments on CLH carcinogenicity-Len Levy-2019-11-20.pdf (2).pdf

Dossier Submitter's Response

In your point of view, the interpretation of the available data as presented in the CLH Report and its use in its conclusion in relation to the CLP criteria and CLH Guidelines would lead to a classification Carc. 2 – suspect human carcinogen, restricted to inhalation only and not to the dermal and oral routes, as we proposed. Your proposal is mainly based on the following arguments:

1. *"A number of published papers (Assem and Levy, 2009; Starr et al. 2012) have clearly demonstrated that there is no increase in lung tumours in either male of female rats in the NTP 2002 study when the data from the appropriate historical background rates are applied and the data are analysed statically. In any case, there never was a statistical increase in lung tumours, even in the original NTP 2002 publication. "*

Regarding male rat results, even if the increase of the incidence of alveolar/bronchiolar adenoma or carcinoma is not statistically significant compared to concurrent control, it exceeds the NTP historical control at 0.5 and 2 mg/m^3. Incidence of lung tumours in the control group is within historical control but somewhat at the upper end of the range. This may explain that the difference is not statistically significant between the groups. However, since the incidences at 0.5 and 2 mg/m3 are outside the historical control ranges, this should be considered as related to V2O5 exposure. At 1 mg/m3, the incidence is at the very upper end of the historical control range (adjusted rate = 14%).

In male rats fed the NTP-2000 diet, only 2 of 609 control rats developed alveolar/ bronchiolar carcinomas, and there was never more than one of this neoplasm in a study. In contrast, there are 3 males with carcinoma at 0.5 and 2 mg/m3 of V2O5.

In addition, analysis of genetic alterations (K-ras) suggest that the mechanism of lung carcinogenesis following divanadium pentaoxide exposure was different than that of spontaneously induced lung neoplasms. This is in favour of an association between lung tumours and exposure to V2O5. In addition, mouse alveolar/bronchiolar adenomas and carcinomas are similar to human adenocarcinomas in histomorphology and molecular characteristics including activation of the *K-ras* gene[7] [8]

2. "*There were no tumour increases at sites, other than the lung in mice, in the rats or mice in the NTP 2002 study but there is ample evidence to show that the inhaled divanadium pentaoxide, reached all tissues and organs (measured as vanadium) over the two-year exposure duration. In terms of marked inflammatory responses at the respiratory epithelium, this demonstrates that the exposure was clearly greatly in excess of the maximum tolerated dose (MTD) in rat and mice for the whole of the exposure duration at all exposure levels yet no systemic tumours or even tissue pathology were reported. For*

[7] 25. Meuwissen R, Berns A. Mouse models for human lung cancer. Genes & Development. 2005;19:643–664.

[8] Nikitin AY, Alcaraz A, Anver MR, Bronson RT, Cardiff RD, Dixon D, Fraire AE, Gabrielson EW, Gunning WT, Haines DC, Kaufman MH, Linnoila RL, Maronpot RR, Rabson AS, Reddick RL, Rehm S, Rozengurt N, Schuller HM, Shmidt EN, Travis WD, Ward JM, Jacks T. Classification of proliferative pulmonary lesions of the mouse recommendations of the mouse models of human cancers consortium. Cancer Res. 2004;64:2307–2316.

this reason, I propose that any carcinogenicity classification is restricted to inhalation, even in the absence of studies via the oral and dermal route."

According to the CLP Guidance: "*In certain instances, route-specific classification may be warranted, **if it can be conclusively proved that no other route of exposure exhibits the hazard**.*
*The classification shall take into consideration whether or not the substance is absorbed by a given route(s); or whether there are only local tumours at the site of administration for the tested route(s), **and adequate testing by other major route(s) show lack of carcinogenicity**."*
To the best of our knowledge, no adequate testing by other major route of exposure is available and consequently, lack of carcinogenicity for other relevant routes cannot be demonstrated which precludes a carcinogenicity classification to the inhalation route only.

Secondly you considered that: "*the exposure was clearly greatly in excess of the maximum tolerated dose (MTD) in rat and mice for the whole of the exposure duration at all exposure levels* yet no or even *tissue pathology were reported".*
We agree that some systemic toxicity was observed especially at the highest tested concentration level since a decrease of mean body weight values was observed. However it should be noticed that the lowest tested concentration level of 1 mg/m^3, showing an increase of lung tumor incidence, did not exhibit a decrease of the body weight. Lastly, the animal survival was kept at a good level in this NTP study (with > 25 animals at the end of the study).

3. "It is noted that the increases in lung cancer in mice at the three exposure levels in the NTP 2002 study were almost maximal and at the same level in the low, medium and high exposure groups (Starr and Macgregor 2014). This lack of a dose response curve is both puzzling and problematical. Normally, a positive dose-response curve gives confidence to the researcher and regulator of a causal link between the exposure to a substance and an effect. This is not possible here. However, there was a marked inflammatory response to the lungs (principally alveolar epithelial bronchiolar epithelial hyperplasia) which was dose related but moderate to severe in all three groups. This response was also seen in all three rat groups where it was even more marked."

In the NTP 2002 study, the shape of the dose response relationship is more comparable to a "plateau or a flat dose response" rather than to a absence of a dose response curve. Secondly, the aim of the CLP process is not to derive a N(L)OAEL but to characterise the hazard of a chemical substance. Since no further studies are made available, it is rather difficult to explain the dose response shape. Lastly, it should be highlighted that the $T_{e1/2}$ (elimination half-time (days))[9] from 2.26 to 13.9 days is well below the value recommended by the OECD Guidance 116 which discourages the use of concentrations leading to an elimination half-time ≥ 365 days in chronic toxicity and carcinogenicity studies.

[9] Table K10 - NTP 2002 study - Lung Deposition and Clearance Parameters with Error Estimates and 95% Confidence Intervals for Female Mice in the 2-Year Inhalation Study of Vanadium Pentoxide,

TABLE K10
Lung Deposition and Clearance Parameters with Error Estimates and 95% Confidence Intervals for Female Mice in the 2-Year Inhalation Study of Vanadium Pentoxide

Parameter[a]	Exposure Concentration (mg/m^3)	Estimated Value	Standard Error	95% Confidence Interval	
				Lower Limit	Upper Limit
$t_{e1/2}$					
	1	6.26	1.3	3.6	9.0
	2	10.7	1.8	7.1	14.3
	4	13.9	2.7	8.4	19.4

[a] k_d=initial deposition (µg V/day), k_r=deposition rate change (day^{-1}), k_e=elimination rate (day^{-1}), AUC_T=area under the lung burden (trapezoids) versus time curve at termination (µg • day), AUC_T/Exposure Concentration=area under the lung burden (trapezoids) versus time curve at termination normalized to exposure concentration, $t_{r1/2}$=deposition half-time (days), $t_{e1/2}$=elimination half-time (days)
[b] For these data, the estimates for k_r are small with relatively large standard errors resulting in wide confidence intervals on the half-times. Thus, the estimated half-times reported above are imprecise and should be interpreted with some caution.

4. *"A major drawback in the interpretation of the similar degree of lung tumour increase in the low, medium and high exposure groups of mice in the NTP 2002 study is that the low, medium and high groups all experienced marked and persistent inflammatory damage to the bronchial and alveolar epithelium, presumably for the full experimental exposure duration. This is not surprising as it is well known that divanadium pentaoxide is highly irritating and points to a poor dose selection for the NTP 2002 study. This is surprising as the 16-day and 3-month range finding studies, reported in NTP 2002, give evidence that marked inflammatory changes could be expected in the two-year study at the selected doses. It is important to note that both the OECD and the USEPA give guidance about the selection of doses used in chronic studies and note that excessive toxicity may "compromise the usefulness of the study and/or quality of the data generated" (OECD 2012). In general, international guidance for the design and conduct of hazard evaluation explicitly state that doses, where irritation or inflammation occur at the site of contact should be avoided (OECD 2012). In the case of chronic studies (as is NTP 2002) it states: "Principle 5 - Physicochemical factors (e.g., solubility, vapour pressure), the bioavailability of the compound, the palatability of the compound in food or drinking water, and other factors such as the potential for the substance to cause adverse effects at the site of administration (e.g., irritation, erosion, and ulceration) will influence the selection of the highest dose for chronic rodent bioassays. It is recommended that doses for chronic rodent bioassays be selected* ***to minimize or avoid adverse*** *nutritional, physical, organoleptic, and* ***irritant effects."*** *More recently, the OECD test guidelines for chronic toxicity/carcinogenicity testing state that potentially corrosive or irritating substances need to be diluted to avoid severe local effects (OECD 2018a,b). Clearly, these important study design factors were not adhered to in the case of the NTP 2002 study in rats and mice, as evidenced by the generally severe and persistent inflammatory changes, seen in both tested rodent species and which presumably occurred over the whole exposure duration. I believe this both "comprises the usefulness of the study" and confounds a clear interpretation of the lung tumour findings in the mice".*

We acknowledge that V2O5 is classified as irritant for respiratory tract and this should be taken into account in the design of chronic inhalation studies. Concentrations tested in the 2-year NTP studies are based on the results of the 16-day and 3-month study.

In the 16-day study in mice: absolute and relative lung weights were increased in males from 4 mg/m3 and at all concentrations tested (from 2 mg/m3) in females.

In the 3-month study in mice: Absolute and relative lung weights of males and females exposed to 4 mg/m3 or greater were significantly greater. Some mice exposed to 2 or 4 mg/m3 had inflammation of the lung. These results totally justify the choice of the concentrations tested in the 2-year study i.e. 0, 1, 2 or 4 mg/m3. In addition, the mortality and body weight data but also the T1/2 elimination confirm that the concentrations tested are adequate in the 2-year study (see response to comment 6).

5. "*A further concern is the lack of concordance between the mice and rats in the NTP 2002 study. It has been proposed that when a compound is non-genotoxic and associated with an increase in lung tumours in mice only, the relevance of the finding for human lung cancer risk is greatly diminished (Smith and Anderson 2017; Smith et al. 2018). In this context, non-genotoxic is intended to mean non-DNA-reactive.* "

For this comment and overall conclusion, please refer to previous answer to comment 6.

RAC's response

Thank you for your comments. RAC agrees that NTP study in rats remained inconclusive for carcinogenicity. However, in mice, increased incidence of lung tumours was seen at all doses in both males and females. According to current classification criteria, an increased incidence of tumours in both sexes of a single species in a well-conducted study can provide sufficient evidence for classification to cat 1B. Aspects, which may be considered to decrease the concern for humans, including the high background incidence in male mice, lack of direct genotoxicity, general toxicity/lung inflammation, and dose selection resulting in flat dose-response, have been taken into account and discussed in RAC opinion. Careful evaluation of the data did not, however, provide enough evidence to decrease the concern.

According to CLP regulation the route of exposure should be stated if it is conclusively proven that no other routes of exposure cause the hazard. In practise, this means negative data via the oral route. Although it is acknowledged that the main concern in the case of vanadium pentoxide is related to the lung cancers in inhalation exposure, it is not possible conclusively to exclude e.g. GI-tract carcinogenicity after oral exposure and therefore, route of exposure cannot to be limited to inhalation.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2019	Germany		MemberState	8

Comment received

The French MSCA proposes to change the current Annex VI entry from Muta. 2 (H341) to Muta. 1B (H340).

The DS states that there is no heritable germ cell mutagenicity test in mammals performed by physiological route but refers to a positive dominant lethal test (Altamirano-Lozano et al., 1996) performed with intraperitoneal injection. However, the reliability of

this test is questionable, because concurrent positive controls, which "should always be used unless the laboratory has demonstrated proficiency" (OECD TG 478), were not included in this test. Laboratory proficiency is not given. A firm assessment of results is thus questionable. No other in vivo heritable germ cell mutagenicity test in mammals judged to be reliable is available in the dossier.

Available in vivo somatic cell mutagenicity tests in mammals in the dossier, which were judged to be reliable (Klimisch 1 or 2) by the DS (in vivo micronucleus assays by Anonymous 2001 and NTP 2002) are negative. These tests were performed using physiological routes (inhalation, oral).

All available positive in vivo somatic cell tests (two comet assays) considered to be reliable by the DS are genotoxicity (indicator) tests and are performed by the intraperitoneal route.

Thus, the criteria for classification as Muta 1B are not fulfilled.

Moreover, all in vivo mutagenicity/genotoxicity tests judged to be reliable by the DS with positive results (Altamirano-Lozano et al., 1996; Altamirano-Lozano et al., 1999) used the intraperitoneal exposure route. All in vivo tests using oral and inhalation exposure judged to be reliable by the DS (micronucleus assays by Anonymous 2001, NTP 2002, Comet assay by Schuler 2011) are negative. According to Guidance on the Application of the CLP criteria (2017): "In summary, classification as a Category 2 mutagen would generally apply if only intraperitoneal in vivo tests show mutagenicity/genotoxicity and the negative test results from the in vivo tests using other routes of application are plausible."
The negative test results from the in vivo tests using other routes of application (inhalation, oral) are plausible (tests considered valid) and only intraperitoneal in vivo tests show mutagenicity/genotoxicity.
According to the Guidance on the Application of the CLP Criteria, classification of Cat. 2 would apply in this case.
The DS states on p. 49 that "In addition to the positive dominant lethal assay performed by intraperitoneal route, in vivo micronucleus assays and Comet assays in somatic cells are positive after respiratory exposure", however, all these tests were judged to be not reliable (Klimisch 3, Klimisch 4) by the DS and should be disregarded from a firm assessment of the data. All in vivo mutagenicity/genotoxicity tests applying physiological routes considered reliable by DS were negative.

All in all, the DE CA does not support to change the current Annex VI entry from Muta. 2 (H341) to Muta. 1B (H340).

Dossier Submitter's Response

Altamirano-Lozano et al., 1996: The argument related to an absence of positive control would be true if the results of this test would have been negative. But in this case, the result is positive and negative control was well included.

Regarding in vivo mutagenicity study performed according to OECD guideline, there are only 2 studies rated as Klimisch score 1 or 2: micronucleus assays performed by Anonymous (2011) and NTP (2002). There was no effect on PCE/NCE in the NTP study by inhalation thus, it cannot be confirmed that the bone marrow was adequately exposed.
Regarding Anonymous (2011): see remark below on the oral route of exposure. Due to

these limitations, the classification proposal has been rather concluded based on a weight of evidence approach.

"*In summary, classification as a Category 2 mutagen would generally apply if only intraperitoneal in vivo tests show mutagenicity/genotoxicity and the negative test results from the in vivo tests using other routes of application are plausible*."Increased micronucleus frequency was found after inhalation (Rojas-Lemus et al., 2014; Sun et al., 1987), even if we acknowledge that the results were issued from studies not rated as Klimisch score 1 or 2. In contrast, negative results were found after oral administration. Differences in toxicokinetics may explained the difference observed after oral or inhalation exposure. Since the major route of expected exposure is by inhalation, studies performed by this route can be more reliable than oral administration. For germ cell mutagenicity, there is no physiological route of exposure tested to confirm or not, the contradictory results obtained in the 2 dominant lethal tests (one positive by intraperitoneal route with a Klimisch score of 2 - Altamirano-Lozano et al. (1996) and one negative by subcutaneous route (Sun et al., 1987 with a Klimisch score of 4).

In conclusion, we aknowledge that this is a borderline case between category 2 and 1B. Category 1B can be fulfilled based on positive in vivo somatic cell mutagenicity test (increased micronucleus observed by Rojas-Lemus (2014) and Sun (1987)) in combination with some evidence that the substance has potential to cause mutations in germ cells (confirmed in toxicokinetics data)). Positive result was also found in a dominant lethal by intraperitoneal route. There is no dominant lethal (DL) performed *via* inhalation route. However, it is not very surprising since we note that dominant lethal (DL) assay are often carried out by non physiological route. Thus this positive finding can hardly be confirmed due to the lack of DL study by a physiological route.

See also response to comment 10

RAC's response

Thank you for your comments. RAC agrees that positive genotoxicity studies have severe limitations and are not sufficient to upgrade classification to 1B.

Date	Country	Organisation	Type of Organisation	Comment number
21.11.2019	United States		Individual	9

Comment received

The Muta 1B classification is neither warranted nor justified for V2O5.

In the most stringent and relevant in vivo genotoxicity studies in which the genotoxicity of V2O5 was investigated using high, tumorigenic concentrations, no evidence for either mutagenicity or chromosomal damage or aneugenicity was shown. These studies include Manjanatha et al. (2015), Banda et al. (2015), Black et al. (2015), Schuler et al. (2011) and NTP (2002). A number of other studies claimed positive findings for in vivo genotoxicity. In general, several of these latter studies suffer from methodological issues. The important question to ask in this context is whether to give any weight to these deficient studies, especially when much higher quality studies are already available from which to make an independent assessment. In the opinion of this reviewer, given the preponderance of weight available from those studies that either followed a validated protocol or were conducted according to the state-of-the-art methodology, it is reasonable to conclude that V2O5 is not likely to be an in vivo genotoxicant.

Consequently, the Muta 1B classification is neither warranted nor justified. In this case, the most appropriate, worst-case scenario, classification for V2O5 is Category 2.

This review's conclusion that V2O5 is not likely an in vivo genotoxicant strengthens the argument that this substance is not a genotoxic carcinogen and DNA reactivity can be excluded in the mode of action for mouse lung tumours. This conclusion was based on the absence of genotoxicity in the tumour target tissue (i.e., lung) using multiple, well-established endpoints.

This information is further expounded upon in the attached report.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment CLH mutagenicity-comments by Gollapudi Bhaskar-2019-11-21.pdf

Dossier Submitter's Response

See responses to comments 8 and 10

Regarding genotoxicity at lung level, NTP (2002) reported K ras mutations, Devereux et al. (2002), elevated phospho-MAPK, Paramanik et al (2013) and Altamirano-Lozano et al. (1999), increased length of DNA migration and Schuler et al (2011), increased 8-oxodGuo. Therefore, conclusion of an absence of genotoxicity in the tumour target tissue (i.e., lung) is untrue.

RAC's response

Thank you for your comments. RAC agrees that positive genotoxicity studies have severe limitations and are not sufficient to upgrade classification to 1B.

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2019	Germany	Vanadium Consortium / Vanitec	Industry or trade association	10

Comment received

Lack of evidence of a primary genotoxic mechanism of action for divanadium pentaoxide:
The analysis of the available data as employed in the CLH report does not appear to transparently weigh the relevance, reliability and adequacy of the data sources. The selection of the studies to be compared against the CLP criteria remains unclear and unexplained, so that the decision on the classification lacks transparency.
The Registrant has undertaken a thorough evaluation of all available data and has compared the data against the classification criteria as laid down in the Guidance on the Application of the CLP criteria (ECHA, 2017) in a weight-of-evidence analysis. A detailed analysis is presented in chapter 2.5 below. The outcome of this weight-of-evidence analysis can be summarised as follows:
• No evidence for in vitro mutagenicity in bacteria
• Equivocal evidence for in vitro clastogenicity/aneugenicity
• No evidence for in vitro mutagenicity in mammalian cells
• No evidence for in vivo mutagenicity in transgenic rodents
• No evidence for site of in vivo contact genotoxicity after inhalation
• No evidence for in vivo clastogenicity, positive findings stem largely from unreliable studies with unphysiological route of exposure
• Positive findings were largely obtained from studies published by one and the same

working group of E. Rojas and M. A. Altamirano-Lozano (University of Mexico City), whose study design and reporting shows recurring deficiencies
In contrast to the CLH report, the Registrant finds that the genetic toxicity studies actually support the conclusion that divanadium pentaoxide does not elicit any mutagenic activity.
The weight-of-evidence analysis of the entire genotoxicity database does not show any clear evidence of germ cell mutagenicity. Consequently, divanadium pentaoxide should not be classified in Category 1B. On the basis of the information obtained from relevant and reliable studies but also considering the remaining studies with all their limitations, the current classification with "Mutagenicity Category 2 – H341" already appears overtly conservative.

Please refer for a more detailed argumentation to the attachment "Vanadium Consortium-Vanitec_Comments on CLH proposal of V2O5_2019-11-22.pdf".

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Vanadium Consortium-Vanitec_Comments on CLH proposal of V2O5_2019-11-22.pdf

Dossier Submitter's Response

Even if not detailed, some justifications of the Klimisch scores are available from the tables (for example: information on purity, presence or not of positive and negative controls, GLP and OECD guideline followed or not etc).

In vitro:

Roldan and Altamiro (1990) and Rodriguez-Mercado (2011): contrary to your comment, we rate these studies with Klimisch score = 3.

Zhong et al., 1994: the purity of the substance is specified, V79 are commonly used cell lines, different concentrations are tested, positive control is included, test done in duplicate, indication of cytotoxicity provided. In particular, for the micronucleus assay, the test is in general accordance with OECD test guideline. In this context, a Klimisch score = 2 is appropriate.

We recognize the limitations you note concerning Kleinsasser et al. (2003) and Ramirez et al. (1997) studies, which do not followed any current recognized guideline. Please note that these studies are not used in the weight of evidence for the proposal as Muta. 1B.

In vivo:

There is no positive control included by Manjanatha et al. 1999. Therefore, the adequate sensivity of the test system cannot be confirmed in particularly in case of negative result.

Comments on Altamiro-Lozano et al., 1996: we understand your questioning about the adequacy of Comet assay protocol for germ cells. Regarding your remark on the weight of mice: according to OECD, weight of mouse is about 0.02 kg which is consistent with the mice weight in the study. Information on body weight was reported in table II of the publication. You point to unsufficient reporting for very precise details: even if we agree that the publication is not very detailed, it cannot be as detailed as a full study report.

We remind you that a CLH report should contain all information available for a substance for the endpoint considered and not only guideline studies. Mechanistic data can also be used as supportive data to conclude on a weight of evidence approach (in particular when there is a lot of studies of varying quality) and/or to identify a mechanism of action.

Regarding the data with non-physiological route: The results obtained from these studies should not be totally excluded. Indeed, they maximise the expected systemic exposure and thus may have limited relevance for risk assessment. In contrast, it is also generally recognized that there is no threshold for mutagenicity unless there is a proof the existence of such a threshold. Classification is only hazard-based. Therefore, these studies can be taken into account as information to be considered in a weight of evidence for hazard assessment and thus for classification purpose.

Human data:

Human data available with divanadium pentaoxide are not adequate to conclude on mutagenicity.

Overall:

Divanadium is a clear clastogen agent in vitro based on reliable studies (Anonymous, 2010 and Zhong et al. 1994).

In vivo, a lot of studies available present deficiencies: no follow current OECD guideline or not adequate route of exposure. For example, regarding micronucleus assays: divanadium is negative when administered by oral route. By inhalation route, it is negative in NTP (2002) study, however, there was no effect on PCE/NCE thus, it cannot be confirmed that bone marrow was adequately exposed. In contrast, increased micronuclei frequency was observed by Rojas-Lemus (2014) and Sun (1987) via the same route of exposure but with deviations from OECD guideline. Consistent positive effects were reported with intraperitoneal or subcutaneous exposures (Garcia-Rodriguez et al., 2016 and Sun et al., 1987). Non-guideline studies suggest that the mutagenicity can be due to oxidative stress (Schuler et al., 2011 and Cano-Gutierrerez et al., 2012). The fact that negative results were obtained from oral route whereas positive results were observed with inhalation - which is the major route of human exposure - and other routes investigated, can be due to difference in kinetics. Regarding germ cell mutagenicity, contradictory results were found with 2 studies performed with non-physiological route (one positive by intraperitoneal route with a Klimisch score of 2 - Altamirano-Lozano et al. (1996) and one negative by subcutaneous route (Sun et al., 1987 with a Klimisch score of 4). There is no dominant lethal (DL) performed *via* inhalation route. However, it is not very surprising since we note that dominant lethal (DL) assay are often carried out by non-physiological route. Thus this positive finding can hardly be confirmed due to the lack of DL study by a physiological route.

Toxicokinetics data by inhalation route confirm that divanadium pentaoxide or its metabolites reach the germ cells after this route of exposure.

In conclusion, we acknowledge that this is a borderline case between category 2 and 1B. Category 1B can be fulfilled in case of positive in vivo somatic cell mutagenicity test (increased micronucleus observed by Rojas-Lemus (2014) and Sun (1987)) in

combination with some evidence that the substance has potential to cause mutations in germ cells (confirmed in toxicokinetics data).
RAC's response
Thank you for your comments. RAC agrees that positive genotoxicity studies have severe limitations and are not sufficient to upgrade classification to 1B.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2019	Germany	Vanadium Consortium / Vanitec	Industry or trade association	11
Comment received				
Effects on fertility and effects via lactation: The Registrant contends that taking the information from several publications together, there is only limited and weak evidence from these studies that divanadium pentaoxide exposure by the inhalation and oral route may affect male and female fertility. This evidence is not considered strong enough to support classification in Cat 1B. It is therefore proposed to retain the already existing Category 2 – H361f. For adverse effects on development, the Registrant is of the opinion that the current harmonised classification with Repro. Category 2 H361d for development is still justified and should be retained. This is supported by the available information on developmental toxicity. For adverse effects on or via lactation, the publications referred to by the CLH report are all of low reliability (RL=3) , but the CLH report nevertheless comes to the conclusion that classification as H362: May cause harm to breast-fed children is warranted. This conclusion is derived from a limited data set without taking relevant uptake routes into consideration; however, an appropriate assessment is not possible due to a lack of any reported (no) effect level. The data summarised by the DS are not considered to represent clear evidence of adverse effects in the offspring due to transfer in the milk or adverse effect on the quality of the milk. There are also no reliable data that indicate the likelihood that the substance is present in potentially toxic levels in breast milk. Overall, the available data do not justify classification for lactation effects. In a very recent publication by US NTP, three-month toxicity studies of tetravalent and pentavalent vanadium compounds in Hsd:Sprague Dawley SD rats and B6C3F1/N mice via drinking water exposure are described (Roberts et al. 2019). Data on the number of offspring from treatments, post implantation in utero and live births, birth weights, and weight gains were generated. The results for vanadium in its pentavalent form indicate that fetal effects are only seen at levels that are toxic to mothers, so that vanadate ions do not appear to be selectively affecting live births or to be selectively toxic to neonates. The full findings of these sub-chronic studies are not available yet, but are expected to be published in 2020. They can reasonably be expected to provide valuable information regarding conclusions on the effect of vanadium in its pentavalent form on fertility and effects via lactation, thereby closing a data gap. Since the Registrant has been aware of these studies, a testing proposal was previously not submitted in the REACH registration of divanadium pentaoxide to avoid duplicate animal testing. The Registrant suggests that any decision on the classification for effects of divanadium pentaoxide on reproduction and via lactation should be deferred until the full study reports will be available for rats and mice.				

Please refer for a detailed argumentation to the attachment "Vanadium Consortium-Vanitec_Comments on CLH proposal of V2O5_2019-11-22.pdf".

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Vanadium Consortium-Vanitec_Comments on CLH proposal of V2O5_2019-11-22.pdf

Dossier Submitter's Response

Our proposal for the fertility classification is predominantly based on adverse effects on the male reproductive system, such as impaired sperm motility in mice after inhalation (NTP, 2002), atrophy of the secondary reproductive organs, hypospermia of the testis and atypical cells of the epididymis in rats after 90 days inhalation (NTP, 2002). These effects have been associated with marked body weight loss and general debilitation. Adverse effects on male fertility have been observed in several other studies in the absence of overt toxicity signs or weight change (e.g., Fortoul et al., 2007: necrosis of spermatogonia, spermatocytes and Sertoli cells). In addition, similar adverse effects on testes and sperm were observed for structural similar compounds, ammonium metavanadate and sodium metavanadate (NaVO3). Moreover, an increase of vanadium concentration in testes was observed following V2O5 inhalation exposure as well as with NaVO3 IP administration. With respect to the specific adverse effects on male reproductive organs originating consistently from several studies, fertility classification as Repr. 1B (H360F) is justified.

Developmental effects, such as delayed ossification, reduced foetal body weight, increased foetal mortality have been documented in several studies with V2O5, ammonium metavanadate and sodium metavanadate, all of which have methodological shortcomings and are hence not regarded as reliable. Since most effects have been observed consistently in the different studies, we deem that classification as Repr. Cat. 2 for development is justified.

Our proposal to add classification as H362: May cause harm to breast-fed children in Annex VI cannot be based on studies with V2O5 since no studies evaluating effects on or via lactation is available with this substance. However, five studies with sodium metavanadate (NaVO3, post-natal i.p. injection) and one study with Pentavanadate (not further specified) are available. These studies are limited in study design (e.g., only one dose level) and/or in reporting (e.g., no purity information) resulting in Klimisch score of 3. Due to qualitative limitations, results of one of these studies would not be sufficient to conclude on adverse effects on or via lactation. However, consistent neurotoxic effects demonstrated in these studies (e.g., demyelination in two studies and astrogliosis in three studies) make the conclusion of effects via lactation adequate in a weight of evidence approach.

Additionally, a read across justification has been provided by the dossier submitter demonstrating that read-across from sodium metavanadate to V2O5 is justified (e.g., because V2O5 releases the same vanadium ions as sodium metavanadate in water or body fluids and bioaccessibility studies indicate comparable absorption rates). Moreover, similar neurotoxic effects of the target substance V2O5 are observed in adult animals (Avila-Costa et al., 2005; Colín-Barenque et al., 2008; Colín-Barenque et al., 2015; Fortoul et al., 2014). In those studies with adult animals exposed to divanadium pentaoxide, identical brain areas (most relevant: hippocampus) are altered as with sodium metavanadate which support the read-across and the neurotoxic potential of V2O5 via lactation. Therefore, the classification proposal as H362: May cause harm to breast-fed children is justified.

Regarding your comment on the very recent publication by US NTP, three-month toxicity studies of tetravalent and pentavalent vanadium compounds in Hsd:Sprague Dawley SD rats and B6C3F1/N mice via drinking water exposure (Roberts et al., 2019). To our knowledge, this study is not published yet in a scientific review but only in a poster format (SOT, 2019). This study was performed with tetravalent (+4; vanadyl sulfate) and pentavalent (+5; sodium metavanadate) compounds in rats and mice but no treatment was conducted with V2O5 itself. Time-mated F0 Hsd:Sprague Dawley SD rats (n=16/group) were exposed to vanadyl sulfate or sodium metavanadate via drinking water beginning on GD6, and then during lactation and in post-weaning for 13-weeks (5 animals for biological sampling).
Data on the number of offspring from treatments, live births, birth weights, and weight gains were generated. However, no information about in utero post implantation loss can be retrieved from these studies either for the vehicle control group or treated animals contrary to your statement.

The results for sodium metavanadate indicate that percentage of live birth, number of live pups at PND 4 and pup survival was decreased from PND 1-4, 4-7 and 7-10 and additionally that pup toxicity during lactation resulted in insufficient animals in the 500 mg/L group to populate the biological sampling cohort for plasma and urine vanadium (V) concentration measurement. All of these information pointed out to a pup toxicity observed during lactation. However, the lack of detailed information about dams moribundity during parturition and throughout lactation in the 250 and 500 mg/L groups prevents a comprehensive understanding of the origin of this letality (eg. dystocia). Therefore, without a full set of information on Roberts et al., 2019, these results cannot be considered for classification purpose.

Regarding males and females B6C3F1/N mice exposed to vanadyl sulfate or sodium metavanadate for 13 weeks via drinking water, results about organ weights or histopathology are not included in this poster which prevents further consideration.

A request for further information on this NTP study was made by the DS at the end of February, 2020. No response from the NTP has been received yet.

RAC's response

Thank you for your comments. RAC agrees that available studies on sexual function and fertility are not sufficient to upgrade classification to 1B. Regarding lactational effects, there are evidence on the transfer of vanadium in milk and although the studies on adverse effects are limited, they raise a concern on lactational effects. New NTP study was not available for evaluation.

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2019	Germany		MemberState	12

Comment received

The French MSCA proposes to change the current Annex VI entry from Repr. 2 (H361d***) to Repr. 1B (H360Fd) and to add Lact. (H362).

The proposal for the fertility classification is predominantly based on adverse effects on the male reproductive system, such as impaired sperm motility in mice after inhalation (NTP, 2002), atrophy of the secondary reproductive organs, hypospermia of the testis and

atypical cells of the epididymis in rats after 90 days inhalation (NTP, 2002). These effects have been associated with marked body weight loss and general debilitation. Adverse effects on male fertility have been observed in several other studies in the absence of overt toxicity signs or weight change (e.g., Fortoul et al., 2007: necrosis of spermatogonia, spermatocytes and Sertoli cells). Similar adverse effects on testes and sperm were observed for ammonium metavanadate and sodium metavanadate. With respect to the specific adverse effects on male reproductive organs originating from several studies, fertility classification as Repr. 1B (H360F) is justified. Developmental effects, such as delayed ossification, reduced foetal body weight, increased foetal mortality have been documented in several studies, all of which have methodological shortcomings and are hence not regarded as reliable. Since most effects have been observed consistently in the different studies, we agree that classification as Repr. Cat. 2 for development is justified. The French MSCA proposes to add classification as H362: May cause harm to breast-fed children in Annex VI. No studies with V2O5 are available which evaluate effects on or via lactation. This proposal is based on five studies with sodium metavanadate (NaVO3, post-natal i.p. injection) and one study with Pentavanadate (not further specified). However, all studies are limited in study design (e.g., only one dose level) and/or in reporting (e.g., no purity information) resulting in Klimisch score 3. Due to qualitative limitations, results of one of these studies would not be sufficient to conclude adverse effects on or via lactation. However, consistent neurotoxic effects demonstrated in these studies (e.g., demyelination in two studies and astrogliosis in three studies) make the conclusion of effects via lactation credible in a weight of evidence approach. Additionally, a read across justification has been provided by the dossier submitter demonstrating that read-across from sodium metavanadate to V2O5 is justified (e.g., because V2O5 releases the same vanadium ions as sodium metavanadate in water or body fluids and bioaccessibility studies indicate comparable absorption rates). Moreover, similar neurotoxic effects of the target substance V2O5 observed in adult animals support the read-across and the neurotoxic potential of V2O5. Therefore, we agree with classification as H362: May cause harm to breast-fed children.
Dossier Submitter's Response
Thank you for your comments and support to the upgraded classification for fertility Cat. 1B, to maintain existing classification as Repr. Cat. 2 for development and to add a classification as H362: May cause harm to breast-fed children.
RAC's response
Thank you for your comments. Regarding sexual function and fertility effects, RAC considers available studies limited and partly contradictory. Therefore, classification to cat 2 for sexual function and fertility effects was considered more appropriate.

RESPIRATORY SENSITISATION

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2019	Germany		MemberState	13
Comment received				
No potential for respiratory sensitisation was found for V2O5 in one sub-chronic inhalation study with monkeys (Knecht et al., 1992) and in four case-control studies with workers				

from vanadium processing industries. Thus, we agree that no classification is required for this endpoint.
Dossier Submitter's Response
Thank you for your comment.
RAC's response
Noted.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2019	Germany	Vanadium Consortium / Vanitec	Industry or trade association	14
Comment received				
Acute toxicity via the oral route: The DS has selected an LD50 using the findings of an acute oral study in female rats with a pulverised product of technical grade (Leuschner, 1991a), thereby disregarding data available both for the pure substance, i.e. pulverised, analytical grade (Leuschner, 1991c) or the technical fused product as marketed (Leuschner, 1991b). The Registrant contends that for the assessment of the true intrinsic toxicity of a substance, the results of the oral toxicity of the pulverised "pure" analytical grade in female rats are more relevant and appropriate. Thus, based on the study by Leuschner (1991c), divanadium pentaoxide is already conservatively classified as Acute Oral Toxicity Category 4 – H302. This existing classification should be retained. Acute toxicity via inhalation: In his classification proposal, the DS has chosen an unusual approach in deriving an LC50 by using only one gender-specific finding in only one acute inhalation study in mice, thereby disregarding reliable data for male mice in the same study (Sullivan, 2011a). This sex-specific derivation is not considered reasonable and is not in accordance with OECD guideline 436, since both male and female animals were tested and therefore results of both sexes should be pooled together. In contrast, in the opinion of the Registrant the acute toxicity findings obtained in rats supported by further findings in mice (Sullivan 2011a,b) document that very fine divanadium pentaoxide powder should instead be classified as: Acute Inhalation Toxicity Category 2 "Fatal if inhaled" – H330". However, the above mentioned very fine divanadium pentaoxide powder was artificially generated in a laboratory by milling, whereas commercially available grades are far coarser (<3% of particles (w/w) < 10 µm). In the studies by Sullivan (2011a,b), granular divanadium pentaoxide was milled to produce a very fine powder (>96% of particles (w/w) < 10 µm) to conduct the inhalation exposures in rodents for comparative research purposes. These tests were not carried out with the substance in the form in which it is placed on the market and in which it can reasonably be expected to be used. According to the CLP Regulation Article 9(5), "when evaluating the available information for the purposes of classification, the manufacturers, importers and downstream users shall consider the forms or physical states in which the substance or mixture is placed on the market and in which it can reasonably be expected to be used." Thus, based on the study by Leuschner (1991d,e,f), divanadium pentaoxide in the forms in which it is actually placed on the market and is used, is already adequately and conservatively classified as Acute Inhalation Toxicity Category 4 – H332. The existing				

classification should be retained.

Please refer for a more detailed argumentation to the attachment "Vanadium Consortium-Vanitec_Comments on CLH proposal of V2O5_2019-11-22.pdf".

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Vanadium Consortium-Vanitec_Comments on CLH proposal of V2O5_2019-11-22.pdf

Dossier Submitter's Response

Acute toxicity via oral route:
We note that in your comment for inhalatory route you quote an article of CLP regulation "*when evaluating the available information for the purposes of classification, the manufacturers, importers and downstream users shall consider the forms or physical states in which the substance or mixture is placed on the market and in which it can reasonably be expected to be used*". Despite for the oral route, your position is to retain the study performed with the analytical grade. We disagree with this approach and we are in favour to consider all the studies.
In your comment for the inhalatory route, you say that deriving an LC50 by using only one gender-specific finding is « unusual ». For classification purpose, we have to consider different factors including sex. In case there are different sex-sensitivities, the most conservative LD50 should be used and not the pooled male and female LD50. Finally, differences observed in purity seem negligeable in our opinion.

Acute toxicity via inhalation route:
The study was published indicating LC50 separately for each sex. It appears protective to select the LC50 of females, which seems in most of the studies more sensitive.
Moreover, in the OECD GL 436, it is said "*If evidence is provided that one sex is more susceptible than the other, then the test may be continued with the more susceptible sex only.*", therefore, as you said in your comment "*sex-specific derivation is not considered reasonable and is not in accordance with OECD guideline 436*" is not true per se.
Concerning the particle size, the results of the studies by Leuschner et al. 1994, with 3 differents median particle size (3.0-3.9 µm; 10.5 µm and 2.9 µm) seem to indicate that the particle size is not a main issue in the acute inhalation toxicity of V2O5. Anyway, classification for acute toxicity is based on OECD protocol in which it is recommended to generate particle sizing with MMAD from 1 to 4 µm in order to expose relevant regions of the resporatory tract of the rodent.Therefore, as indicated in the CLP guidance, we selected the lowest LC50 in the most sensitive species and sex.

RAC's response

Acute oral toxicity: Based on the available information, it is not evident that the technical grade pulverised from of vanadium pentoxide tested in Leuchner et al. (1994) could not be on the market. In addition, as harmonised classification should be based on all of the data available for the endpoint in question, RAC agrees with the DS that the data regarding all tested forms should be taken into account. RAC also notes that the degree of analytical purity of the different forms tested is similar, and even higher for the technical grade pulverised form (calculated V2O5 99.3%) than for the analytical grade pulverised form (calculated V2O5 97.86%) of vanadium pentoxide.

Acute inhalation toxicity: As noted by the DS, indeed already in the original study report LC50 values were reported separately for males and females. RAC agrees with the DS that combining the LC50 values is not acceptable in this case, as the difference between

the sexes was so large. However, RAC notes that according to the TG 436, interpretation of the Anonymous (2011) acute inhalation toxicity test result should not be based on calculated LC50 values. Please see RAC's response to comment number 15 for further explanation. Regarding the particle sizes used for testing, we concur with the DS's response, and would like to note that the same requirement for particle size applies to all substances tested for acute inhalation toxicity.

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2019	Germany		MemberState	15
Comment received				
The French MSCA proposes to change the current Annex VI entry from Acute Tox. 4* (H332, H302) to Acute Tox. 1 (H330) and Acute Tox. 3 (H301). The proposal for the Acute Tox. oral classification (Cat. 3, H301) is based on eight of the twelve available studies resulting in an LD50 between 50-300 mg/kg bw, resulting in Acute toxicity hazard Category 3. The two studies resulting in LD50 < 50 mg/kg bw are considered not assignable (Klimisch score 4, Izmerov et al. 1982, mouse, 23 mg/kg bw) or not reliable (Klimisch score 3, Massmann, 1956, rat, LD50: 10.4 mg/kg bw) due to insufficient details on the protocol and substance tested. Therefore, we agree that Acute Tox. 3 – H301 is warranted and that an ATE of 100 mg/kg bw should be applied. The proposal for the Acute Tox. inhalation classification (Cat. 1, H330) is based on one study resulting in an LC50 for female mice of <0.056 mg/l (Anonymous 2011, lethality females: 2/3 and 3/3 at 0.056 and 0.5 mg/l respectively, Klimisch score 2), which is (practically) below the threshold for Acute Tox. 1 classification (≤0.05 mg/l). Five more acute tox. inhalation toxicity studies with rats are available resulting in LC50 ≥ 0.25 mg/l, indicating that rats are less sensitive than mice. One study with rabbits is available with LC50 = 0.205 mg/l. Since classification should be based on the lowest LC50 value available, we agree that classification as Acute Tox. 1 – H330 and an ATE of 0.005 mg/l are warranted.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted. However, according to OECD TG 436, which was used for the Anonymous 2011 study, "*the lower boundary estimates of the toxic class should be based on 6 animals per test concentration group, regardless of sex"*. These 6 animals can be either 3 of each sex, or 6 of the more sensitive sex. In addition, according to the OECD TG 436, the interpretation of the test result should not be based on calculated LC50 values. Instead, OECD TG 436 is a test procedure, where GHS classification is derived based on lethality at pre-fixed concentration levels. Based on the data from Anonymous (2011) and following the OECD TG 436 test procedure for aerosols (dusts and mists), illustrated in Annex 3c of the OECD TG 436, the results of Anonymous (2011) for both Fischer 344 rats and B6C3F1 mice clearly indicate acute inhalation toxicity category 2. The DS based their proposal on the apparently more sensitive sex (females) of the B6C3F1 mice in Anonymous (2011). While OECD TG 436 suggests that testing could be conducted in the more sensitive sex alone, if a sex difference is indicated, this was not done in Anonymous (2011). Therefore, at such low number of female animals in Anonymous (2011) (n=3/dose level instead of 6), it is difficult to establish whether the B6C3F1 females were indeed considerably more sensitive than males. In a 16-day repeated-dose study by NTP (2002), B6C3F1 female mice were not more sensitive to				

vanadium pentoxide by inhalation than males, when tested at a dose level of almost 60% of that in Anonymous (2011) (0.032 vs. 0.056 mg/L), and for 6 h/day instead of 4 h. Please refer to the RAC opinion document for further details. Based on the results from Leuschner et. al. (1994), also in Sprague-Dawley rats a sex difference existed (females were more sensitive), but it was much less pronounced. On the other hand, in the Fisher 344 rat in Anonymous (2011), a sex difference was not evident.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2019	Germany		MemberState	16
Comment received				
The French MSCA proposes to update the current Annex VI entry from STOT RE 1 (H372**) to STOT RE 1 (H372 (respiratory tract, inhalation)) to include the target organ and the route of exposure. We agree that the current harmonized classification is still justified based on the available studies but that an update is warranted.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

PUBLIC ATTACHMENTS
1. Vanadium Consortium-Vanitec_Comments on CLH proposal of V2O5_2019-11-22.pdf [Please refer to comment No. 1, 6, 10, 11, 14]
2. Contribution V2O5 CLH Public Consultation_FC.pdf [Please refer to comment No. 2]
3. Contribution V2O5 CLH Public Consultation_IPC.pdf [Please refer to comment No. 3]
4. CLH-V2O5 chemistry comments by David White-2019-11-21.pdf [Please refer to comment No. 4]
5. CLH mutagenicity-comments by Gollapudi Bhaskar-2019-11-21.pdf [Please refer to comment No. 9]
6. Comments on CLH carcinogenicity-Len Levy-2019-11-20.pdf (2).pdf [Please refer to comment No. 7]