Committee for Risk Assessment

RAC

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

P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

10 December 2020

CLH-O-0000006927-60-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **divanadium pentaoxide; vanadium pentoxide**

EC Number: **215-239-8**

CAS Number: **1314-62-1**

The proposal was submitted by **France** and received by RAC on **7 August 2019.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

France has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **23 September 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **22 November 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Tiina Santonen**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **10 December 2020** by **a simple majority of all members present and having the right to vote.**

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	023-001-00-8	divanadium pentaoxide; vanadium pentoxide	215-239-8	1314-62-1	Muta. 2 Repr. 2 Acute Tox. 4 * Acute Tox. 4* STOT SE 3 STOT RE 1 Aquatic Chronic 2	H341 H361d*** H332 H302 H335 H372** H411	GHS07 GHS08 GHS09 Dgr	H341 H361d*** H332 H302 H335 H372** H411			
Dossier submitters proposal	023-001-00-8	divanadium pentaoxide; vanadium pentoxide	215-239-8	1314-62-1	**Add** Carc. 1B Lact. **Modify** Muta. 1B Repr. 1B Acute Tox. 1 Acute Tox. 3 STOT RE 1	**Add** H350 H362 **Modify** H340 H360Fd H330 H301 H372 (respiratory tract, inhalation)	**Add** GHS06 **Retain** GHS08 Dgr **Remove** GHS07	**Add** H350 H362 **Modify** H340 H360Fd H330 H301 H372 (respiratory tract, inhalation)		**Add** inhalation: ATE = 0,005 mg/L (dusts or mists) oral: ATE = 100 mg/kg bw	
RAC opinion	023-001-00-8	divanadium pentaoxide; vanadium pentoxide	215-239-8	1314-62-1	Carc. 1B Muta. 2 Repr. 2 Lact. Acute Tox. 3 Acute Tox. 2 STOT RE 1	H350 H341 H361fd H362 H301 H330 H372 (respiratory tract, inhalation)	GHS06 GHS08 GHS09 Dgr	H350 H341 H361fd H362 H301 H330 H372 (respiratory tract, inhalation)		inhalation: ATE = 0,05 mg/L (dusts or mists) oral: ATE = 220 mg/kg bw	
Resulting Annex VI entry if agreed by COM	023-001-00-8	divanadium pentaoxide; vanadium pentoxide	215-239-8	1314-62-1	Carc. 1B Muta. 2 Repr. 2 Lact. Acute Tox. 3 Acute Tox. 2 STOT RE 1 STOT SE 3 Aquatic Chronic 2	H350 H341 H361fd H362 H301 H330 H372 (respiratory tract, inhalation) H335 H411	GHS06 GHS08 GHS09 Dgr	H350 H341 H361fd H362 H301 H330 H372 (respiratory tract, inhalation) H335 H411		inhalation: ATE = 0,05 mg/L (dusts or mists) oral: ATE = 220 mg/kg bw	

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The current Annex VI entry for vanadium pentoxide regarding acute toxicity is Acute Tox. 4* (H302) and Acute Tox. 4* (H332). The DS proposed to modify these to Acute Tox. 3 (H301) and Acute Tox. 1 (H330).

Much of the data for this endpoint originates from handbooks or other secondary sources with very few details on the study design and the substance tested. Therefore, the reliability of the studies could not be assessed by the DS (Klimisch score 4) or was assessed as not reliable (Klimisch score 3). RAC notes that only studies with a reliability score of 1 or 2 should be used as a basis for classification. The remaining studies can be used as supporting studies in a weight of evidence approach.

Oral route

The available data includes 12 studies; 6 were performed in rats, 4 in mice and 2 in rabbits. Only three studies (reported in the same publication by Leuschner *et al.* (1994)) were assigned A reliability score of 1. These are summarised below in Table A1. The remaining 9 studies, including all those performed in mice and rabbits, were assigned reliability scores 3 or 4 (short summaries with information on species and resulting LD50 values available in the CLH dossier).

The reported LD50 values from all the 12 studies cover a broad dose range, 10.4-714.7 mg/kg bw (Massmann, 1956; Leuschner *et al.*, 1994, respectively). The DS summarised the LD50 values obtained as follows:

- 2 derived LD50s that fall into category 2 (5-50 mg/kg) *
- 8 derived LD50s that fall into category 3 (50-300 mg/kg) **
- 2 derived LD50s that fall into category 4 (300-2000 mg/kg)

** RAC notes that the DS assessed one of these studies as not reliable (Klimisch 3), and for the other, reliability was not assignable (Klimisch 4)*

*** RAC notes that the DS assessed 7/8 of these studies as not reliable (Klimisch 3), and that for 1/8 studies, the LD50 value leading to Cat. 3 concerned only females. For males in the same study, the LD50 would fall into Cat. 4.*

In the three reliable studies in Sprague-Dawley rats by Leuschner et al. (1994), the LD50 values ranged from 221.1 to 715.7 mg/kg bw vanadium pentoxide. Three forms of vanadium pentoxide were tested. The technical grade pulverised vanadium pentoxide with a purity of 99.3 % showed a higher toxicity compared to the other forms of vanadium pentoxide (analytical grade pulverised, and technical grade fused). Based on the lowest LD50 value obtained in these studies, observed in females with technical grade pulverised vanadium pentoxide, the DS proposed to classify vanadium pentoxide as Acute Tox. 3; H301. They considered also the results reported in Sun *et al.* (1987; Klimisch score 4), to support this classification. This publication included studies in male and female rats (2 studies), male and female mice (2 studies) and male rabbits (1 study), all indicating Cat. 3 with LD50 values between 64 and 137 mg/kg bw, but with no further information available.

In addition, they proposed an ATE of 100 mg/kg bw based on a conservative approach using the converted acute toxicity point estimate at 100 mg/kg bw as indicated in CLP regulation. The DS's

reasoning for this approach was the lack of details reported in Sun *et al.* (1984) and the steep dose-response shown in Leuschner *et al.* (1994), shown below in Table A2.

Table A1. *Summary table of the acute oral toxicity studies, for which the reliability score was assessed by the DS as 1.*

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral toxicity, standard acute method OECD TG 401 Minor deviations GLP: yes Reliability (Klimisch score): 1	Sprague-Dawley rats 5 male and 5 female animals per dose group	Divanadium pentaoxide analytical grade pulverised Degree of analytical purity: Vanadium: 56.05 % (calculated V_2O_5: 97.86 %)	Single application *via* gavage Vehicle: 0.8 % aqueous hydroxypropyl-methylcellulose gel 215, 316, 464, 681, and 1000 mg /kg bw	Males: 474.2 mg/kg bw Females: 466.93 mg/kg bw **Acute Tox. 4**	(Leuschner *et al.*, 1994) (also study no. 001 from ECHA Dissemination, 2017) Details in Annex I
	Sprague-Dawley rats 5 male and 5 female animals per dose group	Divanadium pentaoxide technical grade fused Degree of analytical purity: Vanadium: 56.25 % (calculated V_2O_5: 100.04 %)	Single application *via* gavage Vehicle: 0.8 % aqueous hydroxypropyl-methylcellulose gel 316, 464, 681, 1000, and 1470 mg/kg bw	Males: 715.7 mg/kg bw Females: 658.4 mg/kg bw **Acute Tox. 4**	(Leuschner *et al.*, 1994) (also study no. 002 from ECHA Dissemination, 2017) Details in Annex I
	Sprague-Dawley rats 5 male and 5 female animals per dose group	Divanadium pentaoxide technical grade pulverised Degree of analytical purity: Vanadium: 55.6 % (calculated V_2O_5: 99.3 %)	Single application *via* gavage Vehicle: 0.8 % aqueous hydroxypropyl-methylcellulose gel 147, 215, 316, and 681 mg/kg bw males and females; 464 mg/kg bw males only	Males: 313.8 mg/kg bw **Acute Tox. 4** Females: 221.1 mg/kg bw **Acute Tox. 3**	(Leuschner *et al.*, 1994) (also study no. 003 from ECHA Dissemination, 2017) Details in Annex I

Table A2. Acute oral toxicity of technical grade pulverised vanadium pentoxide in rats in Leuschner *et al.* (1994).

Doses (mg/kg b.w.)	Mortality		Symptoms, pathology and histology
	male	female	
147	0/5	0/5	
215	0/5	1/5	
316	3/5	5/5	moderate inhibition of body weight gain (males)
464	5/5	-	enlarged stomach (1/5 males)
681	5/5	5/5	light reddened intestinal walls (1/5 males)

Results taken from disseminated database-file and registration dossier.

Dermal route

For acute toxicity via the dermal route, there is one publication available by Leuschner *et al.* (1994), consisting of three studies performed with the same three vanadium pentoxide forms as above: analytical grade pulverised (analytical purity Vanadium 56.05%, calculated vanadium pentoxide 97.86%), technical grade fused (analytical purity vanadium 56.25%, calculated vanadium pentoxide 100.04%) and technical grade pulverised (analytical purity vanadium 55.6%, calculated vanadium pentoxide 99.3%). The studies were performed according to OECD TG 402 with minor deviations under GLP and have been assigned reliability score 1 by the DS. 5 male and 5 female Sprague-Dawley rats were used in each study. Each vanadium pentoxide form was tested at dose levels of 2000 and 2500 mg/kg bw with 24 h application. Neither mortality nor signs of toxicity were observed in any of the studies up to the highest dose tested. Therefore, the DS did not propose a classification for this exposure route.

Inhalation route

The DS proposed to modify the current Annex VI entry Acute Tox. 4* (H332) to Acute Tox. 1 (H330). The data available include 8 studies in rats, mice and rabbits. Of these, 5 studies (4 in rats one in mice) have been assessed by the DS as reliable with restrictions (Klimisch score 2), summarised below in Table A3. In addition, one study in rabbits was assessed as not reliable due to relevant uncertainties regarding the control of the aerosol atmosphere and the actual exposure concentrations in the study (Sjöberg(1950), LC50 (7 h) 0.205 mg/L). Two further studies in rats could not be assessed for reliability (Sax and Lewis (1989), LCLo (2 h) 70 mg/m³ = 0.07 mg/L; US EPA, cited from IPCS (2001), LC67 (1 h) 1440 mg/m³ = 1.4 mg/L).

The DS summarised the LC50 values available from 6/8 of these studies as follows:

- 1 derived LC50 that fall into category 1 (≤ 0.05 mg/L, aerosolised powder exposure)*
- 2 derived LC50s that fall into category 2 (0.05 < LC50 ≤ 0.5 mg/L, aerosolised powder exposure in one of these studies and no information available on the test atmosphere in the other study)**
- 3 derived LC50s that fall into category 4 (1.0 < LC50 ≤ 5.0 mg/L, dust exposure)***
- For 2 studies, no conclusion on classification can be reached (dust exposure in one of these studies and no information available on the test atmosphere in the other study).
-

**RAC notes that in this study, the LC50 value resulting Cat. 1 (0.056 mg/L) was obtained only in female mice (n=3), of which 2/3 died. In male mice of the same study, the LC50 value was > 0.5 mg/L, for which the DS summarised: no conclusion on classification. However, RAC notes that it was reported in the study results that at 2.0 mg/L, all 10 mice (males and females) died within 4 days of exposure. Therefore, the LC50 value for males in this study seems to have been between > 0.5 mg/L and < 2.0 mg/L, falling into either Cat. 3 or Cat. 4 (likely Cat. 3, considering that 1/3 male mice died at 0.5 mg/L).*

*** RAC notes that DS assessed one of these studies as not reliable (Klimisch 3)*

**** RAC notes that for 2/3 of these studies, the LC50 value leading to Cat. 4 classification was only for females. In the same studies, the LC50 values for males were > 10 mg/L, which would lead to no classification*.

The DS proposed to classify vanadium pentoxide as Acute Tox. 1 "fatal if inhaled" – H330. Their reasoning was that *"According to CLP guidance, classification is based on the lowest LC50 value available."* In addition, they proposed an ATE of 0.005 mg/L for dusts and mists, taking into account that the lowest LC50 was below 0.056 mg/L Anonymous (2011) and was not further explored, and therefore the derivation of the ATE value should be based on a conservative approach using the converted acute toxicity point estimate at 0.005 mg/L, as indicated in CLP regulation.

***Table A3**. Summary of the animal studies on acute inhalation toxicity considered adequately reliable for classification. All values are expressed in mg/L V_2O_5, unless otherwise indicated.*

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value** **LC_{50}**	**Reference**
Acute inhalation toxicity, standard acute method OECD TG 403 Minor deviations GLP: yes Reliability (Klimisch score): 2	Sprague-Dawley rats 5 male and 5 female animals per dose group	Divanadium pentaoxide analytical grade pulverised Degree of analytical purity: Vanadium: 56.05 % (calculated V_2O_5: 97.86 %) Physical state: beige, solid (powder) Dust Median particle size: 3.0 - 3.9 µm	Single nose-only application for 4 h 0.90 +/- 0.39, 2.42 +/- 0.38, 4.72 +/- 1.45 mg/L air	Males: 11.09 mg/L air **No classification** Females: 4.29 mg/L air **Acute Tox. 4**	(Leuschner *et al.*, 1994) (also study no. 001 from ECHA Dissemination, 2017) Details in Annex I
	Sprague-Dawley rats 5 male and 5 female animals per dose group	Divanadium pentaoxide technical grade fused Degree of analytical purity: Vanadium: 56.25 % (calculated V_2O_5: 100.04 %) Physical state: beige, solid (powder) Dust Median particle size: 10.5 µm	Single nose-only application for 4 h 0.97 +/- 0.62, 2.71 +/- 1.94, 6.0 +/- 0.57 mg V_2O_5/L air	Males: 16.19 mg/L air **No classification** Females: 4.04 mg/L air **Acute Tox. 4**	(Leuschner *et al.*, 1994) (also study no. 002 from ECHA Dissemination, 2017) Details in Annex I
	Sprague-Dawley rats 5 male and 5 female animals per dose group	Divanadium pentaoxide technical grade pulverised Degree of analytical purity: Vanadium: 55.6 % (calculated V_2O_5: 99.3 %) Physical state: beige, solid (powder) Dust Median particle size: 2.9 µm	Single nose-only application for 4 h 1.11 +/- 0.08, 1.62 +/- 0.27, 5.2 +/- 1.52 mg V_2O_5/L air	Males: 4.40 mg/L air Females: 2.21 mg/L air **Acute Tox. 4**	(Leuschner *et al.*, 1994) (also study no. 003 from ECHA Dissemination, 2017) Details in Annex I

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Acute Inhalation Toxicity Study - Acute Toxic Class Method OECD TG 436 Minor deviations GLP: yes Reliability (Klimisch score): 2	Fischer 344 rats 2 mg/L: 5 male and 5 female animals 0.5 mg/L: 3 male and 3 female animals 0.056 mg/L: 3 male and 3 female animals	Divanadium pentaoxide (granular) Purity is confidential information Physical state: Yellow-orange granular powder. The milled test substance was aerosolised as a powder. MMAD: 2.00 mg/L: 2.71 µm (GSD: 2.05 to 2.58) 0.50 mg/L: 2.75 µm (GSD: 1.73 to 2.21) 0.056 mg/L: 1.88 µm	Since all animals died within 4 days after exposure to 2 mg/L, lower concentrations of 0.056 and 0.5 mg/L were tested as well. Exposure time: 4 h ; nose-only; aerosolisation of powder	LC_{50} = 0.25 mg/L (male and female rats) Lethality observed in: - males: 0/3 and 3/3 at 0.05 and 0.5 mg/l respectively. - females, 0/3 and 2/3 at 0.05 and 0.5 mg/l respectively. **Acute Tox. 2**	Anonymous, 2011 from (ECHA Dissemination, 2017) Study: 006 (section specific investigation, study report Details in Annex I
Acute Inhalation Toxicity Study - Acute Toxic Class Method OECD TG 436 Minor deviations GLP: yes Reliability (Klimisch score): 2	B6C3F1 mice 2 mg/L: 5 male and 5 female animals 0.5 mg/L: 3 male and 3 female animals 0.056 mg/L: 3 male and 3 female animals	Divanadium pentaoxide (granular) Purity is confidential information Physical state: Yellow-orange granular powder The milled test substance was aerosolised as a powder. MMAD: 2.00 mg/L: 2.71 µm (GSD: 2.05 to 2.58) 0.50 mg/L: 2.75 µm (GSD: 1.73 to 2.21) 0.056 mg/L: 1.88 µm (GSD: 2.28 to 2.40)	Since all animals died within 4 days after exposure to 2 mg/L, lower concentrations of 0.056 and 0.5 mg/L were tested as well. Exposure time: 4h ; nose-only; aerosolisation of powder	LC_{50} > 0.5 mg/L (in males) **No conclusion on classification** LC_{50} < 0.056 mg/l (in females) **Acute Tox. 1** Lethality observed in: - males: 0/3 and 1/3 at 0.056 and 0.5 mg/l respectively. - females: 2/3 and 3/3 at 0.056 and 0.5 mg/l respectively.	Anonymous, 2011 from (ECHA Dissemination, 2017) Study: 007 (section specific investigation, study report Details in Annex I

Comments received during consultation

One MSCA commented on the proposed classification and agreed with the proposals for both routes of exposure.

One Industry/ trade association commented on the proposal, disagreeing with it. Concerning the acute tox oral classification, they believed the already conservative acute oral toxicity classification to Cat. 4 should be retained. They viewed 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 than those in female rats with a pulverised product of technical grade.

Similarly, concerning acute inhalation toxicity, the industry/trade association was of the opinion that based on the study by Leuschner, vanadium pentoxide, 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, and that the existing classification should be retained. They found the DS's choice of deriving an LC50 by using only one gender-specific finding in only one acute inhalation study in mice unusual. They considered this approach neither reasonable nor in accordance with OECD TG 436. In their opinion, the acute toxicity findings obtained in rats supported by further findings in mice document that very fine vanadium pentoxide powder should instead be classified as: Acute Inhalation Toxicity Category 2 "Fatal if inhaled" – H330". They added that however, the very fine vanadium pentoxide powder was artificially generated in a laboratory by milling, whereas commercially available grades are far coarser (<3% of particles (w/w) < 10 µm). They mentioned that 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.*"

Assessment and comparison with the classification criteria

Oral route

RAC agrees with the DS that only the studies performed in rats by Leuschner *et al.* (1994) can be used as a basis for classification, because the reliability of the rest of the studies were either low or could not be assessed due to only minimal information on the studies being available. The studies by Leuschner *et al.* (1994) were performed in Sprague-Dawley rats (F+M) according to the OECD TG 401 with minor deviations and under GLP.

The three studies resulted in three sets of LD50 values: technical grade pulverised LD50 was the lowest: 221.1 mg/kg bw in females and 313.8 mg/kg bw in males. Analytical grade pulverised LD50 was 466.93 mg/kg bw in females and 474.2 mg/kg bw in males. Technical grade fused LD50 was the highest: 658.4 mg/kg bw in females and 715.7 mg/kg bw in males. Therefore, in all three studies, the females appeared somewhat more sensitive than males. The degree of analytical purity of vanadium was similar in all three studies, 55.6-56.25% (calculated vanadium pentoxide 97.86-100.04%).

In all 3 studies by Leuschner *et al.* (1994), the LD50 values from males would warrant Cat. 4 classification. In 2/3 of the studies, also the LD50 values from females would warrant Cat. 4, while 1/3 LD50 values from females would warrant Cat. 3. The DS proposed to classify vanadium pentoxide in Cat. 3 instead of Cat. 4, based on the lowest LD50 value from females.

The CLP guidance states that "*If there is a wide range of ATE values from the same species, it may be informative to consider the studies collectively, to understand possible reasons for the different results obtained. This would include consideration of factors such as the sex and age of the animals, the animal strains used, the experimental protocols, the purity of the substance and form or phase in which it was tested (e.g. the particle size distribution of any dusts or mists tested), as well as exposure mode and numerous technical factors in inhalation studies. This assessment may aid selection of the most appropriate study on which to base the classification.*"

In all three studies, the study design and animals used (strain, age) were similar, as well as the analytical purity of vanadium (55.6-56.25%). Therefore, the LD50 values appear to be related to the form of vanadium pentoxide used and the sex. It is also noteworthy that in the study with vanadium pentoxide technical grade pulverised (resulting in the overall lowest LD50 values), the mean LD50 value of males and females combined would be 267.45 mg/kg bw, also warranting

Cat. 3. In addition, although RAC considers that caution should be used with the results of the rest of the available studies of either poor or not assessable reliability, in general a weight of evidence assessment based on them would also support Cat. 3 for vanadium pentoxide. All in all, RAC finds the DS's proposal to classify vanadium pentoxide in Cat. 3 for acute oral toxicity acceptable.

However, considering that the approach for assigning the category is already rather conservative, and that there are several LD50 values available from reliable studies, RAC considers it unnecessarily cautious to use the converted acute toxicity point estimate as proposed by the DS for the ATE value (100 mg/kg bw as indicated in CLP regulation). According to the criteria, "*The acute toxicity estimate (ATE) for the classification of a substance is derived using the LD50/LC50 where available*". Therefore, RAC considers that an ATE value of 220 mg/kg bw is adequate, based on the lowest LD50 value observed in rats in reliable studies (221.1 mg/kg bw).

In conclusion, RAC is of the opinion that **Acute Tox. 3, H301** is warranted for vanadium pentoxide. In addition, RAC proposes an **ATE value of 220 mg/kg bw.**

Dermal route

There are three reliable studies performed on different vanadium pentoxide forms. As the LD50 was > 2500 mg/kg bw in all of them, classification via the dermal route is not warranted.

Inhalation route

The dataset for acute inhalation toxicity included five studies that were sufficiently reliable (reliable with restrictions, according to the DS). They were performed in rats (4 studies) and mice (1 study) according to OECD TG 403 or 436 with minor deviations and under GLP.

Leuschner *et al.* (1994) examined the same three forms of vanadium pentoxide in Sprague-Dawley rats as in the oral and dermal toxicity experiments. The three studies were performed according to OECD TG 403 with minor deviations and under GLP. The obtained LC50 values were in the range between 2.21-16.19 mg/L and appear to be related to the sex and the form tested. As with acute oral toxicity, females appeared to be more sensitive than males. Also here, the technical grade pulverised vanadium pentoxide with a purity of 99.3 % showed a higher toxicity compared to the other forms of vanadium pentoxide (analytical grade pulverised and technical grade fused) with LC50 values of 4.40 mg/L in males and of 2.21 mg/L in females.

In the other sufficiently reliable study, reported in the disseminated REACH registration dossier Anonymous (2011), acute inhalation toxicity was investigated in Wistar rats and B6C3F1 mice according to OECD TG 436. LC50 values were 0.25 mg/L in male and female Wistar rats, > 0.5 mg/L in male B6C3F1 mice and < 0.056 mg/L in female B6C3F1 mice.

The LC50 values vary greatly between studies, within species (between two different rat strains) and between sexes. The latter is particularly noticeable in mice, although also in Sprague-Dawley rats (but not in Fischer 344) females were more sensitive than males.

According to Annex I: 3.1.2.2.1. of the Guidance on the classification criteria: "*The preferred test species for evaluation of acute toxicity by the oral and inhalation routes is the rat … When experimental data for acute toxicity are available in several animal species, scientific judgement shall be used in selecting the most appropriate LD50 value from among valid, well-performed tests"*.

Moreover, the guidance states: "*Where several experimentally determined ATE values (i.e. LD50, LC50 values or ATE derived from studies using signs of non-lethal toxicity) are available, expert judgement needs to be used to choose the most appropriate value for classification purposes. Each study needs to be assessed for its suitability in terms of study quality and reliability, and also for its relevance to the substance in question in terms of technical specification and physical*

form. Studies not considered suitable on reliability or other grounds should not be used for classification.

In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested. However, expert judgement may allow another ATE value to be used in preference, provided this can be supported by a robust justification. If there is information available to inform on species relevance, then the studies conducted in the species most relevant for humans should normally be given precedence over the studies in other species. If there is a wide range of ATE values from the same species, it may be informative to consider the studies collectively, to understand possible reasons for the different results obtained. This would include consideration of factors such as the sex and age of the animals, the animal strains used, the experimental protocols, the purity of the substance and form or phase in which it was tested (e.g. the particle size distribution of any dusts or mists tested), as well as exposure mode and numerous technical factors in inhalation studies. This assessment may aid selection of the most appropriate study on which to base the classification."

In an attempt to select the most appropriate LC50 value, the results from Leuschner *et al.* (1994) and Anonymous (2011) are discussed below (available in tabulated form in Table A3). All studies reported in these two publications were performed according to an OECD TG (403 in Leuschner *et al.* (1994) and 436 in Anonymous (2011)) with only minor deviations and under GLP. All exposures in all the studies were made via the nose-only and for 4 h.

The Leuschner *et al.* (1994) study was performed in Sprague-Dawley rats. The study protocol was the same in each of the three tests. The LC50 values ranged from 2.21 mg/L air in females (technical grade pulverised) to 16.19 mg/L air in males (technical grade fused). The group size in each of the three studies was 5 males and 5 females. The test material was a powder dust in each test. The median particle sizes (MMAD, GSD not available) varied from 2.9 µm to 10.5 µm and did not appear to greatly impact the resulting toxicity (Table A3, reported in more detail in Annex I). Also, the analytical purity of the test compounds were similar (~56% Vanadium, calculated vanadium pentoxide 98-100%). Therefore, it appears that the vanadium pentoxide form tested, and the sex are the most likely contributors to the different LC50 values observed within the Sprague-Dawley strain. Regardless of the differences, the resulting classification would be similar. 2/3 of the LC50 values from males would lead to no classification. 1/3 LC50 value from males and 3/3 LC50 values from females would lead to Acute Tox. 4.

In Anonymous (2011) performed in Fischer 344 rats and B6C3F1 mice, the study protocol was the same in both tests. The LC50 value for Fischer 344 rats was 0.25 mg/L (males and females). For male B6C3F1 mice, the LC50 was between 0.5 mg/L and 2 mg/L, and for females < 0.056 mg/L. The group size in each test was 3 males and 3 females, except for the highest dose level (2 mg/L), where 5 males and 5 females were included. In Anonymous (2011), the test compound was milled and aerosolised as a granular powder. The particle sizes were slightly smaller than in Leuschner *et al.* (1994), ranging from 1.88 µm (highest doses; MMAD) to 2.75 (mid-dose), which could contribute to the higher observed toxicity. The purity of the test compound was known to RAC but is confidential information. Based on the LC50 values, the resulting classification for Fischer 344 rats would be Cat. 2. For female B6C3F1 mice, the result would be Cat 1., while for male B6C3F1 mice, the classification would likely be Cat. 3 (or Cat. 4; an exact LC50 was not determined).

The results of Leuschner *et al.* (1994) and Anonymous (2011) are markedly different, one (including 3 studies) indicating Acute tox Cat. 4 for the Sprague-Dawley strain, and the other Cat. 2 for the Fischer 344 strain. Furthermore, the result in female mice, indicating Cat. 1, deviates from both the results from rats and male mice (Cat. 3). The available information, discussed above, does not help to understand possible reasons for the different results obtained.

The most evident differences within and between the studies appear to be the species and/or strains and the form of the test compound used.

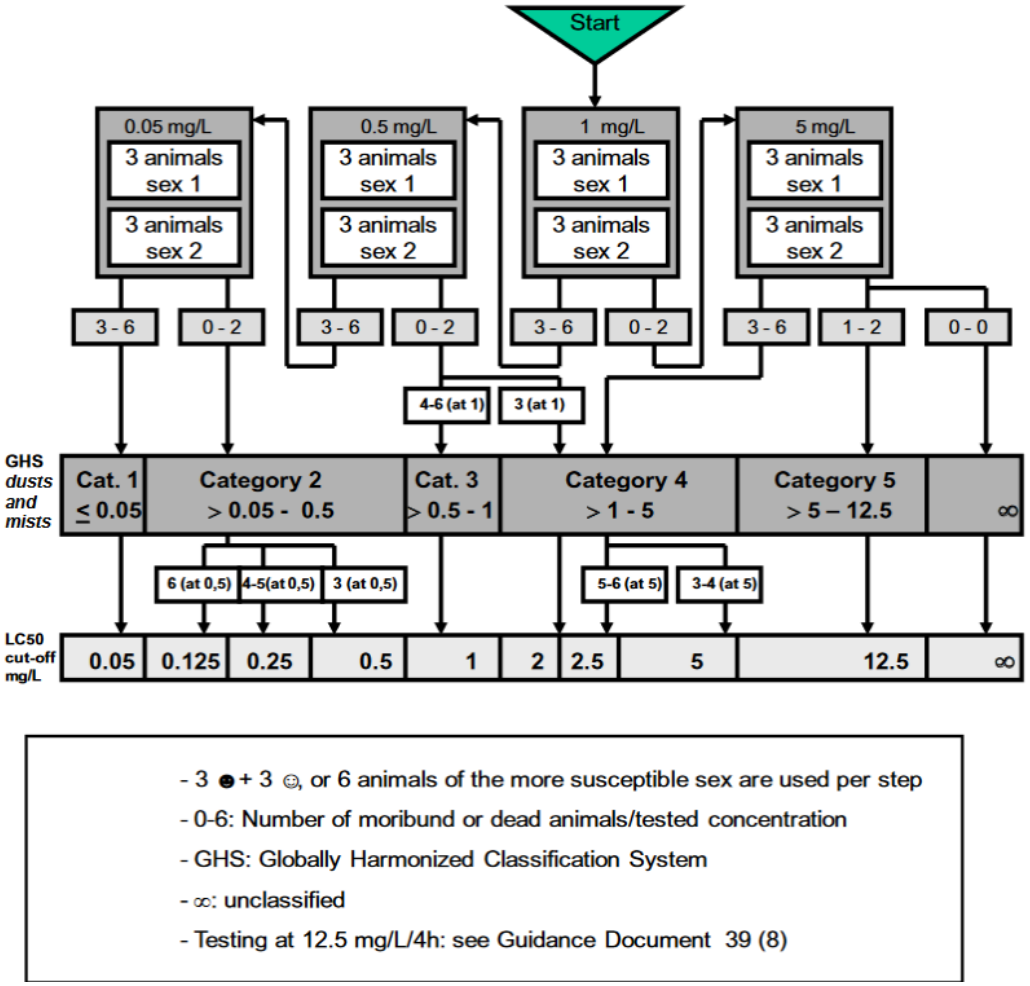
The DS proposed to classify vanadium pentoxide for acute inhalation toxicity Cat. 1 according to the more sensitive sex (females) of the most sensitive species (mouse). While it appears evident that the female mice were the most susceptible in these studies, it should be noted that there is only one acute inhalation toxicity mouse study available, and the number of animals in Anonymous (2011) was small (n=3/sex at the two lower dose levels), although acceptable according to the TG. However, it is important to note that according to OECD TG 436, testing requires 6 animals/test concentration, either 3 of each sex or 6 of the more susceptible sex. According to the TG, "*the lower boundary estimates of the toxic class should be based on 6 animals per test concentration group, regardless of sex"*. Therefore, it appears questionable to consider the results from males and females separately, resulting in only n=3/dose level.

In rats, there are 4 rat studies available. Furthermore, according to the CLP criteria, OECD TG 403 and OECD TG 436, the rat is the preferred species, not mouse, and if mice are used, it should be justified. No such justification is provided. In addition, neither the CLP criteria nor the guidance imply that the more sensitive sex of one species or within an experiment should automatically dictate the classification. The OECD TGs would allow performing the study only in the more susceptible sex, though.

Combining the male and female mouse results from Anonymous (2011) would lead to a combined LC50 value of around 0.28 mg/L for mice. This value is obtained by assuming that the LC50 for males was 0.5 mg/L (in reality it was higher than that) and that the LC50 for females was 0.056 (in reality it was lower than that). This combined LC50 value would lead to Cat. 2. However, RAC considers that such combination of the LC50 values is not acceptable in this case, as the difference between the sexes was so substantial.

Nevertheless, it should be noted that, according to the OECD TG 436, the interpretation of the test result should not be based on calculated LC50 values at all. Instead, TG 436 is a test procedure where GHS classification is derived based on lethality at pre-fixed concentration levels (Figure A1). According to the TG, lethality of all 6 animals/concentration level should be considered (the 6 animals can be either 3 of each sex, or 6 of the more sensitive sex). According to Annex 3c of TG 436, the results of Anonymous (2011) for both Fischer 344 rats and B6C3F1 mice clearly indicate acute inhalation toxicity category 2. Based on the data shown in table A3 and following the TG 436 test procedure for aerosols (dusts and mists) shown in Figure A1: because at the highest dose level tested (2 mg/L) all animals of both species died, the dose level of 0.5 mg/L was tested. At 0.5 mg/L, 4/6 B6C3F1 mice died (3 females, 1 male), and 5/6 of Fischer 344 rats died (3 males, 2 females). Therefore, testing at the dose level of 0.05 mg/L was performed. At the (actual) concentration of 0.056 mg/L, 2/6 B6C3F1 mice (2 females) died. According to the TG, this indicates GHS Cat. 2 for dusts and mists (Cat 1 would result if 3-6 of the animals had died at 0.05 mg/L). With respect to the Fischer 344 rat, at 0.056 mg/L, no lethalities were observed, also indicating Cat. 2 (lethality in 0-2 animals). The tested dose level of 0.056 mg/L was about 10% greater than that required by the TG of 0.05 mg/L, but it is assumed that this had little impact on the result.

Figure A1. *Annex 3c of the OECD TG 436, illustrating the test procedure and the interpretation of the result.*



On the other hand, 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 a 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. 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. It should also be noted that 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 what was used in Anonymous (2011) (0.032 vs. 0.056 mg/L), and for 6 h/day instead of 4 h. In this 16-day range finding test, conducted prior to an inhalation Mammalian bone marrow chromosome aberration test, groups of five male and five female B6C3F1 mice were exposed to particulate aerosols of vanadium pentoxide at concentrations of 0, 2, 4, 8, 16 and 32 mg/m^3 (=0.032 mg/L) by inhalation, 6 hours per day, 5 days per week for 16 days. All males exposed to 32 mg/m^3 and one 8 mg/m^3 male died or were killed moribund before the end of the study, whereas all females survived all treatments (although the 32 mg/m^3 females lost weight during the study). In the study, the vanadium pentoxide aerosol sizes ranged from 1.0 to 1.3 µm (MMAD;

GSD 2.3-2.8). The toxicity in males observed in the (NTP, 2002) study could derive from effects purely related to repeated-dose toxicity. Mortalities occurred on day 6.

All in all, RAC notes the sex difference in the toxic effects observed in the B6C3F1 mouse, but considers that it is difficult to base the classification only on the apparently more sensitive female mice, because only 3 females were tested, while the TG would require testing of 6 females in case only one sex was included in the study. On the other hand, in the (NTP, 2002) study, up to the dose levels of 0.032 mg/L, females were not more sensitive than males. This highest dose tested in NTP, 2002 did not result in mortality in females, with repeated 6 h/day exposures (16 day-study), and was almost as high as the lowest dose tested in the Anonymous (2011) acute tox study (0.032 mg/L and 0.056 mg/L, respectively, with 6 h/day and 4 h/day exposures). Therefore, RAC considers that the result from the (NTP, 2002) study lessens the concern raised regarding the susceptibility of the female B6C3F1 mice in the Anonymous (2011) acute tox inhalation study. Following the TG 436, the available data from Anonymous (2011) for both B6C3F1 mice and Fischer 344 rat indicate Cat. 2 for acute inhalation toxicity.

Taking everything described above into account, RAC is of the opinion that Acute Tox. 2 is warranted for vanadium pentoxide, based on the results for both B6C3F1 mice and Fischer 344 rats in Anonymous, 2011.

For acute inhalation toxicity, the ATE value of 0.005 mg/L proposed by the DS appears unreasonably conservative, considering that even in two-year carcinogenicity studies using inhalation exposure (NTP, 2002), the highest dose levels were 0.004 mg/L for mice and 0.002 mg/L for rats. Instead, and considering the large range of experimental LC50 values obtained, RAC would prefer to use the appropriate conversion value from Annex I Table 3.1.2 that relates to the classification category 2, 0.05 mg/L (dust/mist).

In conclusion, RAC is of the opinion that for acute inhalation toxicity, **classification as Acute Tox. 2, H330 is warranted for vanadium pentoxide, with an ATE of 0.05 mg/L (dusts or mists).**

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed not to classify vanadium pentoxide for respiratory sensitisation. The data available for assessing this endpoint included one non-guideline sub-chronic inhalation test in cynomolgus monkeys and four studies on workers from vanadium processing industries.

Overall, the DS noted that respiratory irritation was frequently found in the available publications which was considered consistent with the current classification as STOT SE 3. Results from a cytological immunology test in cynomolgus monkey (Knecht *et al.*, 1992) and in workers exposed to vanadium pentoxide did not show significant difference in the IgG levels in the Cynomolgus monkey (Knecht *et al.*, 1992) or in IgE levels in cynomolgus monkey and exposed workers. Therefore, the DS concluded that the reported respiratory symptoms appear not to be linked to a sensitisation mechanism, but rather to an irritating mechanism thus no classification and labelling for respiratory sensitisation was proposed.

Comments received during consultation

One MSCA commented the proposal and agreed with no classification for respiratory sensitisation.

Assessment and comparison with the classification criteria

Animal data

The only *in vivo* study available for this endpoint was a non-guideline sub-chronic study in adult male cynomolgus monkeys (*Macaca sascicularis;* Knecht *et al.* 1992). No information was available on the GLP conditions. The study assessed pulmonary reactivity to vanadium pentoxide with provocation challenges and compared vanadium pentoxide reactivity before and after subchronic vanadium pentoxide exposure with pulmonary function testing. In addition, bronchial lavage fluid was analysed. The DS evaluated the reliability of the study as 2 in the Klimisch score. The monkeys were exposed to vanadium pentoxide dust by whole-body inhalation for 6 h/day and 5 d/week for 26 weeks. The purity of the test compound was >99.6%. There were three groups of animals:

- Group 1 (9 animals): 0.1 mg/m^3 on three days a week (Mon, Wed, Fri) and peak doses of 1.1 mg/m^3 on two days a week (Tue, Thu)
- Group 2 (9 animals): constant doses of 0.5 mg/m^3 (Mon-Fri)
- Group 3 (8 animals): controls exposed to the vehicle (filtered, conditioned air)

The study design also included 6 h/d vanadium pentoxide challenge doses of 0.5 mg/m^3 and 3 mg/m^3 (at two-week intervals, respectively) both before and after the sub-chronic exposures. Pulmonary function tests were conducted on the day following each dust challenge.

After the sub-chronic exposure, pulmonary reactivity was not increased on either vanadium pentoxide group in comparison with the control group. Instead, a trend towards a decrease in pulmonary reactivity was found in both exposure groups. Acute effects after the pre-exposure challenge with 0.5 and 3 mg vanadium pentoxide/m^3 were concentration-dependent impairment in pulmonary function, characterized by airway obstructive changes (increased resistance and decreased flow). Analysis of respiratory cells recovered from the lung by bronchoalveolar lavage demonstrated that airway obstruction was accompanied by a significant influx of inflammatory cells into the lung. Cytological immunological results test (IgE and IgG analysis) did not indicate allergic sensitization.

Human data

Four occupational studies are available from vanadium processing industries, summarised below.

1) A cross-sectional case-control study from Finland with 63 male workers from a vanadium pentoxide producing company, reported in three publications (Kiviluoto *et al.,* 1979 and 1981a, and Kiviluoto, 1980). The average exposure was 11 years. Measured exposures between 1970-1975 were 0.2-0.5 mg vanadium/m^3, corresponding to 0.26-0.89 mg vanadium pentoxide/m^3. Due to technical changes at the factory, exposure was reduced in early 1976 to 0.01-0.04 mg vanadium/m^3, corresponding to 0.018-0.071 mg vanadium pentoxide/m^3. The control group consisted of 63 male dust-exposed matched individuals, who worked at a nearby mine (except for Kiviluoto *et al.,* 1981a, where the control group consisted of only 22 workers). The performed tests included rhinoscopy, analysis of sputum cells, measurement of pulmonary ventilation, analysis of nasal secretion smear cells, respiratory questionnaire, x-ray analysis of the lung, and hematologic and serum chemical laboratory tests.

   In Kiviluoto *et al.* (1979), cases of self-reported subjective symptoms of respiratory tract irritation were reported, as well as clear signs of non-allergic inflammation (a significantly increased number of neutrophils but not eosinophils in the nasal smears of the exposed group). In histopathology, a significantly higher number of plasma cells were reported in

nasal mucosa samples. Also, an increase in the number of "round cells" in mucous membranes from nasal turbinates was reported. Respiratory symptoms reported in Kiviluoto (1980) included significantly more wheezing in the exposure group, but X-ray analysis did not show exposure related differences, and there were no differences observed between the groups in ventilation measurements. In Kiviluoto *et al.* (1981), significant differences between the groups were not observed in the hematologic parameters. In serum, significant differences were observed for albumin (↓), chloride (↓), urea (↑), bilirubin (↓) and conjugated bilirubin (↑).

2) A case-control study from South Africa (Irsigler *et al.*, 1999) included 12 vanadium plant workers that were exposed to vanadium pentoxide concentrations of <0.15-1.53 mg/m^3. These subjects had diagnosed bronchial hyperreactivity (by lung function and bronchoprovocation tests). The control subjects (n=12) worked in the same company but did not show bronchial hyperreactivity. None of the subjects were exposed to toxic levels of SO_2 and NH_3, but co-exposure to SO_2 and NH_3 could be excluded only for 3/12 workers.

   In the bronchial hyperreactivity group, 7/12 subjects had cough and breathing difficulties developed within 6 months of starting to work at the factory. In the control group, 2/12 subjects experienced the same symptoms within the same time period. The IgE levels between cases and controls were not significantly different.

3) A case-control study from USA (Lewis, 1959a, b) included 24 men working in vanadium plants, 13 in Colorado and 11 in Ohio. All had been exposed to vanadium (as vanadium pentoxide) via inhalation for at least six months. The exposure concentrations in the Colorado plant were 0.097-0.243 mg/m^3 vanadium pentoxide (mass respirable vanadium: 16.6-51 %; particle size < 5 µm: 92.5-99 %), and in the Ohio plant 0.018-0.925 mg/m^3 vanadium pentoxide (mass respirable vanadium: 2-100%; Particle size < 5 µm: 96.3-100 %). 45 control subjects that did not work in the vanadium industry were matched for age, economic status and job activities.

   Symptoms with significant increases in the exposure *vs.* control group included cough, sputum, eye, nose and throat irritation, nosebleed, wheezing, rales, injected pharynx or green tongue.

4) A retrospective cohort study from Peru (Vintinner *et al.*, 1955) included 78 workers engaged in the processing of vanadium-bearing ore, and 37 controls. The vanadium concentrations in the air varied from 0.01-58.80 mg/m^3. In the control areas the concentration range was 0.000-0.007 mg/m³. These concentrations do not seem to refer to vanadium pentoxide. All dust particles were below 5 µm in diameter. The concentration of SO_2 in the air in various workplaces ranged 0.0-2.0 ppm.

   An abnormally high prevalence of signs and symptoms indicative of irritation to the upper respiratory tract and to the eyes was reported among the workers exposed to vanadium-bearing dusts, compared with the workers not exposed to such dust.

According to the CLP Regulation, *"Substances shall be classified as respiratory sensitisers (Category 1) where data are not sufficient for sub-categorisation in accordance with the following criteria:*

(a) if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity; and /or

(b) if there are positive results from an appropriate animal test".

On human evidence, the CLP regulation furthermore states: *"Evidence that a substance can lead to specific respiratory hypersensitivity will normally be based on human experience. In this context, hypersensitivity is normally seen as asthma, but other hypersensitivity reactions such as rhinitis/conjunctivitis and alveolitis are also considered. The condition will have the clinical character of an allergic reaction. However, immunological mechanisms do not have to be demonstrated."*

And on animal evidence: *"Data from appropriate animal studies (11) which may be indicative of the potential of a substance to cause sensitisation by inhalation in humans (12) may include:*

(a) measurements of Immunoglobulin E (IgE) and other specific immunological parameters in mice;

(b) specific pulmonary responses in guinea pigs."

All in all, although the data are limited, upper respiratory track symptoms were reported in the occupational studies. However, they appear to be caused by irritation rather than sensitisation. The only potentially relevant finding regarding sensitisation was reported in the occupational study by Kiviluoto *et al.* (1979), as a significantly higher number of plasma cells in nasal mucosa samples. This finding is interesting, because plasma cells derived from B-lymphocytes produce IgE antibodies. However, there is no more information available on this finding, and as the available studies did not report symptoms appearing to be related to hypersensitivity, the plasma cell finding on its own is not considered significant.

Regarding the sensitising property of vanadium pentoxide in general, there are no experimental data available in the REACH registration dossier for skin sensitisation. However, vanadium pentoxide was considered by the registrant as not skin sensitising, based on read-across from vanadium oxide sulphate pentahydrate (vanadyl sulphate) and sodium metavanadate, for which GPMT studies are available.

In conclusion, RAC agrees with the DS that neither the *in vivo* animal nor human data available indicate respiratory sensitisation potential for vanadium pentoxide. Therefore, **classification is not warranted**.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS proposed to update the current Annex VI entry from STOT RE 1 (H372**) to STOT RE 1 (H372) and to include the target organ and the route of exposure (respiratory tract, inhalation). This was based on both human and animal data for vanadium pentoxide.

There are seven animal studies available, of which four were assessed as reliable (Klimisch 1; NTP, 2002: 2-year study in mice and rats, 90-day study in mice and 90-day study in rats; Schuler *et al.*, 2011), another was assessed as reliable with restrictions (Klimisch 2; Knecht *et al.*, 1992), and two were assessed as not reliable (Klimisch 3; García *et al.*, 2004; Mountain *et al.*, 1953). The studies assessed as reliable or reliable with restrictions are summarised below in Table S1. In addition, there are seven studies available in humans, summarised in Table S2.

In good quality animal studies, non-neoplastic changes were observed in the respiratory system (lung, larynx and nose) in both rats and mice, namely lung fibrosis and hyperplasia. They were

observed in sub-chronic 90-day studies in rats and mice from the dose level of 2 mg/m^3 (corresponding to 0.002 mg/L/6h/day; NTP, 2002). Similar lung effects were observed in 2-year studies from the dose levels of 0.5 mg/m^3 in rats and 1 mg/m^3 in mice (NTP, 2002).

The DS noted that the experimental findings are also supported by the available human data. Findings included respiratory tract irritation, increased number of neutrophils in nasal smears, significantly higher number of plasma cells in nasal mucosa samples and other signs of upper respiratory tract inflammation (Kiviluoto *et al*., 1979; Kiviluoto, 1980).

There is only one study available on repeated dose toxicity after oral exposure, assessed by the DS as not reliable (Mountain *et al.*, 1953). The most significant finding was a dose-related decrease in erythrocyte counts and haemoglobin levels in the two lower dose groups (LOEL 6.25 mg V_2O_5/kg bw/d). However, no values were reported for these endpoints for the high-level vanadium groups and no statistical evaluation was performed. Due to these deficiencies and because the study design does not allow a conclusion on the definite dose level at the two lowest doses (dose levels were increased after 35 days of exposure) the study was considered not reliable. The other study assessed by the DS as not reliable (García *et al.*, 2004) studied central nervous system effects in rats after subacute (repeated 5 day) i.p. dosing. Only one dose level was included. RAC notes that in addition, the i.p. exposure route is not particularly relevant for humans.

No relevant repeated dose toxicity studies with dermal exposure to vanadium pentoxide could be identified.

Therefore, the DS noted that the current harmonized classification STOT RE 1 for vanadium pentoxide remains justified and should be updated to include the target organ and the route of exposure: STOT RE 1 – H372 (respiratory tract; inhalation).

***Table S1**. Summary of the animal studies of adequate quality available for evaluating STOT RE, as assessed by the DS (reliable and reliable with restrictions). All doses/concentrations given as vanadium pentoxide unless indicated otherwise).*

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Inhalation route:			
16-day subacute study in female B6C3F1 mice (5	Divanadium pentaoxide, purity: 99.8 %	In the lowest exposure group, no histological alterations were observed.	(Schuler *et al.*, 2011)
groups of 48 mice) GLP: yes Reliability (Klimisch score): 1	0, 0.25, 1, 4 mg V_2O_5/m^3 (nose-only inhalation, aerosol, 6 h/d for 16 days) Parameters assessed: concentration of V in lung and blood, lung weight, lung histopathology Lung samples were analysed for DNA strand breaks using the comet assay and analysis of 9 specific DNA-oxo-adducts in lung tissue	At 1 and 4 mg/m^3 lung weights were increased dose-dependently, multifocal /diffuse alveolar histiocytosis, multifocal sub-acute alveolitis and increased cell proliferation rate were observed dose-dependently in addition to multifocal granulocytic infiltration. Results for mutagenicity assay reported in the corresponding section.	Details in Annex I "germ cell mutagenicity"

90-day study 10 male and 10 female F344 rats per dose group GLP: yes Reliability (Klimisch score): 1	Divanadium pentaoxide, purity: 99% 0, 1, 2, 4, 8, or 16 mg V_2O_5/m^3 (6 h/d + T_{90} (15 min), 5 d/w for 3 months,whole body inhalation to particulate aerosol).	The highest concentration was lethal to several rats (7 males and 3 females). No indication on the time of death. Bw and bw gain were decreased from 4 mg/m³ in males and at 16 mg/m³ in females. Significant exposure-related changes in pulmonary function were observed in male and female rats exposed to 4, 8, or 16 mg/m³, evidenced by reduced lung compliance, changes in breathing measurements, impaired capacity to diffuse carbon monoxide, reduced static and dynamic lung volumes, and exaggerated flows. The respiratory effects were more intense with increased exposure time, as indicated by increased lung weights and a greater spectrum and increased severity of proliferative and inflammatory lesions in the lungs of most exposed rats. Lung: Minimal to moderate fibrosis of the lung occurred in rats exposed to 2 mg/m³ or greater as well as lung inflammation. Alveolar/bronchiolar epithelial hyperplasia was present in all rats exposed to 2 mg/m³ or greater. Hyperplasic alveoli cells often contained one or two cells that were very large and occasionally binucleate. Squamous metaplasia (a single focus) was observed within an area of hyperplasia in one female exposed to 16 mg/m³. Nose: Hyperplasia and metaplasia of the nasal respiratory epithelium were significantly increased in animals exposed to 4 mg/m³ and greater. The ventral portion of the nasal septum, the vomeronasal organ, and, to a lesser extent, the ventral lateral walls of the anterior portion of the nasal cavity were involved. Nasal inflammation was also observed. Changes in hematology, depletion of lymphocytes in the spleen, thymus, and lymph nodes, atrophy of metaphyseal bone of the femur, and atrophy of the secondary reproductive organs were observed with testis hypospermia and atypical cells of the epididymis in males at 16 mg/m³. Results on reproductive effects are discussed in section 10.10. NOAEC local = 1 mg/m³ air (males and females) based	(NTP, 2002) from (ECHA Dissemination, 2017) Study: 002, publication Details in Annex I
		on increased lung weights and epithelial hyperplasia, inflammation and fibrosis in lungs at 2 mg/m³ and above. $NOAEC_{systemic}$ was 8 mg/m³ in rats.	

90-day study 10 male and 10 female B6C3F1 mice per dose group GLP: yes Reliability (Klimisch score): 1	Divanadium pentaoxide, purity: 99% 0, 1, 2, 4, 8, or 16 mg V_2O_5/m^3 (6 h/d + T90 (15 min), 5 d/w for 3 months, Whole body inhalation to particulate aerosol).	The highest concentration was lethal to one male mouse. The mouse that died early appeared thin. There were no other clinical findings related to divanadium pentaoxide exposure. Final mean body weights and body weight gains from 8 mg/m^3 in males and from 4 mg/m^3 in females were significantly less vs. controls. The respiratory tract was clearly the primary site of toxicity in mice exposed to divanadium pentaoxide. The respiratory effects were more intense with increasing exposure time and started at 2 mg/m³, as indicated by increased lung weights and a greater spectrum and increased severity of proliferative and inflammatory lesions in the lungs of most exposed mice. The absolute lung weight was significantly increased in males exposed to 2 mg/m^3. Inflammation and alveolar/bronchiolar epithelial hyperplasia was present in mice exposed at and above 2 mg/m³. All mice at and above 8 mg/m³ had lung inflammation and epithelial hyperplasia. The epididymal spermatozoal motility of males exposed from 8 mg/m^3 was significantly decreased. Results on reproductive effects are detailed in section 10.100. $NOAEC_{local}$ = 1 mg/m³ air (males and females) based on increased absolute lung weights and epithelial hyperplasia and inflammation in lungs at 2 mg/m³. $NOAEC_{systemic}$ was 2 mg/m³ in mice based on decreased body weight.	(NTP, 2002) from (ECHA Dissemination, 2017) Study: 006 Details in Annex I
Subchronic (26 weeks) inhalation study in monkeys Adult, male cynomolgus monkeys (*Macaca fascicularis*) 8 -9 animals per exposure group The study assessed pulmonary reactivity to V_2O_5 with provocation challenges, and compared V_2O_5 reactivity before and after subchronic V_2O_5	Divanadium pentaoxide, > 99.6 % Whole body inhalation for 6 h/d, 5 d/week for 26 weeks Group 1 (9 animals): 0.1 mg/m³ (Mon, Wed, Fri) and 1.1 mg/m³ (Tue, Thurs) Group 2 (9 animals): 0.5 mg/m³ Group 3 (8 animals): control goup, exposed against vehicle clean air Provocation challenge before and after	In none of the two exposure groups, pulmonary reactivity to V_2O_5 was increased by subchronic V_2O_5 exposure in comparison to control group. Instead, a decrease was found in both exposure groups. This result indicates that the subchronic exposure may induce tolerance under the exposure conditions used in this study. One animal in the control group was removed from the study because of a parasitic infestation. One animal in the peak exposure group died unexpectedly of an effect unrelated to the exposure. **Effects after pre-exposure challenge (acute effects):** Pre-exposure challenges with 0.5 and 3 mg V_2O_5/m^3 produced a concentration-dependent impairment in pulmonary function, characterized by airway obstructive changes (increased resistance and decreased flow). Analysis of respiratory cells recovered from the lung by	(Knecht *et al.*, 1992) Also study 010 from (ECHA Dissemination, 2017)

exposure with pulmonary function testing. In addition to pulmonary function testing, bronchial lavage fluid was analysed. GLP: no information Reliability (Klimisch score): 2	subchronic exposure: - 6 h/d with 0.5 mg V_2O_5 and 2 weeks later: - 6 h/d with 3 mg V_2O_5	bronchoalveolar lavage demonstrated that airway obstruction was accompanied by a significant influx of inflammatory cells into the lung. Cytological immunological results test (IgE and IgG analysis) did not indicate allergic sensitization.	

2-year carcinogenicity study in B6C3F1 mice and F344/N rats 50 male and 50 female animals per dose group GLP: yes Reliability (Klimisch score): 1	Divanadium pentaoxide, purity: 99% (6 h/d, 5 d/w, 104 weeks) Mice: 0, 1, 2, 4 mg V_2O_5/m^3 *via* inhalation to particulate aerosol Rats: 0, 0.5, 1, 2 mg V_2O_5/m^3 *via* inhalation to particulate aerosol; whole body Diet: NTP-2000 MMAD = 1.0-1.3 µm	**Rats:** Survival of rats in the exposure groups was comparable to animals in control group. Decreased body weight gain was observed at 2 mg/m³. In the respiratory tract (lungs) lesions were observed: inflammation, interstitial fibrosis, histiocytosis and hyperplasia (alveole and bronchiole) mostly in a dose dependent manner starting from 0.5 mg/m³ with squamous metaplasia of the alveoli at 2 mg/m³. In the other part of the respiratory tract, inflammation, fibrosis, degeneration, hyperplasia and squamous metaplasia of the respiratory epithelium of the epiglottis were observed in larynx from 0.5 mg/m³ as well as hyperplasia of the respiratory epithelium of goblet cells in the nose. The incidences (but not severity) of chronic nephropathy were significantly increased in male rats exposed to 1 or 2 mg/m³ (46/50 and 47/50 respectively vs. 37/50 in the control group). Although the NTP doesn't have a formal historical control database for nonneoplastic lesions, a review of recent studies indicates that the incidence in the male control group in the current study is low. Overall, it is not clear if the increased incidences of nephropathy were related to exposure to V_2O_5 or were a reflection of the low incidence in the control group. LOAEC $_{local}$ = 0.5 mg/m³ air (males and females) based on non-neoplastic changes (epithelial hyperplasia, squamous metaplasia, chronic inflammation, fibrosis, degeneration) in the respiratory system (lung, larynx, and nose) of male and female rats. **Mice:** Survival of males at 4 mg/m³ was significantly less than controls. Abnormal breathing was observed particularly in those animals exposed to 2 or 4 mg/m³. Decreased body weight gain was observed from 2 mg V_2O_5/m^3. In the respiratory tract (lungs), lesions were observed: inflammation, interstitial fibrosis, histiocytosis and	(NTP, 2002) Also studies no. 001 and 005 reported in (ECHA Dissemination, 2017) Details in Annex I

		hyperplasia (alveolar and bronchiole) mostly in a dose dependent manner starting from 1 mg/m³. In the other parts of the respiratory tract, suppurative inflammation of the nose, degeneration and squamous metaplasia of the respiratory epithelium in the nose. as well as squamous metaplasia of the respiratory epithelium of the epiglottis were observed in larynx from 1 mg/m³ A $LOAEC_{local}$ of 1 mg/m³ air (males and females) based on non-neoplastic changes (epithelial hyperplasia, squamous metaplasia, chronic inflammation, fibrosis, degeneration) in the respiratory system (lung, larynx, and nose) of male and female mice. No NOAEL can be derived. Results on neoplastic lesions are reported in section 10.9.	

Table S2. *Summary of the human data by the DS on STOT RE. Most of the studies were also included for Resp. Sens*.

Type of data/report	**Test substance**	**Route of exposure** **Relevant information about the study (as applicable)**	**Observations**	**Reference**
Cross-sectional case-control study with 63 workers from a V_2O_5-producing company in Finland.	Divanadium pentaoxide	- 63 male exposed workers were examined (on average exposed for 11 years). The control group consisted of 63 male dust-exposed matched individuals (operators of a nearby mine). - Exposure against 0.2 – 0.5 mg V/m³ (measured between 1970 and 1975, determined from total dust). This corresponds to 0.36 – 0.89 mg V_2O_5/m³ - In early 1976, exposure was reduced to 0.01 - 0.04 mg V/m³ due to technical changes at the factory. This corresponds to 0.018 – 0.071 mg V_2O_5/m³ **Performed tests:** - Rhinoscopy - Sputum cells were analysed - Pulmonary ventilation measured - Nasal secretion smear cells were analysed	Cases self-reported subjective symptoms of respiratory tract irritation **Rhinoscopy:** no differences between the groups **Cytology:** Number of neutrophils significantly increased in nasal smears of exposed group. **Histopathological findings:** Significantly higher number of plasma cells in nasal mucosa samples. Increase in the number of "round cells" in mucous membranes from nasal turbinates → clear signs of inflammation (not related to allergy, since number of eosinophils not significantly changed in exposed group) No results on pulmonary ventilation measurements reported in the publication	(Kiviluoto *et al.*, 1979) (ECHA Dissemination, 2017) "epidemiological data" Study: 001

		Same collective as indicated above in Kiviluoto *et al.*, (1979) **Performed tests (testing in 1975):** - Respiratory questionnaire - X-ray analysis of the lung - Pulmonary ventilation measured	**Respiratory symptoms:** significantly more wheezing in the exposure group. **X-ray analysis:** no exposure related differences observed **Ventilation measurement:** no differences observed	(Kiviluoto, 1980)
		Same collective exposure as indicated above (Kiviluoto *et al.*, 1979). However the control group consisted of only 22 men. Whether these men were part of the "collective control" mentioned by Kiviluoto *et al.*(1979) is not described. **Performed tests:** - Hematologic and serum chemical laboratory tests	No significant differences were observed for the hematologic results of exposed and non-exposed workers. In the serum chemical test, significant differences were observed for serum albumin (↓), chloride (↓), urea (↑), bilirubin (↓) and conjugated bilirubin (↑).	(Kiviluoto *et al.*, 1981a) (ECHA Dissemination, 2017) "epidemiological data" Study: 003
Case-control study with 12 workers from a vanadium plant in South Africa.	Divanadium pentaoxide	12 workers chronically exposed against < 0.15 - 1.53 mg V_2O_5/m^3 with diagnosed bronchial hyperreactivity (by lung function and bronchoprovocation tests). Control subjects (12) worked in the same company but did not show bronchial hyperreactivity. **Performed tests/observations:** - Onset of symptoms - Serum IgE and atopy	**Onset of symptoms** in 7/12 subjects, symptoms of cough and breathing difficulties developed within 6 months after start of the work in the factory. In the control group only 2/12 experienced the same symptoms within this time period. **Serum IgE and atopy** IgE levels between cases and controls were not significantly different. None of the subjects was exposed against toxic levels of SO_2 and NH_3. For 3/12 workers co-exposure against SO_2 and NH_3 could be excluded.	(Irsigler *et al.*, 1999)

Case-control study with 24 men working in vanadium plants in the USA (13 men from Colorado and 11 men from Ohio)	Divanadium pentaoxide	24 workers were exposed to vanadium (as V_2O_5) *via* inhalation (at least for 6 months) against the following concentrations: Colorado plant: 0.097 - 0.243 mg V_2O_5 /m³ (mass respirable vanadium: 16.6 % to 51 %; Particle size < 5 µm: 92.5 to 99 %) Ohio plant: 0.018 - 0.925 mg V_2O_5/m³ (mass respirable vanadium: 2 % to 100 %; Particle size < 5 µm: 96.3 to 100 %) 45 control subjects matched for age, economic status and job activities, not coming from the vanadium industry. **Performed tests/observations:** - physical examination, - history (incl. detailed occupational history and a subjective evaluation of alcohol and fat intake), - electrocardiogram, urinalysis, hematocrit, serum cholesterol, and analysis of urine for its content of vanadium	**Symptoms with significant differences increased in exposure vs. control group:** Cough, sputum, eye, nose, throat irritation, epistaxia, wheezing, rales, or injected pharynx, green tongue After an analysis of variance and the geographical effects were removed, the cholesterol levels of the exposed subjects are found to be significantly lower than those of the controls ($p< 0.05$). No significant differences were found for haematocrit urinalysis and electrocardiogram results	(Lewis, 1959a; b) (ECHA Dissemination, 2017) Study: 004
Experimental study in 24 volunteers (12 workers of a vanadium plant and 12 students)	Divanadium pentaoxide	8 subjects (4 workers and 4 students) were attributed to each of three different exposure zones (high: 0.028 - 0.062 mg V/m³; medium: 0.004 - 0.019 mg V/m³; low: 0.008 - 0.019 mg V/m³). The subjects had to be present during the 8 hours lasting working day for 5 days. **Performed tests:** - psychological, neuropsychological, psychosomatic and behaviour toxicological performance tests	No behaviour toxicological changes were observed between the different groups. The variation of exposure to vanadium pentoxide had no influence on eye-hand coordination and on performance in fine motor response → No influence of variation of exposure to V_2O_5 on neurobehavioural performances was found. → No correlation between neuropsychological performances and concentration of metabolized vanadium was found.	Hörtnagel *et al.*, 1994 cited from (ECHA Dissemination, 2017) Study: 005
Retrospective cohort study with 78 workers engaged in the processing of vanadium-bearing ore, and 37 controls in Peru	Divanadium pentaoxide	Vanadium concentrations in air varied from 0.01 - 58.80 mg/m³. In the control areas the concentration range was 0.000 to 0.007 mg/m³. Concentrations do not seem to refer to V_2O_5. All dust particles were below 5 µm in diameter. The concentration of sulphur dioxide in air in various work places ranged between 0.0 and 2.0 ppm.	Abnormally high prevalence of signs and symptoms indicative of irritation to the upper respiratory tract and to the eyes among workers exposed to vanadium-bearing dusts as compared with workers not exposed to such dust. Vital capacity, circulation, neurological findings, muscular strength: No significant differences among the three groups of workers were observed.	(Vintinner *et al.*, 1955) (ECHA Dissemination, 2017) Study: 006

Comments received during consultation

One MSCA commented on the classification proposal and agreed with the proposed update.

Assessment and comparison with the classification criteria

Several good quality studies are available for assessing STOT RE by vanadium pentoxide. Effects targeting the respiratory tract have been consistently demonstrated *in vivo* both in rats and in mice.

In a 90-day study in F344 rats (NTP, 2002), several effects in the respiratory tract were observed. Changes in pulmonary function were seen at 4, 8, and 16 mg/m^3. Minimal to moderate lung fibrosis was present in rats exposed to 2 mg/m^3 or greater. Lung inflammation was also observed. Alveolar/bronchiolar epithelial hyperplasia was present in all rats exposed to 2 mg/m^3 or greater. Hyperplasic alveoli cells often contained one or two cells that were very large and occasionally binucleate. Hyperplasia and metaplasia of the nasal respiratory epithelium were significantly increased in animals exposed to 4 mg/m^3 and greater. The ventral portion of the nasal septum, the vomeronasal organ, and, to a lesser extent, the ventral lateral walls of the anterior portion of the nasal cavity were involved. Nasal inflammation was also observed. NOAEC local = 1 mg/m³ air (males and females) is based on increased lung weights and epithelial hyperplasia. Inflammation and fibrosis in lungs was observed at 2 mg/m^3 and above. The highest concentration was lethal to several rats (7 males and 3 females). Bw and bw gain were decreased from 4 mg/m^3 in males and at 16 mg/m^3 in females.

In mice the effects were similar. In a 90-day study in B6C3F1 mice (NTP, 2002), the respiratory tract was considered the primary site of toxicity. The respiratory effects were more intense with increasing exposure time and started at 2 mg/m³, as indicated by increased lung weights and a greater spectrum of increased severity of proliferative and inflammatory lesions in the lungs of most of the exposed mice. The absolute lung weight was significantly increased in males exposed to 2 mg/m3. Inflammation and alveolar/bronchiolar epithelial hyperplasia was present in mice exposed at and above 2 mg/m^3. All mice at and above 8 mg/m3 had lung inflammation and epithelial hyperplasia.

n a study using adult male cynomolgus monkeys, considered as reliable with restrictions (Knecht *et al.*, 1992), pre-exposure challenges with 0.5 and 3 mg vanadium pentoxide/m^3 produced a concentration-dependent impairment in pulmonary function, characterized by airway obstructive changes (increased resistance and decreased flow). Analysis of respiratory cells recovered from the lung by bronchoalveolar lavage demonstrated that airway obstruction was accompanied by a significant influx of inflammatory cells into the lung. However, the sub-chronic 26-week exposure itself did not produce changes regarding the endpoints investigated indicative for airway hyperreactivity.

In a two year carcinogenicity study in F344 rats (NTP, 2002), lesions were observed in lungs: inflammation, interstitial fibrosis, histiocytosis and hyperplasia (alveoli and bronchiole); mostly in a dose dependent manner starting from 0.5 mg/m^3 with squamous metaplasia of the alveoli at 2 mg/m^3. In the other part of the respiratory tract, inflammation, fibrosis, degeneration, hyperplasia and squamous metaplasia of the respiratory epithelium of the epiglottis were observed in larynx from 0.5 mg/m^3 as well as hyperplasia of the respiratory epithelium of goblet cells in the nose. The LOAEC local = 0.5 mg/m³ air (males and females) is based on non-neoplastic changes (epithelial hyperplasia, squamous metaplasia, chronic inflammation, fibrosis, degeneration) in the respiratory system (lung, larynx, and nose) of male and female rats. Similar effects were also observed in B6C3F1 mice, mostly in a dose dependent manner starting from 1 mg/m^3.

Although the data available from humans is limited, the findings support the view that the respiratory tract is a target organ. Findings in humans included respiratory tract irritation, increased number of neutrophils in nasal smears, significantly higher number of plasma cells in nasal mucosa samples and other signs of upper respiratory tract inflammation (Vintinner *et al.*, 1955; Lewis, 1959a,b; Kiviluoto *et al*., 1979; Kiviluoto, 1980; Irsigler *et al.*, 1999).

RAC agrees with the DS that based on the available data, the inhalation route is relevant, and that the respiratory tract can be identified as the target organ.

Minimal to moderate lung fibrosis and hyperplasia were observed in rats in 90-day sub-chronic studies, starting from 2 mg/m^3 (=0.002 mg/L/6h/day). This is clearly within the guidance value range for Cat. 1. (≤0.02 mg/L/6h/day). Also, in mice the respiratory tract was clearly the target organ in a 90-day study, and effects grew more intense with increasing exposure time. Inflammation and alveolar/bronchiolar epithelial hyperplasia was present in mice exposed at and above 2 mg/m^3. Similar effects were further observed in a repeated 2-year study. The respiratory tract effects are considered toxicologically significant and can be presumed to have the potential to produce significant toxicity also in humans following repeated exposure.

Therefore, RAC agrees with the DS that classification as **STOT RE 1, H372 (respiratory tract; inhalation)** is warranted for vanadium pentoxide.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Vanadium pentoxide has an existing classification of germ cell mutagenicity category 2 (H341). The DS proposes to upgrade this classification to mutagenicity category 1B. This proposal was based on the following evidence:

- Although vanadium pentoxide was not mutagenic in *in vitro* studies, positive results from several *in vitro* micronucleus tests and comet assay have been published.
- Although *in vivo* genotoxicity using physiological routes of exposure (inhalation, oral) were mainly negative, positive results in micronucleus assays were obtained in one study in peripheral blood reticulocytes after inhalation, and in other study (by the same research group) in polychromatic erythrocytes after i.p. injection. Still from the same research group, comet assay studies in several organs (including testes) and dominant lethal assay showed positive results in mice after i.p. administration.
- *K-ras* mutations observed in alveolar/bronchiolar carcinomas in NTP 2002 study and elevated phospho-MAPK in carcinoma tissues with *K-ras* mutations were used as supporting evidence.

Toxicokinetic information showing that vanadium is distributed to testes following inhalation and intra-tracheal route of exposure was also used as supporting evidence and classification to mutagenicity category 1B was proposed.

Comments received during consultation

Comments were received from one MSCA, one industry association and one individual. None of these supported the DS proposal to classify vanadium pentoxide to category 1B for germ cell mutagenicity but proposed instead category 2. The main justifications for this alternative proposal were:

- Lack of evidence for *in vitro* mutagenicity in bacteria or mammalian cells

- Equivocal evidence for *in vitro* clastogenicity/aneugenicity
- Lack of evidence for *in vivo* mutagenicity in a study in transgenic rodents
- Lack of evidence for site of contact genotoxicity after inhalation in recent well conducted studies
- Lack of evidence for *in vivo* clastogenicity in studies using physiological routes of exposure
- The only positive findings reported were obtained largely from studies not using a physiological route of exposure, published by the same working group, whose study design and reporting showed deficiencies.

Assessment and comparison with the classification criteria

Vanadium pentoxide have not shown direct mutagenicity in *in vitro* studies. Negative findings have been observed in Ames test (OECD TG 471) with and without S9 mix, in Mouse lymphoma Assay (OECD TG 476) with and without S9 mix, in Gene mutation assay in Chinese hamster V79 cells without metabolic activation. Only one *in vitro* bacterial mutagenicity test (Sun *et al.*, 1987, cited in WHO, 1988) showed positive response in *E. Coli* but not in *S. typhimurium*. However, there is only limited information available from this study and due to the secondary reporting full evaluation of the study could not be done. Overall, it can be concluded that vanadium pentoxide is not directly mutagenic *in vitro*.

Regarding clastogenic/aneugenic effects, there are some evidence on these effects *in vitro*. Table M1 lists studies with vanadium pentoxide considered reliable (Klimisch score 1 or 2) by the DS.

Table M1

Method	Cell type and doses	Results	Reference
Mammalian cell micronucleus test OECD TG 487, no deviations (Klimisch 1)	Human peripheral blood lymphocytes and human lymphoblastoid TK6 cells, several doses from 1-100 µg V_2O_5/mL, includes also range-finding study	Positive (+/- S9 mix)	Anonymous, 2010 from (ECHA Dissemination, 2017)
Mammalian cell micronucleus test (Klimisch 2)	Syrian hamster embryo (SHE) cells 0, 10, 15, 20, and 25 µg V_2O_5/mL without metabolic activation	Negative	Gibson *et al.*, 1997)
Mammalian chromosome aberration test (Klimisch 2)	Lymphocytes: human peripheral blood 0, 1, 2, 4, or 8 µg V_2O_5/mL	Negative	(Rodríguez-Mercado et al., 2010)
Micronucleus test with cytochalasin B and SCE assay (Klimisch 2)	Chinese hamster V79 cells (lung fibroblast cells) 0, 1, 2, 3 µg V_2O_5/mL for 24h	Positive (positive also for kinetochrore + MN)	(Zhong *et al.*, 1994)
Comet Assay (Klimisch 2)	Human nasal epithelia and lymphocytes 0, 0.06, 0.12, 0.24, and 0.47 mM V_2O_5	Positive in lymphocytes negative in nasal cells	(Kleinsasser *et al.*, 2003)
A study on the induction of aneuploidy measured by 1) in situ hybridization (DNA probes for chromosomes 1 and 7) 2) immunostaining of lymphocyte spindle apparatus 3) measurement of polymerisation and depolymerisation of tubulin (Klimisch 2)	Human primary lymphocytes 0, 0.001, 0.01, 0.1 µM V_2O_5	FISH: increase in frequencies of nuclei exhibiting 3 or more hybridization regions Immunostaining: disruption of microtubules in spindle apparatus at all doses Tubulin polymerization: polymerization inhibited/depolymerization stimulated only at lowest concentration.	(Ramírez *et al.*, 1997)

In addition, the following studies (derived from the same research laboratory) with some limitations in the reporting or the conduct of the study (e.g. lack of positive control) are reported:

- Negative chromosome aberration (CA) and sister-chromatid exchange assay with human lymphocytes in human primary lymphocytes (Roldán and Altamirano, 1990)
- Positive Comet Assay Human blood leucocytes (Rodríguez-Mercado *et al.*, 2011)
- Positive Comet assay in human leucocytes and primary lymphocytes (Rojas *et al.*, 1996)

Taken together, these data suggest that vanadium pentoxide can cause chromosomal damage *in vitro*. One of the mechanisms could involve disruption of cell division resulting in aneuploidy.

In vivo data shows varying results. In general, majority of *in vivo* studies using physiological routes of exposure (inhalation or oral administration) remain negative, whereas studies using the i.p. route of administration show positive responses. Table M2a lists *in vivo* genotoxicity studies using inhalation/oral administration with the most reliable (Klimisch 1 and 2) in the beginning. Table M2b lists *in vivo* genotoxicity studies using i.p. administration. In addition, there are *in vivo* studies by Sun *et al.* (1987), which cannot to be evaluated due to the unavailability of the study reports. These include positive MN tests via i.p., s.c. and inhalation exposure, negative oral MN and DNA synthesis inhibition assay and negative dominant lethal test via s.c. administration. These have not been listed in the tables and are not considered in the evaluation. There are also some data on the toxicokinetics of vanadium after exposure to vanadium pentoxide suggesting that vanadium is able to reach testes. Increased vanadium levels were measured after oral administration of vanadium pentoxide (Anonymous, 2011, Klimisch 1) and inhalation exposure (Fortoul *et al.*, 2007; Mussali-Galante *et al.*, 2005 and related study reports, Klimisch 3).

***Table M2a**. Inhalation/oral genotoxicity studies.*

Method	Animals and exposure	Results	Reference
Bone marrow micronucleus assay OECD TG 474 Klimisch: 1	6 male Sprague-Dawley rats per dose group Dose levels in the main study: 0, 30, 60, 120 mg V_2O_5/kg/ bw/day Gavage	Negative No effect on PCE/NCE V levels in bone marrow and testes increased in dose-dependent manner	(Anonymous, 2011)
DNA damage and/or repair in lungs Klimisch: 1	Female B6C3F1 mice 0, 0.25, 1, 4 mg V_2O_5/m³ (nose-only inhalation, 6 h/d for 16 days)	Comet assay: Negative Concentration dependent increase in 8-oxodGuo DNA lesions with significant effects at 1 and 4 mg/m³	(Schuler *et al.*, 2011)
Bone marrow micronucleus assay OECD TG 474 Klimisch: 2	10 Male and female B6C3F1 mice per dose group, 0, 1, 2, 3, 8, 16 mg V_2O_5/m³ whole body inhalation, 6 h/d, 5 d/w, for 3 months	Negative No effect on PCE/NCE	(NTP, 2002)
K-ras mutation in the development of lung tumours Klimisch: 2	6 male transgenic Big Blue mice per dose and exposure group 0, 0.1, 1 mg V_2O_5/m³ nose-only inhalation, 6 h/d 5 d/w for 4 or 8 weeks	Negative	(Banda *et al.*, 2015)
Transgenic rodent Somatic Cell Gene Mutation Assay OECD TG 488 Klimisch: 3 (or 2**)	the same as above	Negative	(Manjanatha *et al.*, 2015)

Method	Animals and exposure	Results	Reference
Micronucleus assay in peripheral blood reticulocytes (Klimisch: 3)	6 male, 6 female CD-1 mice 1.4 mg V_2O_5/m^3 via inhalation, 2 h/twice a week; blood samples collected at 24 h and every week until the end of the 4-week exposure period	Positive in males at all time points Negative in females	(Rojas-Lemus *et al.*, 2014)
Immunohisto-chemical changes in actin testicular cytoskeleton (Klimisch: 3)	Male CD-1 mice 1.4 mg V_2O_5/m^3 via inhalation 1 h/twice a week, for a total of 12 weeks. Every week 5 exposed animals and 5 control animals were sacrificed.	Structural DNA damage microscopically observed	(Rodríguez-Lara *et al.*, 2016)
Observation of nuclear changes via electron microscopy in spleen cells of exposed mice. (Klimisch: 3)	Male CD-1 mice 1.4 mg V_2O_5/m^3 via inhalation) 1 h/twice a week, for a total of 3 months. Every week 3 exposed animals and 3 control animals were sacrificed.	Spleen lymphocytes showed time dependent (up to 5 weeks) nuclear changes (lobulations, invaginations, deep evaginations, chromatin redistribution with increased heterochromatin, pronounced perinuclear cisterns)	(Rodríguez-Lara *et al.*, 2013)
Analysis of morphological changes, liver function test (LFT), and oxidative stress damage in exposed mice (Klimisch: 3)	Male CD-1 mice 1.4 mg V_2O_5/m^3 via inhalation) 1 h/twice a week, for a total of 6 weeks.	Oxidative stress (lipid peroxidation and inflammatory infiltration) was observed as well as an increase in the size of the nuclei of hepatocytes and binucleated cells	(Cano-Gutiérrez *et al.*, 2012)
Evaluation of DNA migration in agarose gels (Klimisch: 3)	6 male albino Wistar rats per group 70 mg/kg single application via gavage	Significant increase in length of DNA migration in liver, kidney, heart, lung, spleen and brain.	(Paramanik and Rajalakshmi, 2013)

**It remains unclear why DS has classified this as Klimisch score 3 and the related study by Banda *et al.* (2015) as Klimisch score 2. The study reports are available and include clear description of the methodology.

Table M2b. i*.p. studies*

Method	Animals and exposure	Results	Reference
Comet assay in lung, kidney, spleen, heart, liver and bone marrow (Klimisch: 2)*	4 CD1 mice per group 0, 5.75, 11.5 or 23 mg V_2O_5/kg bw	Positive results in all tissues with varying sensitivity	(Altamirano-Lozano *et al.*, 1999)
Bone marrow SCE assay (Klimisch: 2)**	see above	Negative	(Altamirano-Lozano *et al.*, 1993)
Comet assay in testicular cells (Klimisch: 2)***	2 CD1 mice per group, doses see above	Positive	(Altamirano-Lozano *et al.*, 1996)
Dominant lethal test (Klimisch: 2***)	15-30 male CD-1 mice per group (mated 1:2) 0 and 8.5 µg V_2O_5/g bw (8.5 mg/kg bw) every 3rd day for 60 days	Positive (no of resorptions was increased but increase in dead fetuses was not statistically significant)	(Altamirano-Lozano *et al.*, 1996)
Micronucleus test in polychromatic erythrocytes (Klimisch: 3)	Hsd:ICR mice 0, 40 mg V_2O_5/kg bw single application, evaluation of MN 0, 24, 48 and 72 h after the dosing	Positive (regardless of the high dose, only marginal but statistically significant increase at all time points). Numbers of apoptotic and necrotic cells increased.	(García-Rodríguez *et al.*, 2016)

**It remains unclear why DS has classified this as Klimisch score 2 even though the study includes several deficiencies, including deficient reporting of applied methodology and controls, and small no of analysed cells. In addition, sampling*

time and analysis of comets do not follow the modern standards. E.g., it seems that hedgehogs were included in migration data and untailed cells were excluded in migration data. It is also unclear whether the samples were randomized and analysed blindly.

***SCE assay is not anymore considered appropriate for genotoxicity assessment.*

****It remains unclear why DS has classified this as Klimisch score 2 even though the study uses i.p. administration and has similar deficiencies as many other studies from the same group (in this case: only one dose, limited reporting of methodology, inadequate number of implantations studied, no positive control, see also deficiencies related to comet assay listed in the case of Altamirano-Lozano et al., 1999).*

The DS has also used NTP (2002) and Deveraux (2002) data showing a high frequency (73%) of *K-ras* mutations and elevated phospho-MAPK in vanadium pentoxide-induced alveolar/bronchiolar lung carcinomas in NTP (2002) cancer study as supporting evidence for genotoxicity. This data supports the role of *K-ras* activation in the vanadium pentoxide-induced carcinogenic process and can give mechanistic evidence which suggests that vanadium pentoxide may cause generation of reactive oxygen species and oxidant-dependent *K-ras*/MAP kinase pathway activation. However, it does not tell if these mutations in tumors are caused by direct genotoxic action of vanadium pentoxide or via indirect/secondary mechanisms during the carcinogenic process. Negative inhalation study by Banda *et al*. (2015) suggests that these *K-ras* mutations seen in mice lung tumours occur at a later stage with chronic vanadium pentoxide exposure and are likely not an early event in vanadium pentoxide-induced mouse lung carcinogenesis. This is also supported by the gene expression study of Black *et al.* (2015) (reported under carcinogenicity in CLH report), which show no evidence for enrichment of pathways associated with cell cycle arrest/proliferation, DNA damage, oxidative stress after 13 weeks inhalation exposure to 2 mg/m^3 of vanadium pentoxide.

As can be seen from table M2a, all those inhalation and oral genotoxicity studies classified as reliable (Klimisch score 1 or 2) were negative. A small (two-fold) induction of oxidative lesions (8-oxodGuo) was seen in the study by Schulter *et al.* (2011) without accompanying induction of DNA strand breaks measured in a Comet assay. With the exception of the study of Paramanik and Rajalakshmi (2013), all the positive studies (Rojas-Lemus *et al.*, 2014; Rodríguez-Lara *et al.*, 2013; Rodríguez-Lara *et al.*, 2016 and Cano-Gutiérrez *et al.*, 2012) come from the same laboratory. One of these was the Rojas-Lemus *et al.* (2014) study on peripheral blood reticulocyte micronuclei using one dose level and intermittent exposure (2 hours, 2 times per week). Its positive results in males (but not in females) contrast to the negative results from the NTP (2002) study by showing MN induction at far lower exposure levels accompanied with a steady increase in MN with time. Since reticulocyte MN indicates recent genotoxic insult in recently divided reticulocytes (which mature within 24-48 h as erythrocytes) this increase with time is difficult to explain, especially when taking into account that vanadium is not accumulating in the body. The Comet assay performed by the Altaminaro-Lozano *et al.* (1999) include also severe deficiencies in reporting methodology and controls (no information on controls provided), small number of animals/analysed cells, late sampling time and deficiencies in the analysis of comets which do not follow the modern standards (e.g. it seems that hedgehogs were included in migration data and not analysed separately, untailed cells were excluded from migration data and it is unclear whether the samples were randomized and analysed blindly or not). The methods used by Rodríguez-Lara *et al.* (2013) and Rodríguez-Lara *et al.* (2016) to assess potential genotoxicity in different tissues are not standard (validated) assays for genotoxicity assessment and are therefore difficult to assess for their relevancy.

Thus, the main evidence used by the DS to upgrade vanadium pentoxide mutagenicity classification from cat 2 to cat 1B comes from the studies employing i.p. administration. All these studies come from the same laboratory and include comet assay in different tissues, micronucleus test in polychromatic erythrocytes and a dominant lethal test. It should be noted that repeated i.p. administration approach used in the dominant lethal test by Altamirano-Lozano *et al.* (1996) is likely to result in high local levels of the substance in peritoneal cavity and testes, the relevance

of which can be questioned. The same applies to testicular comet assay reported in the same study report which used only 2 mice/dose group. In this test, small cell group of the analysed testicular cells did not actually show any dose dependency in the DNA damage. In addition, the same deficiencies as reported in the case Altamirano-Lozano *et al.* (1999) apply in this case. In the fertility assessment/dominant lethal study there was a statistically significantly increased incidence of resorptions and small, non-significant increase in the number of dead foetuses in addition to the lower number of pregnant females and implantations. The exposure resulted in lower bw of the exposed males by day 60. In addition to the i.p route of exposure, only one dose level, the lack of positive control, limited number of pregnant females (resulting in limited number of implantations), the limited documentation of the conduct study and the results of the study itself makes its interpretation difficult and questions the Klimisch score of 2 proposed by the DS.

There are also two research reports which have evaluated genotoxicity in vanadium pentoxide exposed workers. The first one, Ivancsits *et al.* (2002) did not see significant differences in comet assay, 8-OHdG or SCE between exposed and non-exposed test persons. The second one, Ehrlich *et al.* (2008) reports increased number of MN and nucleoplasmic bridges (NPBs) and nuclear buds (Nbuds) in 23 and 53 vanadium pentoxide factory workers when compared to the controls. DNA migration was, however, unaffected. No correlation between genotoxicity markers and plasma vanadium levels were seen. Possible co-exposures to other impurities were not discussed. No far-reaching conclusions can be made based on these studies.

Comparison with the criteria

The classification in Germ Cell Mutagenicity Category 1A is based on positive evidence from human epidemiological studies. Since there is no such evidence on vanadium pentoxide, Cat. 1A is not applicable.

The classification in Category 1B is based on: 1) positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or 2) positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells or 3) positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny. In case there are also negative or equivocal data, a weight of evidence approach using expert judgement has to be applied.

In the case of vanadium pentoxide, there is one *in vivo* heritable germ cell mutagenicity test in mice (dominant lethal study) showing positive response. The same study includes also positive comet assay in testicular cells. Another study performed by the same group reported positive responses in comet assay in different tissues. These studies were performed using repeated i.p. administration, included only one dose level and included several deficiencies in the study conduct and reporting (see above), which question the reliability of the study. In addition, i.p. administration is known to result in high local peritoneal and testicular concentrations of the applied substance. High quality *in vivo* studies in somatic cells using physiological routes of exposure (oral or inhalation) have been generally negative. When taking into account that:

- Vanadium pentoxide does not seem to be directly mutagenic in *in vitro* and *in vivo* studies
- Although it has caused chromosomal effects *in vitro*, *in vivo* genotoxicity in well-conducted studies using physiological routes of exposure has been negative
- Positive findings *in vivo* were obtained largely from studies with unphysiological route of exposure, published by the same working group whose study design, analysis and reporting show deficiencies and are contradicting the data obtained from other, high-quality studies

changing the existing cat 2 classification to 1B does not seem justified based on the available data. Although there are toxicokinetic evidence showing that vanadium can reach testes, this is not considered sufficient for cat 1B classification when taking into account that there is no clear evidence on *in vivo* genotoxicity. **Therefore, RAC recommends to retain the existing classification Muta. 2, H341.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS proposed to classify vanadium pentoxide as carcinogenic Cat. 1B on the basis of NTP inhalation carcinogenicity studies in rat and in mice. In the mice study, the incidence of alveolar/bronchiolar carcinoma was 12/50, 29/50, 30/50, 35/50 in male mice and 0/50, 23/50, 18/50, 22/50 in female mice, for each dose (0, 1, 2 or 4 mg/m^3), respectively. The incidence of alveolar/bronchiolar adenoma or carcinoma was 22/50, 42/50, 43/50, 43/50 in male mice and 1/50, 32/59, 35/50, 32/50 in female mice, for each dose respectively. These increases were statistically significant in all groups and were considered dose-related and exceeding the historical control data. In the rat study, the incidence of alveolar/bronchiolar adenoma was 4/50, 8/49, 5/48, 6/50 in male rats and 0/49, 3/49, 1/50, 0/50 in female rats, for doses 0, 0.5, 1 and 2 mg/m^3 respectively. The incidence of alveolar/ bronchiolar adenoma or carcinoma was 4/50, 10/49, 6/48, 9/50 in male rats and 0/49, 3/49, 1/50, 1/50 in female rats, for each dose, respectively. Mice data showing clear increases in lung tumours in both sexes were considered sufficient to classify vanadium pentoxide in carcinogenicity category 1B. A tumour promotion study conducted in male A/J, BALB/cJ and C57BL/6J mice provided supporting evidence. Epidemiological studies in humans on the carcinogenicity of vanadium pentoxide are not available.

Since there are no studies using other routes of exposure, the DS considered that tumours after exposure via e.g. oral route cannot to be excluded and therefore, the criteria for specifying the route of exposure are not met.

Comments received during consultation

Comments were received from one MSCA, one industry association and one individual. The commenting MSCA supported classification to carcinogenicity category 1B although uncertainties related with the possible high background incidence of mice lung tumours and the fact that clear increase in the number of tumours was observed only in mice were recognized.

Industry association and a commenting individual disagreed with the proposal for carcinogenicity cat 1B classification and supported classification to category 2 instead. In addition, specification of inhalation as the route of exposure was proposed. The main arguments to support this were:

- the carcinogenicity was restricted to a single experiment in mice without clear dose-response relationship
- the tumours induced in mice are a local, site-specific response (no tumours at other sites in mice (or rats) in spite of evidence of systemic exposure to the vanadate moiety
- tumour induction is likely to be caused by secondary mechanisms and the tumour formation in mice is accompanied by long-standing chronic inflammation
- high inflammatory response in all dose groups compromises the usefulness of NTP studies
- there are questions whether the genomic responses of mice to inflammation are comparable to those of humans

- lack of concordance in mice and rats and low human relevance of bronchioalveolar lung tumours induced only in mice by non-genotoxic chemicals
- absence of reliable human data

Assessment and comparison with the classification criteria

There is one full carcinogenicity study with vanadium pentoxide performed in rats and mice (NTP 2002). The cancer incidences observed in this study are listed in tables C1 (rats) and C2 (mice) together with information on the general toxicity and historical control rates.

Table C1. *Carcinogenicity study in rats*

dose	0 mg/ m^3	0.5 mg/ m^3	1 mg/ m^3	2 mg/ m^3	HC*
Males					
lung adenomas	4/50	8/49	5/48	6/50	0-12%
lung carcinomas	0/50	3/49	1/48	3/50	0-6%#
No of animals surviving to the end of the study	20	29	26	27	
Mean survival (d)	668	680	692	671	
BW	no effect in exposed group compared to the controls				
Females					
lung adenomas	0/49	3/49	1/50	0/50	0-8%#
lung carcinomas	0/49	0/49	0/50	1/50	0-2%
No of animals surviving to the end of the study	33	24	29	30	
Mean survival (d)	688	678	679	680	
BW	reduced at 2 mg/m^3 when compared to the controls				

**According to analysis by Starr et al. (2012) using data from animals fed with NTP-2000 diet.*

#In NTP report, only a limited number of HC data from animals fed with NTP-2000 diet were available and HC incidence was somewhat lower, for male lung carcinomas the range was 0%-2% and female lung adenomas it was 0%-6%.

As can be seen from the data compiled in table C1, in female rats no tumour induction was seen. In male rats, slight increases in lung adenomas and carcinomas were seen when compared to the current control groups but these were not dose-dependent and only adenomas in the low dose group in males exceeded the HC incidences reported by Starr *et al.* (2012). Carcinomas in low and high group (but not in the mid-dose group) were on the upper end of HC when compared to the HC data presented by Starr *et al.* (2012) but exceeded the more limited HC data available at the time the study was conducted. No increased incidences of tumours were seen in other organs. The exposure had no impact on the survival or body weights of the animals, but the animals in all dose groups showed chronic active inflammation and alveolar and bronchiolar epithelium hyperplasia at all doses. In addition, at the highest doses it also showed increased incidences of interstitial fibrosis and alveolar and bronchiolar squamous metaplasia. Overall, the rat study does not provide clear evidence on the carcinogenicity of vanadium pentoxide at the dose levels causing inflammation and fibrosis in the lungs. Based on the analysis of lung vanadium burden, the total lung doses for rats exposed to 0.5, 1, or 2 mg/m^3 were estimated to be 130, 175, and 308 µg vanadium, respectively.

Table *C2*: *Carcinogenicity study in mice*

dose	0 mg/m^3	1 mg/m^3	2 mg/ m^3	4 mg/ m^3	HC*
Males					
lung adenomas	13/50	16/50	26/50	15/50	4-26%
lung carcinomas	12/50	29/50	30/50	35/50	4-24%
No of animals surviving to the end of the study	39	33	36	27	
Mean survival (d)	710	692	704	668	
BW	reduced at 2 and 4 mg/m^3 when compared to the controls				
Females					
lung adenomas	1/50	17/50	23/50	19/50	0-12%
lung carcinomas	0/50	23/50	18/50	22/50	0-6%
No of animals surviving to the end of the study	38	32	30	32	
Mean survival (d)	692	655	653	688	
BW	reduced at all doses when compared to the controls				

**HC data from controls given NTP-2000 diet available at the time of the study.*

In the mice study, the incidences of alveolar/bronchiolar carcinoma were significantly increased in all groups of exposed male and female mice. The incidences of alveolar/ bronchiolar adenoma were significantly increased only in mid-dose males exposed to 2 mg/m^3 but for exposed females the incidences were significantly increased in all dose groups. These increased incidences exceeded the historical ranges for controls (in all routes) given NTP-2000 diet and for chamber controls given NIH-07 diet (inhalation studies). Many exposed animals had multiple adenomas and/or carcinomas. Survival of 4 mg/m^3 males was significantly less than that of the chamber controls. Also mean body weights of 4 mg/m^3 males and all exposed groups of females were generally less than those of the chamber controls throughout the study, and those of males exposed to 2 mg/m^3 were less from week 85 to the end of the study. According to the study report, abnormal breathing was observed in some mice at doses 2 and 4 mg/m^3. Incidences of chronic inflammation, alveolar and bronchiolar hyperplasia and histiocytic cellular infiltrate were significantly increased in all exposed groups of mice. The incidence of interstitial fibrosis was increased in mice exposed to 2 or 4 mg/m^3. Overall, this data shows clear increase in lung tumour incidence in mice at the dose levels causing lung inflammation. However, significant general toxicity (seen as reduced survival and/or reduced body weights and abnormal breathing) was observed at mid and high dose, although females showed lower body weights already at the lowest dose when compared to the controls. Based on the analysis of lung vanadium burden the total lung doses for mice exposed to 1, 2, or 4 mg/m^3 were estimated to be 153, 162, and 225 µg vanadium, respectively. The lung doses being very close to each other may explain the flat dose response seen in mice.

According to the analysis of mice lung tumours by NTP (2002), a high frequency (73%) of *K-ras* mutations were identified in vanadium pentoxide-induced alveolar/bronchiolar carcinomas compared to those in spontaneous alveolar/bronchiolar carcinomas from untreated B6C3F1 mice (30%). This supports the role of *K-ras* activation in the vanadium pentoxide induced carcinogenic process and the mechanistic evidence suggesting that vanadium pentoxide causes the generation

of reactive oxygen species and oxidant dependent Ras/MAP kinase pathway activation. The most frequent mutations were GGT to GAT transitions and GGT to GTT transversions.

However, the inhalation study by Banda *et al.* (2015) shows lack of significant changes in the levels of *K-ras* mutations and the gene expression study of Black *et al.* (2015) (reported under carcinogenicity in the CLH report) shows no evidence for enrichment of pathways associated with cell cycle arrest/proliferation, DNA damage or oxidative stress after 13 weeks inhalation exposure to 2 mg/m^3 of vanadium pentoxide. These results suggest that these *k-ras* mutations seen in mice lung tumours occur later in the carcinogenic process and are likely not an early event in vanadium pentoxide-induced mouse lung carcinogenesis.

The same is suggested also by the study of Manjanatha *et al.* (2015) (see mutagenicity section, table M2a) evaluating cII mutations in lung of male Big Blue mice exposed by inhalation to vanadium pentoxide.

The conduct and dose selection of NTP (2002) study has recently been criticised in the McGregor *et al*. (2020) study provided by Industry after the end of the general consultation. The main points raised were:

1. Dose-finding studies made in two different laboratories showed differing responses in lung inflammation of exposed animals. The reason for this difference remains unclear. However, it was speculated that it is related to the differences in aerosol generation system. This complicated the dose selection for the NTP (2002) 2-year study, which was not very successful, especially considering dose-response analysis and the use of results in risk assessment (e.g. no NOAEL was identified and tumour response at the lowest dose level in mice was already high). The authors criticised NTP for the selection of Battelle Nortwest Laboratories (BNL) (showing higher inflammatory response in dose-finding studies) for the conduct of 2-year study.

2. According to the same study there were major flaws made by BNL: 1) The vanadium pentoxide levels were controlled in test chambers mainly by measuring elemental vanadium by ICP; 2) the control of vanadium pentoxide stability was performed by XRD analyses only before the start of study and then only once more during the rat study and once more during the mice study.

   According to the authors, more frequent XRD analyses would have been needed to confirm the stability of the compound throughout the study. In this respect they refer to the paper by Duffus (2007), which speculates on the possibility of generation of reactive intermediates (ROS) due to catalytic properties of vanadium pentoxide in contact with organic matter (e.g. excreta) in chambers and e.g. possible VOCs formed in the chambers (due to animals, ventilation system and test material).

3. The selection of test compound was also criticised: NTP (2002) used orthrorhombic crystalline form of vanadium pentoxide and not fused flakes which is the most commonly used form.

Regarding point 1 RAC agrees that dose-selection of NTP 2-year study was not very successful and complicates the use of data for the risk assessment purposes. However, for hazard assessment the doses were sufficient to demonstrate the carcinogenic activity of vanadium pentoxide in mice. Although the reason for selecting BNL (showing higher inflammatory response) for the conduct of 2-year carcinogenicity study is not explained in the NTP report, RAC notes that BNL results seem to be in line with more recent inhalation studies by Schuler *et al.* (2011), Banda *et al.* (2015), Manjanatha *et al*. (2015). The BNL results are also in line with other studies which

also showed inflammation, Schuler *et al.* (2011) and increased lung weight already at 1 mg/m^3 after 16 weeks exposure in mice (Banda *et al.*, 2015; Manjanatha *et al*., 2015 and Schuler *et al.*, 2011).

Regarding points 2 and 3, it needs to be highlighted that the orthorhombic crystalline form of vanadium pentoxide is on the market and there is no information which would put in question its relevance for the hazard assessment of V_2O_5. Speculations by Duffus (2007) on the instability of the vanadium pentoxide are not substantiated by the data. In the ITRII test system vanadium pentoxide seemed to remain stable during the 90 day inhalation study period and, although BTL had a different aerosol generation system, in the BTL study, XRD analyses confirmed the stability of the test compound on days 3 and 10 in mice and rats studies, respectively.

On the vanadium pentoxide catalytic properties and possible generation of carcinogenic ROS in contact with organic material in the inhalation chamber, RAC considers that this speculation does not rule out relevance for humans. Generation of ROS in contact with human body is a rather common mechanism for the tumorigenicity of metal compounds. Although it is a threshold indirect mechanism, often associated with respiratory tract inflammation, it does not make it irrelevant for humans. On the other hand, Schuler *et al*. (2011) found only limited evidence on the oxidative stress in lungs of mice exposed 16 weeks to vanadium pentoxide via inhalation.

Additionally, during the RAC meeting, the validity of the NTP study was questioned by the industry representative because of the high blood vanadium levels observed in control mice and rats in 2-year study. Indeed, blood levels in control animals were ≥10-times higher than reported in other studies, for example in Schuler *et al.* (2011). Also, the blood levels in mice exposed to 4 mg/m^3 of vanadium pentoxide were >6 times higher than the blood levels reported in Schuler *et al.* (2011) after similar exposure. In addition, LOQ for blood vanadium analysis in NTP (2002) was 10-times higher than e.g. in Schuler *et al*. (2011). Industry expressed their concern that these higher levels are caused by another source of vanadium during the study. According to industry this seriously questions the validity of NTP (2002). However, RAC notes that vanadium lung burdens were below the limit of detection (0.17 µg V/g lung) in control animals, and the levels in exposed animals were in the same order of magnitude as in other studies, like in Schuler *et al.* (2011). For example, in NTP (2002) maximum lung burdens reported in mice were 42 µg/g tissue after exposure to 4 mg/m^3, whereas in Schuler *et al.* (2011) maximum levels after 16 d exposure to 4 mg/m^3 were 62 µg/g tissue. Excess intake of vanadium from food and drinking water sources is not likely either. It should be noted that the vanadium blood levels were approximately 5 times higher than those observed in NTP drinking water studies with the highest doses (representing MTD and an intake of ~2 mg V/day) of vanadyl sulphate and sodium metavanadate. Thus, if correct, they represent a substantial exposure to vanadium.

According to NTP (2020) and Prestart report (1996) (full references added in "Additional references" section), similar vanadium levels (0.273 ± 0.014 µg vanadium/g blood) were measured also in blood that was obtained from commercial sources to validate the analytical method and run calibration curves.

Collectively the data point to unknown sources contributing to the reported levels in both blood from commercial sources and the control study group when the ICP-AES method with a wavelength of 309.311 nm was used.

Based on this, and contrary to industry's theory, RAC does not consider that these blood vanadium levels measured in the animals in the NTP (2002) study represent the real levels. Also, RAC does not consider that these analytical issues in blood vanadium measurements invalidate the cancer findings in mice.

Additionally, there is one cancer promotion study performed in male A/J, BALB/cJ and C57BL/6J mice. After injection of methylcholanthren (MCA) animals were exposed for 5 weeks to V_2O_5. After 20 weeks the tumour rate was determined. Table C3 shows the results of the study.

Table C3:

	Corn oil (control)		MCA-treated	
Strain	PBS	V_2O_5	PBS	V_2O_5
A/J	0.0 ± 0.0	0.5± 0.5	3.3 ± 0.75	10 ± 1.4
BALB	0.0 ± 0.0	0.0 ± 0.0	0.78 ± 0.28	2.2 ± 0.36

Results taken from (Rondini et al., 2010)

These data suggest tumour promotion activity of vanadium pentoxide. No tumours were observed in C57BL/6J mice.

In the study by Yao *et al.* (1986) male and female mice were exposed for 1 year to vanadium pentoxide at the dose of 0.5, 2 and 8 mg/m^3. Papillomatous and adenomatous tumours in the lungs were reported in 2/79 and 3/62 mice at 2 and 8 mg/m^3, respectively whereas no tumours reported in control animals and at 0.5 mg/m^3. No further data is available from this study.

Comparison with the criteria

A substance is classified into Category 1A if it is known to have a carcinogenic potential in humans. Category 1A is largely based on human evidence. In the case of vanadium pentoxide, no human data exists and therefore Category 1A is not applicable.

Category 1B is indicated in the case of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in at least two species or in two independent studies in one species. Alternatively, 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.

In the case of vanadium pentoxide, NTP study in rats did not provide clear evidence on the carcinogenicity of the substance. However, in mice, increased incidence of lung tumours was seen at all doses in both males and females. Since the NTP study can be considered a well conducted study and have been performed under GLP, criteria for Category 1B is considered to be fulfilled. There are, however, few aspects which may decrease the concern for humans. First of these is the fact that these tumours occur normally at high frequency especially in male mice. However, according to the NTP molecular oncology study, tumours observed in mice showed different mutational spectrum with a high frequency of *K-ras* mutations compared to the tumours observed in control animals. Also, a reduced tumour latency was observed in all exposed groups in mice (in controls first tumours were observed in 667-731 days, in exposed mice in 281-522 days, see further details in the CLH dossier). The second aspect which may decrease the concern, is the general toxicity and high incidence of inflammatory effects in lungs in mice. Inflammation may increase the risk for lung cancer by inducing oxidative stress and reactive oxygen species and secondary genotoxicity. G to T transformations in *K-ras* gene (as observed also with vanadium pentoxide) have been considered as indicative for oxidative damage. Inflammation and secondary genotoxicity have been considered to play a role in many of metal induced respiratory tract cancers. This mechanism may have an impact on the shape of the dose response and the existence of the MoA based threshold for the carcinogenic effects. However, although this decreases the concern for cancer at low exposure levels it doesn't make the cancers observed

in animals non-relevant for humans. In the mice study high incidence of malignant lung tumours were seen both in males and females already at the lowest dose level and, although also at this dose level inflammatory lesions were recorded in the majority of the animals, they were primarily minimal to mild in severity. The flat dose-response seen in mice is likely to be related to the lung vanadium burdens, which were very close to each other in the different dose groups. Regardless of the concerns expressed by the industry and discussed above in detail, RAC considers NTP (2002) a generally well conducted study which is sufficient for classification purposes.

Overall, RAC concurs with the Dossier Submitter that **vanadium pentoxide fulfils the criteria for carcinogenicity category 1B.**

There is no data on the carcinogenicity via other routes of exposure. 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. Although in inhalation studies no systemic tumours could be observed, even though systemic absorption of vanadium moiety could be demonstrated, induction of local tumours after oral exposure (seen e.g. in oral studies with hexavalent chromium) cannot be excluded due to the lack of oral data. Therefore, according to the criteria, it is not possible to define the route of exposure. Nevertheless, it is acknowledged that the main concern is related to the inhalation exposure.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

DS has proposed to classify vanadium pentoxide for fertility cat 1B based on the following evidence:

- NTP (2002) showing impairment of sperm motility at 8 and 16 mg/m^3 in male mice and effects on oestrous cycle from 8 mg/m^3.
- Effects on sex organ weights in males, disturbing cycle, dystocia and fertility effects (reduced mating index, fertility index) without body weight reductions in a study by Morgan and El-Tawil (2003), after exposure to ammonium metavanadate via the drinking water.
- I.p. studies by Altamirano *et al.* (1991), Altamirano-Lozano *et al.* (1996), Uche *et al.* (2008) with vanadium pentoxide, and Chandra *et al.* (2007a, b, c) with sodium metavanadate showing effects on sperm and reproductive function of male mice or rats.

Regarding developmental effects, the DS proposes to retain existing classification to cat 2. based on the following evidence:

- Developmental effects (including decreased foetal body weight, embryofoetal mortality and malformations) reported with vanadium pentoxide in studies with low reliability (Klimisch score 3 or 4).
- Adverse developmental effects in a drinking water study with ammonium metavanadate (NH_4V0_3) by Morgan and El-Tawil (2003) and intra-gastric study by Domingo *et al.* (1986) and i.p. study by Gómez *et al.* (1992) which all show deficiencies and are therefore not considered to provide fully reliable evidence justifying Cat 1B for development.
- Despite the limitations in the studies reduce the confidence in the results, since the results still raise a concern on developmental effects, the DS proposes to retain classification to cat 2 for development.

In addition, the DS proposes classification for effects via lactation. This proposal cannot be based on the data on vanadium pentoxide since there are no appropriate studies available. However,

read across to other pentavalent vanadium species has been made. There are five publications available on sodium metavanadate (post-natal i.p. injection) suggesting neurotoxic effects via lactation. The studies showing neurotoxic effects in pups have used i.p. as route of administration and have limitations in the study design (e.g. only one dose level) and/or in reporting (e.g. no purity information) resulting in Klimisch score of 3. However, consistent neurotoxic effects were considered adequate in a weight of evidence approach for classification. Additionally, there are toxicokinetic data showing excretion of vanadium into the milk. Vanadium has also been found in the tissues of the offspring supporting uptake of vanadium via the milk.

The full presentation of the data can be found under "Assessment and comparison with the classification criteria".

Comments received during consultation

Comments were received from one MSCA and from one industry association. The commenting MS supported classification for reproductive category 1B for sexual function and fertility and category 2 for development. It also supported classification for H362 (May cause harm to breast-fed children). Industry was in favour of retaining existing classification of reproductive category 2 for both fertility and development without classification for lactation effects. The main reasoning presented by the industry to support category 2 for fertility was:

- Studies using i.p. administration are not equally relevant and adequate for classification purposes as studies using physiological routes of exposure and no guideline-conforming reproduction toxicity studies are available specifically for V_2O_5.
- In the only reliable study concerning female fertility (NTP 90-day study), there was no effect on estrous cycle length in female mice and the statistically significant effect on estrous cycle length in female rats was without clear relation to dose and treatment. The other study, Morgan and El-Tawil (2003), used to assess female fertility effects with ammonium metavanadate (NH_4VO_3) in female Sprague-Dawley shows limitations and is categorized with a reliability score of 3. In an i.p. study there was an effect on the ovulation rate, but not on the oestrus cycle. Therefore, the reported findings clearly lack consistency and may be incidental.
- Regarding male fertility study, NTP (2002), it is considered to provide only limited evidence for assessing vanadium induced effects on male reproductive organs, and it is doubtful whether this would result in any functional deficit in fertility. In addition, the findings are not consistent between species and there was no clear relation to dose and treatment.
- The other inhalation data comes from the same group and it is unclear how many different studies (possibly only one or two) the published data are referring to. All four publications have severe limitations. Other studies from the same group use IP route and should be therefore disregarded from the analysis. Three studies with other vanadates using oral route have also limitations and can be used only as supporting evidence.
- Regarding lactation, industry acknowledges that there are data showing excretion of vanadium into the milk of lactating mothers and that it is taken up by the suckling pups but questions the relevance of the i.p. studies (with reliability score 3) for the classification.
- Regarding developmental and lactational effects, industry also refers to a recent publication by US NTP on three-month toxicity studies with tetravalent and pentavalent vanadium compounds in Hsd:Sprague Dawley SD rats and B6C3F1/N mice via drinking water exposure, Roberts *et al.* (2019). According to the preliminary results for vanadium in its pentavalent form (published only as an abstract) fetal effects are only seen at levels that are toxic to mothers, so that vanadate ions do not appear to selectively affect live

births or to be selectively toxic to neonates. The full findings of these sub-chronic studies are expected to be published in 2020.

Assessment and comparison with the classification criteria

Sexual function and fertility

There are no guideline-based one or two generation reproductive toxicity studies available on vanadium pentoxide. However, there are NTP (2002) studies in rats and mice giving information on the effects to reproductive organs. In addition, there are a number of research reports evaluating the effects of vanadium pentoxide (or other pentavalent vanadium compounds like ammonium and sodium metavanadates on reproductive organs or fertility. Unfortunately, many of these contain several deficiencies and some have been performed using i.p. administration, which is not considered appropriate for reproductive toxicity testing. The studies with vanadium pentoxide have been presented in Table F1 and studies with ammonium or sodium metavanadates, used as supporting evidence by the DS, have been presented in table F2.

Table F1*. Data on sexual function and fertility on vanadium pentoxide.*

Method	**Animals and exposure**	**Results**	**Reference**
Inhalation / Oral studies			
90-day study Klimisch score: 1	10 male and 10 female F344 rats per dose 0, 1, 2, 4, 8, or 16 mg V_2O_5/m^3 (0.56-9 mg V/m3) 6 h/d, 5 d/w for 3 months, whole body inhalation	*Males:* At the highest dose 7/10 males died. No deaths at other doses. Final body weights at 4, 8 and 16 mg significantly reduced. Atrophy of the secondary reproductive organs, hypospermia and atypical cells in the epididymis were observed in 16 mg/m^3. No other effect on reproductive organs (weight of cauda epididymis, epididymis and testis, spermatid heads/g testis, spermatid count, sperm motility and concentration). *Females:* At the highest dose 3/10 females died, no deaths at other doses. Final body weights at the high dose significantly reduced. Atrophy of the secondary reproductive organs in 16 mg/m^3. Estrous cycle length of females exposed to 8 mg/m^3 was increased compared to the controls (controls: 5.00 ± 0.00 d, 4 mg/m^3: 5.00 ± 0.08 d, 8 mg/m^3: 5.50 ± 0.14 d, 16 mg/m^3: 5.25 ± 0.25 d). Number of females in diestrus was significantly elevated (control: 39.2% in diestrus vs. 4 mg/m^3: 40.8%, 8 mg/m^3: 49.2%, 16 mg/m^3: 71.9%) In addition, local effects were reported in both males and females with a NOAEC of 1 mg/m^3 (see section on STOT RE for further details).	(NTP, 2002)

90-day study Klimisch score:1	10 male and 10 female B6C3F1 mice per dose 0, 1, 2, 4, 8, or 16 mg V_2O_5/m^3 6 h/d 5 d/w for 3 months, whole body inhalation	*Males:* At the highest dose 1/10 males died, no other deaths. Final body weights at 8 and 16 mg/m³ significantly reduced. Absolute and relative lung weights of males and females exposed to 4 mg/m³ or greater were significantly greater than those of the controls. No effects on weight of cauda epididymis, epididymis and testis, number of spermatid heads/g testis, spermatid count, or concentration. At 8 and 16 mg/m³, sperm motility from was significantly reduced: (control: 88.63 ± 0.9%, 4 mg/m³: 86.23 ± 1.64%, 8 mg/m³: 77.10 ± 3.15% and 16 mg/m³: 83.11 ± 2.48%) *Females:* No deaths. Final body weights started to be reduced from 4 mg/m³. No significant differences in estrous cycle. Local effects were reported in both males and females from 1 mg/m³ air (in males and females) (see section on STOT RE for further details).	(NTP, 2002)
Testicular effects after vanadium inhalation Klimisch score: 3	Male CD-1 mice 8 animals sacrificed at each time point (3 controls, 5 exposed) 0; 0.02 M V_2O_5 (1436 µg V_2O_5/m^3 via inhalation) 1 h/twice a week, for a total of 12 weeks, every week animals were sacrificed.	No overt toxicity signs or body weight or testicular weight changes were detected in the vanadium pentoxide exposed animals compared with controls. Vanadium concentration in testes increased drastically after 1 week of exposure and remained stable during the study. The average concentration was 0.05±0.02 µg/g of dry tissue in the controls vs. 1.63±0.15 µg/g in exposed animals. Necrosis of spermatogonium, spermatocytes and Sertoli cells was observed as well as pseudo-nuclear inclusion and disruption of cellular junctions. See also three mechanistic studies from the same research group described in the text below (Mussali-Galante *et al.*, 2005, Bizarro-Nevares *et al.,* 2016 and Rodríguez-Lara *et al.,* 2016).	(Fortoul *et al.*, 2007)
Intra-peritoneal studies			
Study of reproductive function in male mice (dominant lethal test, see mutagenicity section) Klimisch score: 2*	CD-1 male mice 15-30 animals per group 0, 8.5 mg V_2O_5/kg bw i.p. injection (4.7 mg V/kg) Group 1: 20 control animals Group 2: 15 animals received vanadium pentoxide every 3rd day for 60 days On day 61, animals mated with unexposed females) and sacrificed 5 days later.	V_2O_5treatment resulted in decrease in fertility rate (85% vs 33%). Sperm count, motility, and morphology were impaired. The effects were getting more severe as exposure time increased. The final body weight of V_2O_5-treated animals for 60 days was lower than controls. According to the publication text, differences were not observed in animals sacrificed at earlier time points (from day 10 to 50) but according to table II in the publication, body weights of animals at 30 wk group was far lower than in controls or in other groups. Implantation sites, live foetuses, and foetal weight were significantly decreased (no information on maternal parameters, e.g. maternal weights were provided). The number of resorptions/dam and of dead foetuses was increased.	(Altamirano-Lozano *et al.*, 1996)

Study of histological and sperm parameters Klimisch score: 2**	male guinea pigs (5/dose) Experiment 1: 0, 4.5, 6.5, 8.5, 10.5, 12.5 mg V_2O_5/kg single i.p. injection (ad. 7 mg V/kg) Experiment 2: 0, 8.5 mg V_2O_5/kg bw i.p Testicular tissue evaluated after 24, 48, 72 and 96 h.	Experiment 1: Statistically significant increase in percentage basal cell death, reduction in sperm motility, reduction in sperm count and alteration in the spermatic cell morphology. (Only data on sperm count, % of dead and motile sperm presented in publication). A significant dose dependent reduction in spermatogonia, formation of hyperplastic seminiferous tubules and epididymis, vacuolar dilatation, severe bleeding of numerous blood vessels and mild necrosis of testicular tissue were also reported (no data provided, only figures on histopathology). No information on general toxicity is given. Experiment 2: According to the authors testicular cells showed different degrees of response in a time-dependant way: Significant decrease in spermatogonia, alterations or destruction of seminiferous tubules of testicular cells, severe bleeding of vessels and vascular dilatation. No data provided, only figures on histopathology.	(Uche *et al.*, 2008)
Study of sex differences in the effects of vanadium pentoxide Klimisch score: 3	prepubertal CIIZ rats 0, 12.5 mg V_2O_5/kg bw i.p. (7 mg V/kg) Experiment 1: Newborn male (n=5) and female (n=9) rats were treated every second day from birth to 21 days. Controls (n=9) received saline. Experiment 2: Female rats (n=6) were treated from day 21 to the day of the first vaginal oestrus Controls (n=10) received saline	Experiment 1 (males): Increase in weight of seminal vesicles, thymus and submandibular glands in treatment group Experiment 1 (females): Ovulation rate was lower in treated animals. No difference in age of vaginal opening, first vaginal oestrus, weight of ovaries, uterus, adrenals or pituitary, thymus, liver, kidneys and submandibular glands. Experiment 2 (females): Increase in the weight of thymus, submandibular glands and liver.	(Altamirano *et al.*, 1991)

*It remains unclear why DS has classified this as Klimisch score 2 even though the study uses only one dose, includes deficiencies and inconsistencies in reporting and employs i.p. administration.

**It remains unclear why DS has classified this as Klimisch score 2 even though the study includes severe deficiencies in the reporting of the study (e.g. no information on general toxicity, quantitative data provided only on sperm count, % of dead and motile sperm) and uses i.p. administration.

In a guideline-based 90-day studies in rats and mice, NTP (2002), effects on reproductive organs and parameters were also evaluated. In male rats, no effects were seen even at the highest dose, whereas in male mice sperm motility was statistically significantly decreased at the two highest dose levels but without clear dose response. These doses resulted also in reduction in bw. In females, no effects in reproductive parameters were seen in mice whereas in rats the estrous cycle of females exposed to 8 mg/m^3 (but not to 16 mg/m^3) was significantly longer than that of the controls, and the number of cycling females in the 16 mg/m^3 group was reduced. 16 mg/m^3 resulted in death of 3/10 animals. Because of the general toxicity and lack of consistency between the species and dose response, no definitive conclusions on fertility effects can be made based on these studies.

In a study by Fortoul *et al.* (2007) mice were exposed by inhalation 1 h/twice a week for a total of 12 weeks to ~1.4 mg/m^3 vanadium pentoxide and three controls and 5 exposed animals were sacrificed every week. In the microscopical evaluation, increased numbers of necrotic

spermatogonium, spermatocytes and Sertoli cells were observed as well as pseudo-nuclear inclusion and disruption of cellular junctions. Vanadium levels in testes were increased. The same group has published also some immunohistochemical studies on the testicular toxicity of vanadium pentoxide. The same dose and exposure pattern was used. It remains unclear if these represented the same or different animals as in Fortoul *et al.* (2007). These studies are as follows:

- Mussali-Galante *et al.* (2005) showing decrease of the percentage of gamma-tubulin in all analysed testicular cells (Sertoli, Leydig and germ cells) starting with the first week of treatment
- Bizarro-Nevares *et al.* (2016) showing reduced membrane and increased cytoplasmic connexin 43 in seminiferous tubules starting at 8 and 4 weeks of exposure, respectively.
- Rodríguez-Lara *et al.* (2016) showing time-dependent reduction in actin content in testicular cells starting from 3 weeks.

The authors hypothesise that effects of vanadium on microtubules and cell cytoskeleton result in reproductive toxicity. It should be noted that these results are in contrast with NTP (2002), which did not show effects on sperm parameters even at significantly higher total exposure.

The remaining studies with vanadium pentoxide have been performed using i.p. route of exposure which reduce their usability in the assessment of reproductive toxicity of vanadium pentoxide. Two of these studies describe decreases in sperm counts and motility (Altamirano-Lozano *et al.*, 1996 and Uche *et al.*, 2008). Altamirano-Lozano *et al.* (1996) also report decreases in implantation sites, live foetuses, and foetal weights and increased numbers of resorptions/dam and dead foetuses after repeated i.p administration (see also chapter Mutagenicity). Both of these studies were scored by DS to Klimisch score 2 regardless of deficiencies in the conduct and reporting of the studies (e.g. only one dose group and limited/contradictory information on general toxicity in Altamirano-Lozano *et al.* (1996), no information on general toxicity in Uche *et al.* (2008)) and i.p. route of exposure. Altamirano *et al.* (1991), on the other hand, report lower ovulation rates in females dosed from birth to the age of 21 days.

Because of the limited data available on vanadium pentoxide itself, the DS has made read across to other pentavalent vanadium compounds and used data on sodium metavanadate and ammonium metavanadate as supporting evidence. The studies used for read across by DS are listed in table F2.

Table F2. *Studies used by DS for read-across.*

Method	**Animals and exposure**	**Results**	**Reference**
Oral/intragastric studies ammonium and sodium metavanadate			
One generation reproductive study Klimisch score: 3	Sprague Dawley rats 200 ppm ammonium metavanadate in drinking water (males: 10 mg NH_4VO_3/kg bw/d) (females: 11.43 mg NH_4VO_3/kg bw/d, about 4-5 mg V/kg/ bw/d) Exposure 14 days premating, during mating, till weaning of pups (21 days of age) Group 1: Treated male group (n=10) mated with untreated females (n=20) Group 2: Treated female group (n=20) mated with untreated males (n=10)	Mating and fertility index reduced in treated males and treated females: Reduction of the number of female rats with regular estrous cycle (12 cycling females (60%) versus 20 cycling females (100%) in the control group) Mating index: Control: 100%, Group 1: 65%; Group 2: 70% Fertility index: 95%, 46.15%, 71.43% Reduced weight of testes, epididymis, prostate gland, seminal vesicles, ($p<0.05$), with no reduction in body weight between control and treated males. Estrous cycle disturbed in treated females, total number corpora lutea reduced (Control: 220, Group 1: 54; Group 2: 94), Signs of dystocia (no. of dams: 0, 1, 4), delayed birth date (no. of dams: 0, 3, 5).	Morgan and El-Tawil, (2003)
Reproductive toxicity study Klimisch score: 3	Sprague-Dawley albino rat Sodium metavanadate Males: 60d before mating Females: 14d before mating, throughout gestation and lactation Dosing: 0, 5, 10, 20 mg $NaVO_3$ /kg/ bw/d (0.6-5.6 mg V/kg/ bw/d) Intra-gastric administration About one half of the fertilized animals were sacrificed on day 14 of gestation with the following examinations: number of corpora lutea, total implantations, living and dead fetuses and number of resorptions. The remaining females were allowed to deliver and to nurse their pups to 21 days.	No maternal effects reported. No significant adverse effects could be observed on the number of corpora lutea, implantations, live and dead fetuses, and resorptions. In animals sacrificed on day 14 of gestation, an increase in the number of dead fetuses and of resorptions was observed in animals treated with 10 and 20 mg/kg bw/day $NaVO_3$ when compared to the control group. But these increases were not significant ($P > 0.05$). Further developmental endpoints analysed in this study are described under developmental toxicity	(Domingo *et al.*, 1986)
Intraperitoneal studies, sodium metavanadate			
Study on fertility and reproductive toxicity in exposed male rats Klimisch score: 2-3 (DS has classified Chandra et al.(2007a) as 2, and (2007b) and (2010) as 3 probably because in two latter publications data from only one dose group were presented.	Chandra *et al.*2007a: 8 male Sprague-Dawley rats for each dose group Experiment 1: 13 days exposure Experiment 2: 26 days exposure 0, 0.2, 0.4, 0.6 mg V/kg bw/d I.p. injection Chandra *et al.* (2007b and 2010): only dose group 0.4 mg/kg bw/d was included in the analyses	Significantly reduced organ weights (testis, seminal vesicles, ventral prostate, coagulating gland, epididymis) at 0.4 and 0.6 mg/kg bw/d Epididymal sperm count significantly reduced, percentage of abnormal sperm significantly increased Dose-dependent reduction of Δ53β- and 17β-hydroxysteroid dehydrogen activity, serum testosterone levels and serum gonadotropins Decrease of superoxide dismutase and catalase activity Dose-dependent increase in lipid peroxidation Increased weight of adrenals, and significant elevation of serum concentrations of corticosterone Testicular lesions, significant reduction of spermatogonia, preleptotene spermatocytes, mid-pachytene spermatocytes and step 7 spermatids (essentially the same effects are reported in all three publications)	Chandra *et al.* (2007a) Chandra *et al.* (2007b) Chandra *et al.,* (2010)

The same research group, who has published the study by Domingo *et al.* (1986) has also published a fertility study, in which male Swiss mice were exposed to sodium metavanadate at doses of 0, 20, 40, 60, and 80 mg/kg bw/day given in the drinking water for 64 days and mated with untreated females (Llobet *et al.*, 1993, Toxicology 80; 2-3, 199-206). For some reason this study was not included in the classification proposal although it has been evaluated e.g. by IPCS (CICAD 29 on vanadium). In this study, decreases relative to the controls in the number of pregnant females were reported in some of the vanadium-treated group without clear dose–response relationship (CICAD 29). No information was given on mating behaviour. There was no significant difference between the groups regarding the numbers of implantations, early or late resorptions, or dead or live fetuses. Decreased body weight was observed in the 80 mg/kg bw/d group. Epididymis weight was also reduced at this dose level, but testicular weights were not altered. Sperm count was significantly decreased at 40, 60, and 80 mg/kg bw/d, but the sperm motility and morphology was unaffected. In histopathology examinations testes were normal.

Overall, from these studies with ammonium or sodium metavanadates, the main study providing support for the reproductive effects of vanadium pentoxide is the study by Morgan and El-Tawil (2003). On the other hand, in the studies by Domingo *et al.* (1986) and Llobet *et al.* (1993) effects were less clear regardless of similar/higher doses. When compared to inhalation studies, the doses used by Morgan and El-Tawil (2003) were only two times higher than maximum inhaled dose (as mg/kg) in NTP inhalation study in rats. Although also i.p. studies by Chandra *et al.* (2007 a,b and 2010) showed clear effects on testis, it should be noted that i.p. administration may result in high local exposure of reproductive organs, which is less relevant for exposures using physiological routes of exposure.

There are additionally three studies in humans analysing association between sperm concentrations and vanadium levels in Pakistani and Japanese men (Katayama *et al.,* 2013; Katayose *et al.,* 2004; Zafar *et al.,* 2015). These studies provide contradictory results as one suggests an association and two showing no association between the vanadium content in seminal plasma and sperm concentration. No other human data are available.

Comparison with the criteria – Sexual function and fertility

Category 1A (Known human reproductive toxicant) classification is not applicable in this case since the classification of a substance in this category is largely based on evidence from humans, and there are no such human information on the sexual function and fertility effects of vanadium pentoxide.

Category 1B (Presumed human reproductive toxicant) is usually proposed on the basis of the data from animal studies, which provide clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects or, if occurring together with other toxic effects, the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

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

In the case of vanadium pentoxide the data on the substance itself is limited. Whereas the inhalation study by Fortoul *et al.* (2007) showed severe histopathological lesions and cell necrosis in mice testis, the NTP (2002) study showed only a decrease in male mice sperm motility (but not in rats) at the two highest doses but without a clear dose response. In female rats, but not in mice, estrous cycle of females exposed to 8 mg/m^3 (but not to 16 mg/m^3) was significantly longer than that of the controls, and the number of cycling females in the 16 mg/m^3 group was reduced, the dose was clearly toxic to the animals. No oral studies have been performed with vanadium pentoxide but the study by Morgan and El-Tawil (2003) showed significant sex organ weight reductions (testes, epididymis, prostate, seminal vesicles) and reduced mating and fertility index without significant body weight reduction after 70 days of oral exposure to a one dose of ammonium metavanadate (200 ppm in drinking water, 8.4 mg V/kg bw/day) in Sprague-Dawley rats. This is the study providing the strongest support for the classification. On the other hand, in the studies by Domingo *et al.* (1986) and Llobet *et al.* (1993) with sodium metavanadate, the findings were less clear regardless of using up to ~2-4 times higher doses of vanadium. In addition, there are few studies using i.p. route of administration. Two of these report decreases in sperm counts and motility with vanadium pentoxide (Altamirano-Lozano *et al.*, 1996 and Uche *et al.*, 2008). Altamirano-Lozano *et al.* (1996) also reports decreases in implantation sites, live foetuses, and foetal weights and increased numbers of resorptions/dam and dead foetuses after repeated i.p administration. Because of the i.p. route of exposure, which may result in high local concentrations of vanadium in peritoneal cavity, these results can be used only as supporting evidence.

Overall, the data on the effects of vanadium pentoxide on fertility is considered limited since there are no proper reproductive toxicity studies using an appropriate route of administration. However, the oral studies performed with ammonium and sodium metavanadates together with slight effects in reproductive parameters in NTP (2002) study raises a concern on the fertility effects also via physiological routes of exposure. Because of the limitations in the studies and partly contradicting findings, in contrast to the DSs evaluation, RAC considers that the data is not sufficient for category 1B classification. Instead, RAC concludes that classification of vanadium pentoxide in **category 2 for sexual function and fertility (suspected reproductive toxicant, H361f) is warranted**.

Developmental toxicity

There is one developmental toxicity (teratogenicity) study using i.p. administration and one using i.v. administration available in literature on vanadium pentoxide. These are presented in table D1.

Table D1.

Method	**Animals and exposure**	**Results**	**Reference**
Teratogenicity study Klimisch score: 3	15 pregnant CD-1 mice in exposure group 13 animals in control group 0, 8.5 mg V_2O_5/kg bw/d from GD 6-15 i.p.	Significant reduction of foetal weight/litter. Significant change in sex ratio towards the female animals. An increase in litters with abnormal foetuses (control: 3%, 8.5 mg/kg: 9%) and an increase in number of abnormal foetuses (control: 3%, 8.5 mg/kg: 15%) Short limbs the most frequent alteration (control: 0%, 8.5 mg/kg: 8%). Malformations at other sites were not significantly elevated. Number of ossification centres in forelimbs was lower. No information on maternal toxicity	(Altamirano-Lozano *et al.*, 1993)
Developmental toxicity study Klimisch score: 3	20/28 female NMRI mice in exposure/control groups Dose: 0, 1 mM V_2O_5 in 0.15 mL single i.v. injection corresponding to 0 and 27.3 µg V_2O_5or 0.9 mg V_2O_5/kg (0.5 mg V/kg) for a 30 gram mouse (default value) - on day 3 of pregnancy (i) or - on day 8 of pregnancy (ii) Animals were sacrificed on GD 17	No effects on resorption frequency, foetal weight, frequencies of foetal hemorrhages. The number of foetuses with less mature skeletons (no ossification of three of four elements: supraoccipital bone, sternum, metatarsalia, all caudal vertebrae) significantly increased in exposure group (ii) (control: 30%, treated: 71%) No information on maternal toxicity	(Wide, 1984)

In addition, there are three studies, which have been cited in earlier reviews, but full reports are not available. The first of these studies is a study by Yang *et al.* (1986) which indicates delayed ossification in rats orally exposed to 1 and 3 mg V_2O_5/kg bw/d (i.e. ≥ 0.6 mg V/kg). At higher dose levels (9 and 18 mg/kg bw/d) skeletal abnormalities were significantly increased but there were also significant decreases in body weight gain (75% and 40% of control values) in dams. In the i.p. studies in rats using different dosing regimens (dosing at different days of the organogenesis) more severe developmental effects, including embryo/fetal mortality and malformations, were observed. Some developmental effects (skeletal malformations, delayed ossification of bone) were also reported to be observed without maternal toxicity. Since these studies are reported only in Chinese language (Zhang *et al.*, 1991; Zhang *et al.*, 1993 a and b) they have not been fully evaluated. In addition, i.p. route of administration makes them less relevant for the assessment. Last study referred in the existing reviews, but not available for evaluation, is the study by Sun *et al.* (1987), which shows increased incidence of resorbed and

dead foetuses at two highest dose levels and wavy ribs at the highest dose level after s.c. administration of vanadium pentoxide. No information on maternal toxicity is available.

Since the data specific for vanadium pentoxide is limited, the DS has evaluated data from other pentavalent vanadium compounds, including ammonium and sodium metavanadates. These data have been summarized in table D2. All these studies have limitations and many of them have applied i.p. administration, only one study has used oral administration and two studies have used intragastric administration.

Table D2.

Method	**Animals and exposure**	**Results**	**Reference**
Oral/intra-gastric studies			
Study on fertility and prenatal developmental toxicity in exposed rats (Klimisch: 3) (no of females: 20 per group, no of pregnant females 6 and 10 for groups 1 and 2)	Ammonium metavanadate 200 ppm in drinking water (approximately 5 mg V/kg in females) Exposure: Males: 70 days Females: 14 days premating, during mating, till weaning of pups (21 days of age) Group 1: mating with treated males; females untreated **Group 2: mating with untreated males, females treated**	**Results from Group 2:** **Adult treated females:** Body weight/dam at termination: 209.5 g vs. 252.1 g Gravid uterine weight/dam: 30.35 g vs. 67.50 g **Foetuses:** Number of dead foetuses/dam: 1.16 vs. 0.15 (control) Number of live foetuses/dam: 3.38 vs. 11.32 (control) Mean foetal body weight (PND 21): 10.34 (n=2) vs. 22.51 (n=213) (control) Live/birth index: 100% vs. 100% (control) Survival index: 85.71% vs. 100% Viability index: 74.28% vs. 99.07% Foetuses with visceral anomalies: 9/ 12 vs. 3/72 in controls Foetuses with skeletal anomalies: 15/23 vs. 1/144 in controls Data not provided on a litter base	(Morgan and El-Tawil, 2003)
Reproductive toxicity study Klimisch score: 3	Sodium metavanadate (purity unknown) Sprague-Dawley albino rat Males: 60d before mating; Females: 14d before mating, throughout gestation and lactation Dosing: 0, 5, 10, 20 mg NaVO3 /kg/ bw/day (0.6-5.6 mg V/kg bw/d) by intra-gastric administration.	Maternal effects: No adverse effects In animals sacrificed on day 14 of gestation, a statistically non-significant increase in the number of dead fetuses and resorptions with 10 and 20 mg/kg bw/d. In animals allowed to birth, the development of the offspring was significantly decreased from birth and during all the lactation period for animals treated at 10 and 20 mg/kg bw/d. Significant decreases in the relative weights of liver, spleen and kidneys of the pups whose mothers received NaVO3 during the lactation from 5 mg/kg bw/day. Decreases in body weight, body length, tail length were also reported in offspring.	(Domingo *et al.*, 1986)

Developmental toxicity study Klimisch score: 3	Sodium metavanadate (purity unknown) Sprague-Dawley rat. Exposure from GD 6-14, cesarean sections at day 20. Dosing: 0, 5, 10, 20 mg NaVO3/kg bw/d (0, 2.1, 4.2, 8.4 mg V/kg/ bw/d in distilled water) intragastrically. No of litters produced: 14,14,12,8	Maternal effects: No significant adverse effects (according to WHO 2001 there were no information on maternal toxicity) Developmental effects: Increased number of abnormal foetuses from 5 mg/kg bw/d by non-dose- response-related. From 10 mg/kg bw/d, an increase of the number of resorptions and number of dead foetuses was observed although no significant effect on the resorption rate could be demonstrated. No skeletal abnormalities. The incidence of visceral abnormalities at 20 mg/kg bw/d was remarkably higher compared to the controls. At high dose only: -hydrocephaly (2(2)/98 foetuses vs. 0(0)/196 foetuses. -hemorrhage in facial area 18(18)/ 98 foetuses vs.2(1)/196 foetuses -hemorrhage in dorsal area (10(10)/98 foetuses vs.2(1)/196 foetuses. WHO (2001) "no clear evidence of direct developmental toxicity"	(Paternain *et al.*, 1987) (study by the same research group as Domingo *et al.*, 1986)
I.p. studies			
Developmental toxicity study Klimisch: 2 (according to DS evaluation, regardless of i.p. route)	Ammonium metavanadate Syrian golden hamster Treatment from gestation day 5-10 0, 0.47, 1.88, 3.75 mg NH_4VO_3/kg bw/d (0, 0.2, 0.8, 1.6 mg V/kg bw/d) I.p. Pregnant females were killed at day 15 (20 females/dose level)	Maternal effects: Maternal body weight and weight gain not significantly different in treatment groups from control Developmental effects: Skeletal abnormalities; "minor abnormalities." significant ($p<0,01$) for all exposed groups Although not statistically significant, external anomalies included meningocoele, one fetus with multiple anomalies and the presence of a molar pregnancy	(Carlton *et al.*, 1982)

Developmental toxicity study Klimisch score: 3	Sodium metavanadate ($NaVO_3$) – analytical grade Swiss mice Exposure from GD 6-15 Dosing: 0, 2, 4, 8 and 16 mg $NaVO_3$/ kg /d (0, 0.8, 1.7, 3.3 mg V/kg/ bw/d) i.p.	Because of the excessive maternal mortality (92%) at 16 mg/kg bw/d, this group was excluded. Maternal effects: Decreased weight gain in all the other tested doses. A statistically significant decrease of the gravid uterine weight was observed from the lowest tested dose level. The body weight of mothers at sacrifice minus gravid uterine weight was not statistically significantly affected. Development effects: Reduced foetal weight, increased embryo and foetolethality with a reduced number of live foetuses per litter (4 and 8 mg/kg bw/d), cleft palate statistically significant at high dose with an apparent dose-response relationship for cleft palate across all doses.	(Gómez *et al.*, 1992)
Behavioural study on early postnatal neurological effects Klimisch score: 3	Sodium metavanadate Sprague-Dawley rat pups (n=5) 3 mg NaVO3/kg bw/day (1st PND-14th PND) i.p.ly, litter mate controls (n=5) received PBS.	Exposed pups significantly impaired motor functioning in Rotarod test ($p < 0.01$) *In vitro* survival analysis of neurological cells oligodendrocyte progenitor cells (OPCs) most sensitive; depletion of OPCs from NaVO3. Significant influence of iron chelator on OPCs viability. Vanadium OPCs interaction suggested as important mechanism of hypomyelination.	(Todorich *et al.*, 2011)

Overall, there are indications on the developmental effects of vanadium also from the studies performed with ammonium and sodium metavanadates. Unfortunately, all these studies have limitations; either they have been performed using i.p. route of exposure, employ only one dose level, have small number of pregnant animals/litters produced, do not contain adequate data on the purity of the substance, or on maternal toxicity, or contain other limitations in the reporting of the study. E.g. the study by Morgan and El-Tawil (2003) reporting skeletal and visceral anomalies employ only one dose group, have less pregnant animals than required by OECD TG 414 and do not contain information on the clinical observations in dams. However, as can be seen from table D2, all these suggest some developmental effects, which in some cases seem to occur without severe maternal effects.

There is one epidemiological nested case control study suggesting an association between environmental vanadium exposure (measured by biomonitoring) and increased risk of low birth weight, Jiang *et al.* (2016). Vanadium exposure was measured just once during pregnancy and may not be representative for the entire pregnancy period. The same research group has provided supporting results also on the population-based cohort study from Hubei, China (Hu *et al.*, 2017; not included in CLH proposal but full reference added in "Additional references" section). However, contribution of other pollutants to the findings cannot be excluded and further studies are needed to show the causality of this observed association.

Comparison with the criteria – Developmental toxicity

Category 1A (Known human reproductive toxicant) classification is not applicable in this case since the classification of a substance in this category is largely based on evidence from humans, and there are no such human information on the developmental effects of vanadium pentoxide.

Category 1B (Presumed human reproductive toxicant) is usually proposed based on data from animal studies, which provide clear evidence of an adverse effect on development in the absence of other toxic effects, or, if occurring together with other toxic effects, the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

Category 2 (Suspected human reproductive toxicant) should be considered when there is some evidence from humans or experimental animals, possibly supplemented with other information, on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects should have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Vanadium pentoxide has an existing classification to category 2 for developmental toxicity. The DS has proposed to retain this existing classification. There are only few, limited studies available on vanadium pentoxide or studies not available for full evaluation. In addition, data is available from ammonium and sodium metavanadates. Although these data support each other and suggest developmental effects (foetal mortality, visceral and skeletal anomalies, developmental delays) in exposed animal, they suffer from significant limitations in the conduct and reporting of the study. For example, half of the studies have used i.p. route of administration, which is not considered an appropriate route of exposure for developmental study. One study has used i.v. route and only three have used oral/intra-gastric administration.

According to CLP criteria, if deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. However, based on the findings all pointing out to the developmental effects in these limited studies, RAC concurs with DS and concludes to **retain the cat 2 (suspected developmental toxicant, H361d) classification for development**.

Lactation

There are no specific data available on the vanadium pentoxide itself. Therefore, the DS has used read-across to pentavalent vanadates to reach conclusion on the effects via lactation. The main studies used by DS for the classification are listed in table L1.

***Table L1**. Studies used to justify lactational classification*

Method	**Animals and exposure**	**Results**	**Reference**
Toxicokinetics			
Kinetic study in rats Klimisch score: 3	^{48}V pentavanadate 3 nursing Sprague Dawley rats received 0.1 µg V/rat (2nd PND, single IV injection)	Two days after injection to nursing rats milk content of V was 34 ng/g x 10^3. 2d after injection, Vanadium in pups' intestines was mainly present in form of low molecular weight (LMW) components (95%), this amount decreased to 19% after 2 weeks (80% then was bound to high molecular fractions; LMW-^{48}V may represent an easily absorbed and mobile form. Tissue concentration of ^{48}V was higher in suckling rats (10d after injection) than in weanling rats (18d after injection, 7d post lactation), e.g., vanadium content in brain: 0.18 ± 0.05 (suckling) vs. 0.09 ± 0.05 ng ^{48}V/g x 103 (weanling); kidney: 4.7±0.87 (suckling) vs. 1.12 ± 0.26 (weanling) Highest levels were seen in intestine, in liver and kidneys.	(Edel and Sabbioni, 1989)
Effects in suckling pups			
Study on postnatal developmental toxicity in suckling rats Reliability Klimisch score: 3	4 dams and their pups per group Sodium metavanadate 0, 3 mg $NaVO_3$/kg bw i.p. injection to dams from PND 10 -21 Pups were observed for behavioral alterations until PND 21 and sacrificed on PND 21.	Eye opening mean day in treated animals was significantly delayed. A progressive decrease in righting time reflex was less marked for exposed animals (non-significant). On exposure day 20 a significant decrease in forelimb support latency was observed in exposure group. Exposed animals showed a significant decrease of locomotor activity (expressed as number of crosses) in the open field test on PND 21. No difference was observed in the number of rearing, grooming and fecal pellets. Microscopic analysis showed decrease in myelin fiber density in different brain areas.	(Soazo and Garcia, 2007)
		In cerebellum HSP70 activation was detected in exposed pups (heat shock protein, indicates vulnerability in brain after neurotoxic injury, e.g., in response to cellular oxidative stress) Astrogliosis: Glial fibrillary acidic protein (GFAP)-positive astrocytes were larger in exposure group Conclusion: signs of oxidative damage in the brain	(Cuesta *et al.*, 2013)

Study on neurobehavioral toxicity in weaned rats after lactational exposure Klimisch: 3	Sodium metavanadate, Sprague-Dawley rats; Dams (n=12) received 3 mg $NaVO_3$ /kg bw/d, i.p. or distilled water, altogether 36 pups studied Pups were tested in the rotarod test (motor coordination) at PND60 and brain biochemical analyses performed	Performance in rotarod test (motor coordination) was significantly impaired in weaned male rats after lactational exposure to NaVO3 (latency to fall from rotarod, coordination index). The neurotrophin growth factor BDNF protects for neuronal survival, synaptic plasticity and learning and memory. Plasma and cerebellar BDNF levels were decreased significantly in the vanadium exposed group. Activation of TrkB receptor was significantly influenced by treatment.	(Wang *et al.*, 2015)
Postnatal developmental toxicity study Klimisch score: 3	Sodium metavanadate, 2 nursing CD-1 mice with their pups (on average 6)/group 0, 3 mg $NaVO_3$ /kg bw/d, i.p.ly injected to dams for 14 days or 21 days starting on PND 1 Mice were sacrificed on PND 15 or 22 Control group received sterile water, i.p.)	In behavioral tests (performed PND 15 and PND 22) a reduction in locomotor activity and negative geotaxis were seen in most instances in pups. "Center square duration" and "stretch attend posture" results were significant at PND22. Immunohistochemistry of brain tissue showed astrocytic activation and demyelination in pups (duration dependent changes)	(Mustapha *et al.*, 2014)
Postnatal developmental toxicity study Klimisch score: 3	Sodium metavanadate 1 nursing Albino rat with its pups/ group 0, 3 mg $NaVO_3$ /kg bw/d, i.p. injected to dams for 14 days starting on PND1. Rats were sacrificed on PND 15 or 22.	Pups exhibited behavioural deficits in most tests, a significant reduction in body weight gain and absolute brain weight. Immunohistochemistry showed reactive astrogliosis induced by vanadium exposure.	(Olopade *et al.,* 2011)
Study on fertility and prenatal developmental toxicity in exposed rats Klimisch: 3	Ammonium metavanadate 200 ppm in drinking water Sprague Dawley rats Exposure of female rats 14 days premating, during mating, till weaning of pups (21 days of age)	During lactation, the pups behavioral responses (such as learning and memory responses) were decreased. However, no data on this were provided. (for other parts of this study not relevant for this section see further fertility/developmental toxicity sections)	(Morgan and El-Tawil 2003)

Regarding excretion of vanadium into the milk, there is one study (Edel and Sabbioni, 1989) showing elevated milk levels and tissue concentrations in suckling rat pups after exposure to radiolabelled pentavanadate via the mother's milk. In addition, there is another toxicokinetic study in rats showing elevated levels of vanadium in the livers of pups whose mothers had been fed with a diet containing sodium metavanadate (V content in livers 1.1 µg/g in vanadium treated vs 0.24 µg/g in control pups) (Elfant and Keen, 1987). In humans, Anke (2004) reports that "lactating women secrete 17% of their vanadium intake into the milk". This statement seems to be based on the observed lower excretion of lactating females to faeces and urine (79% in faeces and 4% in urine) in comparison to non-lactating woman (91% in faeces, 9% in urine). Overall, although the DS has classified studies as low reliability, they are still considered to give enough evidence on the excretion of vanadium into the mother's milk and its uptake by the suckling pups.

There are four different studies (Soazo and Garcia, 2007 and Coasta *et al.*, 2013 seem to represent the same study) suggesting neurobehavioral effects in rodents after exposure of nursing dams with sodium metavanadate. As pointed out by the DS, although maternal exposure from i.p. route may be higher than it would be from physiological application, suckling pups were only exposed via lactation. Therefore, in this case i.p. administration can be considered acceptable in the absence of studies using other routes of exposure. Although all these studies show limitations (including the use of 1 dose only, purity of the applied compound not given, no documentation of general toxicity), all of these suggest deficits in behavioural tests and damage to brain tissue/cells. Although Morgan and El-Tawil (2003) mention in their study that "during lactation, the pups behavioural responses (such as learning and memory responses)…were decreased" it is not possible to draw conclusions on this study since they provide no data to support this observation and because of the study design it cannot to be concluded whether these possible effects were specifically caused by the exposure via the milk.

In addition, DS also referred to the studies in adult rodents or pups directly exposed to pentavalent vanadates showing neurological effects as supporting evidence (see CLH report for references).

Comparison with the criteria- Lactation

According to the classification criteria substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned based on:

(a) human evidence indicating a hazard to babies during the lactation period; and/or

(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or

(c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

In the case of vanadium pentoxide there is no specific data on the substance itself. Therefore, assessment is based on the data on other pentavalent vanadium compounds. There are toxicokinetic data in rodents showing excretion of vanadium to the milk and its uptake by the suckling pups resulting in higher tissue levels of vanadium when compared to the non-exposed pups. What comes to the adverse effects caused by the exposure to vanadium via lactation, there are four studies available suggesting neurotoxic effects. Although all these studies have limitations, including the use of only one dose, use of i.p. administration of vanadium and poor reporting, these together with toxicokinetic information raise a concern on possible lactation effects. Although i.p. administration was not considered an appropriate route of exposure for

fertility or developmental effects, in the case of lactational effects when suckling pups are exposed via the milk, it can be accepted in the absence of other data. In addition, since there is no cut-off criteria regarding quantitative exposure for lactation, potential higher bioaccessibility of used vanadium compounds compared to vanadium pentoxide is less important here. Overall, RAC concurs with the DS that **classification for lactational effects, H362: May cause harm to breast-fed children,** is warranted.

Additional references

Llobet JM, Colomina MT, Sirvent JJ, *et al.* (1993) Reproductive toxicity evaluation of vanadium in male mice. Toxicology 80/2-3, 199-206.

McGregor JA., White DJ., Williams AL. (2020) The limitations of using the NTP chronic bioassay on vanadium pentoxide in risk assessments. Regulatory Toxicology and Pharmacology, Volume 113, June 2020, https://doi.org/10.1016/j.yrtph.2020.104650.

NTP (2020): Brian Berridge, National Toxicology Program. Letter (NTP 2020), December 3, 2020 to John W. Hilbert III, President Vanadium Producers & Reclaimers Association.

Prestart Report (1996): Vanadium Pentoxide, V2O5, CAS# 1314-62-1, C61427B)]+A, Method Performance Evaluation and Prestart Report for Toxicokinetic Studies – Determination of Vanadium in Blood" (Prestart Report, June 28, 1996)

Hu *et al.* (2017): Association of adverse birth outcomes with prenatal exposure to vanadium: a population-based cohort study. The Lancet Planetary Health. 1. e230-e241. 10.1016/S2542-5196(17)30094-3.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).