Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

divanadium pentaoxide; vanadium pentoxide

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

CLH-O-0000006927-60-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

10 December 2020

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DIVANADIUM PENTAOXIDE; VANADIUM PENTOXIDE

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

Divanadium pentaoxide

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

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

Index Number: **023-001-00-8**

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CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Divanadium pentaoxide
Other names (usual name, trade name, abbreviation)	Divanadium pentaoxide; Vanadium pentoxide; Vanadium(V) oxide Dioxovanadiooxy(dioxo)vanadium
ISO common name (if available and appropriate)	*Not applicable*
EC number (if available and appropriate)	215-239-8
EC name (if available and appropriate)	Divanadium pentaoxide
CAS number (if available)	1314-62-1
Other identity code (if available)	ICSC number: 0596 RTECS number: YW2460000 UN Number: 2862
Molecular formula	V_2O_5
Structural formula	Not relevant for an inorganic substance
SMILES notation (if available)	Not relevant for an inorganic substance
Molecular weight or molecular weight range	181.878 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	Concentration range ≥ 80 wt % - 100% Typical purity ≥ 98% (no further indication available on impurities or additives)

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self- classification and labelling (CLP)**
Divanadium pentaoxide	≥ 80 wt % (mono-constituent)	Acute Tox. 4 (H302)* Acute Tox. 4 (H332)* STOT SE 3 (H335) Muta 2 (H341) Repr. 2 (H361d)*** STOT RE 1 (H372)** Aquatic Chronic 2 (H411)	Lead registrants report the harmonised classification. In addition, in their CSR (2016), they proposed an alternative classification for V_2O_5 analytical grade : Acute Tox. 4 (H302), Acute Tox. 4 (H332), Eye Dam. 1 (H318), STOT SE 3 (H335), Repr. 2 (H361)[#], STOT RE 1 (H372)(respiratory tract, inhalation), Aquatic Chronic 2 (H411) 25 additional notifications (525 notifiers, 16/8/2017) are available, see below this table.

*) The classification as obtained from Annex VII shall then substitute the minimum classification indicated in this Annex if it differs from it.
**) The classification under 67/548/EEC indicating the route of exposure has been translated into the corresponding class and category according to this Regulation, but with a general hazard statement not specifying the route of exposure as the necessary information is not available.
***) In order not to lose information from the harmonized classification for fertility and developmental effects under Directive 67/548/EEC, the classifications have been translated only for those effects classified under that Directive.
[#]) H361 without suffix (f,d, or fd): a substance classified in Repr Cat 2 but the effects cannot be specified with respect to fertility and/or developmental toxicity (ECHA, 2015).

The following C&L inventory information for self-classification is available for the general entry of divanadium pentaoxide (CAS 1314-62-1) on 15/08/2017.

Classification	**Number of notifiers**
Not classified	0
Acute Tox 4 – H302, H312, H332	504
STOT SE3 – H335	519
Muta. 2 – H341	499
Repr. 2 – H361	524
STOT RE 1 - H372	522
Eye Dam 1 – H318	142

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

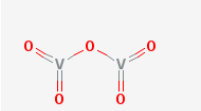
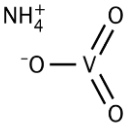
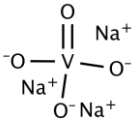
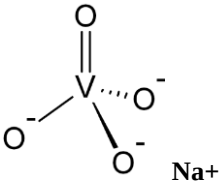
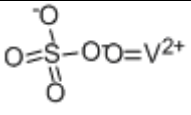
Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
No data available				

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

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
No relevant additives					

Table 5: Test substances (non-confidential information) :

Further data on vanadium compounds:

Identification of test substance	Molecular Formula:	Structure	Valency
Vanadium **CAS N°7440-62-2**	**V**		**0**
Divanadium pentaoxide: **CAS N°1314-62-1**	**V2O5**		**+5**
Ammonium metavanadate **CAS N°7803-55-6**	**NH4VO3**		**+5**
Sodium orthovanadate **CAS N° 13721-39-6**	**Na3VO4**		**+5**
Sodium metavanadate **CAS N°13718-26-8**	**NaVO3**		**+5**
Vanadyl sulfate **CAS N°27774-13-6**	**VOSO4**		**+4**

2 SEE SPECIFIC STUDY RECORDS BELOW FOR FURTHER INFORMATION.PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard 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	GHS08 GHS07 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)	**Retain** GHS08 Dgr **Add** GHS06 GHS09 **Remove** GHS07	**Add** H350 H362 **Modify** H340 H360Fd H330 H301 H372 (respiratory tract, inhalation		**Add** inhalation: ATE = 0.005 mg/L (dusts and mists) oral : ATE = 100 mg/kg bw	
Resulting Annex VI entry if agreed by RAC and COM	023-001-00-8	divanadium pentaoxide; vanadium pentoxide	215-239-8	1314-62-1	Carc. 1B Muta. 1B Repr. 1B Lact. Acute Tox. 1 Acute Tox. 3 STOT SE 3 STOT RE 1 Aquatic Chronic 2	H350 H340 H360Fd H362 H330 H301 H335 H372 (respiratory tract, inhalation) H411	GHS06 GHS08 GHS09 Dgr	H350 H340 H360Fd H362 H330 H301 H335 H372 (respiratory tract, inhalation H411		inhalation: ATE = 0.005 mg/L (dusts and mists) oral: ATE = 100 mg/kg bw	

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

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier	No
Oxidising gases	Hazard class not assessed in this dossier	No
Gases under pressure	Hazard class not assessed in this dossier	No
Flammable liquids	Hazard class not assessed in this dossier	No
Flammable solids	Hazard class not assessed in this dossier	No
Self-reactive substances	Hazard class not assessed in this dossier	No
Pyrophoric liquids	Hazard class not assessed in this dossier	No
Pyrophoric solids	Hazard class not assessed in this dossier	No
Self-heating substances	Hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	No
Oxidising liquids	Hazard class not assessed in this dossier	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not assessed in this dossier	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity *via* oral route	**Proposed update of the harmonised classification: Acute Tox 3 – H301**	**Yes**
Acute toxicity *via* dermal route	**Data conclusive but not sufficient for classification**	**Yes**
Acute toxicity *via* inhalation route	**Proposed update of the harmonised classification: Acute Tox 1 – H330**	**Yes**
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Hazard class not assessed in this dossier	No
Respiratory sensitisation	**Data conclusive but not sufficient for classification**	**Yes**
Skin sensitisation	Hazard class not assessed in this dossier	No
Germ cell mutagenicity	**Proposed update of the harmonised classification: Muta 1B – H340**	**Yes**
Carcinogenicity	**Harmonised classification proposed: Carc. 1B – H350**	**Yes**
Reproductive toxicity	**Proposed update of the harmonised classification: Repr. 1B – H360 Fd Lact – H362**	**Yes**
Specific target organ toxicity-single exposure	Already classified as STOT SE 3 – H335 Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	**Proposed update of the harmonised classification proposed: STOT RE 1 – H372**	**Yes**
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Already classified as Aquatic Chronic 2 – H411 Hazard class not assessed in this dossier	No
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Divanadium pentaoxide has been classified according to the Classification and Labelling of Dangerous Substance Directive (Dir. 67/548/EEC) and then adapted to Regulation (EC) No. 1272/2008 (CLP Regulation) (CLP00). Therefore, the current status of the CLP classification is:

- Acute Tox. 4* - H302
- Acute Tox. 4* - H332
- STOT SE 3 – H335
- Muta. 2 – H341
- STOT RE 1 – H372**
- Repr. 2 – H361d***
- Aquatic Chronic 2 – H411

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

- **CMR endpoints:**

There is no requirement for justification that action is needed at Community level.

- **Acute Tox. and STOT RE endpoints:**

The reason for a need for action at Community level is "*Change in existing entry due to changes in the criteria*". Update of the current entry set under Directive 67/548/EEC is justified because the criteria does not correspond directly to the classification in a hazard class and category under CLP regulation.

5 IDENTIFIED USES

Divanadium pentaoxide is used for the production of vanadium compounds and as an intermediate in the production of vanadium and steel alloys. Besides it is used as a catalyst e.g. in developing solutions or for the oxidation of sulfide to sulfate (ATSDR, 2012; IARC, 2006; NTP, 2002). It is discussed, if vanadium is essential for several species of green algae, fungi and nitrogen-fixing microorganisms (Anke, 2004), but essentiality in mammals has not been conclusively shown (ATSDR, 2012). Some vanadium compounds are in use for pharmaceutical purposes (Assem and Levy, 2012; Michibata, 2012).

6 DATA SOURCES

Starting point for data searches for this report have been recent reviews and monographs with toxicological risk assessments on divanadium pentaoxide or pentavalent vanadium compounds (specifically ammonium

metavanadate, sodium metavanadate, sodium orthovanadate). Most relevant reviews used are ATSDR (2012), NTP (2008), IARC (2006), WHO (2001) and EPA (2011). As a draft, EPA (2011) was not used to analyse assessment and conclusions directly, but was helpful for retrieval of relevant studies.

Furthermore, REACH registration dossiers for divanadium pentaoxide and vanadates, available from ECHA's disseminated database (ECHA Dissemination, 2017) have been analysed for study references, which then have been considered as data sources for this CLH report.

Publication compilations on vanadium toxicity until June 2015 have been performed by VANITEC[1] and were used for data search.

Documents submitted by the Vanadium Producers and Reclaimers Association (VPRA) to the U.S. Environmental Protection Agency provided two relevant documents used in this CLH report (VPRA, 2010; 2011).

Furthermore, ECHA guidance documents on the application of CLP criteria and on the preparation of dossiers for harmonised classification and labelling were used to compile this report (ECHA, 2014; 2017).

Systematic searches for publications and other relevant data were performed based on the following databases:

- U.S. National Library of Medicine, Pubmed.gov[2]
- TOXNET, ChemID*plus*[3], IPCS[4], eChemPortal[5]
- Chemical Abstracts (at host STN International Europe[6])
- SciSearch, Biosis, CAB Abstracts, Embase (at host Deutsches Institut für Medizinische Dokumentation und Information, DIMDI[7])
- Experimental data on specific substances as documented in OECD Application Toolbox (OECD, 2016),

in addition to unspecific databases (e.g., *google scholar*).

[1] http://ieh.cranfield.ac.uk/Vandogra/Recent_publications.aspx assessed at 31.1.2017

Vanitec brings together organizations involved in the mining, processing, manufacture, research and use of vanadium and vanadium-containing products.

[2] https://www.ncbi.nlm.nih.gov/pubmed assessed at 31.1.2017

[3] https://chem.nlm.nih.gov/chemidplus/ assessed at 31.1.2017

[4] http://www.inchem.org/ assessed at 31.1.2017

[5] http://www.echemportal.org/echemportal/page.action?pageID=9 assessed at 31.1.2017

[6] http://www.stn-international.de/index.php?id=123 ; https://www.fiz-karlsruhe.de/ assessed at 31.1.2017

[7] https://www.dimdi.de/static/de/db/ assessed at 31.1.2017

Chemical and biochemical aspects of vanadium compounds were addressed in a separate literature search (e.g., Crans *et al.*, 2004; Michibata, 2012; Rehder, 2013).

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Yellow to rust-brown crystalline solid	(ECHA Dissemination, 2017)	
Melting/freezing point	690 °C	(ECHA Dissemination, 2017)	
Boiling point	-	(ECHA Dissemination, 2017)	According to the reference, divanadium pentaoxide has no boiling point, it decomposes at 1750°C and 101.325 kPa.
Relative density	3.654	(ECHA Dissemination, 2017)	Measured at 21.7 °C
Vapour pressure	No data	(ECHA Dissemination, 2017)	
Surface tension	No data	(ECHA Dissemination, 2017)	
Water solubility	Moderately soluble* (551 ± 25 mg/L)	(ECHA Dissemination, 2017)	Measured, 21°C, pH6, loading 1 mg/L, after 24 hrs.
Partition coefficient n-octanol/water	No data	(ECHA Dissemination, 2017)	
Flash point	No data	(ECHA Dissemination, 2017)	
Flammability	No data	(ECHA Dissemination, 2017)	
Explosive properties	No data	(ECHA Dissemination, 2017)	
Self-ignition temperature	No data	(ECHA Dissemination, 2017)	
Oxidising properties	No oxidising properties	(ECHA Dissemination, 2017)	Measured
Granulometry	Total dustiness: 458.85 mg/g (DMT) MMAD 1 = 7.72 µm, MMAD 2 = 32.56 µm GSD 1 = 2.56 GSD 2 = 1.54		See remarks*
Stability in organic solvents and identity of relevant degradation products	No data	(ECHA Dissemination, 2017)	
Dissociation constant	No data	(ECHA Dissemination, 2017)	
Viscosity	No data	(ECHA	

Property	Value	Reference	Comment (e.g. measured or estimated)
		Dissemination, 2017)	
*remark on identity: divanadium pentaoxide may occur in different forms and granulometry (particles: powder, crystalline, fused). Divanadium pentaoxide in particle form is treated as *one* entity chemical below because of a) a common mode of action and identical consequences for classification, b) limited quantitative differences in physicochemical parameters and subsequent biochemical reactions. For further background see Annex II (Justification for read across).			

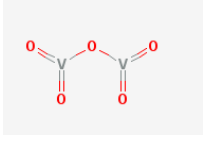
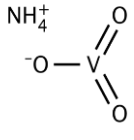
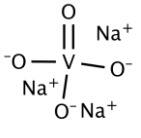
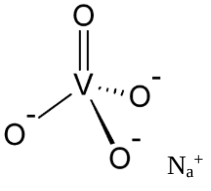
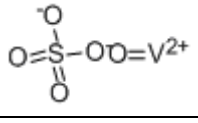
8 EVALUATION OF PHYSICAL HAZARDS

Not performed for this substance.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

In order to give a comprehensive view on the examined compound, the following information: molecular formula, structure, valence and water solubility of vanadium and vanadium compounds are recalled below in Table 9.

Table 9: Identification of vanadium and vanadium compounds including molecular formula, structure, valence and water solubility:

Identification of test substance	**Molecular Formula:**	**Structure**	**Valency**	**Water solubility (Ref)**	
Vanadium CAS N°7440-62-2	V		**0**	0.15 mg/L (20°C; pH 5.8, dissolved after 72h)	(ECHA, 2017) VPRA (2010)
Divanadium pentaoxide: CAS N°1314-62-1	V_2O_5		**+5**	920 mg/L (20°C; pH 2.7; duration not provided)	(ECHA, 2017) VPRA (2010)
Ammonium metavanadate CAS N°7803-55-6	NH_4VO_3		**+5**	7.8 g/L (20°C; pH 6; duration not provided)	(ECHA, 2017)
Sodium orthovanadate CAS N° 13721-39-6	Na_3VO_4		**+5**	soluble	WHO, 2001
Sodium metavanadate CAS N°**13718-26-8**	$NaVO_3$		**+5**	89 g/L (20°C; pH 9.5; duration not provided)	(ECHA, 2017)
Vanadyl sulfate CAS N°27774-13-6	$VOSO_4$		**+4**	Very soluble	WHO, 2001

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

a) Absorption:

Some bioaccessibility and speciation data in several physiological media are available for V_2O_5 (VPRA (2010)). Five synthetic body fluids were used: phosphate-buffered saline (PBS), a standard physiological fluid that mimics the ionic strength of human blood serum, Gamble's solution (GMB, pH 7.4) that mimics the interstitial fluid within the deep lung under normal health conditions, artificial sweat (ASW, pH 6.5), that simulates the hypo-osmolar fluid, linked to hyponatraemia (loss of Na^+ from blood), which is excreted from the body upon sweating, artificial lysosomal fluid (ALF, pH 4.5) which simulates intracellular conditions in lung cells occurring in conjunction with phagocytosis and represents relatively harsh conditions and lastly artificial gastric fluid (GST, pH 1.5) that mimics the very harsh digestion setting of high acidity in the stomach. Although those data may give relevant information on metals bioaccessibility, an incomplete reporting of these data does not allow to assess them more thoroughly.

Respiratory route:

After inhalation exposure to divanadium pentaoxide in particle form, most of the substance is deposited and retained in the lungs. Deposited particles release vanadium ions which may speciate either in cationic (VO_2^+) or anionic (HVO_4^{2-}) forms (IARC, 2006). After intra-tracheal instillation in rats, 30 - 50% of divanadium pentaoxide were still in the lungs after the first hour after exposure and 10-30% after two to three days (Greim, 2006 - report not available).

Indirect evidence in humans (increased elimination of vanadium in urine after occupational inhalation exposure to divanadium pentaoxide) demonstrates systemic uptake of the substance from inhalation (Kiviluoto *et al*., 1981b).

After intratracheal exposure in female rat, 40% of $^{48}V_2O_5$ was recovered in urine by day 3 post-exposure while the skeleton accounted for 30% by day 7 leading to a systemic absorption rate of at least 70% with uptake being much greater *via* the intra-tracheal route than by the oral route (Conklin *et al*., 1982).

V_2O_5 powder (with particle size 0.31 µm, geometric mean) in Gamble's solution, that mimics the interstitial fluid within the deep lung under normal health conditions, was about eight times more soluble than in purified water (pH not given) (Toya *et al*., 2001). However, some fraction of divanadium pentaoxide will be dissolved more slowly in the respiratory tract, as evident from long retention and accumulation (Greim, 2006 report not available).

Oral route:

Administration of $^{48}V_20_5$ by gavage resulted in absorption of 2.6 – 3% of the administered dose 3 days after treatment (Conklin *et al.*, 1982; Greim, 2006; IARC, 2006). However, an analysis of further toxicokinetic studies shows more heterogeneous data on oral absorption: Nielsen (1995) report a range from 1% – more than 10% for the absorption of vanadium compounds (not further specified). Feed composition, fasting, concentration, duration of exposure, age of the tested species and method for quantification, all may influence significantly absorption. Based on a kinetic study with sodium metavanadate, Bogden *et al.* (1982) showed that 39.7 ± 18.5% of ingested vanadium was retained and that 59.1 ± 18.8% of ingested vanadium was excreted in the feces. These authors conclude: "*caution in assuming that ingested vanadium will always be poorly absorbed from the gastrointestinal tract, is suggested"*.

As only evidenced from other pentavalent vanadium compounds, higher levels of vanadium were found in tissues of young rats (21 days postpartum) compared to older animals (115 days after birth), possibly due to the undeveloped gastrointestinal barrier (ATSDR, 2012; Edel *et al*., 1984; Edel and Sabbioni, 1989).

If divanadium pentaoxide is administered in aqueous media, some solution reactions may already have occurred prior to entering the biological system, which, in turn, may influence absorption characteristics and systemic availability. The dissolved fraction of divanadium pentaoxide may be present as vanadate-ions (Crans *et al.*, 2004).

Dermal route:

Dermal absorption of divanadium pentaoxide appears to be minimal (ATSDR, 2012).

b) Distribution

After divanadium pentaoxide **inhalation** exposure of CD-1 mice, the tested compound was distributed to testis (Mussali-Galante *et al*., 2005; Fortoul *et al*., 2007). Vanadium concentration drastically increased in testes 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 *versus* 1.63±0.15µg/g in exposed animals. Lastly, vanadate (V) anions (HVO_4^{2-}; VO_4^{3-}) and the vanadyl (IV) cations (VO^{2+}) reversibly bind to transferrin (Gorzsás *et al*., 2006; Greim, 2006) and, possibly, to albumin (Edel and Sabbioni, 1989).

After **intra-tracheal** instillation of 200 ng/kg body weight of ^{48}V-labelled pentavalent and tetravalent vanadium in rat, vanadium was retrieved in the lungs, liver, kidneys, bone, testes and spleen, vanadium being removed from them with time at different rates. The metabolic patterns of both chemical forms of vanadium: pentavalent and tetravalent were similar. Pulmonary vanadium clearance was initially rapid with 80-85% of

the ^{48}Vanadium removed within 3 h. At day 12, about 2% of the element was remaining in the lung. Intracellularly, the major part of liver, kidney and lung vanadium was present in the nuclear fraction (30-40% of the homogenate), followed by cytosol and mitochondrial fractions. Two biochemical pools of vanadium were identified: the first corresponding to protein bound vanadium, which may be involved in the long-term accumulation of the element in the lung, the second one representing a diffusible vanadium form (Edel and Sabbioni, 1988). Further study from Greim, 2006 with single intra-tracheal application of [^{48}V] divanadium pentaoxide confirmed this tissue distribution pattern in male rats. Vanadium was found in kidneys, bone, spleen and liver, and in small amounts also in muscles, heart, brain and testes. Low amounts were also detected in ovary, adrenal, and adipose tissue (< 0.1%). Twenty percent of the substance was detected in blood 1 hour after intra-tracheal application. After 5 days, 30% of the activity was found in skeleton. Two weeks after single intratracheal application, 40% of vanadium was still detected in the body, with 12% remaining in skeleton (Greim, 2006). Repeated inhalation of divanadium pentaoxide showed accumulation in the lung (Greim, 2006).

After **oral** gavage application, divanadium pentaoxide was mainly distributed to bone, liver, muscle, kidney, spleen, and blood (Conklin *et al.*, 1982; IARC, 2006). Additionally, the tissue distribution pattern of ^{48}Vanadium following oral administration did not show significant differences between pentavalent (^{48}V(V)) and tetravalent (^{48}V(IV)) forms (Edel and Sabbioni, 1988 - no further information given on the tested vanadium forms).

Divanadium pentaoxide is shown to cross the placenta barrier (Greim, 2006; NTP, 2002). Edel and Sabbioni (1989) demonstrated that ^{48}V-vanadate (pentavanadate) reaches the foetus. Suckling rats showed a significant absorption of vanadium (^{48}V pentavanadate) taken up by the milk. Vanadium in milk may be transported in the form of a biocomplex with lactoferrin (Edel and Sabbioni, 1989).

c) Metabolism

Metal compounds like divanadium pentaoxide are not metabolised. However, in the mammalian body, the original compound may be dissolved, reduced and/or (re-)oxidated by redox-reactions, and oligomerised or monomerised. The site of transformation (e.g. from vanadate to vanadyl or *vice versa*) may differ from the site of interaction with biological tissues. As a first step in contact with body fluids, pentavalent vanadium compounds (e.g., divanadium pentaoxide) will be dissolved to vanadate ions (Crans *et al.*, 2004). However, divanadium pentaoxide may dissolve slowly at the port of entry and may be present undissolved in significant quantities. In the lung, divanadium pentaoxide should therefore be discriminated from other pentavalent vanadium compounds with higher solubility. For further details see endpoint specific comments and Annex II (Justification for read across).

d) Elimination

Chronic **inhalation** of divanadium pentaoxide by female rats (0.5, 1 or 2 mg V_2O_5/m^3) resulted in calculated elimination half-lives by lung clearance of 37, 59 or 61 days, respectively. For female mice, inhalation of 1, 2 or 4 mg/m^3 led to lung clearance half-lives of 6, 11 or 14 days, respectively (Dill *et al*., 2004; NTP, 2002).

According to tracer measurements by Sabbioni and Maroni (1984) with **intra-tracheal** application, elimination of vanadium (not further specified, but probably V_2O_5) depends on exposure level, with a half-life around 20 hours for high exposure (at that level significant accumulation was observed), and a half-life around 15 hours for low exposure. Other studies report that 85% of the pentavalent (V^{5+}) form of vanadium is cleared from the lungs 3 hours after intra-tracheal exposure in male albino rats (Edel and Sabbioni, 1988) and 90% of divanadium pentaoxide was eliminated from the lungs of female rats three days after exposure (Conklin *et* al., 1982). After intra-tracheal application of divanadium pentaoxide to rats for two weeks, about 40% of the dose was eliminated in urine (Greim, 2006).

In workers exposed to vanadium dust, a biphasic elimination of vanadium (no further speciation provided) was shown. Elevated concentrations of vanadium in urine were still observed 16 days after the end of exposure compared to controls (Kiviluoto *et al.*, 1981b). Concentrations in urine from inhalation exposure have been correlated with air concentrations of divanadium pentaoxide for workers and respective "biological exposure limits" have been established (Drexler and Greim, 2006).

Vanadium compounds which were deposited in the bones will be eliminated very slowly (see distribution section).

After inhalation exposure, pentavalent vanadium compounds are primarily excreted *via* the urine (Greim, 2006). Whereas after **oral** administration, most of the vanadium intake is eliminated in the feces.

Since vanadium is poorly absorbed in the gastrointestinal tract, a large percentage of vanadium is excreted unabsorbed in the feces in rats following oral exposure (ATSDR, 2012). More than 80% of the administered dose of ammonium metavanadate or sodium metavanadate accumulated in the feces after 6 or 7 days (Adachi *et al*. 2000; Patterson *et al*. 1986). After 2 weeks of exposure, 59.1±18.8% of sodium metavanadate was found in the feces (Bogden *et al.* 1982). Approximately 0.9% of ingested vanadium was excreted in the urine of rats exposed to sodium metavanadate in the diet for 7 days (Adachi *et al.* 2000). An elimination half-time of 11.7 days was estimated in rats exposed to vanadyl sulfate in drinking water for 3 weeks (Ramanadham *et al.* 1991).

According to a study in humans (49 non-nursing and 49 nursing women; for 7 of the nursing women, concentration of vanadium in mothers' milk was quantified), nursing women excret 17% of the vanadium oral intake to the milk, 79% *via* faeces and 4% *via* urine, whereas non-nursing women excreted 91% of

vanadium *via* faeces and 9% *via* urine (Anke, 2004). No further details on the background of this study are available.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Table 10: Summary table of animal studies on acute oral toxicity (all values are expressed in mg V_2O_5/kg bw, if not indicated otherwise)

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

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
Acute oral toxicity study No guideline followed GLP: no Reliability (Klimisch score): 3	7 rats per dose group (no further information given)	Divanadium pentaoxide, dissolved in aqueous sodium carbonate, analytical controls ensured degree of purity (no further information given)	Single peroral application Dose levels not given	10.4 mg/kg bw **Acute Tox. 2**	(Massmann, 1956) Details in Annex I
Acute oral toxicity data No further information available Reliability (Klimisch score): 4	Mouse (no further information available)	Divanadium pentaoxide (no further information available)	No information available	23 mg/kg **Acute Tox. 2**	(Izmerov *et al.*, 1982)
Acute oral study No further information available Reliability (Klimisch score): 4	Male rabbits (no further information available)	Divanadium pentaoxide (no further information available)	No information available	64 mg/kg bw **Acute Tox. 3**	US EPA, cited from IPCS 2001[8]
Acute oral study No further information available Reliability (Klimisch score): 4	Mice (no further information available)	Divanadium pentaoxide (no further information available)	No information available	64 - 117 mg/kg bw **Acute Tox. 3**	Yao *et al.*, 1986, cited from IPCS 2001[9]
Acute oral study No further information available Reliability (Klimisch score): 4	Male rats (no further information available)	Divanadium pentaoxide (no further information available)	Vehicle: oil suspension No information available	86 mg/kg bw **Acute Tox. 3**	Sun 1987, cited from (HSE, 2002)
Acute oral study No further information	Male/female rats (no further information available)	Divanadium pentaoxide (no further information	Vehicle: starch suspension No information	137 mg/kg bw **Acute Tox. 3**	Sun 1987, cited from (HSE, 2002)

[8] http://www.inchem.org/documents/cicads/cicads/cicad29.htm#_29ci8100 (checked on 16/10/2017)

[9] http://www.inchem.org/documents/cicads/cicads/cicad29.htm#_29ci8100 (checked on 16/10/2017)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
available Reliability (Klimisch score): 4		available)	available		
Acute oral study No further information available Reliability (Klimisch score): 4	Male mice (no further information available)	Divanadium pentaoxide (no further information available)	Vehicle: oil suspension No information available	64 mg/kg bw **Acute Tox. 3**	Sun 1987, cited from (HSE, 2002)
Acute oral study No further information available Reliability (Klimisch score): 4	Male/female mice (no further information available)	Divanadium pentaoxide (no further information available)	Vehicle: starch suspension No information available	117 mg/kg bw **Acute Tox. 3**	Sun 1987, cited from (HSE, 2002)
Acute oral study No further information available Reliability (Klimisch score): 4	Male rabbit (no further information available)	Divanadium pentaoxide (no further information available)	Vehicle: water No information available	64 mg/kg bw **Acute Tox. 3**	Sun 1987, cited from (HSE, 2002)

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

There is a range of acute oral toxicity data available, which mostly come from handbooks or other secondary sources (US EPA, Yao *et al.*, 1986; Sun *et al.*, 1987). In particular, for these references, it is not possible to assess the reliability of the results due to absence of details on the protocol used. Study reports or publications with experimental details are available for two studies (Leuschner *et al.*, 1994 and Massmann, 1956).

Reported LD_{50} values from studies in rats, mice and rabbits cover a broad range from 10.4 (Massmann, 1956) to 715.7 mg/kg body weight (Leuschner *et al.*, 1994). The only reliable studies available are those published by Leuschner *et al.* (1994), with a protocol similar to OECD TG 401. Three different forms of (highly pure) divanadium pentaoxide were investigated, resulting in LD_{50} values (depending on sex and form) from 221.1 to 715.7 mg V_2O_5/mg bw. The technical grade pulverised divanadium pentaoxide with a purity of 99.3 %

showed a higher toxicity compared to the other forms of divanadium pentaoxide (analytical grade pulverised and technical grade fused).

10.1.2 Comparison with the CLP criteria

Overall, on the 12 studies available:

- 2 derived LD_{50} that fall into category 2 (5-50 mg/kg)
- 8 derived LD_{50} that fall into category 3 (50-300 mg/kg)
- 2 derived LD_{50} that fall into category 4 (300-2000 mg/kg)

According to CLP guidance, classification is based on the lowest LD_{50} value available. However, the two studies concluding on LD_{50} falling into category 2 (Massmann *et al.*, 1956; Izmerov *et al.*, 1982) are not judged adequate for classification purpose because of the lack of details on the protocol and substance tested.

Instead, considering the lowest LD_{50} of 221 mg/kg bw obtained in females exposed to divanadium pentaoxide technical grade (calculated degree of purity in V_2O_5 of 99.3%) in the most reliable study (Leuschner *et al.*, 1994), also supported by the results obtained from Sun *et al.* (1987) (LD_{50} between 64 and 137 mg/kg bw), a classification as Acute Tox. 3 – H301 is warranted.

For the derivation of the Acute Toxicity Estimate (ATE), taking into account the lack of details reported in Sun *et al.*, 1984 and the steep dose-response showed in Leuschner *et al.*, 1994, the derivation of the ATE value of divanadium pentaoxide should be based on the conservative approach using the converted acute toxicity point estimate at 100 mg/kg bw as indicated in CLP regulation.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the available acute oral toxicity studies, divanadium pentaoxide needs to be classified **Acute Tox. 3 "toxic if swallowed"** – **H301** according to Regulation (EC) 1272/2008/EC**.** An **ATE of 100 mg/kg bw** is proposed.

10.2 Acute toxicity - dermal route

Table 11: Summary table of animal studies on acute dermal toxicity (all values are expressed in mg V_2O_5/kg bw, if not indicated otherwise)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
Acute dermal	Sprague-Dawley	Divanadium	Single occlusive	Males and	(Leuschner *et al.*,

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Value LD_{50}**	**Reference**
toxicity, standard acute method OECD TG 402 Minor deviations GLP: yes Reliability (Klimisch score): 1	rats 5 male and 5 female animals per dose group	pentaoxide analytical grade pulverised Degree of analytical purity: Vanadium: 56.05 % (calculated V_2O_5: 97.86 %)	application for 24 h 2000 and 2500 mg/kg bw	females: ≥ 2500 mg/kg bw	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 occlusive application for 24 h 2000 and 2500 mg/kg bw	Males and females: ≥ 2500 mg/kg bw	(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 occlusive application for 24 h 2000 and 2500 mg/kg bw	Males and females: ≥ 2500 mg/kg bw	(Leuschner *et al.*, 1994) (also study no. 003 from ECHA Dissemination, 2017) Details in Annex I

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

One reliable acute dermal toxicity, performed with three different forms of divanadium pentaoxide, is available. Neither mortality nor other signs of toxicity were observed in these studies with any of the forms used up to the highest dose tested (2500 mg/kg).

10.2.2 Comparison with the CLP criteria

The LD_{50} values are above the classification cut-off of 2000 mg/kg bw established for classification under regulation (EC) 1272/2008 criteria.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Based on the available acute dermal toxicity studies, divanadium pentaoxide does not warrant a classification for acute dermal toxicity.

10.3 Acute toxicity - inhalation route

Table 12: Summary table of animal studies on acute inhalation toxicity (all values are expressed in mg V_2O_5/L, if not indicated otherwise)

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
Acute inhalation study No further information available	Rat (no further information available)	Divanadium pentaoxide (no further information available)	2 h (no further information available)	LCl_o = 70 mg/m³ (= 0.07 mg/L) **No conclusion on classification**	(Sax and Lewis, 1989)

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**
Reliability (Klimisch score): 4					
Acute inhalation study No further information available Reliability (Klimisch score): 4	Rat (no further information available)	Divanadium pentaoxide dust (no further information available)	1 h (no further information available)	LC_{67} = 1440 mg/m³ (= 1.4 mg/L) **No conclusion on classification.**	US EPA, cited from IPCS 2001[10]
Acute inhalation study No guideline followed Reliability (Klimisch score): 3	Rabbits (no further information available) No of animals per dose, see column "Dose levels, duration of exposure"	Divanadium pentaoxide, purity 99.9 % Particle size: 30 % < 5 µm 3 % > 5 and < 10 µm 67 % > 10 µm (no further information available)	0.109 mg/L: 2 animals exposed for 240 min (c x t = 26.2) 0.525 mg/L: 4 animals exposed for 60 min (c x t = 31.5) 0.077 mg/L: 6 animals exposed for 420 min (c x t = 32.3) 0.205 mg/L: 4 animals exposed for 420 min (c x t = 86.1)	LC_{50} (7 h) = 0.205 mg/L **Acute Tox. 2**	(Sjöberg, 1950)
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

[10] http://www.inchem.org/documents/cicads/cicads/cicad29.htm#_29ci8100 (checked on 16/10/2017)

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**
		(GSD: 2.28 to 2.40)			
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

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

Apart from two values reported in secondary sources, whose reliability cannot be assessed (Sax and Lewis, 1989 and US EPA cited from IPCS, 2001), there are several experimental studies available.

In the oldest publication (Sjöberg, 1950) sufficient details are given to evaluate the study. Although carefully performed, there are relevant uncertainties regarding the control of the aerosol atmosphere and the actual exposure concentrations in this study (reliability 3).

Leuschner *et al.* (1994) examined three different forms of divanadium pentaoxide in a guideline study (GLP compliant) with Sprague-Dawley rats and obtained LC_{50} values from 2.21 to 16.19 mg/l, depending on sex and the form used. Females appear to be more sensitive than male rats. As with the oral toxicity experiments, the technical grade pulverised divanadium pentaoxide with a purity of 99.3 % showed a higher toxicity compared to the other forms of divanadium pentaoxide (analytical grade pulverised and technical grade fused) with LC_{50} values of 4.40 mg/L in males and of 2.21 mg/L in females compared to the other forms.

In a further GLP compliant guideline study, which is reported in the disseminated REACH registration dossier, acute inhalation toxicity was investigated in Wistar rats and B6C3F1 mice according to the Acute Toxic Class Method OECD TG 436. LC_{50} values were of 0.25 mg/L in male and female Wistar rats, greater than 0.5 mg/L in male B6C3F1 mice and strictly below 0.056 mg/L in female B6C3F1 mice. Female mice

appear to be much more sensitive compared to male mice (Anonymous, 2011).

10.3.2 Comparison with the CLP criteria

Overall, on the 8 studies available:

- 1 derived LC_{50} that fall into category 1 (≤ 0.05 mg/L - aerosolised powder exposure)
- 2 derived LC_{50} that fall into category 2 (0.05 - ≤ 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 LC_{50} that fall into category 4 (> 1 - ≤ 5 mg/L – dust exposure)
- 2 for which 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) .

The LC_{50} values obtained by Leuschner *et al.*, 1994 in Sprague-Dawley rats with dust exposure are within the range (1-5 mg/L) established for classification as Acute Tox. 4 – H332 under regulation (EC) 1272/2008 criteria. Further studies conducted according to the Acute Toxic Class Method OECD TG 436 showed LC_{50} values in Wistar rats of both sexes within the range (0.05-0.5 mg/L with aerosolised powder exposure in one of these studies) established for classification as Acute Tox. 2 under regulation (EC) 1272/2008 and LC_{50} in female mice below the threshold for Acute Tox. 1 (≤ 0.05 mg/L with aerosolised powder exposure) (Anonymous, 2011). According to CLP guidance, classification is based on the lowest LC_{50} value available. In this context, a classification as Acute Tox. 1 – H330 is warranted.

For the derivation of the Acute Toxicity Estimate (ATE), taking into account that the lowest LC_{50} is strictly below 0.056 mg/L in Anonymous, 2011 and was not further explored, the derivation of the ATE value of divanadium pentaoxide should be based on the conservative approach using the converted acute toxicity point estimate at 0.005 mg/L as indicated in CLP regulation.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on the available acute inhalation toxicity studies, divanadium pentaoxide needs to be classified: **Acute Tox. 1 "fatal if inhaled"** – **H330.** An **ATE of 0.005 mg/L** for dusts and mists of divanadium pentaoxide is proposed.

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

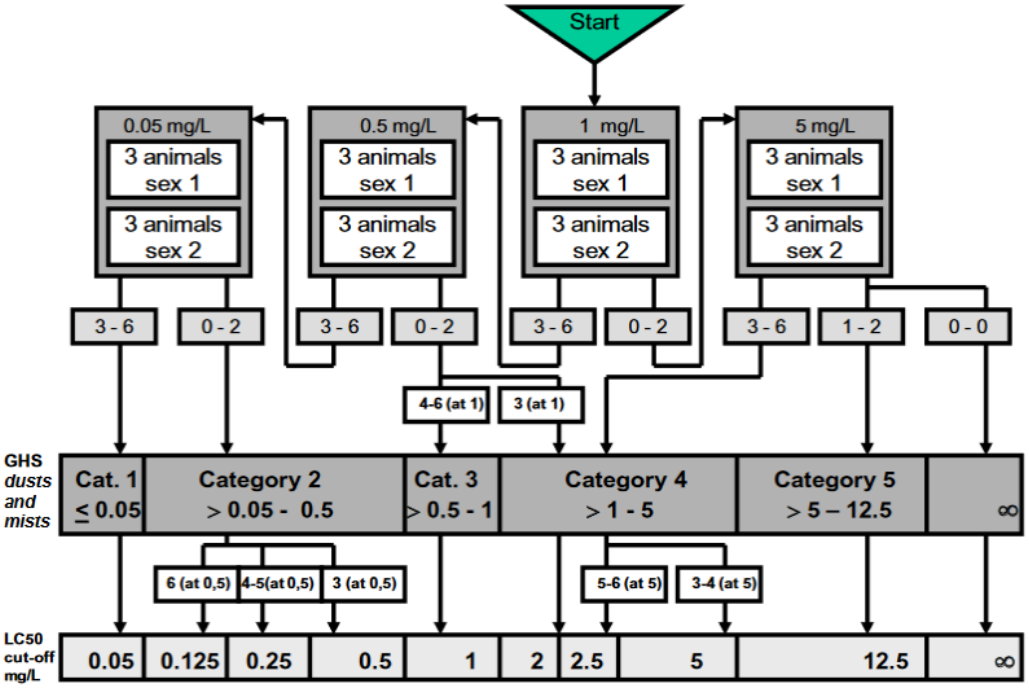
test result should not be based on calculated LC50 values at all. Instead, TG 436 is a test procedure where GHS classification is derived based on lethality at pre-fixed concentration levels (Figure A1). According to the TG, lethality of all 6 animals/concentration level should be considered (the 6 animals 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.*

ANNEX 3 c

Acute Inhalation Toxicity:

Test procedure with a starting concentration of 1 mg/L/4h for aerosols



- 3 ♂ + 3 ♀, or 6 animals of the more susceptible sex are used per step
- 0-6: Number of moribund or dead animals/tested concentration
- GHS: Globally Harmonized Classification System
- ∞: unclassified
- Testing at 12.5 mg/L/4h: see Guidance Document 39 (8)

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).**

10.4 Skin corrosion/irritation

Evaluation not performed for this substance

10.5 Serious eye damage/eye irritation

Evaluation not performed for this substance

10.6 Respiratory sensitisation

Table 13: Summary table of experimental data on pulmonary function

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Subchronic (26 weeks) inhalation study in monkeys 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 exposure with pulmonary function testing. In addition to pulmonary function testing, bronchial lavage fluid was analysed. Adult, male cynomolgus monkeys (*Macaca fascicularis*) 8-9 animals per exposure group GLP: no information Reliability (Klimisch score): 2	Divanadium pentaoxide, > 99.6 % Whole body inhalation for 6 h/ d, 5 d/week for 26 weeks Group 1 (9 animals): 0.1 mg/m^3 (Mon, Wed, Fri) and 1.1 mg/m^3 (Tue, Thurs) Group 2 (9 animals): 0.5 mg/m^3 Group 3 (8 animals): control group, exposed against vehicle clean air Challenge before and after 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	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 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.	(Knecht *et al.*, 1992) (ECHA Dissemination, 2017) Study: 010

Table 14: Summary table of human data on respiratory effects

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 (average exposure 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/m^3 (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/m^3 (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 and throat irritation, epistaxia, wheezing, rales, or injected pharynx, green tongue. After an analysis of variance and the geographical effects 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

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

There is one experimental study in adult male cynomolgus monkey assessing pulmonary reactivity to V_2O_5 with provocation challenges (Knecht *et al.*, 1992). They also compared V_2O_5 reactivity before and after subchronic V_2O_5 exposure with pulmonary function testing. 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). The 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. Subchronic V_2O_5 inhalation did not produce an increase in V_2O_5 reactivity in comparison to an unexposed control group, and cytological immunological test (IgE and IgG analysis) results indicate the absence of allergic response.

In a cross-sectional case-control study with workers exposed to V_2O_5, self-reported cases of subjective symptoms of respiratory tract irritation were observed with clear signs of inflammation observed (Kiviluoto *et al.*, 1979). As number of eosinophils was not significantly changed in exposed group, these effects were not considered as related to allergy (Kiviluoto, 1980). A case-control study with a limited number of workers from a vanadium plant in South Africa (Irsigler *et al.*, 1999) observed also respiratory symptoms (cough and breathing difficulties) in exposed workers without significant difference of the IgE level between cases *versus* controls. In an older case-control study with 24 men working in vanadium plants in the USA (Lewis, 1959a ; b), eye and respiratory (nose and lung) effects: cough, sputum, eye, nose, throat irritation, epistaxia, wheezes, rales, injected pharynx were observed in the exposure *versus* control group. Symptoms indicative of irritation to the upper respiratory tract and to the eyes were also reported by Vintinner *et al.*, (1955) among workers exposed to vanadium-bearing dusts as compared with workers not exposed to such dust.

Overall, respiratory irritation was consistently found in the available publications, which is consistent with the current classification as STOT SE 3. Results from cytological immunological test in cynomolgus monkey (Knecht *et al.*, 1992) and in workers exposed to V_2O_5 did not show significant difference of the IgG levels in

Cynomolgus monkey (Knecht *et al.*, 1992) nor on IgE levels in cynomolgus monkey and exposed workers. Therefore, the reported respiratory symptoms appear not to be linked to a sensitisation mechanism, but rather to an irritating mechanism.

10.6.1 Comparison with the CLP criteria

According to CLP, "*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".

Experimental and human data do not identify respiratory sensitisation potential for V_2O_5.

10.6.2 Conclusion on classification and labelling for respiratory sensitisation

No classification and labelling for respiratory sensitisation is required for V_2O_5 according to CLP regulation.

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**.

10.7 Skin sensitisation

Evaluation not performed for this substance

10.8 Germ cell mutagenicity

Table 15: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Bacterial gene mutation assay OECD TG 471 deviations not specified Ames Test GLP: yes Reliability (Klimisch score): 1	Divanadium pentaoxide, purity 99 %	*Salmonella typhimurium* TA97, TA98, TA100, TA102, TA1535 TA102, TA1535 and TA 97: 0, 0.03, 0.1, 0.3, 1.0, 3.0, 6.0, 10.0 and 33.0 µg V_2O_5/plate TA100 and TA 98: 0, 0.1, 0.3, 1.0, 3.0, 6.0, 10.0, 33.0, 100.0 and 333.0 µg V_2O_5/plate Tested up to cytotoxic concentration No vehicle reported +/- S9 of rat or hamster liver microsomes Positive controls: yes	Negative (+/- S9 mix) for all strains tested	(NTP, 2002) (seems to be identical to study no. 015 from ECHA Dissemination 2017)
Bacterial gene mutation assay Ames test Only limited information available from secondary reporting Reliability (Klimisch score): 4	Divanadium pentaoxide, no information on purity available	*S. typhimurium* TA1535, TA1537, TA98, TA100	Negative (no further details available)	Sun et al, 1987 cited from (WHO, 1988)
		E. coli WP2, WP2uvrA, CM891	Positive (no further details available)	
Mammalian cell gene mutation assay OECD TG 476, No deviations Mouse lymphoma Assay GLP: yes Reliability (Klimisch	Divanadium pentaoxide, purity is confidential information	Mouse lymphoma L5178Y cells Range finding study: 56.84, 113.7, 227.4, 454.8, 909.5, 1819 µg V_2O_5/mL Experiment 1: 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 48 and 64 µg V_2O_5/mL Experiment 2: - with S9-mix: 0, 2, 4, 8, 12, 16, 20, 25, 30, 35 and 40 µg V_2O_5/mL; - without S9-mix: 0, 0.5, 1, 2, 4, 8, 12, 14, 16, 20 and 30 µg V_2O_5/mL. Concentrations selected for the	Negative (+/- S9 mix) at all concentrations tested	Anonymous, 2010 from (ECHA Dissemination , 2017) Study: 001, study report

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
score): 1		mutation experiments were based on the results of cytotoxicity range finding study. The test article was formulated as a suspension in 0.5% w/v methyl cellulose (0.5% MC). +/- S9 mix Positive controls: yes		
Mammalian cell micronucleus test OECD TG 487 No deviations Human lymphocyte cells and TK6 cells GLP: yes Reliability (Klimisch score): 1	Divanadium pentaoxide, purity is confidential information	Human peripheral blood lymphocytes and human lymphoblastoid TK6 cells Range finding study: without and with S9-mix (3+21 hours): 6.599, 11.00, 18.33, 30.55, 50.92, 84.87, 141.4, 235.7, 392.9, 654.8, 1091 and 1819 µg V_2O_5/mL; without S9-mix (24+24 hours): 6.599, 11.00, 18.33, 30.55, 50.92, 84.87, 141.4, 235.7, 392.9, 654.8, 1091 and 1819 µg V_2O_5/mL Experiment 1 (human lymphocyte cells): without and with S9-mix (3+21 hours): 0, 5, 10, 20, 30, 35, 40, 45, 50, 55, 60, 75 and 100 µg V_2O_5/mL; without S9-mix (24+24 hours): 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12.5, 15, 17.5 and 20 µg V_2O_5/mL. Experiment 2 (TK6 cells): without and with S9-mix (3+21 hours): 0, 5, 10, 20, 30, 35, 40, 45, 50, 55, 60, 75 and 100 µg V_2O_5/mL; - without S9-mix (24+24 hours): 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12.5, 15, 17.5 and 20 µg V_2O_5/mL. Concentrations selected for the main experiments were based on the results of cytotoxicity range finding study. The test article was formulated as a suspension in 0.5% w/v methyl cellulose (0.5% MC). +/- S9 mix Positive controls: yes	Positive (+/- S9 mix) in experiment 1 (human lymphocyte cells) at all concentrations tested Positive (+/- S9 mix) in Experiment 2 (TK6 cells) at all concentrations tested	Anonymous, 2010 from (ECHA Dissemination , 2017) Study: 013, study report
Mammalian cell micronucleus test in SHE cells GLP: no	Divanadium pentaoxide, supplied by NTP, no statement on purity	Syrian hamster embryo (SHE) cells 0, 10, 15, 20, and 25 µg V_2O_5/mL Dose levels were selected based on solubility and/or toxicity limits.	Negative (- S9 mix) at all concentrations tested	(Gibson *et al.*, 1997) (ECHA Dissemination , 2017)

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
Reliability (Klimisch score): 2		Vehicle: DMSO Without metabolic activation Positive controls: yes		Study: 014
Mammalian chromosome aberration in human lymphocytes - microscopic evaluation- GLP: not specified Reliability (Klimisch score): 2	Divanadium pentaoxide, no information on purity available	Lymphocytes: human peripheral blood 0, 1, 2, 4, or 8 µg V_2O_5/mL The concentrations for the test item were selected based on preliminary reports Vehicle: distilled water No information on metabolic activation Positive controls: yes	Negative at all concentrations tested	(Rodríguez-Mercado *et al.*, 2010) (ECHA Dissemination , 2017) Study: 016
Gene mutation assay in Chinese hamster V79 cells Reliability (Klimisch score): 2	Divanadium pentaoxide, purity: 99.9%	Chinese hamster V79 cells (lung fibroblast cells) 0, 1, 2, 3, 4 µg V_2O_5/mL for 24h Without metabolic activation Positive control: yes	No increase in the frequency of gene mutation at HPRT locus	(Zhong *et al.*, 1994)
Micronucleus test in Chinese hamster V79 cells, with cytochalasin B Reliability (Klimisch score): 2		Chinese hamster V79 cells (lung fibroblast cells) 0, 1, 2, 3 µg V_2O_5/mL for 24h Without metabolic activation Positive controls: yes	A concentration-dependent significant increase in micronucleus frequency was observed starting at the lowest concentration. The concentration-dependency was also observed in the numbers of kinetochore-positive micronuclei, indicating spindle dysfunction	
Sister chromatid exchange (SCE) test in Chinese hamster V79 cells Reliability (Klimisch score): 2		Chinese hamster V79 cells (lung fibroblast cells) 0, 1, 2, 3, 4 µg V_2O_5/mL for 24h Without metabolic activation Positive controls: yes	No significant increase in SCE was observed at any concentration	
Chromosome aberration (CA) and sister-chromatid	Divanadium pentaoxide, no information	Human primary lymphocytes 0, 2, 4, 6 µg V_2O_5/mL	No increase in structural chromosome aberration (CA) was observed.	(Roldán and Altamirano, 1990)

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
exchange assay with human lymphocytes Reliability (Klimisch score): 3	on purity available	Positive controls: no	Increase in frequency of polyploid cells at all concentrations but not concentration-dependency observed. Mitotic index was statistically significantly decreased and average generation time statistically significantly increased at 4 and 6 µg/mL	
Comet Assay Reliability (Klimisch score): 3	Divanadium pentaoxide, purity ≥ 99.6%	Human blood leucocytes 0, 1, 2, 4, 8 µg V_2O_5/mL for 2,4 or 6 h Positive controls: no	Positive for DNA single strand breaks at all concentrations and time points; no structural aberrations	(Rodríguez-Mercado *et al.*, 2011)
Comet Assay Reliability (Klimisch score): 2	Divanadium pentaoxide, no information on purity available	Human nasal epithelia (n=11) and lymphocytes (n=11) 0, 0.06, 0.12, 0.24, and 0.47 mM V_2O_5 Vehicle: water Positive controls: yes	Positive in lymphocytes (dose-dependent) Negative in mucosal cells	(Kleinsasser *et al.*, 2003)
Comet assay with samples from 4 different human donors Reliability (Klimisch score): 3	Divanadium pentaoxide, no information on purity available	Human leucocytes Human primary lymphocytes 0.3, 30, 3000 µM V_2O_5 No positive control	<u>Results with human leucocytes</u> Concentration-dependent increase of DNA migration observed starting in the lowest concentration <u>Results with human primary lymphocytes</u> Donor 1: no increase in DNA migration Donor 2 and 3: response observed, with significant effect in the highest (or the two highest) concentration(s) Donor 4: significant dose response observed (significant at all concentrations)	(Rojas *et al.*, 1996)
Study of induction of aneuploidy in exposed cells	Divanadium pentaoxide, no information on purity	Human primary lymphocytes 0, 0.001, 0.01, 0.1 µM V_2O_5 - in situ hybridization (FISH) with DNA	<u>Results of FISH:</u> Significant concentration-dependent increase in frequencies of nuclei	(Ramírez *et al.*, 1997)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Reliability (Klimisch score): 2	available	probes for chromosomes 1 and 7 - immunostaining of lymphocyte spindle apparatus with anti-β-tubulin antibody - measurement of polymerisation and depolymerisation of tubulin - negative control: cell culture medium - positive control: colcemid	exhibiting 3 or more hybridization regions Results of immunostaining: At all tested concentrations disruption of microtubules in spindle apparatus was observed Results on tubulin polymerisation: In the lowest concentration tubulin polymerization was inhibited and depolymerisation was stimulated.	

Table 16: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Analysis of 40 alveolar/bronchiolar carcinomas from B6C3F1 mice exposed to vanadium pentoxide for 2 years for mutations in exons 1 and 2 of *Kras* and overexpression of mutant P53 protein Reliability (Klimisch score): 2	Divanadium pentaoxide, purity: 99%	Exposure of mice against 0, 1, 2, 4 mg V_2O_5/m^3 *via* inhalation for 6 h/d, 5 d/w, 104 weeks Lung tissue obtained during the NTP study was analysed	*Kras* mutations identified in 29/40 (73 %) of alveolar/bronchiolar carcinomas. Historical control data (spontaneous alveolar/bronchiolar carcinomas): 30 % According to the authors of the NTP report, the results suggest "that vanadium pentoxide either promotes lesions initiated spontaneously or causes oxidative damage that results in a random pattern of *Kras* mutations"	(NTP, 2002)
Mechanistic Study: Analysis of lung carcinomas from B6C3F1 mice from the NTP study (NTP, 2002) for presence of MAPK (mitogen	Divanadium pentaoxide, purity: 99%	Exposure of mice against 0, 1, 2, 4 mg V_2O_5/m^3 *via* inhalation for 6 h/d, 5 d/w, 104 weeks Lung tissue obtained during the NTP study was analysed (NTP, 2002)	Total MAPK expression levels were similar between normal lung and lung carcinomas. Phospho-MAPK elevated in 5/6 lung carcinoma	(Devereux *et al.*, 2002)

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
activated protein kinase) activity, *Kras* mutations and chromosomal defects Reliability (Klimisch score): 2			samples, in which *Kras* mutations and chromo-some 6 LOH (loss of heterozygosity) were identified and in 4/5 carcinomas with *Kras* mutations but no LOH. no phospho-MAPK was detected in carcinoma tissue without *Kras* mutation and in normal lung tissue	
Kras mutation in the development of lung tumours Non-GLP Reliability (Klimisch score): 2	Divanadium pentaoxide, purity: 100%	6 male transgenic Big Blue mice per dose group and exposure group The levels of two different *Kras* codon 12 mutations [GGT→GAT (G12D) and GGT→GTT (G12V)] were measured in lung DNAs by Allele-specific Competitive Blocker PCR (ACB-PCR). 0, 0.1, 1 mg V_2O_5/m³ nose-only inhalation, 6 h/d 5 d/w for 4 or 8 weeks	Negative for both concentrations and time points	(Banda *et al.*, 2015) (ECHA Dissemination, 2017) Study: 005
Transgenic Rodent Somatic Cell Gene Mutation Assays OECD TG 488 no information on deviations Non-GLP Reliability (Klimisch score): 3	Divanadium pentaoxide, purity: 100%	6 male transgenic Big Blue mice per dose group and exposure group Mutant frequencies for the *CII* gene in the lung as well as lung weights from BB mice were analysed. 0, 0.1, 1 mg V_2O_5/m³ nose-only inhalation, 6 h/d 5 d/w for 4 or 8 weeks Concentrations were selected based on their tumorigenic potential Vehicle: air No positive controls	Negative for both concentrations and time points	(Manjanatha *et al.*, 2015), (ECHA Dissemination, 2017) Study: 004
Micronucleus assay OECD TG 474 deviations: yes Mammalian somatic cell study in bone marrow erythrocytes GLP: yes Reliability (Klimisch score): 1	Divanadium pentaoxide, purity is confidential information	6 male Sprague-Dawley rats per dose group Dose levels-range-finder: 90, 120, 170, 240, 300 mg V_2O_5/kg/day Dose levels - MN study: 0, 30, 60, 120 mg V_2O_5/kg/day Gavage Maximum dose: Maximum tolerated dose based on range-finder data.	Negative at all doses tested Vanadium levels increased in all sampled tissues (bone marrow and testes) in a dose-dependent manner.	Anonymous, 2011 (ECHA Dissemination, 2017) Study: 001, study report Details in Annex I.

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		Vehicle: corn oil Positive controls: yes		
Micronucleus assay OECD TG 475, deviations: not specified Mammalian somatic cell study in bone marrow erythrocytes GLP: yes Reliability (Klimisch score): 2	Divanadium pentaoxide, purity: 99%	10 Male and female B6C3F1 mice per dose group 0, 1, 2, 3, 8, 16 mg V_2O_5/m^3 (whole body inhalation, 6 h/d, 5 d/w, for 3 months) Based on results of a dose range finding study doses were selected. Vehicle: air Positive controls: not stated	Negative for male and female animals at all doses tested No effect on PCE/NCE.	(NTP, 2002) (ECHA Dissemination, 2017) Study: 002 Details in Annex I
Micronucleus assay in peripheral blood reticulocytes Reliability (Klimisch score): 3	Divanadium pentaoxide, purity: 99.99%	6 male, 6 female CD-1 mice 0, 0.02 M V_2O_5 (1.4 mg V_2O_5/m^3, *via* inhalation). V_2O_5 was suspended in saline, containing Tween 20. 2 h/twice a week; blood samples were obtained at 24 h and every week until the end of the 4-week exposure period Authors deemed the level of vanadium used would reflect airborne vanadium exposures that occur routinely among the citizens of many urban centers.	Positive in male animals at all time points Negative in female animals (authors assume a protective influence of oestrogen)	(Rojas-Lemus *et al.*, 2014)
Micronucleus test in polychromatic erythrocytes Reliability (Klimisch): 3	Divanadium pentaoxide, no information on purity available	5 male Hsd:ICR mice per dose group 0, 40 mg V_2O_5/kg bw single application V_2O_5 was dissolved in distilled water. Intraperitoneal injection 0, 24, 48 and 72 h after application blood from caudal vein was taken and analysed for micronuclei. At 48 h the number of apoptotic and necrotic cells was determined.	Number of micronucleated cells was marginally but significantly increased time-dependently beginning at 24 h. In addition a number of early and late apoptotic as well as necrotic cells was increased significantly.	(García-Rodríguez *et al.*, 2016)
Micronucleus test (Only limited information available from secondary source) Reliability Klimisch	Divanadium pentaoxide, no information on purity available	Mice (Kunming albino and 615) 0, 0.17, 2.13, 6.4 mg V_2O_5/kg bw for 5 days Intraperitoneal injection Positive control: yes	In both strains significant levels of induced micronuclei were observed.	Sun et al, 1987 cited from (WHO, 1988)

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
score): 4		Mice (Kunming albino) 1.44, 2.83, 5.65, 11.3 mg V_2O_5/kg bw/d for 6 weeks Oral administration in a solution at 3% of starch solution.	Negative	
		Mice (615) 0.25, 1 or 4 mg V_2O_5/kg bw Subcutaneous injection	Increase in the frequency of micronuclei	
		Mice (615) 0.5, 2 or 8 mg V_2O_5/m³ Inhalation exposure	Increase in the frequency of micronuclei	
Evaluation of DNA migration (representing DNA damage) in tissue of exposed rats Reliability (Klimisch score): 3	Divanadium pentaoxide, no information on purity available	6 male albino Wistar rats per group 70 mg/kg single application *via* gavage After 28 days animals were sacrificed and DNA was isolated	Significant increase in length of DNA migration in liver, kidney, heart, lung, spleen and brain.	(Paramanik and Rajalakshmi, 2013)
Comet assay Reliability (Klimisch score): 2	Divanadium pentaoxide, purity: 99.6%	4 male CD1 mice per dose group 0, 5.75, 11.5 or 23 mg V_2O_5/kg bw Intraperitoneally injected Vehicle: saline DNA damage in several tissues was evaluated according to 3 different parameters: tailed cells vs. untailed cells; DNA migration length values; and number of cells with different nucleus/tail ratios.	<u>Tailed cells:</u> Lung and kidney: Statistically significant decrease at the low and high dose compared with controls Spleen: Statistically significant decrease at the low and mid dose Heart: Dose-related decrease, statistically significant at all dose levels Liver, bone marrow: No statistically significant effects were observed <u>DNA migration:</u> A dose-response related increase on DNA migration was observed for all tissues apart from bone marrow and spleen. <u>Nucleus/tail ratios:</u> Dose-dependent decrease	(Altamirano-Lozano *et al.*, 1999)

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			in percent of cells with low damage in all tissues, with different sensitivities.	
Study on testicular DNA damage in male mice (Comet Assay) Reliability (Klimisch score): 2	Divanadium pentaoxide, purity: 99.6%	2 male CD-1 mice per dose group 0, 5.75, 11.5, 23 mg V_2O_5/bw single intraperitoneal injection 24 h later animals were sacrificed. Cells from testes were used for the Comet assay.	Comet assay revealed significant DNA damage increasing with the dose.	(Altamirano-Lozano *et al.*, 1996)
Mammalian cell study: DNA damage and/or repair GLP: yes Lung tissue Reliability (Klimisch score): 1	Divanadium pentaoxide, purity: 99.8%	Female B6C3F1 mice (5 groups of 48 mice) 0, 0.25, 1, 4 mg V_2O_5/m³ (nose-only inhalation, 6 h/d for 16 days) Lung samples were analysed for DNA strand breaks using the comet assay. Vehicle: air Positive controls: yes	Negative for all doses tested	(Schuler *et al.*, 2011) (ECHA Dissemination, 2017) Study: 003
		Analysis of 9 specific DNA-oxo-adducts in lung tissue Positive controls: yes	Concentration dependent increase in 8-oxodGuo DNA lesions with significant effects at 1 and 4 mg/m³	(Schuler *et al.*, 2011) Details in Annex I.
Sister chromatid exchange assay with bone marrow from exposed mice Reliability (Klimisch score): 2	Divanadium pentaoxide, purity: 99.6%	4 male CD-1 mice per dose group 0, 5,75, 11.5, 23 mg V_2O_5/kg bw Intraperitoneally injected 24 h later animals were sacrificed Vehicle: distilled water	Negative at all concentrations tested	(Altamirano-Lozano *et al.*, 1993)
Immunohisto-chemical changes in actin testicular cytoskeleton Reliability (Klimisch score): 3	Divanadium pentaoxide, no information on purity available	Male CD-1 mice 0, 0.02 M V_2O_5 (1.4 mg V_2O_5/m³ *via* inhalation) 1 h/twice a week, for a total of 12 weeks. Every week 5 exposed animals and 5 control animals were sacrificed. Testicular cells were analysed immunohistochemically.	Structural DNA damage microscopically observed (time dependency was not evaluated)	(Rodríguez-Lara *et al.*, 2016)
DNA synthesis inhibition assay (Only limited information available	Divanadium pentaoxide, no information on purity	Male mice 0, 14.6, 29.2, 58.4 mg V_2O_5/kg bw, orally administered	No significant differences between exposure group animals and controls	Sun et al, 1987 cited from (WHO, 1988)

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
from secondary source) Reliability (Klimisch score): 4	available	Vehicle: 3% starch solution 24 h after dosing, animals were sacrificed and tissue from testes, spleen and liver were analysed		
Observation of nuclear changes *via* electron microscopy in spleen cells of exposed mice. Reliability (Klimisch score): 3	Divanadium pentaoxide, no information on purity available	Male CD-1 mice 0, 0.02 M V_2O_5 (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. The spleen was prepared and lymphocytes were analysed *via* electron microscopy (TEM)	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 Reliability (Klimisch score): 3	Divanadium pentaoxide, purity: 99.99%	20 male CD-1 mice (10 in exposure group, 10 in control group) 0, 0.02 M V_2O_5 (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)
Dominant lethal test Study of reproductive function and testicular DNA damage in male mice Reliability (Klimisch score): 2	Divanadium pentaoxide, purity: 99.6%	15-30 male CD-1 mice per group, mated with unexposed females (1:2) after exposure 0, 8.5 µg V_2O_5/g bw i.p. injection Group 1: 20 control animals received vehicle (saline) every 3rd day for 60 days Group 2: 15 animals received V_2O_5 every 3rd day for 60 days Animals of group 1 and 2: on day 61, animals were subjected to a fertility assessment test (mating with unexposed females) and sacrificed 5 days later. Group 3: 30 animals received V_2O_5 every 3rd day for 60 days, groups of 5 animals were sacrificed every 10 days after the beginning of the treatment.	V_2O_5 treatment resulted in decrease in fertility rate and pregnant females. Implantation sites, live foetuses, and foetal weight were decreased significantly, the number of resorptions/dam and dead foetuses was increased. Sperm count, motility, and morphology were impaired. The effects were getting more severe the longer the exposure time was. Testis weight was reduced.	(Altamirano-Lozano *et al.*, 1996) Details in Annex I.
Dominant lethal mutation assay Only limited information available Reliability (Klimisch	Divanadium pentaoxide, no information on purity available	Male mice (no more details available) 0, 0.2, 1 or 4 mg V_2O_5/kg bw/d for 3 months, *via* subcutaneous injection	Negative result for the induction of dominant lethal mutations (no further details available)	Sun et al, 1987 cited from (WHO, 1988)

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
score): 4		Animals were mated with untreated females and number of foetuses and resorptions were recorded on GD 17		

Table 17: Summary table of human data relevant for germ cell mutagenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Determination of DNA strand breaks (with alkaline comet assay), 8-hydroxy-2-deoxyguanosine (8-OHdG) and frequency of sister chromatid exchange (SCE) in whole blood leukocytes or lymphocytes of exposed workers	Divanadium pentaoxide	49 male workers employed in a vanadium factory and 12 non-exposed controls Mean exposure time: 12.4 years No air concentration is given, vanadium concentration in serum of workers: median 5.38 µg/L (range 2.18 – 46.35 µg/L)	No significant differences in comet assay values, 8-OHdG or SCE between exposed and non-exposed test persons were found.	(Ivancsits *et al.*, 2002)
Determination of DNA strand breaks in leukocytes using the alkaline comet assay and different parameters of chromosomal instability in lymphocytes using the cytokinesis-block micronucleus (MN) cytome method	Divanadium pentaoxide	Experiment 1 (Comet assay): 53 exposed workers and 52 controls Experiment 2 (cytom method): 23 exposed workers and 24 controls (MN method) No air concentration is given, vanadium plasma concentrations in exposed workers 7-times higher than in controls (0.3 µg/L, range 0.24 – 0.39 vs. 2.2 µg/L, range 1.54 – 3.89)	Experiment 1: No difference in DNA migration under standard conditions Formation of oxidised purine bases: elevated by 7% Formation of oxidised pyrimidine bases elevated by 33% Sensitivity of cells towards Bleomycin – induced DNA damage was higher (25%), and DNA repair was only very slightly observed Experiment 2: Number of MN in exposed worker 2.5-fold increased, number of nucleoplasmic bridges 7-fold and number of nuclear buds 3-fold increased. Frequency of necrotic cells: increased by 55%,	(Ehrlich *et al.*, 2008) Details in Annex I.

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			frequency of apoptotic cells increased by 50%	

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

In-vitro testing:

- Similarly to other metal compounds, *in vitro* tests on bacterial gene mutation without and with metabolic activation showed negative results in various strains *of Salmonella typhymurium* (NTP, 2002; Sun *et al.*, 1987). However, some early reports with limited reporting and unknown quality indicated increased reverse mutations from V_2O_5 exposure in *E. Coli* (Sun, 1987).
- The outcome of a mammalian cell gene mutation assay without and with metabolic activation was negative. No increase in the frequency of gene mutations were observed at the HPRT locus of Chinese hamster V79 lung fibroblast cells (Zhong *et al.*, 1994). Negative results were also found in a mammalian cell gene mutation assay (OECD TG 476) (Anonymous, 2010).
- No chromosomal aberrations were found in human lymphocytes and no increases in sister chromatid exchanges (SCE) were detected in V79 hamster lung fibroblast cells (Rodríguez-Mercado *et al.*, 2010; Roldán and Altamirano, 1990; Zhong *et al.*, 1994).
- Several micronucleus tests gave positive results with dose dependent increase in Chinese hamster V79 lung fibroblast cells without metabolic activation (Zhong *et al.*, 1994), human lymphocyte cells and human lymphoblastoid TK 6 cells both with and without metabolic activation (GLP, OECD TG 487 - study; Anonymous, 2010) except a negative finding in Syrian hamster embryo cells without metabolic activation (Gibson *et al.*, 1997).
- Several *in vitro* comet assay studies provided positive results in human blood leucocytes or lymphocytes (Kleinsasser *et al.*, 2003; Rodríguez-Mercado *et al.*, 2011; Rojas *et al.*, 1996). Negative findings were retrieved in human nasal epithelia mucosal cells (Kleinsasser *et al.*, 2003).
- Induction of aneuploidy was also observed in human primary lymphocytes (Ramírez et al., 1997; Roldán and Altamirano, 1990). Additionally, an increased number of kinetochore-positive micronuclei, indicating spindle dysfunction was observed in Chinese hamster V79 cells (lung fibroblast cells) without metabolic activation (Zhong *et al.*, 1994).

In-vivo testing:

- In accordance with *in-vitro* testing, negative results were obtained in a transgenic rodent somatic cell gene mutation assay by inhalation (Banda *et al.*, 2015; Manjanatha *et al.*, 2015) and in a mice SCE test (Altamirano-Lozano *et al.*, 1993).
- Negative results were obtained for DNA strand breaks in lung using a comet assay by inhalation in B6C3F1 mice (Schuler *et al.*, 2011). No significant differences for DNA synthesis inhibition was observed in testes, spleen and liver tissues of mice exposed by oral route (Sun et al, 1987 cited from (WHO, 1988)).
- Among the available genotoxic assays, those conducted on the bone marrow did not generally show genotoxic effects in micronucleus (MN) assay after oral or inhalation administration and sister chromatid exchange (SCE) test after intraperitoneal injection (Anonymous, 2011; NTP, 2002; Altamirano-Lozano *et al.*, 1993). Positive results in micronucleus assays were obtained in peripheral blood reticulocytes after inhalation and polychromatic erythrocytes after intraperitoneal injection (Rojas-Lemus *et al.*, 2014 and MN assay from Garcia *et al.* (2016) respectively). Positive results in MN assay were also reported by Sun *et al.* 1987 after intraperitoneal and subcutaneous administrations, and after inhalation but not after oral administration.
- Structural DNA damage was observed in testicular cells of male CD-1 mice exposed by inhalation (Rodríguez-Lara *et al.*, 2016).
- Comet assays show genotoxic effects in liver, brain, testis, lung, kidney, spleen and heart (Altamirano-Lozano *et al.*, 1999 by intraperitoneal route; Paramanik *et al.* 2013 by gavage, Altamirano-Lozano *et al.*, 1996 by intraperitoneal route).
- Nuclear changes (spleen lymphocytes (Rodríguez-Lara *et al.*, 2013)), and oxidative stress (liver (Cano-Gutiérrez *et al.*, 2012) were also reported.
- Dominant lethal assay in mice (Altamirano-Lozano *et al.*, 1996) gave positive results after intraperitoneal administration when another dominant lethal assay indicated negative outcomes after subcutaneous injection (Sun, 1987, cited from WHO, 1988). However, these latter results were not sufficiently reported which does not allow a proper assessment of these data.
- Concentration dependent increase in 8-oxodGuo DNA lesions were observed in lung tissue following inhalation exposure of B6C3F1 mice (Schuler *et al.*, 2011).
- Lastly, after chronic exposure by inhalation to mice, *Kras* mutation was observed in alveolar/bronchiolar carcinomas and phospho-MAPK were detected in carcinoma tissues when *Kras* mutations were observed (NTP, 2002 and Devereux *et al.*, 2002).

As for other metals, the overall V_2O_5 mutagenic and genotoxic profile that emerges is complex. Overall, negative results were mainly obtained for point mutations in *in-vitro and in-vivo* testings and for *in-vitro* cytogenetic changes. Most of the *in-vitro* micronuclei and comet assays gave positive results and aneugenic effects were observed. *In-vivo* MN, comet and dominant lethal assays indicate a genotoxic hazard for the somatic and germinal cells. Overall, even if all of the available studies were not conducted in accordance with the best European or international technical standards due mainly to the use of only one dose/concentration per assay preventing the identification of a dose-response relationship analysis, these studies were considered in a weight of evidence analysis of the all genotoxic database. In one study with workers exposed to divanadium pentaoxide no changes in the comet assay were observed and the analysis revealed no increase of 8-oxodGuo DNA lesions or SCE (Ivancsits *et al.*, 2002). However, in another study with significantly lower exposure levels, an elevated formation of oxidized purine and pyrimidine bases, an increased number of micronuclei in exposed worker, nucleoplasmic bridges and nuclear buds demonstrated chromosomal instability (Ehrlich *et al.*, 2008).

- Lastly, a higher sensitivity of cells towards Bleomycin–induced DNA damage and a very slight increased DNA repair were observed (Ehrlich *et al.*, 2008). These authors concluded that cancer risk is related to DNA instability from V_2O_5 exposure. In this study, the number of smokers and the number of cigarettes smoked per day in the exposed and in the control group were similar (n=12/52 or 11/52, respectively; no. of cigarettes/day: 7 (0-21) or 5 (0-17), respectively). However this study did not discuss the relative alcohol consumption and other potentially relevant occupational exposures (e.g., other metal compounds).

10.8.2 Comparison with the CLP criteria

For potential classification on germ cell mutagenicity, criteria from CLP Regulation *(EC, 2017)* were applied:

- *"The classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans"* (EC, 2017*).*

There are no epidemiological data to support classification of divanadium pentaoxide in Category 1A.

- *"The classification in Category 1B is based on positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals"* (EC, 2017).

There is no *in vivo* heritable germ cell mutagenicity tests in mammals performed by physiological route. However, there is adequate assay for divanadium pentaoxide by other route of exposure. No effect is reported in a dominant lethal assay performed by subcutaneous route in a study with inadequate reporting (Sun, 1987,

cited from WHO, 1988). Dominant lethality testing (Altamirano-Lozano *et al.*, 1996) performed with intraperitoneal injection was positive.

- Classification in Category 1B can also be based on *"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. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells"* (EC, 2017).

Positive results are obtained from *in vivo* micronucleus assays performed by inhalation (Rojas-Lemus *et al.*, 2014; Sun *et al.*, 1987) and by intraperitoneal route (Garcia-Rodruiguez, 2016; Sun *et al.*, 1987). Furthermore comet assays gave generally positive results with V_2O_5 and in particular, a comet assay conducted by intraperitoneal route, showing positive results in testis of male CD-1 mice (Altamirano-Lozano *et al.*, 1996).

The ability of the substance or its metabolite(s) to interact with the genetic material of germ cells is demonstrated in several studies. Toxicokinetic data show ^{48}V-labelled pentavalent and also tetravalent vanadium are distributed to the testes of rat exposed by intratracheal route (Edel and Sabbioni, 1988 and Greim, 2006). Additionally, inhalation exposure to divanadium pentaoxide leads to drastic increased vanadium concentration in testes of exposed mice (Mussali-Galante *et al.*, 2005; Fortoul *et al.*, 2007) showing that vanadium is distributed to testes following inhalation and intra-tracheal route of exposure. In addition, human seminal plasma analysis has reported vanadium content in seminal plasma and sperm concentration (Zafar *et al.*, 2015). Lastly, results from repeated-dose toxicity studies and studies on reproductive toxicity (see Section 10.10) indicate that the germ cells are reached from exposure to divanadium pentaoxide.

- *"This hazard class is primarily concerned with substances that may cause mutations in the germ cells of humans that can be transmitted to the progeny. However, the results from mutagenicity or genotoxicity tests in vitro and in mammalian somatic and germ cells in vivo are also considered in classifying substances and mixtures within this hazard class"* (EC, 2017). ECHA (2017) further comments: *"Thus, classification as a germ cell mutagen (Category 1A, 1B, and 2) classifies for the hazard heritable genetic damage as well as providing an indication that the substance could be carcinogenic"*

In addition to the *in vivo* mutagenicity studies on somatic and germ cells which reported positive results, V_2O_5 is also genotoxic *in vitro* in several micronucleus tests and comet assays.

As reported in the section 10.9, V_2O_5 is a carcinogenic substance.

Overall, the criteria to classify V_2O_5 as *in vivo* heritable germ cell mutagen in mammals are fulfilled. Therefore, germ cell mutagenicity classification to **Muta. 1B, 'H340: Suspected of causing genetic defects',** is warranted.

- *"The classification in Category 2 is based on positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from somatic cell mutagenicity tests in vivo, in mammals or other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays"* (EC, 2017).

Positive evidence is obtained from *in vitro* assays and from *in vivo* germ cell and somatic cell mutagenicity studies. Thus, a category 2 is not adequate.

- *"Classification as a Category 2 mutagen would generally apply if only intraperitoneal in vivo tests show mutagenicity/genotoxicity and the negative test results from the in vivo tests using other routes of application are plausible"* (ECHA, 2015*).*

There is no *in vivo* heritable germ cell mutagenicity tests in mammals performed by physiological route. In addition to the positive dominant lethal assay performed by intraperitoneal route, *in vivo* micronucleus assays and Comet assays in somatic cells are positive after respiratory exposure (inhalation and intra-tracheal administration). In addition, toxicokinetic data and repeated-dose toxicity studies confirm that V_2O_5 or its metabolite(s) can reach germ cells. **Thus, a category 2 is not adequate.**

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

There is no *in vivo* heritable germ cell mutagenicity tests in mammals performed by physiological route. However, there is adequate assay for divanadium pentaoxide by other route of exposure. No effect is reported in a dominant lethal assay performed by subcutaneous route in a study with inadequate reporting (Sun, 1987, cited from WHO, 1988). Another dominant lethality test (Altamirano-Lozano *et al.*, 1996) was positive. This positive dominant lethality test was performed with intraperitoneal injection which is considered as non-physiological route of exposure. However, even if it can be argued that intraperitoneal administration maximises systemic exposure, it is generally recognised that there is no threshold for mutagenicity unless there is specific proof for the existence of such a threshold (CLP guidance, 2017). In addition, a comet assay, conducted by intraperitoneal route, showed positive results in testis of male CD-1 mice. Positive results are also obtained in somatic cells in *in vivo* micronucleus assays performed by inhalation (Rojas-Lemus *et al.*, 2014) or by intraperitoneal route (Garcia-Rodriguez *et al.*, 2016; Sun *et al.*, 1987) and in *in vivo* comet assay by intraperitoneal route (Altamirano-Lozano *et al.*, 1999).

In experimental animal studies, distribution of ^{48}V-labelled pentavalent and tetravalent vanadium to the testes was highlighted after intra-tracheal route of exposure (Edel and Sabbioni, 1988 and Greim, 2006) and

increased vanadium concentration was also observed in testes after inhalation exposure (Mussali-Galante *et al.*, 2005; Fortoul *et al.*, 2007) or after oral administration (Anonymous, 2011). In addition, human seminal plasma analysis has reported vanadium content in seminal plasma and sperm concentration (Zafar *et al.*, 2015). The distribution of pentavalent or tetravalent vanadium to the testes in human and in experimental animals combined with the observed effects on germ cells in several repeated-dose toxicity or reproductive toxicity studies (see Section 10.10) indicate that the germ cells are reached after exposure to divanadium pentaoxide.

Therefore, germ cell mutagenicity classification to **Muta. 1B, 'H340: Suspected of causing genetic defects',** is warranted.

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	Positive in lymphocytes negative in nasal cells	(Kleinsasser *et al.*, 2003)

	0, 0.06, 0.12, 0.24, and 0.47 mM V_2O_5		
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)
Micronucleus assay in peripheral blood reticulocytes (Klimisch: 3)	6 male, 6 female CD-1 mice 1.4 mg V_2O_5/m³ 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/m³ 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/m³ via inhalation) 1 h/twice a week, for a total of 3 months. Every week 3 exposed animals and 3 control animals were	Spleen lymphocytes showed time dependent (up to 5 weeks) nuclear changes (lobulations, invaginations, deep evaginations, chromatin	(Rodríguez-Lara *et al.*, 2013)

	sacrificed.	redistribution with increased heterochromatin, pronounced perinuclear cisterns)	
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.**

10.9 Carcinogenicity

Table 18: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
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) Rats: 0, 0.5, 1, 2 mg V_2O_5/m³ *via* inhalation (particulate aerosol) Mice: 0, 1, 2, 4 mg V_2O_5/m³ *via* inhalation (particulate aerosol) Diet: NTP-2000	**Rats:** Survival of rats in the exposure groups was comparable to animals in control group. Decreased body weight gain was observed at 2 mg V_2O_5/m³ only in female rats. <u>In male rats:</u> Lung: alveolar/ bronchiolar adenoma (4/50, 8/49, 5/48, 6/50); alveolar/bronchiolar carcinoma (0/50, 3/49, 1/48, 3/50); alveolar/ bronchiolar adenoma or carcinoma (4/50, 10/49, 6/48, 9/50) <u>In female rats:</u> Lung: Alveolar/ bronchiolar adenoma 0/49, 3/49, 1/50, 0/50); alveolar/bronchiolar adenoma or carcinoma (0/49, 3/49, 1/50, 1/50) Although there were no statistically significant increases in the incidences of lung neoplasms in rats, the incidences of alveolar/ bronchiolar adenoma in 0.5 mg/m^3 males and of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) in 0.5 and 2 mg/m^3 males exceeded the historical ranges in controls (all routes) given NTP-2000 diet and in inhalation chamber controls given NIH-07 diet (NTP, report 2002). This response is considered related to exposure to divanadium pentoxide. The incidence of alveolar /bronchiolar adenoma in 0.5 mg/m^3 females was at the upper end of the historical control range for studies using NTP-2000 diet and exceeded the range in the larger database for	(NTP, 2002) (ECHA Dissemination, 2017) Studies: 001 & 002 Details in Annex I

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		inhalation studies using NIH-07 diet. In the respiratory tract (lung, larynx, nose), non-neoplastic lesions were also observed: inflammation, fibrosis, metaplasia and hyperplasia mostly in a dose dependent manner starting in the lowest exposure concentration. Conclusion from NTP: *some* evidence of carcinogenic activity in male rats and *equivocal* evidence of carcinogenic activity in female rats **Mice:** Survival of the highest dosed males (4 mg/m³) was significantly less than controls. Abnormal breathing was observed particularly in those exposed to 2 or 4 mg/m³. Decreased body weight gain was observed from 1 mg V_2O_5/m³ in females and from 2 mg V_2O_5/m³ in males. In male mice: Alveolar/bronchiolar carcinoma incidence was: 12/50, 29/50, 30/50, 35/50. Alveolar/bronchiolar adenoma or carcinoma incidence was 22/50, 42/50, 43/50, 43/50) In female mice: Alveolar/bronchiolar carcinoma incidence: 0/50, 23/50, 18/50, 22/50. Alveolar/bronchiolar adenoma or carcinoma incidence: 1/50, 32/59, 35/50, 32/50. The incidences of alveolar/ bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) were significantly increased in all groups of exposed male and female mice. The incidences of alveolar/ bronchiolar adenoma were significantly increased in males exposed to 2 mg/m^3 and in all groups of exposed females. These increased incidences exceeded the historical ranges for controls (all routes) given NTP-2000 diet and for chamber controls given NIH-07 diet (inhalation studies) and many exposed animals had multiple adenomas and/or carcinomas. [11] Non-neoplastic lesions: There were significant increased incidences of alveolar epithelial hyperplasia and bronchiolar epithelial hyperplasia in the lungs of exposed male and female mice. The	

[11] Note: Focal hyperplasia of the alveolar/bronchiolar epithelium, which is thought to be a precursor to adenoma, was not recognized in this study, possibly because of the overwhelming incidences of neoplasms and the observed bronchiolization.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		hyperplasia was essentially a diffuse change with proliferation of epithelium in the distal terminal bronchioles and immediately associated alveolar ducts and alveoli. The hyperplasia of the alveolar epithelium was pronounced and increased in severity with increasing exposure concentration, while the hyperplasia of the distal bronchioles was minimal to mild with slight increases in severity in mice exposed to 4 mg/m^3. The changes in mice were similar to those observed in rats but were not as pronounced. Other lesions (inflammation, fibrosis, squamous metaplasia) were observed in the respiratory tract (lung, larynx, nose) in a dose dependent manner starting in the lowest exposure concentration. Male and female mice: *clear evidence* of carcinogenic activity (conclusion from NTP)	
Carcinogenicity study in male and female mice (1 year exposure, no further information on strain available) 62 - 84 male and female mice (no further information available) Reliability (Klimisch score):4	Divanadium pentaoxide, no information on purity available. 0, 0.5, 2, or 8 mg V_2O_5 dust/m^3 (particle size not reported) for 4 h/day for 1 year.	Papillomatous and adenomatous tumours in the lungs were reported in 2/79 and 3/62 mice at 2 and 8 mg/m^3, respectively. No tumours reported in control animals and at 0.5 mg/m^3. No further information available.	(Yao *et al.*, 1986, as cited from WHO, 2001)
Tumour promotion study in male A/J, BALB/cJ and C57BL/6J mice After injection of methylcholanthrene (MCA) animals were exposed for 5 weeks. After 20 weeks the tumour rate was determined. Reliability (Klimisch score): 2	Divanadium pentaoxide, purity > 99.9% After injection of MCA, animals were exposed once a week for 5 weeks to V_2O_5 at 4 mg V_2O_5/kg bw. Pulmonary administration was performed *via* oropharyngeal aspiration. After 20 weeks, the tumour rate was determined. Vehicle: PBS (phosphate buffered saline) Control animals were	Treatment of animals with MCA followed by V_2O_5 exposure promoted lung tumours (solid adenomas (80%) and the remaining papillary (20%)) in A/J and BALB mice (significantly compared to controls (treatment with corn oil + V_2O_5 or Treatment with corn oil + PBS)). Tumour promotion was associated with inflammation involving induction of multiple chemokines and transcription factors NFκB and c-Fos as well as activation of extracellular signal-regulated kinases 1 and 2. → This study shows that V_2O_5 acts as an *in vivo* lung tumour promotor in A/J and BALB/cJ mice. No tumours were observed in C57BL/6J mice.	(Rondini *et al.*, 2010) Details in Annex I.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	treated with corn oil instead of MCA and/or PBS Pulmonary tumours were enumerated 20 weeks after the MCA treatment. In addition, pulmonary inflammation was assessed in bronchoalveolar fluid and chemokine production, transcription factor activity and MAPK signalling were assessed in lung homogenates.		

Table 19: Summary table of human data on carcinogenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Retrospective case control study	Divanadium pentaoxide	Male and female employees in vanadium processing facilities (production of V_2O_5) 196 questionnaires were collected and evaluated	No statistically significant association between cancer and exposure to divanadium pentaoxide could be established.	(Fourie, 2010) (Master thesis)

Table 20: Summary table of other *in vivo* studies relevant for carcinogenicity

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Analysis of 40 alveolar/bronchiolar carcinomas from B6C3F1 mice exposed to vanadium pentoxide for 2 years for mutations in exons 1 and 2 of *Kras* and overexpression of mutant P53 protein Reliability (Klimisch score): 2	Divanadium pentaoxide, purity: 99%	Exposure of mice against 0, 1, 2, 4 mg V_2O_5/m^3 *via* inhalation for 6 h/d, 5 d/w, 104 weeks Lung tissue obtained during the NTP study was analysed	*Kras* mutations identified in 29/40 (73 %) of alveolar/bronchiolar carcinomas. Historical control data (spontaneous alveolar/bronchiolar carcinomas): 30 % According to the authors of the NTP report, the results suggest "that vanadium pentoxide either promotes lesions initiated spontaneously or	(NTP, 2002)

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			causes oxidative damage that results in a random pattern of *Kras* mutations"	
Mechanistic Study: Analysis of lung carcinomas from B6C3F1 mice from the NTP study (NTP, 2002) for presence of MAPK (mitogen activated protein kinase) activity, *Kras* mutations and chromosomal defects Reliability (Klimisch score): 2	Divanadium pentaoxide, purity: 99%	Exposure of mice against 0, 1, 2, 4 mg V_2O_5/m^3 *via* inhalation for 6 h/d, 5 d/w, 104 weeks Lung tissue obtained during the NTP study was analysed (NTP, 2002)	Total MAPK expression levels were similar between normal lung and lung carcinomas. Phospho-MAPK elevated in 5/6 lung carcinoma samples, in which *Kras* mutations and chromo-some 6 LOH (loss of heterozygosity) were identified and in 4/5 carcinomas with *Kras* mutations but no LOH. no phospho-MAPK was detected in carcinoma tissue without *Kras* mutation and in normal lung tissue	(Devereux *et al.*, 2002)

Table 21: Summary table of other *in vitro* and *in vivo* studies on gene expression or transformation assay, relevant for carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Genomic analysis of human lung fibroblasts exposed to divanadium pentaoxide Reliability (Klimisch score): 2	Divanadium pentaoxide, no information on purity available Human lung fibroblasts (cell line ATCC 16 Lu) 0 or 10 µg V_2O_5/cm^2 Cells were harvested after 1, 4, 8, 12 and 24 h RNA was isolated and microarray to analyse expressed genes were performed.	More than 1400 genes were altered, 300 induced, >1300 suppressed. Involved genes were associated with cell growth (growth factors), chemokines, oxidative stress response and DNA binding	(Ingram *et al.*, 2007)
Gene expression profiling in lung tissue of female B6C3F1 mice exposed for 13 weeks.	Divanadium pentaoxide, purity: 99.6% 0 or 2 mg V_2O_5/m^3, 6 h/d, 5 d/w for 13 weeks (whole body exposure to respirable particulate aerosol)	In exposure group, 1026 genes were expressed, differently, 483 of these were specific to V_2O_5. These genes are involved in: - hyaluron and sphingolipid	(Thomas *et al.*, 2009) (ECHA Dissemination, 2017)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Reliability (Klimisch score): 2		metabolism - adenylate cyclase functions - c-AMP signaling - PKA activation/signaling - enrichment of lipids /lipoprotein metabolism - inflammatory pathways No evidence for enrichment of pathways associated with cell cycle arrest/proliferation, DNA damage, oxidative stress	Study: 005 (Black *et al.*, 2015)
Syrian hamster embryo Transformation (SHE) assay Microscopic evaluation of morphological transformation Reliability (Klimisch score): 2	Divanadium pentaoxide, no information on purity available Syrian hamster embryo cells isolated from 13-day-gestation embryos <u>Experiment 1:</u> 24 h exposure 0 – 75 µg V_2O_5/mL (5 concentrations + control) <u>Experiment 2:</u> 7 d exposure 0 – 87.5 µg V_2O_5/mL (5 concentrations + control) Highest concentrations tested were determined in a cytotoxicity screening test	<u>Experiment 1:</u> Negative for significant morphological transformation <u>Experiment 2:</u> Positive for significant morphological transformation starting in the lowest concentration	(Kerckaert *et al.*, 1996)

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

As indicated in Table 18, two adequate long-term studies on carcinogenicity with divanadium pentaoxide administered by inhalation are available on two species (NTP, 2002).

The conclusion by NTP with regard to the results with rats is: *"Under the conditions of this 2-year inhalation study, there was some evidence of carcinogenic activity of vanadium pentoxide in male F344/N rats and equivocal evidence of carcinogenic activity of vanadium pentoxide in female F344/N rats based on the occurrence of alveolar/bronchiolar neoplasms. Exposure to vanadium pentoxide caused a spectrum of non-neoplastic pulmonary lesions in the respiratory tract (nose, larynx, and lung) including alveolar and*

bronchiolar epithelial hyperplasia, inflammation, fibrosis, and alveolar histiocytosis of the lung in male and female rats and an unusual squamous metaplasia of the lung in male and female rats."

In this 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 each doses respectively of 0, 0.5, 1 or 5 mg/m^3. 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 doses respectively of 0, 0.5, 1 or 5 mg/m^3. Some authors such as Starr *et al.*, 2012 questioned the use of adequate historical control data and the lack of dose-response. However, according to NTP report 2002, an appropriate comparison was done with their concurrent historical control group either with NTP-2000 or with the previous diet used at NTP (NIH-07 diet). Indeed, although not statistically significant, the increases in the incidences of alveolar/ bronchiolar adenoma in 0.5 mg/m^3 males and of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) in 0.5 and 2 mg/m^3 males exceeded the historical ranges in controls (all routes) given NTP-2000 diet and in inhalation chamber controls given NIH-07 diet (NTP, report 2002). The incidence of alveolar /bronchiolar adenoma in 0.5 mg/m^3 females was at the upper end of the historical control range for studies using NTP-2000 diet and exceeded the range in the larger database for inhalation studies using NIH-07 diet.

The conclusion by the NTP with regard to the results with mice is: "*clear evidence of carcinogenic activity of vanadium pentoxide in male and female B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms. Exposure to vanadium pentoxide caused a spectrum of non-neoplastic lesions in the respiratory tract (nose, larynx, and lung) including alveolar and bronchiolar epithelial hyperplasia, inflammation, fibrosis, and alveolar histiocytosis of the lung in male and female mice. Hyperplasia of the bronchial lymph node occurred in female mice."*

In the second NTP study conducted on mice, 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 respectively of 0, 1, 2 or 4 mg/m^3. The incidence of alveolar/bronchiolar adenoma or carcinoma incidence 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 of 0, 1, 2 or 4 mg/m^3. The increases were dose-related, statistically significant in all groups of exposed males and females and exceeded the historical control data. Many exposed animals had multiple adenomas and/or carcinomas.

A wide range of proliferative lesions in the lungs were observed in rats and mice exposed to divanadium pentoxide for 2 years. The incidence of hyperplasia of the alveolar and bronchiolar epithelium was increased in exposed rats and mice. NTP, 2002 report concluded that this hyperplastic change was striking and appeared more prominent than had been observed in other NTP inhalation studies. Although the exact pathogenesis was not determined in this study, the hyperplasia of the alveolar and bronchiolar epithelium was consistent with bronchiolization, a process in which bronchiolar epithelium proliferates and migrates down

into alveolar ducts and adjacent alveoli. Whether this represented a precursor lesion for development of pulmonary neoplasms is not known. The lung tumour response in rats was not concentration-related and in mice, a flat dose response was observed. Several dose metrics and lung-burden data (see Annex I for details) were used to aid in interpretation of lung pathology in exposed rats and mice. In the case of all dose metrics, rats received more vanadium than mice. In mice, the total 'dose' was similar in the groups exposed to 1 mg/m^3 and 2 mg/m^3 and this may help explain the flat dose response in the lung neoplasms in male and female mice. When the total dose is corrected for body weight, mice received a three- to five-fold higher dose of vanadium than rats at comparable exposure concentrations of 1 and 2 mg/m^3. Therefore, on a body weight basis, mice received considerably more vanadium than rats, and this may help explain the differences in responses between the species (National Toxicology Program, 2002; Ress *et al*., 2003).

In a one-year study in mice, with limited reporting, papillomatous and adenomatous tumours in the lungs were reported at the two highest tested concentrations of 2 and 8 mg V_2O_5/m^3 (Yao *et al.*, 1986).

In addition, in a tumor promotion study conducted in male A/J, BALB/cJ and C57BL/6J mice (Rondini *et al.*, 2010), animals were primarily exposed to methylcholanthrene (MCA) followed by weekly exposure of V_2O_5 *via* oropharyngeal aspiration at the dose of 4 mg/kg bw/d (for 5 weeks). V_2O_5 exposure promoted significantly lung tumours with a majority of solid adenomas and the remaining papillary in A/J and BALB mice. Tumour promotion was associated with inflammation involving induction of multiple chemokines and transcription factors NFκB and c-Fos as well as activation of extracellular signal-regulated kinases 1 and 2. No tumours were observed in C57BL/6J mice. This study shows that V_2O_5 acts as an *in vivo* lung tumour promotor in A/J and BALB/cJ mice.

Pending on the tested species and on the protocol used (*in vitro versus in vivo* inhalation), genomic analyses (Table 20) gave contradictory results. Results from Thomas *et al.* (2009) and Black *et al.* (2015) indicated that there was no evidence for enrichment of pathways associated with cell cycle arrest/proliferation, DNA damage, or oxidative stress from their gene expression profiling in an *in vivo* study analysing lung tissue of B6C3F1 mice. Whereas Ingram *et al.* (2007) found positive evidence for oxidative stress response and increased DNA binding from altered genes in an *in vitro* study with human lung fibroblast cells. Gene expression analysis with sodium metavanadate (data not shown) found changes that are consistent with cellular transformation including anchorage-independent growth and enhanced migration ability or changes in DNA repair and oxidative stress (Clancy *et al.*, 2012; Passantino *et al.*, 2013). Additionally, a transformation assay in Syrian hamster embryo cells with divanadium pentaoxide resulted in positive results with a 7-day exposure (Kerckaert *et al.*, 1996), as shown in Table 20.

The mode of action for carcinogenicity of V_2O_5 is currently not elucidated. From the genotoxicity database, V_2O_5 induced positive results in several micronucleus tests and comet assays. Some influence of secondary genotoxicity may be assumed by induction of oxidative stress, inhibition of DNA repair and interference with the activity of protein phosphatases and kinases (Beyersmann and Hartwig, 2008). The observed induction of aneuploidy by vanadate (V) ((Ramírez *et al.*, 1997; Roldán and Altamirano, 1990; Zhong, 1994), is interpreted by the inhibition of spindle formation and the disruption of microtubule assembly (Beyersmann and Hartwig, 2008). The ability of V_2O_5 to induce a range of genotoxic effects, possibly due to inhibition of microtubule polymerization during spindle assembly, may be hypothesised (Assem and Levy, 2009). Similar interpretation on mode of action has been provided, e.g., by IARC (2006), Assem and Levy (2012), Zwolak (2014) and Rodríguez-Mercado *et al.* (2011). Lastly, VPRA (2011) (data not shown) found indications of an impairment of the repair-capacity due to divanadium pentaoxide exposure in a recent *in vitro* study, possibly an additional mode of action, that contributes to carcinogenicity.

Lastly, a promotor effect of divanadium pentaoxide may be assumed based on the results observed after initiation with MCA (Rondini *et al.* (2010) and the potential amplifying effect of divanadium pentaoxide on prior existent *Kras* mutations (Banda *et al.*, 2015; Black *et al.*, 2015) or on spontaneous lesions.

A retrospective case control study on employees working in vanadium processing facilities (production of V_2O_5) did not show statistically significant association between cancer and occupational exposure to divanadium pentaoxide (Fourie, 2010). A study with environmental exposure to vanadium compounds (limited reliability) close to a mining area provided no indication of increased cancer mortality for the population in the neighbourhood (Boice *et al.*, 2007, data not shown). Lastly, no meaningful epidemiology studies on carcinogenicity of divanadium pentaoxide are available.

Table 22: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
F344/N rats	Alveolar, bronchiolar neoplasms: adenoma and carcinoma	No	Yes	Yes	Some evidence in males Equivocal evidence in females	No excessive toxicity	Inhalation	Not elucidated Genotoxic effects reported Secondary genotoxicity and epigenetic events suggested. Assumed to be relevant to
$B6C3F_1$ mice	Alveolar, bronchiolar neoplasms: adenoma	No	Yes	Yes First incidence (alveolar/bronchiolar adenoma or	Both sexes positive: clear evidence	No excessive toxicity	Inhalation	

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confoun-ding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
	and carcinoma			carcinoma) (male): Control: 667 days; 1 mg/m³: 405 days 2 mg/m³: 534 days 4 mg/m³: 394 days (female): Control: 731 days; 1 mg/m³: 522 days 2 mg/m³: 281 days 4 mg/m³: 478 days				humans

10.9.2 Comparison with the CLP criteria

IARC (2006) classified divanadium pentaoxide: "*possibly* carcinogenic to humans (Group 2B)", based on *sufficient* evidence in animals and *inadequate* evidence in humans, according to the IARC criteria.

For potential classification on carcinogenicity, criteria of the CLP Regulation (EC, 2017) were applied.

- *"Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence"* (EC, 2017)

As indicated, there is inadequate evidence from human studies on exposure to divanadium pentaoxide for classification into Category 1A. **Criteria for a category 1A is thus not fulfilled.**

With regard to animal studies the following criteria have to be discussed:

- "*sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites*" (ECHA, 2017)

There is clear evidence of increased incidence of alveolar/ bronchiolar neoplasms in both sexes in B6C3F1 mice in the NTP (2002) study which is a well-conducted GLP study. Some evidence of carcinogenicity was reported in the NTP (2002) study for male rats and equivocal evidence of carcinogenicity in female rats. This criterion would call for **classification of divanadium pentaoxide as a Category 1B substance.**

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

Evidence of carcinogenicity is clear in mice in a single experiment. Even if the evidence is only defined by the NTP (2002) as only "some" or "equivocal" in the rats, the findings are consistent to those reported in mice. There is no doubt on the adequacy of the design, conduct and interpretation of the NTP (2002) studies. In these studies, both benign and malignant tumours were observed. As detailed below, V_2O_5 should be considered as a complete carcinogen agent. Considering all these elements, **criteria for Category 2 are not fulfilled.**

The CLP Regulation subsequently requests to consider further parameters for classification justification:

- *"tumour type and background incidence":* lung tumours (adenoma and carcinoma) only; statistically significant in mice of both sexes; exceed historical control in mice (both sexes) and male rats.

- *"multi-site responses":* No: lung tumours only

- *"progression of lesions to malignancy":* yes: carcinoma observed

- *"reduced tumour latency":* yes

- *"whether responses are in a single or both sexes"[…] "whether responses are in a single species or several species":* clear evidence in mice of both sexes; some evidence in male rats, equivocal evidence in female rats

- *"structural similarity to a substance(s) for which there is good evidence of carcinogenicity":* No information

- "*route of exposure*": inhalation, no adequate study by other route of exposure

- *"comparison of absorption, distribution, metabolism and excretion between test animals and humans":* no information

- "*the possibility of a confounding effect of excessive toxicity at test doses":* No, only some decreases of body weight at the highest dose

- *mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity"* (EC, 2017)*:* The mode of action of V_2O_5 carcinogenicity is not elucidated. From genotoxicity database, divanadium pentaoxide is considered as a genotoxic agent (see section 10.8). Secondary genotoxicity and epigenetic events are also suggested. By default, the tumours reported are considered relevant for human.

- *"Limited evidence of carcinogenicity should be assigned, if the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. … the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs"* (EC, 2017).

As for other metals, the overall V_2O_5 mutagenic and genotoxic profile is quite complex. In *in vivo* assays, MN, comet and dominant lethal effects were observed showing a genotoxic hazard for the somatic and germinal cells. In addition, some human *in vivo* data showed: DNA damage, oxidized purine and pyrimidine base, necrotic cells, MN in lymphocytes. Hypothesised mechanism of action for divanadium pentaoxide are quite wide from induction of oxidative stress, inhibition of DNA repair, interference with the activity of protein phosphatases and kinases, the inhibition of spindle formation and the disruption of microtubule assembly or a promotor component effect (Rondini *et al.*, 2010). However, an overall weight of evidence supports the current understanding that divanadium pentaoxide is a complete carcinogen.

10.9.3 Conclusion on classification and labelling for carcinogenicity

With a weight of evidence approach, considering the clear evidence for carcinogenicity in mice in both sexes, also supported by results in rats (NTP, 2002) with the various other CLP criteria to be considered, a classification to **Carc. 1B, H350: May cause cancer** is warranted.

Classification should not be limited to a single exposure pathway. Although the data available only relate to inhalation exposure, a decision to restrict carcinogenicity classification to the inhalation pathway of exposure would not be justified, in the absence of carcinogenicity data by the oral or dermal pathway for divanadium pentaoxide.

Because of the flat dose-response relationship for cancer in the respective animal study (NTP, 2002), no extrapolation to derive a threshold has been performed to provide concentration limits for ingredients of a mixture. Therefore no specific concentration limits (SCL) may be defined and the default (concentration limit $\geq 0.1\%$ in mixtures classification) is assigned.

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 of 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.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 23: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
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 (0, 0.56, 1.12, 2.25, 4.50, 8.99 mg V/m^3) 6 h/d + T_{90} (15 min), 5 d/w for 3 months, whole body inhalation Animals were not mated	<u>Effects on male animals:</u> At the highest dose 7/10 males died during the study. At the other dose groups no deaths occurred. Final body weights from animals in dose groups 4, 8 and 16 mg V_2O_5/m^3 were significantly reduced. Atrophy of the secondary reproductive organs was observed in 16 mg/m^3 males and hypospermia of the testis and atypical cells of the epididymis were observed in 16 mg/m^3 males. However, these lesions may have been associated with the marked body weight loss and general debilitation of these rats. No other effect on reproductive organs (weight of cauda epididymis, epididymis and testis, spermatid heads/g testis, spermatid count, sperm motility and concentration). NOAEC $_{repro\ male\ rats}$: >8 mg/m^3 (based on male histopathological lesions at 16 mg/m^3)	(NTP, 2002) from (ECHA Dissemination, 2017) Study: 003, publication Details in Annex I.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		Effects on female animals: At the highest dose 3/10 females died during the study. At the other dose groups no deaths occurred. Final body weights from the high dose group animals were significantly reduced. Atrophy of the secondary reproductive organs was observed in 16 mg/m^3. Estrous cycle length of females exposed to 8 mg/m^3 was significantly longer than that in control group (control: 5.00 ± 0.00 d, 4 mg/m³: 5.00 ± 0.08 d, 8 mg/m³: 5.50 ± 0.14 d, 16 mg/m³: 5.25 ± 0.25 d). In the highest dose groups, normal estrous cycle is disturbed. Number of females in diestrus was significantly elevated (control: 39.2% in diestrus vs. 4 mg/m³: 40.8%, 8 mg/m³: 49.2%, 16 mg/m³: 71.9%) NOAEC $_{\text{repro female rats}}$: 4 mg/m³ (based on increased estrous cycle length). In addition, local effects were reported in both males and females with a NOAEC local of 1 mg/m³ air based on increased lung weights and epithelial hyperplasia, inflammation and fibrosis in lungs at 2 mg/m^3 and above (see section on STOT RE for further details).	
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 (0, 0.56, 1.12, 2.25, 4.50, 8.99 mg V/m^3) 6 h/d + T_{90} (15 min), 5 d/w for 3 months, whole body inhalation Animals were not mated	Effects on male animals: At the highest dose 1/10 males died during the study. At the other dose groups no deaths occurred. Final body weights from the 8 and 16 mg/m³ dose groups were significantly reduced. Absolute and relative lung weights of males and females exposed to 4 mg/m^3 or greater were significantly greater than those of the chamber controls. Divanadium pentaoxide exposure did not significantly affect weight of cauda epididymis, epididymis and testis, number of spermatid heads/g testis, spermatid count, or concentration. However, at 8 and 16 mg/m³, sperm motility from epididymis 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%) Effects on female animals:	(NTP, 2002) (ECHA Dissemination, 2017) Study 004, publication Details in Annex I.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		No deaths occurred. Final body weights started to be reduced in the exposure group of 4 mg/m³. No significant differences were noted in estrous cycle parameters between exposed and chamber control females. NOAEC $_{Repro}$: 4 mg/m³ (based on decreased sperm motility). In addition, local effects were reported in both males and females with a $NOAEC_{local}$ of 1 mg/m³ air (males and females) based on increased absolute lung weights and epithelial hyperplasia and inflammation in lungs at 2 mg/m^3 (see section on STOT RE for further details).	
Testes effects after vanadium inhalation Male CD-1 mice 8 animals sacrificed at each time point (3 controls, 5 exposed) Reliability (Klimisch score): 3	Divanadium pentaoxide, no information on purity available 0; 0.02 M V_2O_5 (1436 µg V_2O_5/m³ *via* inhalation) Concentration based on the average concentration in the 18 filters measurement (0, 0.79 mg V/m³) Inhalation, 1 h/twice a week, for a total of 12 weeks, every week animals were sacrificed. Vehicle: deionized water Testes were analysed immunohistochemically.	During the 12-week exposure period no overt toxicity signs or weight changes (body weight and testicular weight) were detected in the V_2O_5 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	(Fortoul *et al.*, 2007)
Mechanistic study, immunohistochemical analysis of gamma-tubulin in somatic and testicular germ cells after vanadium inhalation Male CD-1 mice 6 animals sacrificed at each time point (3 controls, 3 exposed) Reliability (Klimisch score): 3	Divanadium pentaoxide, no information on purity available 0; 0.02 M V_2O_5 (1.4 mg V_2O_5/m³ *via* inhalation) (0, 0.79 mg V/m³ *via* inhalation) 1 h/twice a week, for a total of 12 weeks, every week animals were sacrificed. Vehicle: deionized water Testicular cells (germinal, Sertoli and Leydig cells) were analysed immunohistochemically.	Decrease of the percentage of gamma-tubulin in all analyzed testicular cells (Sertoli, Leydig and germ cells) starting with the first week of treatment in a time dependent manner. Vanadium accumulated in the testes starting with the initial inhalation (24 h)	(Mussali-Galante *et al.*, 2005)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Mechanistic study, distribution of connexin 43 (part of the gap junctions) in seminiferous tubules of mice after exposure to V_2O_5 60 male CD1 mice in exposure group 24 in control group Reliability (Klimisch score): 3	Divanadium pentaoxide, no information on purity available 0; 0.02 M V_2O_5 (1.4 mg V_2O_5/m^3 *via* inhalation) (0, 0.79 mg V/m³ *via* inhalation) 1 h/twice a week, for a total of 12 weeks, every week animals were sacrificed. Vehicle: saline	Reduced membrane connexin 43 in seminiferous tubules starting at 8 weeks of exposure Cytoplasmic connexin 43 increased continuously starting at 4 weeks of exposure	(Bizarro-Nevares *et al.*, 2016)
Mechanistic study, immunohistochemical changes in actin testicular cytoskeleton Male CD-1 mice, 10 animals sacrificed at each time point (5 controls, 5 exposed) Reliability (Klimisch score): 3	Divanadium pentaoxide, no information on purity available 0; 0.02 M V_2O_5 (1.4 mg V_2O_5/m^3 *via* inhalation) (0, 0.79 mg V/m³ *via* inhalation) 1 h/twice a week, for a total of 12 weeks, every week animals were sacrificed. Testicular cells were analysed immunohistochemically.	Actin content time-dependently reduced in testes cells. Effect is significant starting at 3 weeks.	(Rodríguez-Lara *et al.*, 2016)
Study of reproductive function in male mice (dominant lethal test) CD-1 male mice 15-30 animals per group Reliability (Klimisch score): 2	Divanadium pentaoxide, purity: 99.6% 0, 8.5 mg V_2O_5/kg bw intraperitoneal injection (males only) (0, 4.7 mg V/kg bw per injection) <u>Group 1:</u> 20 control animals received vehicle (saline) every 3rd day for 60 days <u>Group 2:</u> 15 animals received V_2O_5 every 3rd day for 60 days Animals of group 1 and 2: on day 61, animals were subjected to a fertility assessment test (mated with unexposed females) and sacrificed 5 days later.	V_2O_5 treatment resulted in decrease in fertility rate (85% vs 33%). Sperm count, motility, and morphology were impaired. The effects were getting more severe the longer the exposure time was. The final body weight of V_2O_5-treated animals during 60 days was lower than controls, while differences were not observed in animals sacrificed at earlier time points (from day 10 to 50). Implantation sites, live foetuses, and foetal weight were significantly decreased. The number of resorptions/dam and of dead foetuses was increased.	(Altamirano-Lozano *et al.*, 1996) Details in Annex I.
Study of sex differences in the effects of	Divanadium pentaoxide, no information on purity available	<u>Experiment 1 (males):</u> Increase in weight of seminal vesicles,	(Altamirano *et al.*, 1991)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Divanadium pentaoxide administrated to prepubertal rats CIIZ rats Experiment1: 5-9 animals per sex in treatment and control group (saline application) Experiment 2: 10 control animals (saline application), 6 in treatment group Reliability (Klimisch score): 3	0, 12.5 mg V_2O_5/kg bw, intraperitoneal injection (0, 7.0 mg V/kg bw per injection) Experiment 1: Newborn male and female rats were treated every second day from birth to 21 days Experiment 2: Female rats were treated from day 21 to the day of the first vaginal oestrus	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	
Study of histological and sperm parameters after exposure 5 male guinea pigs/dose group Reliability (Klimisch score): 2	Divanadium pentaoxide, analytical grade, no further information available Experiment 1: 0, 4.5, 6.5, 8.5, 10.5, 12.5 mg V_2O_5/kg single intraperitoneal injection in saline solution (0, 2.5, 3.7, 4.8, 5.9, 7.0 mg V/kg bw) Experiment 2: 0, 8.5 mg V_2O_5/kg bw/d intraperitoneally injected. (0, 4.8 mg V/kg bw/d) Testicular tissue of 5 animals 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. In addition 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. No indication on general toxicity is given. Experiment 2: 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	(Uche *et al.*, 2008) Details in Annex I

Table 24: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Analysis of Vanadium in human seminal plasma from Pakistani men	Vanadium, Speciation not indicated	75 semen samples from humans (25 with normospermia = control, 25 with oligospermia and 25	Vanadium levels in oligozoospermic and azoospermic subjects are higher compared to normozoospermic subjects. (normospermic: 2.98 ± 1.51 ppb, oligospermic: 4.66 ± 2.75 ppb,	(Zafar *et al.*, 2015)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		with azospermia)	azospermic: 8.28 ± 4.57 ppb) Correlation values showed that V is negatively correlated with sperm volume (r = -0.38, p<0.01), concentration (r = -0.45, p<0.05) and motility (r = -0.45, p<0.05).	
Analysis of Vanadium in human seminal plasma from Japanese men correlation of vanadium in sperm and sperm concentration, motility, morphology and volume	Vanadium, Speciation not indicated	Semen samples from humans (28 with normospermia = control, 28 with oligospermia and 28 with azospermia)	No correlation between Vanadium content in seminal plasma and sperm concentration was observed. In a multivariance analysis no clear correlation between effects on sperm and vanadium load could be determined.	(Katayama *et al.*, 2013)
Analysis of Vanadium in human seminal plasma from Japanese men correlation of vanadium in sperm and sperm concentration, motility, morphology and sperm number	Vanadium, Speciation not indicated	Semen samples from 128 humans.	No correlation between Vanadium content in seminal plasma and sperm concentration was observed. Linear regression analysis showed no correlation between vanadium concentration and sperm concentration, sperm number and motility.	(Katayose *et al.*, 2004)

Table 25: Summary table of other studies relevant for toxicity on sexual function and fertility – studies performed with other vanadium compounds

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Ammonium metavanadate NH_4VO_3				
Study on fertility [and prenatal developmental toxicity] in exposed rats One-Generation Reproduction Toxicity	Ammonium meta-vanadate, no information on purity available	200 ppm in drinking water (calculated with default factors according to ECHA (2012)): <u>Treated male Sprague Dawley rats:</u> 10 mg NH_4VO_3/kg bw/d, (4.35 mg V/kg bw/d) 70 day exposure <u>Treated female Sprague Dawley rat:</u> 11.43 mg NH_4VO_3/kg bw/d, (4.97 mg V/kg bw/d) 61 days exposure (14 days	<u>Male fertility</u> was investigated by mating exposed (and control) males with virgin untreated females. <u>Female fertility was</u> investigated by mating exposed (and control) females with untreated males Mating and fertility index reduced in treated males and treated females: Mating index: Control: 100%, Group 1: 65%; Group 2: 70% Fertility index: 95%, 46.15%, 71.43% Reduced weight of testes, epididymis,	(Morgan and El-Tawil, 2003) (ECHA Dissemination, 2017) Study: 001 publication Details in Annex I

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Study GLP: no Reliability (Klismich score): 3		premating, during mating, till weaning of pups (21 days of age) Group 1: Treated male group (n=10) mated with untreated females (n=20), fertility assessment (mating, fertility index, delayed birth date, signs of dystocia, number of corpora lutea, implantation sites, [resorbed, dead, live foetuses, pre-/ post-implantation losses], dam's body weight at the end of gestation, [gravid uterine and placental weights, fetal body weight, fetal survival and viability indices during lactation]); endpoints in square brackets: discussed within developmental toxicity, section 10.10.4). Exposed males (n=10) were analysed after mating period for body weight, testes weight, epididymis, prostate, seminal vesicles (n=10 controls). Group 2: Treated female group (n=20) mated with untreated males (n=10), fertility assessment as in Group 1. Exposed females (n=20) were examined for estrous cycle regularity during premating period. Dams were sacrificed with their offspring and endpoints recorded. Group 3: [Examination of foetuses] endpoints in square brackets: discussed within developmental toxicity, section 10.10.4)	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). Developmental effects reported under section 10.10.4.	
Sodium metavanadate $NaVO_3$				
Reproductive toxicity study Reliability (Klimisch score): 3	Sodium meta-vanadate	Sprague-Dawley albino rat Sodium metavanadate (purity not indicated). Males: 60d before mating; Females: 14d before mating, throughout gestation and lactation Dosing: 0, 5, 10, 20 mg $NaVO_3$ /kg/d Intra-gastric administration About one half of the fertilized animals were sacrificed on day 14	Maternal effects: No adverse effects In animals allowed to birth, the development of the offspring was always significantly decreased from birth and during all the lactation period for animals treated at 10 and 20 mg/kg/day. Significant decreases in the relative weights of liver, spleen and kidneys of the pups whose mothers received $NaVO_3$ during the lactation from 5 mg/kg/day. Decreases in body weight,	(Domingo *et al.*, 1986)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		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 young to 21 days.	body length, tail length were also observed in offsprings. 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/day $NaVO_3$ when compared to the control group. But these increases were not significant ($P > 0.05$). LOAEL: 5 mg $NaVO_3$/kg/d (2.1 mg V/kg/d).	
Study on fertility and reproductive toxicity in exposed male rats Reliability (Klimisch score): 2	Sodium meta-vanadate, no information on purity available	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 Intraperitoneal injection Animals were sacrificed 24 h after last treatment	Significantly reduced organ weights (testis, cauda epididymis, ventral prostate, seminal vesicles, coagulating gland) at 0.4 and/or 0.6 mg V/kg bw/d Dose-dependent reduction of $\Delta^5$3β- and 17β-hydroxysteroid dehydrogenase activity 17β-HSD and serum testosterone Dose-dependent decrease of superoxide dismutase and catalase activity Dose-dependent increase in lipid peroxidation Significant reduction of sperm count, spermatogonia Δ, preleptotene spermatocytes, mid-pachytene spermatocytes and step 7 spermatids at 0.4 and/or 0.6 mg V/kg bw/d Dose dependent increase of abnormal sperm	(Chandra *et al.*, 2007b)
Study on fertility and reproductive toxicity in exposed male rats Reliability (Klismisch) : 3	Sodium meta-vanadate, no information on purity available	8 male Sprague-Dawley rats for each group 0, 0.4 mg V/kg bw/d for 26 days Intraperitoneal injection Animals were sacrificed 24 h after last treatment	Significantly reduced organ weights (testis, seminal vesicles, ventral prostate, coagulating gland, epididymis) Epididymal sperm count significantly reduced, percentage of abnormal sperm significantly increased Reduction of $\Delta^5$3β- and 17β-hydroxysteroid dehydroge activity, serum testosterone levels and serum gonadotropins Decrease of superoxide dismutase and catalase activity Increase in lipid peroxidation Increased weight of adrenals, and	(Chandra *et al.*, 2007a)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			significant elevation of serum concentrations of corticosterone Testicular lesions, significant reduction of spermatogonia, preleptotene spermatocytes, mid-pachytene spermatocytes and step 7 spermatids	
Study on fertility and reproductive toxicity in exposed male rats Reliability (Klimisch score): 3	Sodium meta-vanadate, no information on purity available	8 male Sprague-Dawley rats for each group 0, 0.4 mg V/kg bw/d for 26 days Intraperitoneal injection	Significantly reduced organ weights (testis, epididymis, prostate, seminal vesicles) Reduced serum testosterone, LH and FSH levels Reduction of $\Delta^5$3β- and 17β-hydroxysteroid dehydroge activity Significantly increased vanadium concentrations in the testis. Decrease of superoxide dismutase and catalase activity Increase in lipid peroxidation Testicular lesions, significant reduction of spermatogonia, preleptotene spermatocytes, mid-pachytene spermatocytes and step 7 spermatids, pathology changes of cauda epididymis, ventral prostate gland, coagulating gland, seminal vesicles, epididymal sperm count and morphology	(Chandra *et al.*, 2010)

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

Section 10.10.1 provides data on experimental testing of males and females with regard to adverse effects on sexual function and fertility from exposure to divanadium pentaoxide.

Since studies assessing reproductive effects of V_2O_5 are rather limited either because no fertility study was conducted according to OECD technical guideline or because most of the studies were conducted by intraperitoneal route with mainly one dose level and a very few by inhalation route, read-across with other vanadium compounds such as sodium metavanadate and ammonium metavanadate has been considered as supporting evidence.

Human data:

Analysis of human seminal plasma from Pakistani and Japanese men (Table 24) (Katayama *et al.*, 2013; Katayose *et al.*, 2004; Zafar *et al.*, 2015) provide contradictory results on the correlation between Vanadium

content in seminal plasma and sperm concentration. All those studies were of limited reliability. No human data related to female sexual function and fertility due to exposure to V_2O_5 are available. The respective studies are therefore not regarded as relevant for classification.

Experimental studies:

Females

Overall, few experimental studies examined the **effects of V_2O_5** on the female reproductive system:

- 2 NTP studies - 90 days of exposure *via* inhalation on F344 rat and B6C3F1 mice (NTP, 2002) – reliability 1. Both studies analysed effects on reproductive organs and oestrous cycle but do not include any mating between animals.
- 1 study *via* intraperitoneal route conducted with only one dose level in CIIZ rats (Altamirano *et al.*, 1991) - reliability score of 3.

After subchronic inhalation, the estrous cycle of female rats exposed to concentrations from 8 mg V_2O_5/m^3 was significantly longer than that of the chamber control group (control: 5.00 ± 0.00 d, 4 mg/m³: 5.00 ± 0.08 d, 8 mg/m³: 5.50 ± 0.14 d, 16 mg/m³: 5.25 ± 0.25 d), and the number of cycling female rats in the 16 mg/m^3 group was reduced (control: 39.2% in diestrus *versus* 4 mg/m³: 40.8%, 8 mg/m³: 49.2%, 16 mg/m³: 71.9%) (reliability: category 1; NTP, 2002). Atrophy of the secondary reproductive organ was also reported at 16 mg/m^3. Such effects were not observed in mice (reliability: category 1; NTP, 2002). In another study conducted with high i.p. doses of V_2O_5, ovulation rate in prepubertal female rats was reduced with no other reproductive effects observed in females (Altamirano *et al.*, 1991).

With **other pentavalent vanadium compounds, such as ammonium metavanadate (NH_4VO_3)** : 11.4 mg NH_4VO_3/kg bw/d (corresponding to 4.97 mg V/kg bw/d) administered to female Sprague Dawley rats in drinking water for up to 61 days induced a reduction of the number of female rats with regular estrous cycle (12 cycling females (60%) *versus* 20 cycling females (100%) in the control group) (Morgan and El-Tawil (2003)). When the treated females were mated to untreated males, the index for mating and fertility were both decreased 70% *versus* 100% and 71.43% *versus* 95%, respectively. The total number of corpora lutea in females was reduced. Additionally, there were an increased number of delayed birth date (50%, if females were exposed to ammonium vanadate vs. 0% in control) and an increased number of dams showing signs of dystocia (40%, if females were exposed to ammonium vanadate *versus* 0% in control). Specific developmental effects were also reported in this study (see description in section 10.10.4).

Although this study has to be categorized “not reliable” (reliability score: 3) according to Klimisch criteria, the effects observed after exposure to ammonium metavanadate are regarded unambiguous, and should be considered as clear adverse effects on sexual function of the female rats after oral exposure. Additionally

similar effects on the oestrous cycle were reported with V_2O_5 in the 90-day NTP (2002) study in rat which support the relevance of the effects observed with vanadium compounds exposure.

Overall, consistent adverse effects were observed on female rat sexual function after inhalation of V_2O_5 (NTP, 2002) or after oral exposure with ammonium metavanadate (Morgan and El-Tawil, 2003). Due to the structural similarity of ammonium metavanadate to divanadium pentaoxide and the consistent findings reported, the observation of Morgan and El-Tawil (2003) is considered relevant for divanadium pentaoxide classification and labelling.

Males

Overall, several experimental studies examined the effects of V_2O_5 on male reproductive system:

- 2 NTP studies - 90 days of exposure *via* inhalation in F344 rat and B6C3F1 mice (NTP, 2002) – reliability 1. Both studies analysed effects on reproductive organ and sperm but do not include any mating between animals.
- 4 studies - 12 weeks of exposure (1h twice a week) *via* inhalation, on CD-1 mice (Fortoul *et al.*, 2007; Bizarro-Nevares *et al.*, 2016; Mussali-Galante *et al.*, 2005; Rodríguez-Lara *et al.*, 2016) – reliability 3,
- 2 studies *via* intraperitoneal route conducted with only one dose level in CD-1 mice and in CIIZ rats respectively (Altamirano-Lozano *et al.*, 1996; Altamirano *et al.*, 1991) - reliability score of 2 and 3 respectively.
- 1 other study *via* intraperitoneal route with several tested dose levels in guinea pigs (Uche *et al.*, 2008) – reliability score of 2.

The subchronic inhalation NTP studies (cf. Table 23) show that sperm motility from epididymis was decreased in mice from 8 mg V_2O_5/m^3 (4.5 mg V/m^3) or above, but not in rats. Atrophy of secondary reproductive organ was noted in male rats (but not in mice), with hypospermia of the testis and atypical cells of the epididymis at 16 mg/m^3 (reliability: category 1; NTP, 2002). Specific developmental effects were also reported in this study and described in section 10.10.4.

Inhalation of an aqueous aerosol of V_2O_5 (unique concentration tested (1.4 mg V_2O_5/m^3; 0.79 mg V/m^3) for 12 weeks in mice led to more severe effects namely necrosis of spermatogonia, spermatocytes and Sertoli cells, pseudo-nuclear inclusion, disruption of cellular junctions (Fortoul *et al.*, 2007) at a lowest concentration than those tested in the NTP (2002) studies. Additionally, vanadium concentration was drastically increased in testes after 1 week of exposure (Fortoul *et al.*, 2007) which shows that the tested compound reaches the testes. Three additional studies (Bizarro-Nevares *et al.*, 2016; Mussali-Galante *et al.*, 2005; Rodríguez-Lara *et al.*, 2016) conducted also by inhalation for 12 weeks demonstrated that testes effects

are related to a specific mode of action and are not secondary to general toxicity of divanadium pentaoxide. In these series of mechanistic studies, three parameters (actin content, connexin43, and gamma-tubulin) needed for adequate function of the blood-testis barrier (BTB) are shown to be impaired. Additionally, Mussali-Galante *et al.*, 2005 showed that vanadium was retrieved in testes of exposed V_2O_5 animals. Each of these studies are judged of limited reliability mainly due to a poor data reporting of the exposure conditions.

Further studies with repeated intraperitoneal injections of V_2O_5 (between 8.5 and 12.5 mg/kg) also indicate severe effects in the male reproductive function including reduced fertility, histopathological effects in the testes and impaired sperm count, motility and morphology (Altamirano-Lozano *et al.*, 1996; Altamirano *et al.*, 1991; Uche *et al.*, 2008). Although those effects resulted from intraperitoneal exposure, they are consistent with effects reported after inhalation.

With **other pentavalent vanadium compounds, such as sodium metavanadate ($NaVO_3$) or ammonium metavanadate (NH_4VO_3),** adverse effects on male reproductive function were also reported. Adverse effects on testes and sperm parameters after low dose intraperitoneal applications (namely 0.2 to 0.6 mg V/kg bw/d for 26 days) were shown for sodium metavanadate in Sprague-Dawley rats (Chandra *et al.*, 2007a; b; 2010). Significant sex organ weight reductions (testes, epididymis, prostate, seminal vesicles) without significant body weight reduction were also observed after 70 days of oral exposure to 8.4 mg V/kg/d as ammonium metavanadate (200 ppm in drinking water) in Sprague-Dawley rats. When the treated male rats were paired with unexposed females, the fertility index decreased from 95% (control) to 46.15% and the mating index from 100% to 65% (Morgan and El-Tawil, 2003). These latter results are convincing and do not point to an effect secondary to unspecific toxicity.

Overall, several studies performed with V_2O_5 (Altamirano *et al.*, 1991; Fortoul *et al.*, 2007; Bizarro-Nevares *et al.*, 2016; Mussali-Galante *et al.*, 2005; Rodríguez-Lara *et al.*, 2016) are quoted with a reliability score of 3, because only one concentration was tested.

Even if some of the studies present some limitations, mainly related to insufficient reporting on the exposure protocol design or due to the use of only one dose level which does not permit to assess the dose-response relationship, they all point to severe testicular effects.

Anyway, studies quoted with a Klimisch score 1 or 2 are available and indicate male reproductive effects: Altamirano-Lozano *et al.*, 1996 in CD-1 mice or Uche *et al.*, 2008 in guinea pigs reporting sperm count, motility and morphology impairment, spermatogonia decrease as well as the NTP, 2002 study showing decreased sperm motility in mice and atrophy of the secondary reproductive organs in rats.

Studies on ammonium metavanadate and sodium metavanadate also support the results found with divanadium pentaoxide. Studies conducted on $NaVO_3$ showed effects on sperm parameters after low dose intraperitoneal application (Chandra *et al.*, 2007b - Klimisch score 2). Although the study of Morgan and El-

Tawil applied a short period of cohabitance (5 days instead of up to 3 weeks as requested), decreased fertility and mating index were observed after oral exposure of NH_4VO_3 (Morgan and El-Tawil, 2003 - Klimisch score 3). These latter results are convincing and do not point to an effect secondary to unspecific toxicity. The read-across rationale has been considered in Annex II.

10.10.3 Comparison with the CLP criteria

For potential classification on sexual function and fertility, criteria from CLP Regulation (EC, 2017) were applied.

- *"The classification of a substance in Category 1A : "Known human reproductive toxicant" is largely based on evidence from humans"* (EC, 2017).

This category is not applicable to divanadium pentaoxide since the human data are judged inadequate.

- *"The classification of a substance in Category 1B "Presumed human reproductive toxicant" is largely based on data from animal studies. Such data shall 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"* (EC, 2017).
- Such "adverse effects on sexual function and fertility" are further described as *"any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems… 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"* (EC, 2017).

Divanadium pentaoxide impairs sperm motility from epididymis at 8 and 16 mg/m^3 in male mice. The respective exposure concentrations were associated with significant weight reductions and lung effects (NTP, 2002). Although a drastic motility reduction is needed to impact fertility in rodent, these effects on sperm are of particular relevance for (subfertile) humans. In rats exposed for 90 days to 16 mg/m^3, atrophy of the secondary reproductive organs, hypospermia of the testis and atypical cells of the epididymis were observed. However, these lesions may have been associated with the marked body weight loss and general debilitation of these rats (NTP, 2002).

Moreover, there are studies with inhalation of an aqueous aerosol of V_2O_5, which indicate, e.g., necrosis of spermatogonia, spermatocytes and Sertoli cells in the absence of overt toxicity signs or weight change (Fortoul *et al.*, 2007) and provide mechanistic data to demonstrate that V_2O_5 is able to damage the blood-testis barrier (Bizarro-Nevares *et al.*, 2016; Mussali-Galante *et al.*, 2005; Rodríguez-Lara *et al.*, 2016). Additionally vanadium concentration was drastically increased in testes after 1 week of exposure which shows that the tested compound reaches the testes (Fortoul *et al.*, 2007; Mussali-Galante *et al.*, 2005). Effects on male reproductive function were also reported after intraperitoneal administrations (Altamirano *et al.*, 1991; Altamirano-Lozano *et al.*, 1996; Uche *et al.*, 2008) although associated with signs of general toxicity for the longer duration of treatment in Altamirano-Lozano *et al.*, 1996. Overall, these consistent effects on male reproductive performance support the conclusion that they cannot be regarded as an indirect effect from other toxic action of the compound.

Regarding female reproductive performance, effects on oestrous cycle from 8 mg/m^3 (NTP, 2002) and ovulation rate (Altamirano *et al.*, 1991) were reported after exposure to V_2O_5 in rat species (no indication given about general toxicity). In the NTP (2002) study, even if general toxicity (mortality and decreased body weight) was reported at the highest concentration of 16 mg/m^3, this was not found at 8 mg/m^3.

Furthermore, study data on ammonium metavanadate after oral exposure indicate sex organ weight reductions in male, disturbing cycle, dystocia and fertility effects (reduced mating index, fertility index, with a more severe effect when only males were treated compared to only treated females) without body weight reductions (Morgan and El-Tawil, 2003). Effects on testes and sperm, associated with an oxidative stress, were also observed after intraperitoneal administration of sodium metavanadate (Chandra *et al.*, 2007a, b, c). Similarity of these observed effects between divanadium pentaoxide, ammonium metavanadate and sodium metavanadate strengthen the use of read across for effects on sexual function and fertility even though possible differences in the systemic absorption from the gastrointestinal tract may interplay. Because no cut-off criteria regarding quantitative exposure is involved in the classification for reproductive toxicant, a potential higher bioaccessibility of ammonium metavanadate (pentavalent form) or sodium metavanadate will not raise uncertainties for classification of V_2O_5 as a toxicant for development.**Therefore the criteria to classify V_2O_5 in Category 1B *"Presumed human reproductive toxicant"* are fulfilled.**

- *"Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, … 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"* (EC, 2017).

The evidence is sufficient for a classification and labelling Cat. 1B. The effects on sexual function, and fertility are clear and consistent among the available studies. The limitations reported in the studies did not question the relevance of the observed effects on sexual function and fertility. Therefore a Cat. 2 is not judged appropriate for V_2O_5.

10.10.4 Adverse effects on development

Table 26: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Studies on V_2O_5			
Prenatal developmental toxicity study 18-21 female Wistar rats per dose group Reliability (Klimisch score): 4 Original study not available	Divanadium pentaoxide, no information on purity available 0, 1, 3, 9, or 18 mg V_2O_5/kg bw/d (0, 0.56, 1.69, 5.06, 10.11 mg V/kg bw/d) from GD 6 – 15. Examination on GD 20 Application in vegetable oil *via* gavage	Dams: in the two highest dose groups significant decreases in body weight gain (75%, 40% of control values) Pups: No effects on resorptions or dead foetuses were observed At 18 mg/kg bw/d: foetal body weight decreased (87% compared to control), body length (92%) and tail length (94%) in the highest dose group (of control values, respectively) In all dose groups non-ossification or delayed ossification of the sternum was observed. In the highest dose group this was accompanied by delayed occipital ossification. Skeletal abnormalities were significantly increased in the two highest dose groups. No visceral abnormalities were reported Results are not given on a per litter basis	(Yang *et al.*, 1986, internal report as cited in Sun, 1987; WHO, 2001; Yang *et al.*, 1986)
Developmental toxicity study Female rats (no more details available) Reliability (Klimisch score): 4 Original study not available	Divanadium pentaoxide, no information on purity available 0.3, 1 or 3 mgV_2O_5/kg bw/d (0.17, 0.56, 1.69 mg V/kg bw/d) in aqueous solution *via* intraperitoneal injection to pregnant rats 9 mg V_2O_5/kg bw, orally administered No information available on the timing of exposure.	Higher number of dead and resorbed foetuses in intraperitoneally injected animals 0.3 mg/kg bw i.p. and 9 mg/kg bw oral induced an array of skeletal anomalies like wavy ribs, fused sternebrae and vertebrae, supernumerary ribs No information on maternal toxicity is provided.	Sun et al, 1987 cited from (WHO, 1988) Only limited information available

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Prenatal developmental toxicity study Pregnant Wistar rats Reliability (Klimisch score): 4 Original study not available	Divanadium pentaoxide, no information on purity available 0, 0.33, 1.0 and 3.0 mg V_2O_5/kg bw/d from GD 6 – 15. (0, 0.19, 0.56, 1.69 mg V/kg bw/d) No information on time of examination available Intraperitoneally injected	Dams: In the highest dose group maternal toxic symptoms, decrease of weight gain during treatment, decrease of placenta weight were observed (no further details available) Pups: Highest dose group: Increased incidence of embryo- foetal mortality and external or skeletal malformation Foetal growth retardation Middle dose group: Increased incidence of embryo- foetal mortality and external or skeletal malformations. Delayed ossification of bone. No information available on the lowest dose tested.	(Zhang *et al.*, 1993b) Article in Chinese, only abstract available
Prenatal developmental toxicity study Female NIH mice Reliability (Klimisch score): 4 Original study not available	Divanadium pentaoxide, no information on purity available 5 mg V_2O_5/kg bw/d on different gestation days (1-5, 6-15, 7, 8, 9, 10, 11, 14-17). (2.80 mg V/kg bw/d) No information on time of examination available Intraperitoneally injected	Dams: No effects on pre-implantation and implantation Pups: Increased frequency of resorption or foetal death for exposure during gestation days 6-15, 7, 14-17 Delayed ossification (no details available) of bones for exposure during gestation days 6-15, 8, 10, 14-17. The authors suggest, that developmental effects occurred with an A/D - ratio (adult/ developmental toxicity) of 3	(Zhang *et al.*, 1991) Article in Chinese, only abstract available
Prenatal developmental toxicity study Female Wistar rats Reliability (Klimisch score): 4 Original study not available	Divanadium pentaoxide, no information on purity available 5 mg V_2O_5 /kg bw/d on different gestation days (9, 10, and 11 and 9-12) or 3 mg V_2O_5 /kg bw/d on GD 6-15 (2.80 mg V/kg bw/d on different gestation days or 1.69 mg V/kg bw/d on GD 6-15) No information on time of examination available Intraperitoneally injected	Exposure from GD 6-15 or 9-12: (3 mg/kg/d or 5 mg/kg/d): Increased foetal mortality, decreased foetal weight and crown-rump length, retarded ossification, increased incidence of subcutaneous haemorrhage, wavy ribs, dilation of lateral ventricles and renal pelvis. Exposure on GD 11 (5 mg/kg/d): Decreased weight gain, increased incidence of subcutaneous haemorrhage and visceral anomalies Exposure on GD 10 (5 mg/kg/d): Increased incidence of foetal death, retarded ossification	(Zhang *et al.*, 1993a) Article in Chinese, only abstract available

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		Exposure on GD 9 and 10 (5 mg/kg/d): Increased incidence of subcutaneous haemorrhage and visceral anomalies. According to the authors, fetotoxic effects occurred "with or without obvious maternal toxicity", but no data on maternal toxicity are provided in the abstract	
Teratogenicity study 15 pregnant CD-1 mice in exposure group 13 animals in control group Reliability (Klimisch score): 3	Divanadium pentaoxide, purity: 99.6% 0, 8.5 mg V_2O_5 /kg bw/d from GD 6-15 (0, 4.7 mg V/kg bw/d) On GD 18 animals were sacrificed Intraperitoneal injection	Significant reduction of foetal weight/litter (control: 1.39 ± 0.05 g, 8.5 mg/kg: 1.04 ± 0.05 g) Significant change in sex ratio towards the female animals (control: 0.89/1.11, 8.5 mg/kg: 1.25/0.75) No significant effects observed on no of pregnant dams, total number of implants, mean no. of implants/litter, mean no. of live foetuses/litter, mean no. of resorptions/litter and mean no. of dead foetuses/litter. 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%) were observed, with short limbs being 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 (control: 13.45 ± 0.47, 8.5 mg/kg: 8.95 ± 0.33) and hindlimbs (control: 14.66 ± 0.52, 8.5 mg/kg: 6.25 ± 0.41) is lower. Other skeletal malformations were not increased significantly. No further information is provided on maternal toxicity	(Altamirano-Lozano *et al.*, 1993)
Developmental toxicity study Female rats (no more details available) Reliability (Klimisch score): 4 Original study not available	Divanadium pentaoxide, no information on purity available 0, 0.5, 1 or 4 mg V_2O_5/kg bw/d for 10 days from GD 7 -16, *via* subcutaneous injection (0, 0.28, 0.56, 2.25 mg V/kg bw/d)	Incidence of resorbed and dead foetuses significantly increased at 1 and 4 mg/kg bw/d (17 and 27.2% respectively, vs. 3.5% in control) In the highest dose group 52.36% of foetuses showed wavy ribs No effects at 0.5 mg/kg bw/d reported No information on maternal toxicity provided.	Sun *et al*, 1987 cited from (WHO, 1988) Only limited information available
Developmental toxicity	Divanadium pentaoxide,	No effects observed on resorption	(Wide, 1984)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
study 20 female NMRI mice in exposure group 28 animals in control group Reliability (Klimisch score): 3	analytical grade, no further information available 0, 1 mM V_2O_5 in 0.15 mL, single i.v. injection in the tail vein This corresponds to 0 and 27.3 µg V_2O_5 (0, 15.34 µg V) or 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	frequency, foetal weight, frequencies of foetal hemorrhages The number of foetuses with less mature skeletons (definition of the authors: no ossification of three of four elements (supraoccipital bone, sternum, metatarsalia, all caudal vertebrae) examined) significantly increased in exposure group (ii) (control: 30%, treated: 71%)	
Study of sex differences in the effects of divanadium pentaoxide administration to prepubertal rats CIIZ rats <u>Experiment1:</u> 5-9 animals per sex in treatment and control group (saline application) <u>Experiment 2:</u> 10 control animals (saline application), 6 in treatment group Reliability (Klimisch score): 3	Divanadium pentaoxide, no information on purity available 0, 12.5 mg V_2O_5/kg bw, intraperitoneal injection (0, 7.0 mg V/kg bw per injection) <u>Experiment 1:</u> Newborn male and female rats were treated every second day from birth to 21 days	<u>Experiment1 (males):</u> Increase in weight of seminal vesicles, thymus and submandibular glands in treatment group <u>Experiment 1 (females):</u> 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	(Altamirano *et al.*, 1991) See also Table 22
Studies on other vanadium compounds			
Ammonium metavanadate (NH_4V0_3)			
Study on fertility and prenatal developmental toxicity in exposed rats Sprague Dawley rats Male rats: n=30 (10 control; 10 group 1, 10 group 2) Female rats: n=60 (20 control; 20 group 1, 20 group 2) GLP: no	Ammonium metavanadate, no information on purity available 200 ppm in drinking water (calculated with default factors according to ECHA (2012)): male rat: 10 mg NH_4VO_3/kg bw/d, (4,35 mg V/kg bw/d) female rat: 11.43 mg NH_4VO_3/kg bx/d, 4,97 mg V/kg bw/d Sprague Dawley rats Male rats: 70 day exposure	<u>Group 1:</u> *Adult treated males:* no mortality or clinical signs of toxicity in males; no significant deviation in body weight compared to control; Sex organ weights (testes, epididymis, prostate gland, seminal vesicles) reduced, mating and fertility index reduced. No reporting on histological examinations in parental animals *Adult untreated females/dams* : Body weight/dam at termination:	**(Morgan and El-Tawil, 2003)** from (ECHA Dissemination, 2017) Details in Annex I

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Reliability (Klimisch): 3	Female rats: 61 days exposure (14 days premating, during mating, till weaning of pups (21 days of age)) <u>Group 1:</u> mating with treated males; females untreated; fertility and reproductive performance were monitored <u>Group 2:</u> mating with untreated males, females treated; fertility and reproductive performance were monitored	215.1 g vs. 252.1 g (control) Gravid uterine weight/dam: 35.50 g vs. 67.50 g (control) *foetuses/ developmental* Number of dead foetuses/dam: 1.12 vs. 0.15 (control) Number of live foetuses/dam: 3.92 vs. 11.32 (control) mean foetal body weight (PND 21): 15.02 (n=16) vs. 22.51 (n=213) (control) Live/birth index: 100% vs. 100%(control) Survival index: 90% vs. 100% (control) Viability index: 85% vs. 99.07% (control) Foetuses with *visceral* anomalies: 4/ 7 (examined, exposed male P0) vs. 3/72 (examined; control) Foetuses with *skeletal* anomalies: 8/13 (examined, exposed male P0) vs. 1/144 (examined; control) Data not provided on a litter base <u>Group 2:</u> *Adult treated females/dams* (for details, refer to Annex I): No reporting of histological examinations in parental animals Body weight/dam at termination: 209.5 g vs. 252.1 g (control) Gravid uterine weight/dam: 30.35 g vs. 67.50 g (control) *foetuses/ developmental* 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% (control) Viability index: 74.28% vs. 99.07% (control)	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Foetuses with *visceral* anomalies: 9/ 12 (examined, exposed female P0) vs. 3/72 (examined; control) Foetuses with *skeletal* anomalies: 15/23 (examined, exposed female P0) vs. 1/144 (examined; control) Data not provided on a litter base	
Developmental toxicity study on ammonium metavanadate NH_4VO_3 Reliability (Klimisch): 2	Ammonium metavanadate (purity: 99%) Syrian golden hamster Treatment from gestation 5-10 day 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) Intraperitoneally 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)
Sodium metavanadate $NaVO_3$			
Reproductive toxicity study Reliability (Klimisch score): 3	Sprague-DawIey albino rat Sodium metavanadate (purity not given). Males: 60d before mating; Females: 14d before mating, throughout gestation and lactation Dosing: 0, 5, 10, 20 mg $NaVO_3$ /kg/day by intra-gastric administration.	Maternal effects: No adverse effects 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/d $NaVO_3$ when compared to the control group. But these increases were not significant ($P > 0.05$). In animals allowed to birth, the development of the offspring was always significantly decreased from birth and during all the lactation period for animals treated at 10 and 20 mg/kg/d. Significant decreases in the relative weights of liver, spleen and kidneys of the pups whose mothers received $NaVO_3$ during the lactation from 5 mg/kg/day. Decreases in body weight, body length, tail length were also observed in offsprings. NOAEL (developmental toxicity): 5 mg $NaVO_3$/kg/d (2.1 mg V/kg/d).	(Domingo *et al.*, 1986)
Developmental toxicity study Reliability (Klimisch score): 3	Pregnant female Sprague-Dawley rat. Sodium metavanadate (purity not given).	Maternal effects: No significant adverse effects Developmental effects: Number of litters 14, 14, 12, 8.	(Paternain *et al.*, 1987)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	Exposure from GD 6-14 with cesarean sections performed at day 20. Dosing: 0, 5, 10, 20 mg N_aVO_3/ kg /d (0, 2.1, 4.2, 8.4 mg V/kg/d in distilled water). Intragastrically	Increased number of abnormal foetuses was observed from 5 mg/kg bw/d (non-dose-response-related). From 10 mg/kg/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 in foetuses from treated dams at 20 mg/kg/d was remarkably higher compared to the control group. At high dose only: -hydrocephaly (2(2)/98 foetuses) vs. 0(0) among 196 foetuses. -hemorrhage in facial area (18(18) among 98 foetuses) vs.2(1) among 196 foetuses -hemorrhage in dorsal area (10(10) among 98 foetuses vs.2(1) among 196 foetuses. NOAEL for teratogenicity: 10 mg $NaVO_3$/kg bw/d (8.4 mg V/kg bw/d). WHO (2001): "no clear evidence of direct developmental toxicity"	
Developmental toxicity study Reliability (Klimisch score): 3	Pregnant female Swiss mice Sodium metavanadate (N_aVO_3) – analytical grade obtained from Sigma Chemical Co. (purity not given). Exposure from GD 6-15 Dosing: 0, 2, 4, 8 and 16 mg N_aVO_3/ kg /d (0, 0.8, 1.7, 3.3 mg V/kg/d) Intraperitoneally	Because of the excessive maternal mortality (92%) at 16 mg/kg/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 (namely 2 mg/kg bw/d). However the body weight of mothers at sacrifice *minus* gravid uterine weight was not statistically significantly affected compared to the control group. Development effects: Reduced foetal weight, increased embryo and foetolethality with a reduced number of live foetuses per litter (4 and 8 mg/kg/d), cleft palate statistically significant at high dose with an apparent dose-response relationship for cleft palate across all	(Gómez *et al.*, 1992)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		doses. LOAEL: 2 mg NaVO3/kg/d (1.7 mg V/kg/d) *via* IP	
Behavioural test and mechanistic study on early postnatal sensitivity for neurological impairments in the rat Reliability (Klimisch score): 3	Sprague-Dawley rat pups (n=5) were injected 3 mg $NaVO_3$/kg bw/day (1st PND-14th PND) intraperitoneally; Litter mate controls (n=5) received PBS. Sodium metavanadate (purity not provided) PND15: Rotarod test of pups Survival analysis (in vitro) of oligodendrocyte progenitor cells (OPCs) vs. mature oligodendrocytes vs. astrocytes, with increasing $NaVO_3$ concentration Background note: Iron is imported to brain *via* oligodendrocytes and OPCs. The 2nd postnatal week corresponds to an intense oligodendrocyte development, myelination and period for peak iron transport. OPCs are known to be sensitive for metals interfering with iron homeostasis.	Behavioural: exposed significantly impaired motor functioning in Rotarod test ($p < 0.01$) In vitro survival sensitivity: OPCs > mature oligodendrocytes > astrocytes Depletion of OPCs from $NaVO_3$. Significant influence of iron chelator on OPCs viability. ➔ vanadium OPCs interaction suggested as important mechanism of hypomyelination	(Todorich *et al.*, 2011)

Table 27: Summary table of human data on adverse effects on development

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Nested case control study (environmental vanadium pollution)	Vanadium, speciation not provided	Cases: n = 204 low birth weight (LBW) cases; 612 matched controls	2.23-fold increase (95% CI: 1.23, 4.05) of Odds Ratio for low birth weight (LBW) in mothers with a urinary vanadium of ≥ 2.91 µg V/g creatinine compared to controls (≤ 1.42 µg V/g creatinine); correlation trend $p < 0.02$ Still significant, if preterm births excluded. Unchanged in multivariable	(Jiang *et al.*, 2016)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			logistic regression including other metals (arsenic, lead, cadmium and nickel) as potential reasons for LBW Vanadium was measured just once during pregnancy and may not be representative for the entire pregnancy period	

From recent canadian environmental data in Canada, vanadium level exposure of general population is lower than 0.1 µg/g creatinine (median)[12]. According to occupational biomonitoring, it is suggested that 25 µg V/m³ can be correlated with 35 µg V/g creatinine (Schaller, 2007). Therefore, background exposure in the study by Jiang *et al.* is at the high end of environmental exposures. The authors consider environmental pollution as source of vanadium.

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

Human epidemiological data raise some concern about vanadium induced low birth weight (Jiang *et al.*, 2016). However, the observations cannot clearly be related to V_2O_5 (speciation not provided).

Available studies with V_2O_5 regarding developmental effects (Table 26) cannot be used directly for classification, due to insufficient reporting and/or non-physiological route of administration (e.g.: IP, IV or sub-cutaneous administration). The gavage study by Yang *et al.* (1986) indicates delayed ossifications in rats orally exposed to 1 and 3 mg V_2O_5/kg bw/d (i.e. ≥ 0.6 mg V/kg bw/d). Decrease in fetal body weight, body length and tail length was also reported at 18 mg/kg bw/day. More severe developmental effects, including embryofetal mortality and malformations, were observed after i.p. administration by Zhang *et al.* (1993a; 1993b) and Altamirano-Lozano *et al.*, (1993). Experimental results with subcutaneous application of V_2O_5 (Sun, 1987) described similar fetotoxic effects. In some studies, it is not clearly reported whether effects occurred at or below maternally toxic doses and whether the observed effects should be regarded as secondary to effects in the dam. Zhang *et al.* (1991) report fetotoxicity at lower concentrations compared to the adult (A/D ratio close to 3) and Zhang *et al.* (1993a) report developmental effects "with and without maternal toxicity". It should be noted that the developmental effects (increased incidence of embryo- foetal

[12] http://www.ec.gc.ca/ese-ees/B0FA951B-51CE-451F-B561-B48DDEE05BD4/SciAD%20Biomonitoring%20Based%20Approach%201%20EN.pdf assessed at 31.1.2017

mortality and external or skeletal malformation, delayed ossification of bone and decreased placenta weight) reported by Zhang *et al.* (1993b) at the middle dose were not associated with maternal toxicity. However, as indicated above, reporting quality is insufficient to base classification decisions on one of these studies on V_2O_5. Study results have to be included into "weight of evidence" considerations.

One study, on reproductive and developmental toxicity of **ammonium metavanadate** has been published (Morgan and El-Tawil, 2003). The authors demonstrate serious foetotoxic and developmental effects in rats after oral exposure to NH_4VO_3 including increased foeto-lethality, reduced foetal birth weight, and gross, skeletal and visceral anomalies, some of which are to be regarded as malformations or abnormalities causing high concern (Moore *et al.*, 2013). Furthermore, in the study by Morgan and El-Tawil (2003), the pups behavioural responses (learning and memory responses) and survival and viability indices were decreased. All these effects occurred in a similar way when parent males or females had been treated with ammonium metavanadate. The authors discuss that this effect could be a "consequence of the vanadium from milk" since treated dams were exposed until post-natal day 21.

Even though the results from the study by Morgan and El-Tawil (2003) are convincing and do not point to a secondary effect, the reliability of this study has to be rated category 3 ("not reliable"), because of major deficiencies in methodology and significant deviations from OECD guideline standards: OECD 415 or OECD 414, respectively, demands a sample size of 20 pregnant females (this study: 6 or 10 for both exposure groups – however, since effects are already reported in this small sample, this deficiency is not considered as major). At least three dose groups (or a limit test) are requested (this study: 1 dose group). At least clinical observations on dams (if not histopathological examinations) should have been provided. Effects on the foetuses were not reported with attribution to the litters. Therefore, potential clusters cannot be excluded. Impaired learning and memory responses are reported in the results section, but no methodology and details of the outcome are provided. No analytics on the drinking water vanadium concentration is given (not explicitly demanded, but quality criterion). Finally, the purity of the tested substance is not given.

However, from the study results it can be concluded that foetotoxic effects occurred in absence of unspecific parental toxicity. In addition, the effects reported in this study are consistent with those found with V_2O_5. Therefore, this study can be used in a weight of evidence approach to conclude that vanadium compounds induce developmental toxicity.

Other studies on reproductive and developmental toxicity have been published on **sodium metavanadate** ($NaVO_3$) in Sprague Dawley rat by oral route (Domingo *et al.*, 1986; Paternain *et al.*, 1987) and by intraperitoneal route (Todorich *et al.*, 2011) and in Swiss mice by intraperitoneal route (Gómez *et al.*, 1992). These studies were quoted with a Klimisch score of 3 mainly due to the absence of indication given on the purity of the tested compound or the route of administration chosen namely intraperitoneal route. Incidence of fetal visceral abnormalities was increased from 5 mg/kg bw/d without a dose-response relationship.

However, it should be noticed the highly increased of fetal visceral abnormalities (Paternain *et al.*, 1987) at 20 mg/kg/d with hydrocephaly, facial and dorsal hemorrhage without any significant maternal adverse effects. In Domingo *et al.*, 1986, in animals allowed to birth, the development of the offspring was always significantly decreased from birth and during all the lactation period for animals treated from 10 mg/kg/d. Incidence of living young per litter was statistically decreased but at 10 mg/kg/d only a non-statistically significant increase in dead fetuses and resorption was also reported from 10 mg/kg bw/day in animals sacrificed on gestation day 14. Lastly, developmental effects were also observed by Gómez *et al.*, (1992) with a reduced foetal weight, an increased embryo and foetolethality - with a reduced number of live foetuses per litter from 4 mg/kg/d) - and cleft palate statistically significant at 8 mg/kg bw/d with an apparent dose-response relationship across all doses.

10.10.6 Comparison with the CLP criteria

For potential classification on developmental toxicity, criteria from CLP Regulation (EC, 2017) were applied.

- *The classification of a substance in Category 1A : "Known human reproductive toxicant" is largely based on evidence from humans* (EC, 2017).

This category is not applicable to divanadium pentaoxide since there is no adequate human data.

- *"The classification of a substance in … Category 1B is largely based on data from animal studies. Such data shall 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"* (EC, 2017).

Developmental effects (including decreased foetal body weight, embryofoetal mortality and malformations) were reported with V_2O_5 in studies of low reliability (Klimisch score 3 or 4). Observations from the experimental drinking water study with ammonium metavanadate (NH_4V0_3) (Morgan and El-Tawil, 2003) also demonstrate adverse developmental effects (e.g., number of dead foetuses/dam, significantly reduced foetal body weight, increased incidences of visceral and skeletal anomalies in the foetus, partly to be classified as malformations). Observations from the oral reproductive toxicity study (Domingo *et al.*, 1986) with sodium metavanadate (N_aVO_3) showed in animals allowed to birth, a significant developmental decrease (decreases in body weight, body length, tail length) of the young from birth and during all the lactation period. Additionally, developmental effects were also observed by Gómez *et al.*, (1992) with reduced foetal weight, increased embryo/foetolethality and statistically significant increased incidence of cleft palate. Those effects are considered not to be secondary to other toxic effects (see further details below).

Similarity of these observed developmental effects with ammonium and sodium metavanadate but also with divanadium pentaoxide strengthen the use of read across for developmental effects even though possible differences in the systemic absorption from the gastrointestinal tract may interplay. Because no cut-off criteria regarding quantitative exposure is involved in the classification for reproductive toxicant, a potential higher bioaccessibility of ammonium metavanadate (pentavalent form) or sodium metavanadate will not raise uncertainties for classification of V_2O_5 as a toxicant for development. Consistent developmental effects in studies performed with V_2O_5 and ammonium metavanadate or sodium metavanadate support this statement. However, the low reliability of the available studies do not allow a classification in Category 1B. A Category 2 is judged more appropriate.

- *Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on … development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects* (EC, 2017) .

All the available studies present methodological limitations. However as most of the developmental effects – including mortality and malformation - are consistent between the different experiments, a Category 2 for developmental effects is judged the most appropriate classification proposal which is consistent with the current harmonized classification of V_2O_5. **Therefore, the current harmonised classification as Repro. Cat 2 for development is still justified and should be maintained.**

Regarding maternal toxicity, the CLP Regulation provides additional comments:

- *"Based on pragmatic observation, maternal toxicity may, depending on severity, influence development via non-specific secondary mechanisms, producing effects such as depressed foetal weight, retarded ossification, and possibly resorptions and certain malformations in some strains of certain species. However, the limited number of studies which have investigated the relationship between developmental effects and general maternal toxicity have failed to demonstrate a consistent, reproducible relationship across species. Developmental effects, which occur even in the presence of maternal toxicity, are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic*

effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies" (EC, 2017).

In most studies, it is not clearly demonstrated whether effects occurred at or below maternally toxic doses and if the observed effects should be regarded as secondary to effects in the dam. No information on maternal general toxicity was reported by Sun *et al.* (1987), Zhan *et al.* (1993a); Altamirano-Lozano *et al.* (1993) and Wide (1984). Developmental effects reported by Yang *et al.* (1986) with V_2O_5 occurred in the presence of decreased body weight gain (40% of control). However, the developmental effects (increased incidence of embryo- foetal mortality and external or skeletal malformation, delayed ossification of bone and decreased placenta weight) reported by Zhang *et al.* (1993b) with V_2O_5 at the middle dose were not associated with maternal toxicity. Zhang *et al.* (1991) suggest that developmental effects occurred with an A/D - ratio (adult/ developmental toxicity) of 3.

Developmental effects in absence of parental effects in the study by Morgan and El-Tawil (2003) performed with ammonium metavanadate are highly probable, but are not definitely shown. In particular, it can be noted that developmental effects (including lethality and abnormalities) were also reported in the group where dams were not treated (only males treated). Minor but significant abnormalities occurred in the absence of maternal effect in Carlton *et al.* (1982) study performed with ammonium metavanadate.

Regarding exposure to sodium metavanadate, no maternal toxicity reported in Domingo *et al.,* 1986 and Paternain *et al.,* 1987 studies. Excessive maternal toxicity was noted at 16 mg/kg bw/day in Gómez *et al.*, 1992 study, so this dose was excluded from further analysis. Developmental effects reported at lower concentration in this study occurred in the presence of decreased maternal body weight gain. Although a maternal weight gain was decreased from 2 mg/kg bw/d associated with a statistically significant decrease of the gravid uterine weight, when the body weight of mothers at sacrifice was corrected by gravid uterine weight, it was not statistically significantly affected compared to the control group. Therefore these severe developmental effects (lethality and cleft palate) cannot be considered consecutive to maternal toxicity. Overall, considering the severity of the developmental effects reported with both V_2O_5 and ammonium metavanadate, it is considered unlikely that they were secondary to maternal toxicity.

- *"Classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally-mediated mechanism has been demonstrated. In such a case, classification in Category 2 may be considered more appropriate than Category 1. However, when a substance is so toxic that maternal death or severe inanition results, or the dams are prostrate and incapable of nursing the pups, it is reasonable to assume that developmental toxicity is produced solely as a secondary consequence of maternal toxicity and discount the developmental effects"* (EC, 2017).

Observed developmental toxicity occurred in ranges well below maternal death and below any indications of severe inanition.

- *Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity* (EC, 2017).

Severe developmental effects were observed with V_2O_5, ammonium metavanadate (e.g., visceral and skeletal abnormalities, some of which may be regarded as malformations) and sodium metavanadate (e.g. lethality, visceral abnormalities and cleft palate) and no maternal toxicity is to be assumed as a relevant factor for the observed effects.

ECHA further comments: "*Adverse effects on postnatal survival and growth seen only at dose levels causing maternal toxicity may be due to lack of maternal care or other causes such as adverse effects on or via lactation or developmental toxicity.""…(ECHA Guidance, 2015).*

In the study by Morgan and El-Tawil (2003) some postnatal effects (altered behavioural responses (learning and memory responses) and decrease of survival and viability indices) were described and discussed by the authors as possibly induced *via* lactation exposure. In fact, in addition to the effects described above, focusing on developmental toxicity with prenatal or early postnatal effects, further postnatal effects from ammonium metavanadate and sodium metavanadate are regarded as "adverse effects *via* lactation" and are discussed separately (Section 10.10.7). However, prenatal developmental toxicity studies are available and show foetal or embryo toxic effects consecutive to *in utero* exposure only. This is thus to be covered by classification as Reprotoxic for development.

10.10.7 Adverse effects on or *via* lactation

Some data are available with pentavanadate (no further specified) (Edel and Sabbioni, 1989) and with sodium and ammonium metavanadate for which a read-across with V_2O_5 is considered acceptable.

Table 28: Summary table of animal studies on effects on or *via* lactation

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Sodium metavanadate ($NaVO_3$)			
Study on postnatal developmental toxicity in suckling	Sodium metavanadate, no information on purity available 0, 3 mg $NaVO_3$/kg bw	Eye opening mean day in treated animals was significantly delayed. A progressive decrease in righting time reflex	(Soazo and Garcia, 2007) Details in

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
rats New-born Wistar rats grouped in litters of 8 pups/dam (4 male and 4 female pups). 4 dams and their pups in exposure group and control group, respectively Reliability (Klimisch score): 3	(1.25 mg V/kg bw/d) intraperitoneal injection to dams from PND 10 -21 Pups were observed for behavioural alterations until PND 21. Pups were sacrificed on PND 21 and microscopic analysis of brain tissue was performed	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.	Annex I.
Study on postnatal developmental toxicity in suckling rats New-born Wistar rats grouped in litters of 8 animals/dam (4 male and 4 female pups). 4 dams and their pups in exposure group and control group, respectively Reliability (Klimisch score): 3	Sodium metavanadate, no information on purity available 0, 3 mg $NaVO_3$ /kg bw (1.25 mg V/kg bw/d) intraperitoneal injection to dams from PND 10 - 21 Pups were sacrificed on PND 21 and biochemical and microscopic analysis was performed	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 → Signs of oxidative damage in the brain	(Cuesta *et al.*, 2013) Details in Annex I.
Study on neurobehavioral toxicity in weaned rats after lactational exposure and mode of action Sprague-Dawley rats; Dams (n=12) received $NaVO_3$ intraperitoneally or distilled water 36 male pups; Reliability (Klimisch score): 3	Sodium metavanadate, no information on purity available 0, 3 mg $NaVO_3$ /kg bw (1.25 mg V/kg bw/d), intraperitoneal injection to dams from PND 1 - 21, pups were exposed *via* lactation Pups were tested in the rotarod test (motor coordination) at PND60. Expression of the brain-derived neurotrophic factor (BDNF) and its tropomyosin-related kinase B (TrkB) receptor were analysed, with or without prior treadmill running as exercise.	Performance in rotarod test (motor coordination) was significantly impaired in weaned male rats after lactational exposure to $NaVO_3$ (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)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Postnatal developmental toxicity study with nursing mice 2 nursing CD-1 mice with their pups/ group Each dam had an average of 6 pups Reliability (Klimisch score): 3	Sodium metavanadate, no information on purity available 0, 3 mg $NaVO_3$ /kg bw/d, (1.25 mg V/kg bw/d) intraperitoneally injected to dams for 14 days or 21 days starting on PND 1 Mice were sacrificed on PND 15 or 22 Control groups for 14 days and 21 days exposures (received sterile water, i.p.)	In behavioural 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) Details in Annex I
Postnatal developmental toxicity study with nursing mice 1 nursing Albino rat with its pups/ group (no information on number of pups available) Reliability (Klimisch score): 3	Sodium metavanadate, no information on purity available 0, 3 mg $NaVO_3$ /kg bw/d, (1.25 mg V/kg bw/d) intraperitoneally 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 analysis showed reactive astrogliosis induced by vanadium exposure.	(Olopade *et al.*, 2011)
Ammonium vanadate (NH_4VO_3)			

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Study on fertility and prenatal developmental toxicity in exposed rats Sprague Dawley rats Male rats: n=30 (10 control; 10 group 1, 10 group 2) Female rats: n=60 (20 control; 20 group 1, 20 group 2) GLP: no Reliability (Klimisch): 3	Ammonium metavanadate, no information on purity available 200 ppm in drinking water (calculated with default factors according to ECHA (2012)): male rat: 10 mg NH_4VO_3/kg bw/d, (4,35 mg V/kg bw/d) female rat: 11.43 mg NH_4VO_3/kg bx/d, 4,97 mg V/kg bw/d Sprague Dawley rats Male rats: 70 day exposure Female rats: 61 days exposure (14 days premating, during mating, till weaning of pups (21 days of age)) <u>Group 1:</u> mating with treated males; females untreated; fertility and reproductive performance were monitored <u>Group 2:</u> mating with untreated males, females treated; fertility and reproductive performance were monitored	<u>Group 2</u>: Adult treated females/dams During lactation, the pups behavioral responses (such as learning and memory responses) were decreased. Results for group 1 are not relevant for this section related to effect on or *via* lactation. The results are nevertheless detailed in section related to effect on fertility and development.	(Morgan and El-Tawil, 2003) Details in Annex I.
Pentavanadate (no further specified)			
Kinetic study in rats Reliability (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: 34 ng ^{48}V/g x 10^3 milk content. 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 much 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 10^3 (weanling); kidney: 4.7±0.87 (suckling) vs. 1.12 ± 0.26 (weanling)	(Edel and Sabbioni, 1989)

Table 29: Summary table of human data on effects on or *via* lactation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No human data are available				

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

There are no human data available on either divanadium pentaoxide or other vanadium compounds to address this exposure pathway and respective effects to the infant.

Experimental data are available with pentavanadate (Edel and Sabbioni, 1989) addressing milk excretion or with other vanadium compounds such as sodium and ammonium metavanadate (for which a read-across with V_2O_5 is considered acceptable on effects on lactation or *via* lactation). These studies identified neonates or sucklings as sensitive exposure group.

As demonstrated in Table 28, five studies have been performed with $NaVO_3$, in which the nursing dams (rats as well as mice) were exposed by intraperitoneal injection of 3 mg $NaVO_3$/kg bw (different durations). Suckling pups were only exposed *via* lactation. Therefore, even if maternal exposure from intraperitoneal route may be higher than it would be from physiological application, the relevant pathway of exposure to the neonate *via* suckling, i.e., physiological uptake, remains. Relevant indication of general (unspecific) toxicity is neither reported from nursing dams nor from sucklings.

All those studies demonstrate deficits of the sucklings in behavioural tests and show myelin damage and/or astrogliosis in the brain of the exposed animals. Even though most of the studies are limited in study design and/or in reporting (1 dose only, purity of the applied compound not given, no documentation of general – unspecific – toxicity), based on weight of evidence, these observations provide clear evidence of an adverse effect of sodium metavanadate to the pup *via* lactation.

Similar effects (behavioral changes, myelin damage and astrogliosis) have been observed if the newborn was directly exposed *via* intraperitoneal injection to sodium metavanadate (Todorich *et al.*, 2011) or if adult rodents were treated accordingly (García *et al.*, 2004) see Table 30.

Similar results were also provided in further studies on this substance (Aschner *et al.*, 2010; Azeez *et al.*, 2016; Folarin *et al.*, 2017a; Folarin *et al.*, 2017b; Sun *et al.*, 2017; Usende *et al.*, 2016 - not further detailed in the present CLH report).

Thus, these results support that effects observed *via* lactation are actually related to vanadium exposure of neonates.

Based on kinetic data with ^{48}V-pentavanadate, the suckling absorbs higher fractions of the vanadate compared to the adult (Edel and Sabbioni, 1989) and therefore should be assumed to be at higher risk.

These studies are consistent with regard to the mode of action of the observed neurotoxicity (e.g., the relevance of the myelin damage and the interference with iron homeostasis) and the specific higher sensitivity of the newborn, e.g., because of the maturation of the oligodendrocytes during this period (Todorich *et al.*, 2011) or the differences in toxicokinetics between neonates and adults (Edel and Sabbioni, 1989).

Elfant and Keen (1987) report an increased vanadium absorption from the gastrointestinal tract of the suckling, "*bioavailability of vanadium added to milk is greater than 70% in suckling rat pups"*. However, the authors do not provide the data or a reference to support this observation. Edel and Sabbioni (1989) demonstrate that vanadium is transported to the brain complexed with lactoferrin and discuss possible reasons why higher levels of vanadium were observed in young rat tissues. Similar to Elfant and Keen (1987), they consider a higher gastro-intestinal absorption either specifically from milk or a greater non-selective permeability of the undeveloped intestinal barrier in young animals. Furthermore, the authors discuss a possible higher retention capacity of the undeveloped tissues for vanadium (Edel *et al.*, 1984).

Even though there are few data on the concentration of vanadium in milk, human data indicate that this exposure pathway is relevant. Anke (2004) reports that "*lactating women secret 17% of their vanadium intake into the milk*". However, no detailed sources for this figure are provided. Overall, the observed effect data on sodium metavanadate and the kinetics from animal studies at least on pentavanadate indicate that this pathway is relevant for humans.

Most data documented above have been reported for sodium metavanadate. Few studies on neurotoxicity have been performed with V_2O_5, also indicating neurotoxic potential (Avila-Costa *et al.*, 2005; Colín-Barenque *et al.*, 2008; Colín-Barenque *et al.*, 2015; Fortoul *et al.*, 2014). Those studies are not discussed within this section, because they are based on observations with adult animals and not with neonate's exposure. However, in those studies with adult animals exposed to divanadium pentaoxide, identical brain areas (most relevant: hippocampus) are altered as with sodium metavanadate (but there are no mechanistic studies comparing neurotoxicity of $NaVO_3$ and V_2O_5).

In a study on ammonium metavanadate presented above addressing developmental toxicity (Morgan and El-Tawil, 2003; see section 10.10.4), the authors also discuss a higher sensitivity of the sucklings to vanadium effects and specifically mention "*during lactation, the pups behavioral responses (such as learning and memory responses)…were decreased*". However, they provide no data to support this observation. From the study design of this study (exposure of the dam prior to and during pregnancy and during lactation) it may not firmly be concluded that effects to the neonates were only directly *via* mother's milk.

10.10.9 Comparison with the CLP criteria

Based on the criteria from CLP Regulation (EC, 2017) supported by guidance on the application of the CLP criteria (version 5.0; July 2017), classification for adverse effects on or *via* lactation was assigned:

- *Ideally, studies will be available which inform directly on whether the substance causes adverse effects in the offspring due to an adverse effect on lactation. One generation or multi-generation reproductive toxicity studies, which involve direct exposure or exposure via the milk of the offspring postnatally, usually provide information on this*.
- The CLP Regulation just defines: "*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*" (EC, 2017) as one of the classification criteria.

For sodium metavanadate, adverse effects in the offspring due to an exposure *via* lactation have been directly demonstrated, as nursing dams were only exposed during lactation to the pentavalent vanadium compound and effects (from behavioural testing and immunohistochemical or histological analyses) have been shown in the pups, which were only exposed *via* lactation (Cuesta *et al.*, 2013; Mustapha *et al.*, 2014; Olopade *et al.*, 2011; Soazo and Garcia, 2007; Wang *et al.*, 2015).

- The CLP Regulation just defines: "*absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk*" (EC, 2017) as one of the classification criteria.
- *"There may be toxicokinetic and toxicodynamic reasons why neonates may potentially be more or less vulnerable to a particular adverse effect than adults…. Therefore, the relative sensitivity of neonates and adults to a substance must be judged on a case by case basis using expert judgement"* .

Evidence is provided that absorption of vanadium *via* milk in neonates is higher than oral absorption of vanadium in water or foodstuff in adults (Edel and Sabbioni, 1989). Additional evidence of bioavailability of vanadium from milk is reported by Elfant and Keen (1987) and Anke (2004).

Moreover, mechanistic studies show that oligodendrocyte progenitor cells develop during a period, when exposure *via* lactation is the only significant route of exposure to the offspring. These progenitor cells are specifically sensitive to vanadium interference during maturation (Todorich *et al.*, 2011).

- The CLP Regulation defines: "*Substances,… 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*" (EC, 2017) as one of the classification criteria.

- *"The mere presence of the substance in the milk alone, without a strong justification for a concern to offspring, would normally not support classification for effects on or via lactation"* .

Behavioural effects and oxidative brain damage reported in the newborns *via* exposure by suckling are regarded as serious health impact. Thus this criterion for non-classification is not relevant in this case.

- *"The criterion indicates that toxicokinetic studies showing that the substance can be present at potentially toxic levels in breast milk can support classification. The implicit assumption behind this clause is that the pups may receive a body burden of the toxic entity through suckling that is sufficient to cause toxicity when the level of the toxic entity in the milk is above a certain threshold level ('a level to cause concern'). There is no robust way to estimate what this threshold is, although the likely body burden expected in the breastfed child may be compared to the toxicity data in adults (e.g. an appropriate NOAEL or BMD) to indicate whether toxicity is likely"*.

Toxicokinetic studies (Edel and Sabbioni, 1989; Elfant and Keen, 1987) and human data (Anke, 2004) indicate that vanadium is transferred to human milk and that relevant absorption occurs. However, absolute effective doses and a dose response relationship have not been established. Potency comparisons to similar doses in the adult have not been performed.

- *"The type and magnitude of the maternal effects and their potential influence on lactation/milk production need to be considered on a case-by-case basis to determine whether classification for effects on or via lactation is necessary"*.

Usually, only one dose has been used for the respective experimental studies either 3 mg/kg bw ($NaVO_3$) *via* intraperitoneal injection in which there is no information on maternal toxicity or 100 ppm (NH_4VO_3) in drinking water to the nursing dam which does not lead to maternal over toxicity.

- *"It should also be noted that some developmental effects resulting from exposure in utero would only manifest postnatally and those should not be used for classification for effects"*.

It could be argued that behavioural effects in the neonate may be due to exposure to sodium or ammonium metavanadate *in utero*, as is possibly the case in the study by Morgan and El-Tawil (2003). However, in the other studies selected for justification of this classification, there was no *in utero* exposure of the suckling since the dams were only exposed during lactation (Cuesta *et al.*, 2013; Mustapha *et al.*, 2014; Olopade *et al.*, 2011; Soazo and Garcia, 2007; Wang *et al.*, 2015). Therefore, this criterion for non-classification is not relevant.

- *"If there is robust evidence to indicate that the effects on lactation are not caused directly by the substance then it should not be classified as such"*.

There is clear evidence that effects in newborns are due to exposure to sodium metavanadate *via* lactation. From general similarity considerations it may be assumed that those effects would also occur from exposure to divanadium pentaoxide. Because no cut-off criteria regarding quantitative exposure is involved in the classification for lactation, a potential higher bioaccessibility of vanadium compounds with sodium metavanadate (pentavalent form) will not raise uncertainties for classification of V_2O_5 for lactation.

However, uncertainties from read-across have to be considered (for further discussion see Annex II, Justification for read across). These uncertainties may lead to potency differences for effects *via* lactation from divanadium pentaoxide compared to sodium metavanadate, which, however, do not eliminate the human relevance of this hazard.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Classification for reproductive toxicity addresses adverse effects on sexual function and fertility, developmental effects and adverse effects on or *via* lactation.

Adverse effects on sexual function and fertility

Divanadium pentaoxide impairs sperm motility from epididymis at 8 and 16 mg/m^3 in male mice exposed for 90 days (NTP, 2002). The respective exposure concentrations were associated with significant weight reductions and lung effects. Although a drastic motility reduction is needed to impact fertility in rodent, these effects on sperm are of particular relevance for (subfertile) humans. Moreover, there are studies with inhalation of an aqueous aerosol of V_2O_5, which indicate severe testicular effects, e.g., necrosis of spermatogonia, spermatocytes and Sertoli cells (Fortoul *et al.*, 2007) in the absence of overt toxicity signs or weight change and provide mechanistic data to demonstrate that V_2O_5 is able to damage the blood-testis barrier (Bizarro-Nevares *et al.*, 2016; Mussali-Galante *et al.*, 2005; Rodríguez-Lara *et al.*, 2016). Additionally vanadium concentration was drastically increased in testes after 1 week of exposure which shows that the tested compound reaches the testes (Fortoul *et al.*, 2007; Mussali-Galante *et al.*, 2005). Effects on male reproductive function were also reported after intraperitoneal administrations (Altamirano *et al.*, 1991; Altamirano-Lozano *et al.*, 1996; Uche *et al.*, 2008). Therefore, these consistent effects on male reproductive performance support the conclusion that they cannot be regarded as an indirect effect from other toxic action of the compound.

Regarding female reproductive performance, effects on oestrous cycle (NTP, 2002) and on ovulation rate (Altamirano *et al.*, 1991) were reported after exposure to V_2O_5 in rat species.

Furthermore, study data on ammonium metavanadate after oral exposure indicate sex organ weight reductions in male, disturbing cycle, dystocia and fertility effects (reduced mating index, fertility index, with a more severe effect when only males were treated compared to only treated females) without body weight

reductions (Morgan and El-Tawil, 2003). Effects on testes and sperm, associated with an oxidative stress, were also observed after intraperitoneal administration of sodium metavanadate (Chandra *et al.*, 2007a, b, c). **A classification of V_2O_5 in Category 1B - H360F *"Presumed human reproductive toxicant"* is therefore warranted.**

Adverse effects on development

Available studies with V_2O_5 regarding developmental effects reported various effects, including foetal mortality and malformations. However, they cannot be used directly for classification, because of insufficient reporting, and/or quality deficiencies. Instead, a relevant study on reproductive and developmental toxicity of ammonium metavanadate *via* drinking water is available (Morgan and El-Tawil, 2003). Even though no histopathology on parental animals is provided, this study demonstrates adverse developmental effects (e.g., number of dead foetuses/dam, significantly reduced foetal body weight, increased incidences of visceral and skeletal anomalies in the foetus, partly to be classified as malformations). Developmental effects were also reported after exposure to sodium metavanadate. Developmental effects are considered not to be secondary to other toxic effects, since no overt maternal toxicity is generally reported, when the data is available. When some maternal toxicity is reported, it cannot explain the severe developmental effect. Similarity of the observed developmental effects with ammonium and sodium metavanadate but also with divanadium pentaoxide strengthen the use of read across for developmental effects even though possible differences in the systemic absorption from the gastrointestinal tract may interplay. Because no cut-off criteria regarding quantitative exposure is involved in the classification for reproductive toxicant, a potential higher bioaccessibility of ammonium metavanadate (pentavalent form) or sodium metavanadate will not raise uncertainties for classification of V_2O_5 as a toxicant for development. However, the low reliability of the available studies does not allow a classification in Category 1B but rather a **Category 2 – H361d**, which is in line with the current harmonized classification of V_2O_5.

In conclusion, because of observed adverse effects of divanadium pentaoxide on sexual function and fertility and on developmental toxicity, supported by the consistent effects reported with ammonium and sodium metavanadate, a classification to **Repr. 1B, H360Fd** is warranted.

Adverse effects on or *via* lactation

There are no data on adverse effects on or *via* lactation available for divanadium pentaoxide. However, adverse effects directly *via* lactation were observed for sodium metavanadate, where offsprings demonstrated impairment in behavioral tests with evidence of myelin damage and astrogliosis (Cuesta *et al.*, 2013; Mustapha *et al.*, 2014; Olopade *et al.*, 2011; Soazo and Garcia, 2007; Wang *et al.*, 2015). In those studies the

dam received sodium metavanadate only after parturition *via* intraperitoneal application, ensuring that the suckling was not exposed prenatally but only *via* suckling. Even if such data on neonates is not available with V_2O_5, neurotoxic potential is reported in adults, supporting the relevance of the effects observed with sodium metavanadate. The observed effect are also strengthened by mechanistic understanding (Garcia *et al.*, 2005; García *et al.*, 2004; Todorich *et al.*, 2011) and toxicokinetic plausibility (Edel and Sabbioni, 1989).

A graduation (suspected or confirmed properties) is not available for this endpoint and read across is well supported from (structural and toxicological) similarity criteria. Therefore, for divanadium pentaoxide classification as **H362: May cause harm to breast-fed children** is warranted.

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.

Additional key elements

Read-across approach used for reproductive endpoints

In the case of reproductive endpoints, the DS has used data from other pentavalent vanadium compounds, including ammonium and sodium metavanadates, for read across the reproductive toxicity of vanadium pentoxide. All these vanadium compounds release vanadium ions in the body. Although vanadium pentoxide is clearly less water soluble than these soluble vanadates, there are bioaccessibility data available showing that vanadium pentoxide shows bioaccessibility similar to that of sodium metavanadate. This is especially relevant in solutions simulating lung fluids such as Gamble's solution and artificial lysosomal fluid (see table below).

Vanadium transforms to its pentavalent form in all media except in artificial lysosomal fluid (see table below). Since bioaccessibility can be considered to reflect bioavailability this data suggests that these readily soluble vanadium substances ($NaVO_3$ and V_2O_5) dissolve practically completely in all physiological media and are expected to have similar bioavailability. It should be noted that good bioavailability does not necessarily mean good absorption. There are some data suggesting poor oral absorption of pentavalent vanadium species, although the data is limited and controversial (especially on oral absorption of vanadium pentoxide). It is possible that there is variability between vanadium compounds due to exposure conditions and therefore, a higher oral absorption of metavanadates compared to vanadium pentoxide cannot be excluded. This should be noted when using read-across for reproductive endpoints.

***Table**: Bioaccessibility and speciation after 2 and 24 hrs in physiological media for pentavalent vanadium compounds and tetravalent vanadyl sulfate (taken from Annex II of CLH report).*

	Phosphate-buffered saline (PBS) (pH 7.4) %		**Gamble's solution (GMB) (pH 7.4) %**		**Artificial lysosomal fluid (ALF) (pH 4.5) %**		**Artificial gastric fluid (GST) (pH 1.5) %**		**Artificial sweat solution (ASW) (pH 6.5) %**	
	2h	24h	2h	24h	2h	24h	2h	24h	2h	24h
V metal	≤ DL	≤1.4 (V)	≤ DL	≤1.2 (V)	≤ 1.2 (IV)	≤1.5(IV)	≤ 1.2 (IV)	≤1.6(IV)	≤ DL	≤0.7(IV) ≤1.3(V)
V_2O_5	≤99(V)	≤98(V)	≤100(V)	100(V)	≤99(IV)	100(IV)	100(V)	100(V)	≤95(V)	≤94(V)
$NaVO_3$	≤100 (V)	100(V)	≤93(V)	100(V)	≤65(IV) ≤40(V)	100(IV)	≤4(IV) ≤90(V)	≤6(IV) ≤90(V)	≤5(IV) ≤89(V)	≤5(IV) ≤99(V)
$VOSO_4$	29 IV) 68 (V)	100(V)	100 (V)	100(V)	94 (IV) 15 (V)	≤90(IV) ≤11(V)	74(IV) 32(V)	≤74(IV) ≤50(V)	54 (IV) 51 (V)	≤32 (IV) ≤72(V)

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	(NTP, 2002)

		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/m³: 5.00 ± 0.08 d, 8 mg/m³: 5.50 ± 0.14 d, 16 mg/m³: 5.25 ± 0.25 d). Number of females in diestrus was significantly elevated (control: 39.2% in diestrus vs. 4 mg/m³: 40.8%, 8 mg/m³: 49.2%, 16 mg/m³: 71.9%) In addition, local effects were reported in both males and females with a NOAEC of 1 mg/m³ (see section on STOT RE for further details).	
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/m^3 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/m³ 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)	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	(Domingo *et al.*, 1986)

	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.	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	
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%,	(Altamirano-Lozano *et al.*, 1993)

		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	
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	(Domingo *et al.*, 1986)

		length were also reported in offspring.	
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 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	Sodium metavanadate, Sprague-Dawley rats; Dams (n=12)	Performance in rotarod test (motor coordination) was significantly impaired in weaned male rats after lactational exposure to NaVO3 (latency to fall from rotarod, coordination	(Wang *et al.*, 2015)

exposure Klimisch: 3	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	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.	
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.

10.11 Specific target organ toxicity-single exposure

Evaluation not performed for this substance

10.12 Specific target organ toxicity-repeated exposure

Table 30: Summary table of animal studies on STOT RE (all doses/concentrations given as V_2O_5, if not indicated otherwise)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Oral route:			
Subchronic mechanistic study investigating cysteine content of the hair of rats 5 male Wistar rats per dose group GLP: no Reliability (Klimisch score): 3	Divanadium pentaoxide, no further information available Feeding study Low level vanadium groups: 25 ppm V and 50 ppm V (first 35 days); 100 ppm V and 150 ppm V (36 - 103 days) High level vanadium groups: 500 and 1000 ppm V for 75 days	At 100 and 150 ppm, lower erythrocyte counts were obtained (at study termination: control group: 7.7 x 10^6 cells/mm^3; 100 ppm group: 6.8 x 10^6 cells/mm^3; 150 ppm group: 6.3 x 10^6 cells/mm^3) accompanied by lower haemoglobin levels (at study termination: control group 15.0 %; 100 ppm V group: 14.5%; 150 ppm V group: 13.7%) Cysteine content of the hair of the control group increased with time, whereas that of the rats fed the 100 ppm vanadium diet remained nearly constant. At 150, 500 and 1000 ppm V, a decrease in the average hair cysteine values occurred. The decreases in cysteine are considered significant on a relative basis, i.e., comparing the vanadium-fed groups with their corresponding controls. Decreased rate of body-weight gain was noted in both high level vanadium treated groups, compared with controls. The LOEL from this study is 100 ppm V, this corresponds to 6.25 mg V_2O_5/kg bw/day	(Mountain *et al.*, 1953) (ECHA Dissemination, 2017) Study: 005 Details in Annex I

Inhalation route:			
16-day subacute study in female B6C3F1 mice (5 groups of 48 mice) GLP: yes Reliability (Klimisch score): 1	Divanadium pentaoxide, purity: 99.8 % 0, 0.25, 1, 4 mg V_2O_5/m³ (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	In the lowest exposure group, no histological alterations were observed. At 1 and 4 mg/m³ 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.	(Schuler *et al.*, 2011) 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/m^3 in males and at 16 mg/m^3 in females. Significant exposure-related changes in pulmonary function were observed in male and female rats exposed to 4, 8, or 16 mg/m^3, 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/m^3 or greater as well as lung inflammation. 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. Squamous metaplasia (a single focus) was observed within an area of hyperplasia in one female exposed to 16 mg/m^3. Nose: 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. 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	(NTP, 2002) from (ECHA Dissemination, 2017) Study: 002, publication Details in Annex I

		hypospermia and atypical cells of the epididymis in males at 16 mg/m^3. Results on reproductive effects are discussed in section 10.10. NOAEC local = 1 mg/m³ air (males and females) based on increased lung weights and epithelial hyperplasia, inflammation and fibrosis in lungs at 2 mg/m^3 and above. $NOAEC_{systemic}$ was 8 mg/m^3 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/m^3. All mice at and above 8 mg/m^3 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/m^3. $NOAEC_{systemic}$ was 2 mg/m^3 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	Divanadium pentaoxide, > 99.6 % Whole body inhalation for 6 h/d, 5 d/week for 26 weeks Group 1 (9 animals): 0.1 mg/m^3 (Mon, Wed, Fri) and 1.1 mg/m^3 (Tue, Thurs) Group 2 (9 animals): 0.5 mg/m^3	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):**	(Knecht *et al.*, 1992) Also study 010 from (ECHA Dissemination, 2017)

with provocation challenges, and compared V_2O_5 reactivity before and after subchronic V_2O_5 exposure with pulmonary function testing. In addition to pulmonary function testing, bronchial lavage fluid was analysed. GLP: no information Reliability (Klimisch score): 2	Group 3 (8 animals): control goup, exposed against vehicle clean air Provocation challenge before and after 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	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 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/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 incidences (but not severity) of chronic nephropathy were significantly increased in male rats exposed to 1 or 2 mg/m^3 (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:**	(NTP, 2002) Also studies no. 001 and 005 reported in (ECHA Dissemination, 2017) Details in Annex I

		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/m³. In the respiratory tract (lungs), lesions were observed: inflammation, interstitial fibrosis, histiocytosis and hyperplasia (alveolar and bronchiole) mostly in a dose dependent manner starting from 1 mg/m^3. 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/m^3 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 31: Summary table of animal studies on STOT RE on other vanadium compounds (sodium metavanadate):

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Study on central nervous system effects from subacute exposure of adult rats Reliability (Klimisch score): 3	34 male rats (strain not documented), 3 months old, were injected intraperitoneally 3 mg NaVO3/kg bw/d (n=17), or saline solution (n=17, control) for 5 consecutive days. Sodium metavanadate (purity not provided) Histochemical and immunohistochemical staining (brain) Biochemical analysis: lipid peroxidation by thiobarbituric acid reaction Behavioral: Open field tests and Rotarod test were performed the day after the last dose had been administered	Decreased myelin fiber density in different brain areas in exposed rats Lipid peroxidation was significantly elevated in cerebellum and hippocampus in the treated group Significant decrease in locomotor activity and grooming response in the treated group. Similar behaviour (exposed/control) in rotarod test	(García *et al.*, 2004)

Table 32: Summary table of human data on STOT RE

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

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

There is information available with divanadium pentaoxide from repeated dose toxicity studies as well as from epidemiological studies on occupationally exposed groups.

Oral exposure

There is limited information on repeated dose toxicity after oral exposure. In the only available experimental study with oral exposure, rats were administered divanadium pentaoxide with the diet at concentrations of 25 ppm (increased to 100 ppm after 35 days) to 1000 ppm vanadium for 75 to 103 days (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 is considered not reliable (reliability 3).

Inhalation exposure

Experimental studies

In a subacute inhalation study, Schuler *et al.* (2011) exposed B6C3F1 mice to aerosols of V_2O_5 for 16 days (6 hours per day) to concentrations of 0, 0.25, 1 and 4 mg/m^3. The observed increase in lung weights, alveolar histiocytosis, multifocal subacute alveolitis, multifocal granulocytic infiltration and cell proliferation rate at concentrations of 1 mg/m^3 and above confirmed the respiratory tract as a target organ after inhalation exposure.

In the subchronic and 2-year studies of the US National Toxicology Program (NTP, 2002), rats and mice were exposed to particulate aerosols of divanadium pentaoxide in concentrations of up to 16 mg/m^3 (subchronic studies) and up to 2 and 4 mg/m^3 for rats and mice respectively (chronic studies). In all studies,

the respiratory tract was the primary target organ. Symptoms observed in these studies in both species were, among others, inflammation, alveolar/bronchiolar hyperplasia and fibrosis of the lung, as described below.

In the subchronic studies of the US National Toxicology Program, the **highest tested concentration was lethal** in rats (10 among 20 animals) and to a limited number of mice (1 death among 20 animals). Body weights were reduced from 4 mg/m^3 (mice and male rats) and at 16 mg/m^3 (female rats). The respiratory effects were more intense with increasing exposure time and started at 2 mg/m^3, as indicated by increased lung weights and increased incidence and severity of proliferative and inflammatory lesions in the lungs of exposed mice and rats. **Inflammation and alveolar/bronchiolar epithelial hyperplasia** was present in mice and rats exposed at and above 2 mg/$m^{3.}$. To be noted that in mice species, hyperplasic alveoli cells often contained one or two cells that were very large and occasionally **binucleated**. In rat species, lung **fibrosis** (**minimal to moderate)** occurred from 2 mg/m^3 or greater as well as lung inflammation. **Lung squamous metaplasia** was observed in one female rat exposed to 16 mg/m^3. Moreover, nasal inflammation was observed as well as hyperplasia and metaplasia of the nasal respiratory epithelium (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 significantly increased in rats exposed to 4 mg/m^3 and greater. $NOAEC_{local}$ is set at 1 mg/m^3 air (mice and rat) based on increased absolute lung weights and epithelial hyperplasia and inflammation in lungs at 2 mg/m^3 and above associated with lung fibrosis in rats from 2 mg/m^3 in mice and rat subchronic studies. $NOAEC_{syst}$ was 2 mg/m^3 in mice and 8 mg/m^3 in rats. Effects on reproductive system were also reported in the subchronic toxicity studies at higher concentrations. Results are detailed in the dedicated section.

In the 2-year studies of the US National Toxicology Program, F344/N rat and B6C3F1 mice were exposed 6h/d, 5 d/w, 104 weeks to V_2O_5 particulate aerosol at 0.5, 1, 2 mg /m^3 and at 1, 2, 4 mg/m^3 respectively. In rats, decreased body weight gain was noted at 2 mg V_2O_5/m^3. In mice, decreased body weight gain was observed from 1 mg V_2O_5/m^3 and survival of the highest dosed male mice (4 mg/m^3) was significantly less than controls. The main targeted tissues are the respiratory tract: lungs, larynx and nose with inflammation, interstitial fibrosis, histiocytosis and hyperplasia (alveolar and bronchiole) mostly in a dose dependent manner. These effects occurred in the lung at all tested concentrations - from 0.5 mg/m^3 in rats and from 1 mg/m^3 in mice, leading to squamous metaplasia of the alveoli at 2 mg/m^3. In the other part of the respiratory tract, inflammation and degeneration of the respiratory epithelium of the epiglottis and squamous metaplasia were observed in larynx at all tested concentrations - from 0.5 mg/m^3 in rats and from 1 mg/m^3 in mice. Additionally, fibrosis and hyperplasia were observed in larynx of exposed rats. According to the NTP, these changes represent a common response to laryngeal injury. In the nose, hyperplasia of the respiratory epithelium of goblet cells was observed in rats and suppurative inflammation, degeneration and squamous metaplasia of the respiratory epithelium in exposed mice. The $LOAEC_{local}$ is of 0.5 mg/m^3 air (males and females) based on non-neoplastic changes in the respiratory system (lung, larynx, and nose) in rats and of 1 mg/ m^3 in mice. No NOAEL can be derived. The incidence of chronic nephropathy was significantly

increased in male rats exposed from 1 mg/m^3. However, it is not clear if the increased incidences in this study were related to exposure to V_2O_5 or were a reflection of the low incidence in the control group. Carcinogenic effects were also reported in the chronic toxicity studies at higher concentrations. Results are detailed in the dedicated section.

Knecht *et al.* (1992) exposed non-human primates (cynomolgus monkeys, *Macaca fascicularis*) by inhalation for 26 weeks in order to investigate effects on hyper-reactivity in the respiratory tract. Provocation tests preceding the subchronic exposure period produced concentration-dependent impairments in pulmonary function, characterized by airway obstructive changes (increased resistance and decreased flow). However, the subchronic exposure itself did not produce changes regarding the endpoints investigated indicative for airway hyper-reactivity.

A series of studies from University of Mexico investigated effects of divanadium pentaoxide on various organ systems is not further discussed in detail here (Avila-Costa *et al.*, 2006; Cano-Gutiérrez *et al.*, 2012; Colín-Barenque *et al.*, 2015; Rodríguez-Lara *et al.*, 2013; Rodríguez-Lara *et al.*, 2016; Rodriguez-Lara *et al.*, 2016; Ustarroz-Cano *et al.*, 2017). These studies are considered to be of limited reliability, as only one exposure concentration was used in each study.

Overall, non-neoplastic changes were observed in the respiratory system (lung, larynx, and nose) in both rats and mice following inhalation exposure of V_2O_5 (particulate aerosol). These effects occurred, in the 90-day studies, from 2 mg/m^3 in both species and in the 2-year studies, from 0.5 mg/m^3 in rats and 1 mg/m^3 in mice.

Epidemiological studies

In a series of publications, effects observed in a group of 63 workers in a Finnish company producing divanadium pentaoxide were investigated (Kiviluoto *et al.*, 1979; 1980, 1981a). Kiviluoto *et al.* (1979) used a case-control study design with controls matched for smoking habits and age. Controls were occupationally exposed to inert dust. Cases were exposed on average for eleven years, with average exposure concentrations of 0.36 – 0.89 mg V_2O_5/m³. Workers from the divanadium pentaoxide production complained about various effects in the upper respiratory tract. The following differences were observed between cases and controls: 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. A second study (Kiviluoto, 1980) confirmed respiratory tract irritation (wheezing), but did not find differences regarding ventilation parameters or in lungs by X-ray analysis. Further, older occupational hygiene studies confirmed the respiratory tract as main target organ after occupational exposure to divanadium pentaoxide (Lewis, 1959a, b; Vintinner *et al.*, 1955).

In summary, human data in combination with reliable animal data (2 studies in rats and 3 studies in mice; Klimisch score: 1) with V_2O_5 (particulate aerosol) support the evidence of local respiratory non-neoplasic effects.

Dermal exposure

No relevant repeated dose toxicity study with dermal exposure to divanadium pentaoxide could be identified.

10.12.2 Comparison with the CLP criteria

Overall, non-neoplastic changes were observed in the respiratory system (lung, larynx, and nose) in both rats and mice following inhalation exposure of V_2O_5 (particulate aerosol). These effects occurred, in the 90-day studies, from 2 mg/m^3 in both species and in the 2-year studies, from 0.5 mg/m^3 in rats and 1 mg/m^3 in mice.

Table 33: Results of toxicity studies relevant for STOT RE in comparison to the CLP criteria

Conclusion	CLP criteria
Animal data Following inhalation exposure of V_2O_5 (particulate aerosol), the target organ of V_2O_5 is the respiratory system: lung, larynx, and nose in 16-day, 90-day and chronic (104 weeks) inhalation studies in F344/N rat and B6C3F1 mice. In the subchronic studies, the respiratory effects were more intense with increasing exposure time and started at 2 mg/m³ with increased lung weights, proliferative and inflammatory lung lesions in both species. Inflammation and alveolar/bronchiolar epithelial **hyperplasia** was observed in both species from 2 mg/m^3. In rat, lung **fibrosis** (minimal to moderate) and lung inflammation occurred from 2 mg/m^3. Lung squamous **metaplasia** was observed in one female rat exposed to 16 mg/m^3. Nasal inflammation, hyperplasia and metaplasia of the nasal respiratory epithelium were observed from 4 mg/m^3 in rats. Systemic toxicity were observed with decreased body weights from 4 mg/m^3 (mice) and at 16 mg/m^3 (rat) and with **lethality** at 16 mg/m^3 in both species although more important in rat species. Effects on reproductive system were also reported in both species (from 8 mg/m³). $NOAEC_{local}$ was 1 mg/m³ air (mice and rat) based on increased absolute lung weights and lung inflammation in lungs from 2 mg/m^3 associated with lung fibrosis in both species in subchronic studies. $NOAEC_{syst}$ was 2 mg/m^3 in mice and 8 mg/m^3 in rats. In the 2-year studies, decreased body weight gain was observed from 2 mg V_2O_5/m³ in both species and decreased survival at the highest dosed male mice (4 mg/m³). The same targeted tissues as in the subchronic toxicity studies were identified: lungs, larynx and nose with inflammation, interstitial **fibrosis**, histiocytosis and **hyperplasia** mostly in a dose dependent manner at all tested concentrations - starting from 0.5 mg/m^3 in rats and from 1 mg/m^3 in mice combined with squamous metaplasia of the alveoli at 2 mg/m^3 in rats. Inflammation and degeneration of the respiratory epithelium in larynx were also observed at all tested concentrations - with squamous **metaplasia** from	STOT RE Category 1 (H372): Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Guidance value to assist on category 1 classification based on 90-day inhalation studies in rat for *dust/mist/fume :* ≤ 0.02 mg/L/6h/day (= 20 mg/m^3)

0.5 mg/m^3 in rats and from 1 mg/m^3 in mice. Fibrosis and hyperplasia were also observed in larynx of exposed rats. In the respiratory epithelium of the nose, hyperplasia of goblet cells was observed in rats and suppurative inflammation, degeneration and squamous metaplasia in mice at these same concentrations. The $LOAEC_{local}$ is of 0.5 mg/m³ air (males and females) based on non-neoplastic changes in the respiratory system (lung, larynx, and nose) in rats and of 1 mg/ m³ in mice. No NOAEL can be derived. Carcinogenic effects in the lung were reported in both species at higher concentrations. <u>Human data</u> The respiratory tract was also confirmed as the target organ of V_2O_5 in several occupational studies. The observed respiratory effects are judged consistent with the description of significant effects reported in the guidance on the application of the CLP criteria (version 5 – July 2017); annex 1 (3.9.2.7.3) "a) *morbidity or death resulting from repeated or long-term exposure. Morbidity or death may result from repeated exposure, even to relatively low doses/concentrations, due to bioaccumulation of the substance or its metabolites, and/or due to the overwhelming of the de-toxification process by repeated exposure to the substance or its metabolites.* *(d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination.* ***(e) multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity.*** In conclusion, respiratory effects namely lung fibrosis in 90-day studies occurring from 2 mg/m^3 (corresponding to 0.002 mg/L/6h/day) in rats and mice is much lower than the classification threshold of ≤ 0.02 mg/L/6h/day for STOT RE 1. Moreover the lung effects were also reported in the 104 week studies: inflammation, interstitial fibrosis, histiocytosis and hyperplasia (alveolar and bronchiole) from 0.5 mg/m^3 (corresponding to 0.0005 mg/L/6h/day) in rats and from 1 mg/m^3 (corresponding to 0.001 mg/L/6h/day) in mice with alveoli squamous metaplasia at 2 mg/m^3 (corresponding to 0.002 mg/L/6h/day) in rats. It should be noted that it cannot be excluded that these effects can occur at lower concentrations since they were noted at all tested concentrations. Applying a conversion factor of 104/12 = 8.7 for the different time of exposure (104 weeks vs. 12 weeks), this leads to a converted value of 8.7 x 0.5 mg/m^3 (corresponding to 0.0005 mg/L/6h/day x 8.7 = 0.004 mg/L/6h/day) in rats which is lower than ≤ 0.02 mg/L/6h/day. Human data confirm the respiratory effects of V_2O_5 reported in experimental studies. In this context, V_2O_5 animal and human data allows to conclude that V_2O_5 **is hazardous to humans regarding the respiratory system after repeated exposure and that there are sufficient evidence to propose a classification as STOT RE 1 (respiratory tract; inhalation) for V_2O_5.**	

10.12.3 Conclusion on classification and labelling for STOT RE

Respiratory effects namely lung fibrosis and hyperplasia in subchronic studies (90 days) were observed from 2 mg/m^3 (corresponding to 0.002 mg/L/6h/day) in rats and mice which is much lower than the threshold for

STOT-RE 1 classification (0.02 mg/L). Similar lung effects, observed in the 104-week study from 0.5 mg/m^3 in rats and from 1 mg/m^3 in mice, also fulfil criteria for classification as STOT RE 1. Experimental findings are also supported by the available human data.

In conclusion, the current harmonized classification of STOT RE 1 for divanadium pentaoxide is still justified and should be updated to include the target organ and the route of exposure: STOT RE 1 – H372 (respiratory tract; inhalation).

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/m^3, 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/m^3. All mice at and above 8 mg/m^3 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/m^3 air (males and females) based on increased absolute lung weights and epithelial hyperplasia and inflammation in lungs at 2 mg/m^3. $NOAEC_{systemic}$ was 2 mg/m^3 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/m^3 (Mon, Wed, Fri) and 1.1 mg/m^3 (Tue, Thurs) Group 2 (9 animals): 0.5 mg/m^3 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/m^3. 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/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 incidences (but not severity) of chronic nephropathy were significantly increased in male rats exposed to 1 or 2 mg/m^3 (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/m^3 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/m^3 was significantly less than controls. Abnormal breathing was observed particularly in those animals exposed to 2 or 4 mg/m^3. 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 Disseminati on, 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 Disseminati on, 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 Disseminati on, 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.

In 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.

10.13 Aspiration hazard

Evaluation not performed for this substance

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Evaluation not performed for this substance

12 EVALUATION OF ADDITIONAL HAZARDS

Evaluation not performed for this substance

13 ADDITIONAL LABELLING

14 ANNEXES

Annex I: Non-confidential annex documenting the key studies for assessment

Annex II: Non-confidential annex: READ ACROSS JUSTIFICATION DOCUMENT

15 REFERENCES

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