

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

Contact details for dossier submitter:

ANSES (on behalf of the French MSCA)

14 rue Pierre Marie Curie

F-94701 Maisons-Alfort Cedex

classification.clp@anses.fr

Version number: 02

Date: August 2019

CONTENTS

1	IDENTITY OF THE SUBSTANCE	4
1.1	NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE	4
1.2	COMPOSITION OF THE SUBSTANCE	5
2	SEE SPECIFIC STUDY RECORDS BELOW FOR FURTHER INFORMATION.PROPOSED HARMONISED CLASSIFICATION AND LABELLING	8
2.1	PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA	8
3	HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING	10
4	JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL	10
5	IDENTIFIED USES	10
6	DATA SOURCES	10
7	PHYSICOCHEMICAL PROPERTIES	13
8	EVALUATION OF PHYSICAL HAZARDS	14
9	TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)	15
9.1	SHORT SUMMARY AND OVERALL RELEVANCE OF THE PROVIDED TOXICOKINETIC INFORMATION ON THE PROPOSED CLASSIFICATION(S).....	16
10	EVALUATION OF HEALTH HAZARDS	20
10.1	ACUTE TOXICITY - ORAL ROUTE.....	20
10.1.1	<i>Short summary and overall relevance of the provided information on acute oral toxicity</i>	22
10.1.2	<i>Comparison with the CLP criteria</i>	23
10.1.3	<i>Conclusion on classification and labelling for acute oral toxicity</i>	23
10.2	ACUTE TOXICITY - DERMAL ROUTE	23
10.2.1	<i>Short summary and overall relevance of the provided information on acute dermal toxicity</i>	24
10.2.2	<i>Comparison with the CLP criteria</i>	24
10.2.3	<i>Conclusion on classification and labelling for acute dermal toxicity</i>	24
10.3	ACUTE TOXICITY - INHALATION ROUTE	25
10.3.1	<i>Short summary and overall relevance of the provided information on acute inhalation toxicity</i>	27
10.3.2	<i>Comparison with the CLP criteria</i>	28
10.3.3	<i>Conclusion on classification and labelling for acute inhalation toxicity</i>	28
10.4	SKIN CORROSION/IRRITATION	28
10.5	SERIOUS EYE DAMAGE/EYE IRRITATION	28
10.6	RESPIRATORY SENSITISATION	29
10.6.1	<i>Comparison with the CLP criteria</i>	34
10.6.2	<i>Conclusion on classification and labelling for respiratory sensitisation</i>	34
10.7	SKIN SENSITISATION.....	34
10.8	GERM CELL MUTAGENICITY	34
10.8.1	<i>Short summary and overall relevance of the provided information on germ cell mutagenicity</i>	45
10.8.2	<i>Comparison with the CLP criteria</i>	48
10.8.3	<i>Conclusion on classification and labelling for germ cell mutagenicity</i>	50
10.9	CARCINOGENICITY	50
10.9.1	<i>Short summary and overall relevance of the provided information on carcinogenicity</i>	56
10.9.2	<i>Comparison with the CLP criteria</i>	59
10.9.3	<i>Conclusion on classification and labelling for carcinogenicity</i>	62
10.10	REPRODUCTIVE TOXICITY	62

10.10.1	<i>Adverse effects on sexual function and fertility</i>	62
10.10.2	<i>Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility</i>	70
10.10.3	<i>Comparison with the CLP criteria</i>	74
10.10.4	<i>Adverse effects on development</i>	76
10.10.5	<i>Short summary and overall relevance of the provided information on adverse effects on development</i> 84	
10.10.6	<i>Comparison with the CLP criteria</i>	86
10.10.7	<i>Adverse effects on or via lactation</i>	89
10.10.8	<i>Short summary and overall relevance of the provided information on effects on or via lactation</i>	93
10.10.9	<i>Comparison with the CLP criteria</i>	95
10.10.10	<i>Conclusion on classification and labelling for reproductive toxicity</i>	97
10.11	SPECIFIC TARGET ORGAN TOXICITY-SINGLE EXPOSURE.....	99
10.12	SPECIFIC TARGET ORGAN TOXICITY-REPEATED EXPOSURE.....	99
10.12.1	<i>Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure</i>	107
10.12.2	<i>Comparison with the CLP criteria</i>	110
10.12.3	<i>Conclusion on classification and labelling for STOT RE</i>	111
10.13	ASPIRATION HAZARD	112
11	EVALUATION OF ENVIRONMENTAL HAZARDS	112
12	EVALUATION OF ADDITIONAL HAZARDS.....	112
13	ADDITIONAL LABELLING.....	112
14	ANNEXES	112
15	REFERENCES.....	113

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 Dioxovanadioxy(dioxo)vanadium
ISO common name (if available and appropriate)	<i>Not applicable</i>
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 ₂ O 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 ₂ O 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


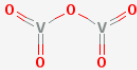
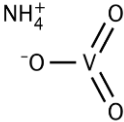
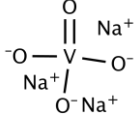
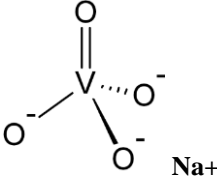
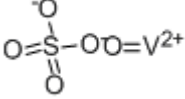
Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
No data available				

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 <i>via</i> oral route	Proposed update of the harmonised classification: Acute Tox 3 – H301	Yes
Acute toxicity <i>via</i> dermal route	Data conclusive but not sufficient for classification	Yes
Acute toxicity <i>via</i> 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 pentoxide 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 VANITEC¹ 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.gov²
- TOXNET, ChemIDplus³, IPCS⁴, eChemPortal⁵
- Chemical Abstracts (at host STN International Europe⁶)
- SciSearch, Biosis, CAB Abstracts, Embase (at host Deutsches Institut für Medizinische Dokumentation und Information, DIMDI⁷)
- 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)	
<p>*remark on identity: divanadium pentaoxide may occur in different forms and granulometry (particles: powder, crystalline, fused). Divanadium pentaoxide in particle form is treated as <i>one</i> 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).</p>			


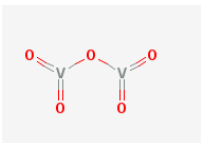
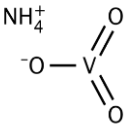
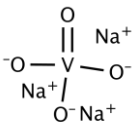
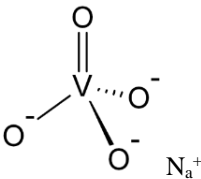
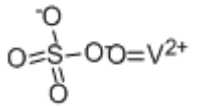
8 EVALUATION OF PHYSICAL HAZARDS

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 ₂ O 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 ₄ VO ₃		+5	7.8 g/L (20°C; pH 6; duration not provided)	(ECHA, 2017)
Sodium orthovanadate CAS N° 13721-39-6	Na ₃ VO 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	VO ₂ SO 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 V₂O₅ (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 (VO₂⁺) or anionic (HVO₄²⁻) 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 ⁴⁸V₂O₅ 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).

V₂O₅ 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 $^{48}\text{V}_2\text{O}_5$ 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 $39.7 \pm 18.5\%$ of ingested vanadium was retained and that $59.1 \pm 18.8\%$ 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 $0.05 \pm 0.02 \mu\text{g/g}$ of dry tissue in the controls *versus* $1.63 \pm 0.15 \mu\text{g/g}$ 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}V 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}V Vanadium following oral administration did not show significant differences between pentavalent ($^{48}\text{V}(\text{V})$) and tetravalent ($^{48}\text{V}(\text{IV})$) 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 V₂O₅/m³) 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/m³ 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 V₂O₅) 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 (V⁵⁺) 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 V₂O₅/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 ₂ O ₅ : 97.86 %)	Single application <i>via</i> 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 <i>et al.</i> , 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 ₂ O ₅ : 100.04 %)	Single application <i>via</i> 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 <i>et al.</i> , 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 ₂ O ₅ : 99.3 %)	Single application <i>via</i> 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 <i>et al.</i> , 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 duration levels, 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 <i>et al.</i> , 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 <i>et al.</i> , 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 duration levels, 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 LD₅₀ 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 LD₅₀ values (depending on sex and form) from 221.1 to 715.7 mg V₂O₅/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 LD₅₀ that fall into category 2 (5-50 mg/kg)
- 8 derived LD₅₀ that fall into category 3 (50-300 mg/kg)
- 2 derived LD₅₀ that fall into category 4 (300-2000 mg/kg)

According to CLP guidance, classification is based on the lowest LD₅₀ value available. However, the two studies concluding on LD₅₀ 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 LD₅₀ of 221 mg/kg bw obtained in females exposed to divanadium pentaoxide technical grade (calculated degree of purity in V₂O₅ of 99.3%) in the most reliable study (Leuschner *et al.*, 1994), also supported by the results obtained from Sun *et al.* (1987) (LD₅₀ 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 V₂O₅/kg bw, if not indicated otherwise)

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

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose duration levels 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 ₂ O 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 ₂ O ₅ : 100.04 %)	Single occlusive application for 24 h 2000 and 2500 mg/kg bw	Males and females: ≥ 2500 mg/kg bw	(Leuschner <i>et al.</i> , 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 ₂ O ₅ : 99.3 %)	Single occlusive application for 24 h 2000 and 2500 mg/kg bw	Males and females: ≥ 2500 mg/kg bw	(Leuschner <i>et al.</i> , 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 LD₅₀ 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 V₂O₅/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 ₂ O 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 <i>et al.</i> , 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 ₂ O 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 ₂ O ₅ /L air	Males: 16.19 mg/L air No classification Females: 4.04 mg/L air Acute Tox. 4	(Leuschner <i>et al.</i> , 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 ₂ O 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 ₂ O ₅ /L air	Males: 4.40 mg/L air Females: 2.21 mg/L air Acute Tox. 4	(Leuschner <i>et al.</i> , 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)	LC ₁₀ = 70 mg/m ³ (= 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/m ³ (= 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 LC₅₀ 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 LC₅₀ 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. LC₅₀ 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 LC₅₀ that fall into category 1 (≤ 0.05 mg/L - aerosolised powder exposure)
- 2 derived LC₅₀ that fall into category 2 ($0.05 - \leq 0.5$ mg/L - aerosolised powder exposure in one of these studies and no information available on the test atmosphere in the other study)
- 3 derived LC₅₀ that fall into category 4 ($> 1 - \leq 5$ 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 LC₅₀ 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 LC₅₀ 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 LC₅₀ 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 LC₅₀ 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 LC₅₀ 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.

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
<p>Subchronic (26 weeks) inhalation study in monkeys</p> <p>The study assessed pulmonary reactivity to V₂O₅ with provocation challenges, and compared V₂O₅ reactivity before and after subchronic V₂O₅ exposure with pulmonary function testing.</p> <p>In addition to pulmonary function testing, bronchial lavage fluid was analysed.</p> <p>Adult, male cynomolgus monkeys (<i>Macaca fascicularis</i>)</p> <p>8-9 animals per exposure group</p> <p>GLP: no information</p> <p>Reliability (Klimisch score): 2</p>	<p>Divanadium pentaoxide, > 99.6 %</p> <p>Whole body inhalation for 6 h/ d, 5 d/week for 26 weeks</p> <p>Group 1 (9 animals): 0.1 mg/m³ (Mon, Wed, Fri) and 1.1 mg/m³ (Tue, Thurs)</p> <p>Group 2 (9 animals): 0.5 mg/m³</p> <p>Group 3 (8 animals): control group, exposed against vehicle clean air</p> <p>Challenge before and after subchronic exposure:</p> <p>- 6 h/d with 0.5 mg V₂O₅ and 2 weeks later:</p> <p>- 6 h/d with 3 mg V₂O₅</p>	<p>In none of the two exposure groups pulmonary reactivity to V₂O₅ was increased by subchronic V₂O₅ exposure in comparison to control group.</p> <p>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.</p> <p>One animal in the control group was removed from the study because of a parasitic infestation.</p> <p>One animal in the peak exposure group died unexpectedly of an effect unrelated to the exposure.</p> <p>Effects after pre-exposure challenge (acute effects):</p> <p>Pre-exposure challenges with 0.5 and 3 mg V₂O₅/m³ produced a concentration-dependent impairment in pulmonary function, characterized by airway obstructive changes (increased resistance and decreased flow).</p> <p>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.</p> <p>Cytological immunological results test (IgE and IgG analysis) did not indicate allergic sensitization.</p>	<p>(Knecht <i>et al.</i>, 1992)</p> <p>(ECHA Dissemination, 2017)</p> <p>Study: 010</p>

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 ₂ O ₅ -producing company in Finland.	Divanadium pentaoxide	<p>- 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).</p> <p>- 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 V₂O₅/m³</p> <p>- 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 V₂O₅/m³</p> <p>Performed tests:</p> <ul style="list-style-type: none"> - Rhinoscopy - Sputum cells were analysed - Pulmonary ventilation measured - Nasal secretion smear cells were analysed 	<p>Cases self-reported subjective symptoms of respiratory tract irritation</p> <p>Rhinoscopy: no differences between the groups</p> <p>Cytology: Number of neutrophils significantly increased in nasal smears of exposed group.</p> <p>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</p> <p>→ clear signs of inflammation (not related to allergy, since number of eosinophils not significantly changed in exposed group)</p> <p>No results on pulmonary ventilation measurements reported in the publication</p>	<p>(Kiviluoto <i>et al.</i>, 1979)</p> <p>(ECHA Dissemination, 2017) “epidemiological data”</p> <p>Study: 001</p>
		<p>Same collective as indicated above in Kiviluoto <i>et al.</i>, (1979)</p> <p>Performed tests (testing in 1975):</p> <ul style="list-style-type: none"> - Respiratory questionnaire - X-ray analysis of the lung - Pulmonary ventilation measured 	<p>Respiratory symptoms: significantly more wheezing in the exposure group.</p> <p>X-ray analysis: no exposure related differences observed</p> <p>Ventilation measurement: no differences observed</p>	<p>(Kiviluoto, 1980)</p>
		<p>Same collective exposure as indicated above (Kiviluoto <i>et al.</i>, 1979)</p> <p>However, the control group consisted of only 22 men. Whether these men were part of the “collective control” mentioned by Kiviluoto <i>et al.</i> (1979) is not described.</p> <p>Performed tests:</p> <ul style="list-style-type: none"> - Hematologic and serum chemical laboratory tests 	<p>No significant differences were observed for the hematologic results of exposed and non-exposed workers.</p> <p>In the serum chemical test, significant differences were observed for serum albumin (↓), chloride (↓), urea (↑), bilirubin (↓) and conjugated bilirubin (↑).</p>	<p>(Kiviluoto <i>et al.</i>, 1981a)</p> <p>(ECHA Dissemination, 2017) “epidemiological data”</p> <p>Study: 003</p>

<p>Case-control study with 12 workers from a vanadium plant in South Africa.</p>	<p>Divanadium pentaoxide</p>	<p>12 workers chronically exposed against < 0.15 - 1.53 mg V₂O₅/m³ with diagnosed bronchial hyperreactivity (by lung function and bronchoprovocation tests). Control subjects (12) worked in the same company but did not show bronchial hyperreactivity.</p> <p>Performed tests/observations:</p> <ul style="list-style-type: none"> - Onset of symptoms - Serum IgE and atopy 	<p>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.</p> <p>Serum IgE and atopy IgE levels between cases and controls were not significantly different.</p> <p>None of the subjects was exposed against toxic levels of SO₂ and NH₃. For 3/12 workers, co-exposure against SO₂ and NH₃ could be excluded.</p>	<p>(Irsigler <i>et al.</i>, 1999)</p>
<p>Case-control study with 24 men working in vanadium plants in the USA (13 men from Colorado and 11 men from Ohio)</p>	<p>Divanadium pentaoxide</p>	<p>24 workers were exposed to vanadium (as V₂O₅) <i>via</i> inhalation (at least for 6 months) against the following concentrations:</p> <p>Colorado plant: 0.097 - 0.243 mg V₂O₅/m³ (mass respirable vanadium: 16.6 % to 51 %; Particle size < 5 µm: 92.5 to 99 %)</p> <p>Ohio plant: 0.018 - 0.925 mg V₂O₅/m³ (mass respirable vanadium: 2 % to 100 %; Particle size < 5 µm: 96.3 to 100 %)</p> <p>45 control subjects matched for age, economic status and job activities, not coming from the vanadium industry.</p> <p>Performed tests/observations:</p> <ul style="list-style-type: none"> - 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 	<p>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.</p> <p>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).</p> <p>No significant differences were found for haematocrit, urinalysis and electrocardiogram results.</p>	<p>(Lewis, 1959a; b) (ECHA Dissemination, 2017) Study: 004</p>

Retrospective cohort study with 78 workers engaged in the processing of vanadium-bearing ore, and 37 controls in Peru	Divanadium pentaoxide	<p>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 V₂O₅.</p> <p>All dust particles were below 5 µm in diameter.</p> <p>The concentration of sulphur dioxide in air in various work places ranged between 0.0 and 2.0 ppm.</p>	<p>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.</p> <p>Vital capacity, circulation, neurological findings, muscular strength: No significant differences among the three groups of workers were observed.</p>	<p>(Vintinner <i>et al.</i>, 1955)</p> <p>(ECHA Dissemination, 2017)</p> <p>Study: 006</p>
---	-----------------------	--	--	--

There is one experimental study in adult male cynomolgus monkey assessing pulmonary reactivity to V₂O₅ with provocation challenges (Knecht *et al.*, 1992). They also compared V₂O₅ reactivity before and after subchronic V₂O₅ exposure with pulmonary function testing. Pre-exposure challenges with 0.5 and 3 mg V₂O₅/m³ 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 V₂O₅ inhalation did not produce an increase in V₂O₅ 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 V₂O₅, 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 V₂O₅ 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 V₂O₅.

10.6.2 Conclusion on classification and labelling for respiratory sensitisation

No classification and labelling for respiratory sensitisation is required for V₂O₅ according to CLP regulation.

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 %	<i>Salmonella typhimurium</i> 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 ₂ O 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 ₂ O ₅ /plate Tested up to cytotoxic concentration No vehicle reported +/- S9 of rat or hamster liver microsomes	Negative (+/- S9 mix) for all strains tested	(NTP, 2002) (seems to be identical to study no. 015 from ECHA Dissemination 2017)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		Positive controls: yes		
Bacterial gene mutation assay Ames test Only limited information available from secondary reporting Reliability (Klimisch score): 4	Divanadium pentaoxide, no information on purity available	<i>S. typhimurium</i> TA1535, TA1537, TA98, TA100	Negative (no further details available)	Sun et al, 1987 cited from (WHO, 1988)
		<i>E. coli</i> 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 score): 1	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 ₂ O ₅ /mL Experiment 1: 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 48 and 64 µg V ₂ O ₅ /mL Experiment 2: - with S9-mix: 0, 2, 4, 8, 12, 16, 20, 25, 30, 35 and 40 µg V ₂ O ₅ /mL; - without S9-mix: 0, 0.5, 1, 2, 4, 8, 12, 14, 16, 20 and 30 µg V ₂ O ₅ /mL. Concentrations selected for the 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	Negative (+/- S9 mix) at all concentrations tested	Anonymous, 2010 from (ECHA Dissemination , 2017) Study: 001, study report
Mammalian cell micronucleus test OECD TG 487 No deviations Human lymphocyte cells and TK6 cells GLP: yes Reliability	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 ₂ O 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 ₂ O ₅ /mL Experiment 1 (human lymphocyte cells): without and with S9-mix (3+21 hours): 0, 5, 10, 20, 30, 35, 40, 45, 50,	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

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
(Klimisch score): 1		<p>55, 60, 75 and 100 µg V₂O₅/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 V₂O₅/mL.</p> <p>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 V₂O₅/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 V₂O₅/mL.</p> <p>Concentrations selected for the main experiments were based on the results of cytotoxicity range finding study.</p> <p>The test article was formulated as a suspension in 0.5% w/v methyl cellulose (0.5% MC).</p> <p>+/- S9 mix</p> <p>Positive controls: yes</p>		
<p>Mammalian cell micronucleus test in SHE cells</p> <p>GLP: no</p> <p>Reliability (Klimisch score): 2</p>	Divanadium pentaoxide, supplied by NTP, no statement on purity	<p>Syrian hamster embryo (SHE) cells</p> <p>0, 10, 15, 20, and 25 µg V₂O₅/mL</p> <p>Dose levels were selected based on solubility and/or toxicity limits.</p> <p>Vehicle: DMSO</p> <p>Without metabolic activation</p> <p>Positive controls: yes</p>	Negative (- S9 mix) at all concentrations tested	<p>(Gibson <i>et al.</i>, 1997)</p> <p>(ECHA Dissemination , 2017)</p> <p>Study: 014</p>
<p>Mammalian chromosome aberration in human lymphocytes - microscopic evaluation-</p> <p>GLP: not specified</p> <p>Reliability (Klimisch score): 2</p>	Divanadium pentaoxide, no information on purity available	<p>Lymphocytes: human peripheral blood</p> <p>0, 1, 2, 4, or 8 µg V₂O₅/mL</p> <p>The concentrations for the test item were selected based on preliminary reports</p> <p>Vehicle: distilled water</p> <p>No information on metabolic activation</p> <p>Positive controls: yes</p>	Negative at all concentrations tested	<p>(Rodríguez-Mercado <i>et al.</i>, 2010)</p> <p>(ECHA Dissemination , 2017)</p> <p>Study: 016</p>
<p>Gene mutation assay in Chinese hamster V79 cells</p> <p>Reliability (Klimisch</p>	Divanadium pentaoxide, purity: 99.9%	<p>Chinese hamster V79 cells (lung fibroblast cells)</p> <p>0, 1, 2, 3, 4 µg V₂O₅/mL for 24h</p> <p>Without metabolic activation</p>	No increase in the frequency of gene mutation at HPRT locus	(Zhong <i>et al.</i> , 1994)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
score): 2		Positive control: yes		
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 ₂ O 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 ₂ O 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 exchange assay with human lymphocytes Reliability (Klimisch score): 3	Divanadium pentaoxide, no information on purity available	Human primary lymphocytes 0, 2, 4, 6 µg V ₂ O ₅ /mL Positive controls: no	No increase in structural chromosome aberration (CA) was observed. 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	(Roldán and Altamirano, 1990)
Comet Assay Reliability (Klimisch score): 3	Divanadium pentaoxide, purity ≥ 99.6%	Human blood leucocytes 0, 1, 2, 4, 8 µg V ₂ O 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 <i>et al.</i> , 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 ₂ O ₅ Vehicle: water Positive controls: yes	Positive in lymphocytes (dose-dependent) Negative in mucosal cells	(Kleinsasser <i>et al.</i> , 2003)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
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 <i>et al.</i> , 1996)
Study of induction of aneuploidy in exposed cells Reliability (Klimisch score): 2	Divanadium pentaoxide, no information on purity available	Human primary lymphocytes 0, 0.001, 0.01, 0.1 μM V_2O_5 - in situ hybridization (FISH) with DNA 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	<u>Results of FISH:</u> Significant concentration-dependent increase in frequencies of nuclei exhibiting 3 or more hybridization regions <u>Results of immunostaining:</u> At all tested concentrations disruption of microtubules in spindle apparatus was observed <u>Results on tubulin polymerisation:</u> In the lowest concentration tubulin polymerization was inhibited and depolymerisation was stimulated.	(Ramírez <i>et al.</i> , 1997)

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 <i>Kras</i> and overexpression of mutant P53 protein Reliability (Klimisch score): 2	Divanadium pentoxide, purity: 99%	Exposure of mice against 0, 1, 2, 4 mg V ₂ O ₅ /m ³ via inhalation for 6 h/d, 5 d/w, 104 weeks Lung tissue obtained during the NTP study was analysed	<i>Kras</i> 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 <i>Kras</i> mutations”	(NTP, 2002)
<u>Mechanistic Study:</u> Analysis of lung carcinomas from B6C3F1 mice from the NTP study (NTP, 2002) for presence of MAPK (mitogen activated protein kinase) activity, <i>Kras</i> mutations and chromosomal defects Reliability (Klimisch score): 2	Divanadium pentoxide, purity: 99%	Exposure of mice against 0, 1, 2, 4 mg V ₂ O 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 <u>similar</u> between normal lung and lung carcinomas. Phospho-MAPK <u>elevated</u> in 5/6 lung carcinoma samples, in which <i>Kras</i> mutations and chromosome 6 LOH (loss of heterozygosity) were identified <u>and</u> in 4/5 carcinomas with <i>Kras</i> mutations but no LOH. <u>no</u> phospho-MAPK was detected in carcinoma tissue without <i>Kras</i> mutation and in normal lung tissue	(Devereux <i>et al.</i> , 2002)
<i>Kras</i> mutation in the development of lung tumours Non-GLP Reliability (Klimisch score): 2	Divanadium pentoxide, purity: 100%	6 male transgenic Big Blue mice per dose group and exposure group The levels of two different <i>Kras</i> 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 ₂ O ₅ /m ³ nose-only inhalation, 6 h/d 5 d/w for 4 or 8 weeks	Negative for both concentrations and time points	(Banda <i>et al.</i> , 2015) (ECHA Dissemination, 2017) Study: 005
Transgenic Rodent	Divanadium	6 male transgenic Big Blue mice	Negative for both	(Manjanatha <i>et</i>

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
<p>Somatic Cell Gene Mutation Assays</p> <p>OECD TG 488</p> <p>no information on deviations</p> <p>Non-GLP</p> <p>Reliability (Klimisch score): 3</p>	<p>pentaoxide, purity: 100%</p>	<p>per dose group and exposure group</p> <p>Mutant frequencies for the <i>CII</i> gene in the lung as well as lung weights from BB mice were analysed.</p> <p>0, 0.1, 1 mg V₂O₅/m³ nose-only inhalation, 6 h/d 5 d/w for 4 or 8 weeks</p> <p>Concentrations were selected based on their tumorigenic potential</p> <p>Vehicle: air</p> <p>No positive controls</p>	<p>concentrations and time points</p>	<p><i>al.</i>, 2015), (ECHA Dissemination, 2017)</p> <p>Study: 004</p>
<p>Micronucleus assay</p> <p>OECD TG 474</p> <p>deviations: yes</p> <p>Mammalian somatic cell study in bone marrow erythrocytes</p> <p>GLP: yes</p> <p>Reliability (Klimisch score): 1</p>	<p>Divanadium pentaoxide, purity is confidential information</p>	<p>6 male Sprague-Dawley rats per dose group</p> <p>Dose levels-range-finder: 90, 120, 170, 240, 300 mg V₂O₅/kg/day</p> <p>Dose levels - MN study: 0, 30, 60, 120 mg V₂O₅/kg/day</p> <p>Gavage</p> <p>Maximum dose: Maximum tolerated dose based on range-finder data.</p> <p>Vehicle: corn oil</p> <p>Positive controls: yes</p>	<p>Negative at all doses tested</p> <p>Vanadium levels increased in all sampled tissues (bone marrow and testes) in a dose-dependent manner.</p>	<p>Anonymous, 2011</p> <p>(ECHA Dissemination, 2017)</p> <p>Study: 001, study report</p> <p>Details in Annex I.</p>
<p>Micronucleus assay</p> <p>OECD TG 475, deviations: not specified</p> <p>Mammalian somatic cell study in bone marrow erythrocytes</p> <p>GLP: yes</p> <p>Reliability (Klimisch score): 2</p>	<p>Divanadium pentaoxide, purity: 99%</p>	<p>10 Male and female B6C3F1 mice per dose group</p> <p>0, 1, 2, 3, 8, 16 mg V₂O₅/m³ (whole body inhalation, 6 h/d, 5 d/w, for 3 months)</p> <p>Based on results of a dose range finding study doses were selected.</p> <p>Vehicle: air</p> <p>Positive controls: not stated</p>	<p>Negative for male and female animals at all doses tested</p> <p>No effect on PCE/NCE.</p>	<p>(NTP, 2002)</p> <p>(ECHA Dissemination, 2017)</p> <p>Study: 002</p> <p>Details in Annex I</p>
<p>Micronucleus assay in peripheral blood reticulocytes</p> <p>Reliability (Klimisch score): 3</p>	<p>Divanadium pentaoxide, purity: 99.99%</p>	<p>6 male, 6 female CD-1 mice</p> <p>0, 0.02 M V₂O₅ (1.4 mg V₂O₅/m³, <i>via</i> inhalation). V₂O₅ was suspended in saline, containing Tween 20.</p> <p>2 h/twice a week; blood samples were obtained at 24 h and every week until the end of the 4-week exposure period</p> <p>Authors deemed the level of</p>	<p>Positive in male animals at all time points</p> <p>Negative in female animals (authors assume a protective influence of oestrogen)</p>	<p>(Rojas-Lemus <i>et al.</i>, 2014)</p>

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		vanadium used would reflect airborne vanadium exposures that occur routinely among the citizens of many urban centers.		
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 ₂ O ₅ /kg bw single application V ₂ O 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 <i>et al.</i> , 2016)
Micronucleus test (Only limited information available from secondary source) Reliability Klimisch score): 4	Divanadium pentaoxide, no information on purity available	Mice (Kunming albino and 615) 0, 0.17, 2.13, 6.4 mg V ₂ O 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)
		Mice (Kunming albino) 1.44, 2.83, 5.65, 11.3 mg V ₂ O 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 ₂ O ₅ /kg bw Subcutaneous injection	Increase in the frequency of micronuclei	
		Mice (615) 0.5, 2 or 8 mg V ₂ O ₅ /m ³ 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 <i>via</i> 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 ₂ O ₅ /kg bw Intraperitoneally injected	<u>Tailed cells:</u> Lung and kidney: Statistically significant decrease at the low and	(Altamirano-Lozano <i>et al.</i> , 1999)

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		<p>Vehicle: saline</p> <p>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.</p>	<p>high dose compared with controls</p> <p>Spleen:</p> <p>Statistically significant decrease at the low and mid dose</p> <p>Heart:</p> <p>Dose-related decrease, statistically significant at all dose levels</p> <p>Liver, bone marrow:</p> <p>No statistically significant effects were observed</p> <p><u>DNA migration:</u></p> <p>A dose-response related increase on DNA migration was observed for all tissues apart from bone marrow and spleen.</p> <p><u>Nucleus/tail ratios:</u></p> <p>Dose-dependent decrease in percent of cells with low damage in all tissues, with different sensitivities.</p>	
<p>Study on testicular DNA damage in male mice (Comet Assay)</p> <p>Reliability (Klimisch score): 2</p>	<p>Divanadium pentaoxide, purity: 99.6%</p>	<p>2 male CD-1 mice per dose group</p> <p>0, 5.75, 11.5, 23 mg V₂O₅/bw single intraperitoneal injection</p> <p>24 h later animals were sacrificed. Cells from testes were used for the Comet assay.</p>	<p>Comet assay revealed significant DNA damage increasing with the dose.</p>	<p>(Altamirano-Lozano <i>et al.</i>, 1996)</p>
<p>Mammalian cell study: DNA damage and/or repair</p> <p>GLP: yes</p> <p>Lung tissue</p> <p>Reliability (Klimisch score): 1</p>	<p>Divanadium pentaoxide, purity: 99.8%</p>	<p>Female B6C3F1 mice (5 groups of 48 mice)</p> <p>0, 0.25, 1, 4 mg V₂O₅/m³ (nose-only inhalation, 6 h/d for 16 days)</p> <p>Lung samples were analysed for DNA strand breaks using the comet assay.</p> <p>Vehicle: air</p> <p>Positive controls: yes</p>	<p>Negative for all doses tested</p>	<p>(Schuler <i>et al.</i>, 2011)</p> <p>(ECHA Dissemination, 2017)</p> <p>Study: 003</p>
		<p>Analysis of 9 specific DNA-oxo-adducts in lung tissue</p> <p>Positive controls: yes</p>	<p>Concentration dependent increase in 8-oxodGuo DNA lesions with significant effects at 1 and 4 mg/m³</p>	<p>(Schuler <i>et al.</i>, 2011)</p> <p>Details in Annex I.</p>

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
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 ₂ O ₅ /kg bw Intraperitoneally injected 24 h later animals were sacrificed Vehicle: distilled water	Negative at all concentrations tested	(Altamirano-Lozano <i>et al.</i> , 1993)
Immunohistochemical 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 ₂ O ₅ (1.4 mg V ₂ O ₅ /m ³ <i>via</i> 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 <i>et al.</i> , 2016)
DNA synthesis inhibition assay (Only limited information available from secondary source) Reliability (Klimisch score): 4	Divanadium pentaoxide, no information on purity available	Male mice 0, 14.6, 29.2, 58.4 mg V ₂ O ₅ /kg bw, orally administered Vehicle: 3% starch solution 24 h after dosing, animals were sacrificed and tissue from testes, spleen and liver were analysed	No significant differences between exposure group animals and controls	Sun <i>et al.</i> , 1987 cited from (WHO, 1988)
Observation of nuclear changes <i>via</i> 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 ₂ O ₅ (1.4 mg V ₂ O ₅ /m ³ <i>via</i> 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 <i>via</i> 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 <i>et al.</i> , 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 ₂ O ₅ (1.4 mg V ₂ O ₅ /m ³ <i>via</i> 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 <i>et al.</i> , 2012)
Dominant lethal test Study of reproductive function and testicular	Divanadium pentaoxide, purity:	15-30 male CD-1 mice per group, mated with unexposed females (1:2) after exposure	V ₂ O ₅ treatment resulted in decrease in fertility rate and pregnant females.	(Altamirano-Lozano <i>et al.</i> , 1996)

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
DNA damage in male mice Reliability (Klimisch score): 2	99.6%	0, 8.5 µg V ₂ O ₅ /g bw i.p. injection Group 1: 20 control animals received vehicle (saline) every 3 rd day for 60 days Group 2: 15 animals received V ₂ O ₅ every 3 rd 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 ₂ O ₅ every 3 rd day for 60 days, groups of 5 animals were sacrificed every 10 days after the beginning of the treatment.	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.	Details in Annex I.
Dominant lethal mutation assay Only limited information available Reliability (Klimisch score): 4	Divanadium pentaoxide, no information on purity available	Male mice (no more details available) 0, 0.2, 1 or 4 mg V ₂ O ₅ /kg bw/d for 3 months, <i>via</i> subcutaneous injection Animals were mated with untreated females and number of foetuses and resorptions were recorded on GD 17	Negative result for the induction of dominant lethal mutations (no further details available)	Sun et al, 1987 cited from (WHO, 1988)

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 <i>et al.</i> , 2002)
Determination of DNA strand breaks in leukocytes using the alkaline comet assay and different	Divanadium pentaoxide	<u>Experiment 1 (Comet assay):</u> 53 exposed workers and 52 controls <u>Experiment 2 (cytom method):</u> 23 exposed workers and 24 controls	<u>Experiment 1:</u> No difference in DNA migration under standard conditions	(Ehrlich <i>et al.</i> , 2008) Details in

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
parameters of chromosomal instability in lymphocytes using the cytokinesis-block micronucleus (MN) cytome method		(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)	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 <u>Experiment 2:</u> 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%, frequency of apoptotic cells increased by 50%	Annex I.

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 typhimurium* (NTP, 2002; Sun *et al.*, 1987). However, some early reports with limited reporting and unknown quality indicated increased reverse mutations from V₂O₅ 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 V₂O₅ 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 V₂O₅ 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-Rodríguez, 2016; Sun *et al.*, 1987). Furthermore comet assays gave generally positive results with V₂O₅ 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 ⁴⁸V-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 pentoxide.

- “*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, V₂O₅ is also genotoxic *in vitro* in several micronucleus tests and comet assays.

As reported in the section 10.9, V₂O₅ is a carcinogenic substance.

Overall, the criteria to classify V₂O₅ 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 V₂O₅ 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 ⁴⁸V-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.

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	Divanadium pentaoxide, purity: 99%	Rats: Survival of rats in the exposure groups was comparable to animals in control group. Decreased body weight gain was observed at 2 mg	(NTP, 2002) (ECHA Dissemination,

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
<p>mice and F344/N rats</p> <p>50 male and 50 female animals per dose group</p> <p>GLP: yes</p> <p>Reliability (Klimisch score): 1</p>	<p>(6 h/d, 5 d/w, 104 weeks)</p> <p>Rats: 0, 0.5, 1, 2 mg V₂O₅/m³ via inhalation (particulate aerosol)</p> <p>Mice: 0, 1, 2, 4 mg V₂O₅/m³ via inhalation (particulate aerosol)</p> <p>Diet: NTP-2000</p>	<p>V₂O₅/m³ only in female rats.</p> <p><u>In male rats:</u></p> <p>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)</p> <p><u>In female rats:</u></p> <p>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)</p> <p>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/m³ males and of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) in 0.5 and 2 mg/m³ 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.</p> <p>The incidence of alveolar /bronchiolar adenoma in 0.5 mg/m³ 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.</p> <p>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.</p> <p>Conclusion from NTP: <i>some</i> evidence of carcinogenic activity in male rats and <i>equivocal</i> evidence of carcinogenic activity in female rats</p> <p>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 V₂O₅/m³ in females and from 2 mg V₂O₅/m³ in males.</p> <p><u>In male mice:</u></p> <p>Alveolar/bronchiolar carcinoma incidence was: 12/50, 29/50, 30/50, 35/50.</p> <p>Alveolar/bronchiolar adenoma or carcinoma incidence was 22/50, 42/50, 43/50, 43/50)</p>	<p>2017)</p> <p>Studies: 001 & 002</p> <p>Details in Annex I</p>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		<p><u>In female mice:</u></p> <p>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.</p> <p>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/m³ 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</p> <p>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 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/m³. 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.</p> <p>Male and female mice: <i>clear evidence</i> of carcinogenic activity (conclusion from NTP)</p>	
Carcinogenicity study in male and female mice (1 year exposure, no further information on strain available)	Divanadium pentaoxide, no information on purity available. 0, 0.5, 2, or 8 mg V ₂ O ₅ dust/m ³ (particle size	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 .	(Yao <i>et al.</i> , 1986, as cited from WHO, 2001)

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
62 - 84 male and female mice (no further information available) Reliability (Klimisch score):4	not reported) for 4 h/day for 1 year.	No further information available.	
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 ₂ O ₅ at 4 mg V ₂ O ₅ /kg bw. Pulmonary administration was performed <i>via</i> oropharyngeal aspiration. After 20 weeks, the tumour rate was determined. Vehicle: PBS (phosphate buffered saline) Control animals were 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.	Treatment of animals with MCA followed by V ₂ O 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 ₂ O 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 ₂ O ₅ acts as an <i>in vivo</i> lung tumour promotor in A/J and BALB/cJ mice. No tumours were observed in C57BL/6J mice.	(Rondini <i>et al.</i> , 2010) Details in Annex I.

Table 19: Summary table of human data on carcinogenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
---------------------	-----------------	--	--------------	-----------

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 ₂ O 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 <i>Kras</i> and overexpression of mutant P53 protein Reliability (Klimisch score): 2	Divanadium pentaoxide, purity: 99%	Exposure of mice against 0, 1, 2, 4 mg V ₂ O ₅ /m ³ <i>via</i> inhalation for 6 h/d, 5 d/w, 104 weeks Lung tissue obtained during the NTP study was analysed	<i>Kras</i> 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 <i>Kras</i> mutations”	(NTP, 2002)
<u>Mechanistic Study:</u> Analysis of lung carcinomas from B6C3F1 mice from the NTP study (NTP, 2002) for presence of MAPK (mitogen activated protein kinase) activity, <i>Kras</i> mutations and chromosomal defects Reliability (Klimisch score): 2	Divanadium pentaoxide, purity: 99%	Exposure of mice against 0, 1, 2, 4 mg V ₂ O ₅ /m ³ <i>via</i> 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 <u>similar</u> between normal lung and lung carcinomas. Phospho-MAPK <u>elevated</u> in 5/6 lung carcinoma samples, in which <i>Kras</i> mutations and chromosome 6 LOH (loss of heterozygosity) were identified <u>and</u> in 4/5 carcinomas with <i>Kras</i> mutations but no LOH. <u>no</u> phospho-MAPK was detected in carcinoma tissue without <i>Kras</i> mutation and in normal lung tissue	(Devereux <i>et al.</i> , 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 ₂ O 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 <i>et al.</i> , 2007)
Gene expression profiling in lung tissue of female B6C3F1 mice exposed for 13 weeks. Reliability (Klimisch score): 2	Divanadium pentaoxide, purity: 99.6% 0 or 2 mg V ₂ O 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 ₂ O ₅ . These genes are involved in: - hyaluron and sphingolipid 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	(Thomas <i>et al.</i> , 2009) (ECHA Dissemination, 2017) Study: 005 (Black <i>et al.</i> , 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 ₂ O ₅ /mL (5 concentrations + control) <u>Experiment 2:</u> 7 d exposure 0 – 87.5 µg V ₂ O ₅ /mL (5 concentrations + control) Highest concentrations tested were	<u>Experiment 1:</u> Negative for significant morphological transformation <u>Experiment 2:</u> Positive for significant morphological transformation starting in the lowest concentration	(Kerckaert <i>et al.</i> , 1996)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	determined in a cytotoxicity screening test		

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/m³. 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/m³. 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/m³ males and of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) in 0.5 and 2 mg/m³ 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/m³ 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/m³. 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/m³. 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/m³ and 2 mg/m³ 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/m³. 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 V₂O₅/m³ (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 V₂O₅ via oropharyngeal aspiration at the dose of 4 mg/kg bw/d (for 5 weeks). V₂O₅ 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 NF κ B 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 V₂O₅ acts as an *in vivo* lung tumour promoter 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 V₂O₅ is currently not elucidated. From the genotoxicity database, V₂O₅ 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 V₂O₅ 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 V₂O₅) 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.
B6C3F ₁ mice	Alveolar, bronchiolar neoplasms: adenoma and carcinoma	No	Yes	Yes First incidence (alveolar/bronchiolar adenoma or carcinoma) <u>(male):</u> Control: 667 days; 1 mg/m ³ : 405 days 2 mg/m ³ : 534 days 4 mg/m ³ : 394 days <u>(female):</u> Control: 731 days; 1 mg/m ³ : 522 days 2 mg/m ³ : 281 days 4 mg/m ³ : 478 days	Both sexes positive: clear evidence	No excessive toxicity	Inhalation	Assumed to be relevant to 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, V₂O₅ 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 V₂O₅ 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 V₂O₅ 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.

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 ₂ O 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 ₂ O 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	(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
		<p>histopathological lesions at 16 mg/m³)</p> <p><u>Effects on female animals:</u></p> <p>At the highest dose 3/10 females died during the study. At the other dose groups no deaths occurred.</p> <p>Final body weights from the high dose group animals were significantly reduced.</p> <p>Atrophy of the secondary reproductive organs was observed in 16 mg/m³.</p> <p>Estrous cycle length of females exposed to 8 mg/m³ 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%)</p> <p>NOAEC_{repro female rats}: 4 mg/m³ (based on increased estrous cycle length).</p> <p>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/m³ and above (see section on STOT RE for further details).</p>	
<p>90-day study</p> <p>10 male and 10 female B6C3F1 mice per dose group</p> <p>GLP: yes</p> <p>Reliability (Klimisch score): 1</p>	<p>Divanadium pentaoxide, purity: 99%</p> <p>0, 1, 2, 4, 8, or 16 mg V₂O₅/m³ (0, 0.56, 1.12, 2.25, 4.50, 8.99 mg V/m³)</p> <p>6 h/d + T₉₀ (15 min), 5 d/w for 3 months, whole body inhalation</p> <p>Animals were not mated</p>	<p><u>Effects on male animals:</u></p> <p>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/m³ or greater were significantly greater than those of the chamber controls.</p> <p>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%)</p>	<p>(NTP, 2002)</p> <p>(ECHA Dissemination, 2017)</p> <p>Study 004, publication</p> <p>Details in Annex I.</p>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		<p><u>Effects on female animals:</u></p> <p>No deaths occurred. Final body weights started to be reduced in the exposure group of 4 mg/m³.</p> <p>No significant differences were noted in estrous cycle parameters between exposed and chamber control females.</p> <p>NOAEC_{Repro}: 4 mg/m³ (based on decreased sperm motility).</p> <p>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/m³ (see section on STOT RE for further details).</p>	
<p>Testes effects after vanadium inhalation</p> <p>Male CD-1 mice</p> <p>8 animals sacrificed at each time point (3 controls, 5 exposed)</p> <p>Reliability (Klimisch score): 3</p>	<p>Divanadium pentaoxide, no information on purity available</p> <p>0; 0.02 M V₂O₅ (1436 µg V₂O₅/m³ <i>via</i> inhalation)</p> <p>Concentration based on the average concentration in the 18 filters measurement (0, 0.79 mg V/m³)</p> <p>Inhalation, 1 h/twice a week, for a total of 12 weeks, every week animals were sacrificed.</p> <p>Vehicle: deionized water</p> <p>Testes were analysed immunohistochemically.</p>	<p>During the 12-week exposure period no overt toxicity signs or weight changes (body weight and testicular weight) were detected in the V₂O₅ exposed animals compared with controls</p> <p>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 <i>vs.</i> 1.63±0.15 µg/g in exposed animals.</p> <p>Necrosis of spermatogonium, spermatocytes and Sertoli cells was observed as well as pseudo-nuclear inclusion and disruption of cellular junctions</p>	(Fortoul <i>et al.</i> , 2007)
<p>Mechanistic study, immunohistochemical analysis of gamma-tubulin in somatic and testicular germ cells after vanadium inhalation</p> <p>Male CD-1 mice</p> <p>6 animals sacrificed at each time point (3 controls, 3 exposed)</p> <p>Reliability (Klimisch</p>	<p>Divanadium pentaoxide, no information on purity available</p> <p>0; 0.02 M V₂O₅ (1.4 mg V₂O₅/m³ <i>via</i> inhalation)</p> <p>(0, 0.79 mg V/m³ <i>via</i> inhalation)</p> <p>1 h/twice a week, for a total of 12 weeks, every week animals were sacrificed.</p> <p>Vehicle: deionized water</p> <p>Testicular cells (germinal, Sertoli and Leydig cells) were analysed immunohistochemically.</p>	<p>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.</p> <p>Vanadium accumulated in the testes starting with the initial inhalation (24 h)</p>	(Mussali-Galante <i>et al.</i> , 2005)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
score): 3			
Mechanistic study, distribution of connexin 43 (part of the gap junctions) in seminiferous tubules of mice after exposure to V ₂ O 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 ₂ O ₅ (1.4 mg V ₂ O ₅ /m ³ <i>via</i> inhalation) (0, 0.79 mg V/m ³ <i>via</i> 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 <i>et al.</i> , 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 ₂ O ₅ (1.4 mg V ₂ O ₅ /m ³ <i>via</i> inhalation) (0, 0.79 mg V/m ³ <i>via</i> 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 <i>et al.</i> , 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 ₂ O 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 3 rd day for 60 days <u>Group 2</u> : 15 animals received V ₂ O ₅ every 3 rd 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 ₂ O 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 ₂ O 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 <i>et al.</i> , 1996) Details in Annex I.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
<p>Study of sex differences in the effects of Divanadium pentaoxide administrated to prepubertal rats</p> <p>CIIZ rats</p> <p><u>Experiment 1:</u> 5-9 animals per sex in treatment and control group (saline application)</p> <p><u>Experiment 2:</u></p> <p>10 control animals (saline application), 6 in treatment group</p> <p>Reliability (Klimisch score): 3</p>	<p>Divanadium pentaoxide, no information on purity available</p> <p>0, 12.5 mg V₂O₅/kg bw, intraperitoneal injection (0, 7.0 mg V/kg bw per injection)</p> <p><u>Experiment 1:</u> Newborn male and female rats were treated every second day from birth to 21 days</p> <p><u>Experiment 2:</u> Female rats were treated from day 21 to the day of the first vaginal oestrus</p>	<p><u>Experiment 1 (males):</u></p> <p>Increase in weight of seminal vesicles, thymus and submandibular glands in treatment group</p> <p><u>Experiment 1 (females):</u></p> <p>Ovulation rate was lower in treated animals</p> <p>No difference in age of vaginal opening, first vaginal oestrus, weight of ovaries, uterus, adrenals or pituitary, thymus, liver, kidneys and submandibular glands</p> <p><u>Experiment 2 (females):</u></p> <p>Increase in the weight of thymus, submandibular glands and liver</p>	(Altamirano <i>et al.</i> , 1991)
<p>Study of histological and sperm parameters after exposure</p> <p>5 male guinea pigs/dose group</p> <p>Reliability (Klimisch score): 2</p>	<p>Divanadium pentaoxide, analytical grade, no further information available</p> <p><u>Experiment 1:</u> 0, 4.5, 6.5, 8.5, 10.5, 12.5 mg V₂O₅/kg single intraperitoneal injection in saline solution</p> <p>(0, 2.5, 3.7, 4.8, 5.9, 7.0 mg V/kg bw)</p> <p><u>Experiment 2:</u> 0, 8.5 mg V₂O₅/kg bw/d intraperitoneally injected.</p> <p>(0, 4.8 mg V/kg bw/d)</p> <p>Testicular tissue of 5 animals evaluated after 24, 48, 72 and 96 h.</p>	<p><u>Experiment 1:</u> Statistically significant increase in percentage basal cell death, reduction in sperm motility, reduction in Sperm count and alteration in the spermatic cell morphology.</p> <p>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.</p> <p><u>Experiment 2:</u> 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</p>	(Uche <i>et al.</i> , 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	Vanadium,	75 semen samples from humans (25 with	Vanadium levels in oligozoospermic and azoospermic subjects are higher compared	(Zafar <i>et al.</i> ,

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
seminal plasma from Pakistani men	Speciation not indicated	normospermia = control, 25 with oligospermia and 25 with azospermia)	to normozoospermic subjects. (normospermic: 2.98 ± 1.51 ppb, oligospermic: 4.66 ± 2.75 ppb, 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 ($r = -0.45$, $p < 0.05$).	2015)
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 multivariate analysis no clear correlation between effects on sperm and vanadium load could be determined.	(Katayama <i>et al.</i> , 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 <i>et al.</i> , 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	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>	<u>Male fertility</u> was investigated by mating exposed (and control) males with virgin untreated females. <u>Female fertility</u> was 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%	(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
Reproduction Toxicity Study GLP: no Reliability (Klimisch score): 3		<p>11.43 mg NH₄VO₃/kg bw/d, (4.97 mg V/kg bw/d)</p> <p>61 days exposure (14 days pre-mating, during mating, till weaning of pups (21 days of age)</p> <p><u>Group 1:</u> 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).</p> <p>Exposed males (n=10) were analysed after mating period for body weight, testes weight, epididymis, prostate, seminal vesicles (n=10 controls).</p> <p><u>Group 2:</u> 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 pre-mating period. Dams were sacrificed with their offspring and endpoints recorded.</p> <p><u>Group 3:</u> [Examination of foetuses] endpoints in square brackets: discussed within developmental toxicity, section 10.10.4)</p>	<p>Fertility index: 95%, 46.15%, 71.43%</p> <p>Reduced weight of testes, epididymis, prostate gland, seminal vesicles, (p<0.05), with no reduction in body weight between control and treated males.</p> <p>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).</p> <p>Developmental effects reported under section 10.10.4.</p>	
Sodium metavanadate NaVO₃				
Reproductive toxicity study Reliability (Klimisch score): 3	Sodium metavanadate	<p>Sprague-Dawley albino rat</p> <p>Sodium metavanadate (purity not indicated).</p> <p>Males: 60d before mating; Females: 14d before mating, throughout gestation and lactation</p> <p>Dosing: 0, 5, 10, 20 mg NaVO₃/kg/d</p>	<p><u>Maternal effects:</u> No adverse effects</p> <p>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.</p> <p>Significant decreases in the relative</p>	(Domingo <i>et al.</i> , 1986)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		<p>Intra-gastric administration</p> <p>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 young to 21 days.</p>	<p>weights of liver, spleen and kidneys of the pups whose mothers received NaVO₃ during the lactation from 5 mg/kg/day. Decreases in body weight, body length, tail length were also observed in offsprings.</p> <p>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 NaVO₃ when compared to the control group. But these increases were not significant (P > 0.05).</p> <p><u>LOAEL</u>: 5 mg NaVO₃/kg/d (2.1 mg V/kg/d).</p>	
<p>Study on fertility and reproductive toxicity in exposed male rats</p> <p>Reliability (Klimisch score): 2</p>	<p>Sodium meta-vanadate, no information on purity available</p>	<p>8 male Sprague-Dawley rats for each dose group</p> <p><u>Experiment 1</u>: 13 days exposure</p> <p><u>Experiment 2</u>: 26 days exposure</p> <p>0, 0.2, 0.4, 0.6 mg V/kg bw/d</p> <p>Intraperitoneal injection</p> <p>Animals were sacrificed 24 h after last treatment</p>	<p>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</p> <p>Dose-dependent reduction of $\Delta^53\beta$- and 17β-hydroxysteroid dehydrogenase activity 17β-HSD and serum testosterone</p> <p>Dose-dependent decrease of superoxide dismutase and catalase activity</p> <p>Dose-dependent increase in lipid peroxidation</p> <p>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</p> <p>Dose dependent increase of abnormal sperm</p>	(Chandra <i>et al.</i> , 2007b)
<p>Study on fertility and reproductive toxicity in exposed male rats</p> <p>Reliability (Klismisch): 3</p>	<p>Sodium meta-vanadate, no information on purity available</p>	<p>8 male Sprague-Dawley rats for each group</p> <p>0, 0.4 mg V/kg bw/d for 26 days</p> <p>Intraperitoneal injection</p> <p>Animals were sacrificed 24 h after last treatment</p>	<p>Significantly reduced organ weights (testis, seminal vesicles, ventral prostate, coagulating gland, epididymis)</p> <p>Epididymal sperm count significantly reduced, percentage of abnormal sperm significantly increased</p> <p>Reduction of $\Delta^53\beta$- and 17β-hydroxysteroid dehydroge activity, serum testosterone levels and serum gonadotropins</p> <p>Decrease of superoxide dismutase and</p>	(Chandra <i>et al.</i> , 2007a)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			<p>catalase activity</p> <p>Increase in lipid peroxidation</p> <p>Increased weight of adrenals, and significant elevation of serum concentrations of corticosterone</p> <p>Testicular lesions, significant reduction of spermatogonia, preleptotene spermatocytes, mid-pachytene spermatocytes and step 7 spermatids</p>	
<p>Study on fertility and reproductive toxicity in exposed male rats</p> <p>Reliability (Klimisch score): 3</p>	<p>Sodium meta-vanadate, no information on purity available</p>	<p>8 male Sprague-Dawley rats for each group</p> <p>0, 0.4 mg V/kg bw/d for 26 days</p> <p>Intraperitoneal injection</p>	<p>Significantly reduced organ weights (testis, epididymis, prostate, seminal vesicles)</p> <p>Reduced serum testosterone, LH and FSH levels</p> <p>Reduction of $\Delta^53\beta$- and 17β-hydroxysteroid dehydroge activity</p> <p>Significantly increased vanadium concentrations in the testis.</p> <p>Decrease of superoxide dismutase and catalase activity</p> <p>Increase in lipid peroxidation</p> <p>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</p>	(Chandra <i>et al.</i> , 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 V₂O₅ are available. The respective studies are therefore not regarded as relevant for classification.

Experimental studies:

Females

Overall, few experimental studies examined the **effects of V₂O₅** 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 V₂O₅/m³ 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/m³ 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/m³. Such effects were not observed in mice (reliability: category 1; NTP, 2002). In another study conducted with high i.p. doses of V₂O₅, 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 (NH₄VO₃)** : 11.4 mg NH₄VO₃/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 V₂O₅ 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 V₂O₅ (NTP, 2002) or after oral exposure with ammonium metavanadate (Morgan and El-Tawil, 2003). Due to the structural similarity of ammonium metavanadate to divanadium pentoxide and the consistent findings reported, the observation of Morgan and El-Tawil (2003) is considered relevant for divanadium pentoxide classification and labelling.

Males

Overall, several experimental studies examined the effects of V₂O₅ 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-Nevarés *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 V₂O₅/m³ (4.5 mg V/m³) 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/m³ (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 V₂O₅ (unique concentration tested (1.4 mg V₂O₅/m³; 0.79 mg V/m³) 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-Nevarés *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 V₂O₅ 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 V₂O₅ (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 (NaVO₃) or ammonium metavanadate (NH₄VO₃)**, 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 V₂O₅ (Altamirano *et al.*, 1991; Fortoul *et al.*, 2007; Bizarro-Nevarés *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 NaVO₃ 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 cohabitation (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 pentoxide 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 pentoxide 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-Nevarés *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/m³ (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/m³, this was not found at 8 mg/m³.

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 pentoxide, 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 V₂O₅.

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 V₂O₅			
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 ₂ O 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 <i>via</i> gavage	<u>Dams:</u> in the two highest dose groups significant decreases in body weight gain (75%, 40% of control values) <u>Pups:</u> 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 <i>et al.</i> , 1986, internal report as cited in Sun, 1987; WHO, 2001; Yang <i>et al.</i> , 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 mg V ₂ O ₅ /kg bw/d (0.17, 0.56, 1.69 mg V/kg bw/d) in aqueous solution <i>via</i> intraperitoneal injection to pregnant rats 9 mg V ₂ O ₅ /kg bw, orally administered	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 sternbrae 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
	No information available on the timing of exposure.		
<p>Prenatal developmental toxicity study</p> <p>Pregnant Wistar rats</p> <p>Reliability (Klimisch score): 4</p> <p>Original study not available</p>	<p>Divanadium pentaoxide, no information on purity available</p> <p>0, 0.33, 1.0 and 3.0 mg V₂O₅/kg bw/d from GD 6 – 15.</p> <p>(0, 0.19, 0.56, 1.69 mg V/kg bw/d)</p> <p>No information on time of examination available</p> <p>Intraperitoneally injected</p>	<p><u>Dams:</u></p> <p>In the highest dose group maternal toxic symptoms, decrease of weight gain during treatment, decrease of placenta weight were observed (no further details available)</p> <p><u>Pups:</u></p> <p><u>Highest dose group:</u></p> <p>Increased incidence of embryo- foetal mortality and external or skeletal malformation</p> <p>Foetal growth retardation</p> <p><u>Middle dose group:</u></p> <p>Increased incidence of embryo- foetal mortality and external or skeletal malformations. Delayed ossification of bone.</p> <p>No information available on the lowest dose tested.</p>	<p>(Zhang <i>et al.</i>, 1993b)</p> <p>Article in Chinese, only abstract available</p>
<p>Prenatal developmental toxicity study</p> <p>Female NIH mice</p> <p>Reliability (Klimisch score): 4</p> <p>Original study not available</p>	<p>Divanadium pentaoxide, no information on purity available</p> <p>5 mg V₂O₅/kg bw/d on different gestation days (1-5, 6-15, 7, 8, 9, 10, 11, 14-17).</p> <p>(2.80 mg V/kg bw/d)</p> <p>No information on time of examination available</p> <p>Intraperitoneally injected</p>	<p><u>Dams:</u> No effects on pre-implantation and implantation</p> <p><u>Pups:</u> Increased frequency of resorption or foetal death for exposure during gestation days 6-15, 7, 14-17</p> <p>Delayed ossification (no details available) of bones for exposure during gestation days 6-15, 8, 10, 14-17.</p> <p>The authors suggest, that developmental effects occurred with an A/D - ratio (adult/ developmental toxicity) of 3</p>	<p>(Zhang <i>et al.</i>, 1991)</p> <p>Article in Chinese, only abstract available</p>
<p>Prenatal developmental toxicity study</p> <p>Female Wistar rats</p> <p>Reliability (Klimisch</p>	<p>Divanadium pentaoxide, no information on purity available</p> <p>5 mg V₂O₅ /kg bw/d on different gestation days (9, 10, and 11 and 9-12) or 3 mg V₂O₅ /kg bw/d on GD 6-15</p>	<p><u>Exposure from GD 6-15 or 9-12:</u> (3 mg/kg/d or 5 mg/kg/d):</p> <p>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</p>	<p>(Zhang <i>et al.</i>, 1993a)</p> <p>Article in Chinese, only abstract available</p>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
score): 4 Original study not available	(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	pelvis. <u>Exposure on GD 11</u> (5 mg/kg/d): Decreased weight gain, increased incidence of subcutaneous haemorrhage and visceral anomalies <u>Exposure on GD 10</u> (5 mg/kg/d): Increased incidence of foetal death, retarded ossification <u>Exposure on GD 9 and 10</u> (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 ₂ O 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 <i>et al.</i> , 1993)
Developmental toxicity study	Divanadium pentaoxide, no information on purity available	Incidence of resorbed and dead foetuses significantly increased at 1 and 4 mg/kg bw/d (17 and 27.2% respectively, vs.	Sun <i>et al.</i> , 1987 cited from

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Female rats (no more details available) Reliability (Klimisch score): 4 Original study not available	0, 0.5, 1 or 4 mg V ₂ O ₅ /kg bw/d for 10 days from GD 7 -16, <i>via</i> subcutaneous injection (0, 0.28, 0.56, 2.25 mg V/kg bw/d)	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.	(WHO, 1988) Only limited information available
Developmental toxicity study 20 female NMRI mice in exposure group 28 animals in control group Reliability (Klimisch score): 3	Divanadium pentaoxide, analytical grade, no further information available 0, 1 mM V ₂ O ₅ in 0.15 mL, single i.v. injection in the tail vein This corresponds to 0 and 27.3 µg V ₂ O ₅ (0, 15.34 µg V) or 0.9 mg V ₂ O 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 observed on resorption 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%)	(Wide, 1984)
Study of sex differences in the effects of divanadium pentaoxide administration to prepubertal rats CIIZ rats <u>Experiment 1:</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 ₂ O 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>Experiment 1 (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 <i>et al.</i> , 1991) See also Table 22
Studies on other vanadium compounds			
Ammonium metavanadate (NH₄V0₃)			
Study on fertility and prenatal developmental toxicity in exposed rats Sprague Dawley rats	Ammonium metavanadate, no information on purity available 200 ppm in drinking water (calculated with default factors)	<u>Group 1:</u> <u>Adult treated males:</u> no mortality or clinical signs of toxicity in males; no significant deviation in body	(Morgan and El-Tawil, 2003) from (ECHA

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
<p>Male rats: n=30 (10 control; 10 group 1, 10 group 2)</p> <p>Female rats: n=60 (20 control; 20 group 1, 20 group 2)</p> <p>GLP: no</p> <p>Reliability (Klimisch): 3</p>	<p>according to ECHA (2012):</p> <p>male rat: 10 mg NH₄VO₃/kg bw/d, (4,35 mg V/kg bw/d)</p> <p>female rat: 11.43 mg NH₄VO₃/kg bx/d, 4,97 mg V/kg bw/d</p> <p>Sprague Dawley rats</p> <p>Male rats: 70 day exposure</p> <p>Female rats: 61 days exposure (14 days pre-mating, during mating, till weaning of pups (21 days of age))</p> <p><u>Group 1</u>: mating with treated males; females untreated; fertility and reproductive performance were monitored</p> <p><u>Group 2</u>: mating with untreated males, females treated; fertility and reproductive performance were monitored</p>	<p>weight compared to control; Sex organ weights (testes, epididymis, prostate gland, seminal vesicles) reduced, mating and fertility index reduced.</p> <p>No reporting on histological examinations in parental animals</p> <p><i>Adult untreated females/dams</i> :</p> <p>Body weight/dam at termination: 215.1 g vs. 252.1 g (control)</p> <p>Gravid uterine weight/dam: 35.50 g vs. 67.50 g (control)</p> <p><i>foetuses/ developmental</i></p> <p>Number of dead foetuses/dam: 1.12 vs. 0.15 (control)</p> <p>Number of live foetuses/dam: 3.92 vs. 11.32 (control)</p> <p>mean foetal body weight (PND 21): 15.02 (n=16) vs. 22.51 (n=213) (control)</p> <p>Live/birth index: 100% vs. 100%(control)</p> <p>Survival index: 90% vs. 100% (control)</p> <p>Viability index: 85% vs. 99.07% (control)</p> <p>Foetuses with <i>visceral</i> anomalies: 4/ 7 (examined, exposed male P0) vs. 3/72 (examined; control)</p> <p>Foetuses with <i>skeletal</i> anomalies: 8/13 (examined, exposed male P0) vs. 1/144 (examined; control)</p> <p>Data not provided on a litter base</p> <p><u>Group 2</u>:</p> <p><i>Adult treated females/dams</i> (for details, refer to Annex I):</p> <p>No reporting of histological examinations in parental animals</p> <p>Body weight/dam at termination: 209.5 g vs. 252.1 g (control)</p> <p>Gravid uterine weight/dam: 30.35 g vs. 67.50 g (control)</p> <p><i>foetuses/ developmental</i></p> <p>Number of dead foetuses/dam: 1.16 vs. 0.15 (control)</p> <p>Number of live foetuses/dam: 3.38 vs. 11.32 (control)</p> <p>Mean foetal body weight (PND 21):</p>	<p>Dissemination, 2017)</p> <p>Details in Annex I</p>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		<p>10.34 (n=2) vs. 22.51 (n=213) (control)</p> <p>Live/birth index: 100% vs. 100%(control)</p> <p>Survival index: 85.71% vs. 100% (control)</p> <p>Viability index: 74.28% vs. 99.07% (control)</p> <p>Foetuses with <i>visceral</i> anomalies: 9/ 12 (examined, exposed female P0) vs. 3/72 (examined; control)</p> <p>Foetuses with <i>skeletal</i> anomalies: 15/23 (examined, exposed female P0) vs. 1/144 (examined; control)</p> <p>Data not provided on a litter base</p>	
<p>Developmental toxicity study on ammonium metavanadate NH₄VO₃</p> <p>Reliability (Klimisch): 2</p>	<p>Ammonium metavanadate (purity: 99%)</p> <p>Syrian golden hamster</p> <p>Treatment from gestation 5-10 day</p> <p>0, 0.47, 1.88, 3.75 mg NH₄VO₃/kg bw/d (0, 0.2, 0.8, 1.6 mg V/kg bw/d)</p> <p>Intraperitoneally</p> <p>Pregnant females were killed at day 15 (20 females/dose level)</p>	<p><u>Maternal effects:</u></p> <p>Maternal body weight and weight gain not significantly different in treatment groups from control</p> <p><u>Developmental effects:</u></p> <p>Skeletal abnormalities; “minor abnormalities.” significant (p<0,01) for all exposed groups</p> <p>Although not statistically significant, external anomalies included meningocele, one fetus with multiple anomalies and the presence of a molar pregnancy.</p>	(Carlton <i>et al.</i> , 1982)
Sodium metavanadate NaVO₃			
<p>Reproductive toxicity study</p> <p>Reliability (Klimisch score): 3</p>	<p>Sprague-Dawley albino rat</p> <p>Sodium metavanadate (purity not given).</p> <p>Males: 60d before mating; Females: 14d before mating, throughout gestation and lactation</p> <p>Dosing: 0, 5, 10, 20 mg NaVO₃ /kg/day by intra-gastric administration.</p>	<p><u>Maternal effects:</u> No adverse effects</p> <p>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 NaVO₃ when compared to the control group. But these increases were not significant (P > 0.05).</p> <p>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 NaVO₃ during the lactation from 5 mg/kg/day. Decreases in body weight, body length, tail length</p>	(Domingo <i>et al.</i> , 1986)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		were also observed in offsprings. <u>NOAEL (developmental toxicity):</u> 5 mg NaVO ₃ /kg/d (2.1 mg V/kg/d).	
Developmental toxicity study Reliability (Klimisch score): 3	Pregnant female Sprague-Dawley rat. Sodium metavanadate (purity not given). Exposure from GD 6-14 with cesarean sections performed at day 20. Dosing: 0, 5, 10, 20 mg NaVO ₃ /kg /d (0, 2.1, 4.2, 8.4 mg V/kg/d in distilled water). Intragastrically	Maternal effects: No significant adverse effects Developmental effects: Number of litters 14, 14, 12, 8. 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”	(Paternain <i>et al.</i> , 1987)
Developmental toxicity study Reliability (Klimisch score): 3	Pregnant female Swiss mice Sodium metavanadate (NaVO ₃) – analytical grade obtained from Sigma Chemical Co. (purity not given). 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/d)	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 <i>minus</i> gravid uterine weight was not statistically significantly affected compared to the	(Gómez <i>et al.</i> , 1992)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	Intraperitoneally	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 doses. LOAEL: 2 mg NaVO ₃ /kg/d (1.7 mg V/kg/d) <i>via</i> 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 (1 st PND-14 th 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 <u>Background note:</u> Iron is imported to brain <i>via</i> oligodendrocytes and OPCs. The 2 nd 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 <i>et al.</i> , 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	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	(Jiang <i>et al.</i> , 2016)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
(environmental vanadium pollution)			<p>with a urinary vanadium of $\geq 2.91 \mu\text{g V/g creatinine}$ compared to controls ($\leq 1.42 \mu\text{g V/g creatinine}$); correlation trend $p < 0.02$</p> <p>Still significant, if preterm births excluded.</p> <p>Unchanged in multivariable logistic regression including other metals (arsenic, lead, cadmium and nickel) as potential reasons for LBW</p> <p>Vanadium was measured just once during pregnancy and may not be representative for the entire pregnancy period</p>	

From recent Canadian environmental data in Canada, vanadium level exposure of general population is lower than $0.1 \mu\text{g/g creatinine}$ (median)¹². According to occupational biomonitoring, it is suggested that $25 \mu\text{g V/m}^3$ can be correlated with $35 \mu\text{g V/g creatinine}$ (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 $\text{V}_2\text{O}_5/\text{kg bw/d}$ (i.e. $\geq 0.6 \text{ mg V/kg bw/d}$). 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

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

(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 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 V₂O₅. 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 NH₄VO₃ 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 V₂O₅. 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_4VO_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 (NaVO_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/foetoletality 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 pentoxide 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 V₂O₅ 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 V₂O₅ 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 V₂O₅ 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 V₂O₅, 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 V₂O₅ 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 (NaVO₃)			
<p>Study on postnatal developmental toxicity in suckling rats</p> <p>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</p> <p>Reliability (Klimisch score): 3</p>	<p>Sodium metavanadate, no information on purity available</p> <p>0, 3 mg NaVO₃/kg bw (1.25 mg V/kg bw/d) intraperitoneal injection to dams from PND 10 -21</p> <p>Pups were observed for behavioural alterations until PND 21.</p> <p>Pups were sacrificed on PND 21 and microscopic analysis of brain tissue was performed</p>	<p>Eye opening mean day in treated animals was significantly delayed.</p> <p>A progressive decrease in righting time reflex was less marked for exposed animals (non-significant).</p> <p>On exposure day 20 a significant decrease in forelimb support latency was observed in exposure group.</p> <p>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.</p> <p>Microscopic analysis showed decrease in myelin fiber density in different brain areas.</p>	<p>(Soazo and Garcia, 2007)</p> <p>Details in Annex I.</p>
<p>Study on postnatal developmental toxicity in suckling rats</p> <p>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</p> <p>Reliability (Klimisch score): 3</p>	<p>Sodium metavanadate, no information on purity available</p> <p>0, 3 mg NaVO₃ /kg bw (1.25 mg V/kg bw/d) intraperitoneal injection to dams from PND 10 - 21</p> <p>Pups were sacrificed on PND 21 and biochemical and microscopic analysis was performed</p>	<p>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)</p> <p>Astrogliosis: Glial fibrillary acidic protein (GFAP)-positive astrocytes were larger in exposure group</p> <p>→ Signs of oxidative damage in the brain</p>	<p>(Cuesta <i>et al.</i>, 2013)</p> <p>Details in Annex I.</p>
<p>Study on neurobehavioral toxicity in weaned rats after lactational exposure and mode of action</p> <p>Sprague-Dawley rats;</p> <p>Dams (n=12) received NaVO₃ intraperitoneally or distilled water</p>	<p>Sodium metavanadate, no information on purity available</p> <p>0, 3 mg NaVO₃ /kg bw (1.25 mg V/kg bw/d), intraperitoneal injection to dams from PND 1 - 21, pups were exposed <i>via</i> lactation</p> <p>Pups were tested in the rotarod test (motor coordination) at PND60.</p> <p>Expression of the brain-derived neurotrophic factor (BDNF) and its tropomyosin-related kinase B</p>	<p>Performance in rotarod test (motor coordination) was significantly impaired in weaned male rats after lactational exposure to NaVO₃ (latency to fall from rotarod, coordination index).</p> <p>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.</p>	<p>(Wang <i>et al.</i>, 2015)</p>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
36 male pups; Reliability (Klimisch score): 3	(TrkB) receptor were analysed, with or without prior treadmill running as exercise.		
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 <i>et al.</i> , 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 <i>et al.</i> , 2011)
Ammonium vanadate (NH₄VO₃)			

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
<p>Study on fertility and prenatal developmental toxicity in exposed rats</p> <p>Sprague Dawley rats</p> <p>Male rats: n=30 (10 control; 10 group 1, 10 group 2)</p> <p>Female rats: n=60 (20 control; 20 group 1, 20 group 2)</p> <p>GLP: no</p> <p>Reliability (Klimisch): 3</p>	<p>Ammonium metavanadate, no information on purity available</p> <p>200 ppm in drinking water (calculated with default factors according to ECHA (2012)):</p> <p>male rat: 10 mg NH₄VO₃/kg bw/d, (4,35 mg V/kg bw/d)</p> <p>female rat: 11.43 mg NH₄VO₃/kg bw/d, 4,97 mg V/kg bw/d</p> <p>Sprague Dawley rats</p> <p>Male rats: 70 day exposure</p> <p>Female rats: 61 days exposure (14 days pre-mating, during mating, till weaning of pups (21 days of age))</p> <p><u>Group 1</u>: mating with treated males; females untreated; fertility and reproductive performance were monitored</p> <p><u>Group 2</u>: mating with untreated males, females treated; fertility and reproductive performance were monitored</p>	<p><u>Group 2</u>: Adult treated females/dams</p> <p>During lactation, the pups behavioral responses (such as learning and memory responses) were decreased.</p> <p>Results for group 1 are not relevant for this section related to effect on or <i>via</i> lactation. The results are nevertheless detailed in section related to effect on fertility and development.</p>	<p>(Morgan and El-Tawil, 2003)</p> <p>Details in Annex I.</p>
Pentavanadate (no further specified)			
<p>Kinetic study in rats</p> <p>Reliability (Klimisch score): 3</p>	<p>⁴⁸V pentavanadate</p> <p>3 nursing Sprague Dawley rats received 0.1 µg V/rat (2nd PND, single IV injection)</p>	<p>Two days after injection to nursing rats: 34 ng ⁴⁸V/g x 10³ milk content.</p> <p>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-⁴⁸V may represent an easily absorbed and mobile form.</p> <p>Tissue concentration of ⁴⁸V 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 ⁴⁸V/g x 10³ (weanling); kidney: 4.7±0.87 (suckling) vs. 1.12 ± 0.26 (weanling)</p>	<p>(Edel and Sabbioni, 1989)</p>

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 V₂O₅ 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 NaVO₃, in which the nursing dams (rats as well as mice) were exposed by intraperitoneal injection of 3 mg NaVO₃/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 secrete 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 pentoxide, 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 (NaVO₃) *via* intraperitoneal injection in which there is no information on maternal toxicity or 100 ppm (NH₄VO₃) 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 V₂O₅ 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/m³ 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 V₂O₅, 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 V₂O₅ is able to damage the blood-testis barrier (Bizarro-Nevarés *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 V₂O₅ 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 V₂O₅ in Category 1B - H360F “Presumed human reproductive toxicant” is therefore warranted.**

Adverse effects on development

Available studies with V₂O₅ 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 fetuses/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 pentoxide 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 V₂O₅ 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 V₂O₅.

In conclusion, because of observed adverse effects of divanadium pentoxide 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 pentoxide. 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 V₂O₅, 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.

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 V₂O₅, 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 ₂ O ₅ /kg bw/day	(Mountain <i>et al.</i> , 1953) (ECHA Dissemination, 2017) Study: 005 Details in Annex I
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 <i>et al.</i> , 2011)

<p>groups of 48 mice) GLP: yes Reliability (Klimisch score): 1</p>	<p>0, 0.25, 1, 4 mg V₂O₅/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</p>	<p>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.</p>	<p>Details in Annex I “germ cell mutagenicity”</p>
<p>90-day study 10 male and 10 female F344 rats per dose group GLP: yes Reliability (Klimisch score): 1</p>	<p>Divanadium pentaoxide, purity: 99% 0, 1, 2, 4, 8, or 16 mg V₂O₅/m³ (6 h/d + T₉₀ (15 min), 5 d/w for 3 months, whole body inhalation to particulate aerosol).</p>	<p>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. Hyperplastic 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</p>	<p>(NTP, 2002) from (ECHA Dissemination, 2017) Study: 002, publication Details in Annex I</p>

		<p>on increased lung weights and epithelial hyperplasia, inflammation and fibrosis in lungs at 2 mg/m³ and above.</p> <p>NOAEC_{systemic} was 8 mg/m³ in rats.</p>	
<p>90-day study</p> <p>10 male and 10 female B6C3F1 mice per dose group</p> <p>GLP: yes</p> <p>Reliability (Klimisch score): 1</p>	<p>Divanadium pentaoxide, purity: 99%</p> <p>0, 1, 2, 4, 8, or 16 mg V₂O₅/m³</p> <p>(6 h/d + T90 (15 min), 5 d/w for 3 months, Whole body inhalation to particulate aerosol).</p>	<p>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.</p> <p>Final mean body weights and body weight gains from 8 mg/m³ in males and from 4 mg/m³ 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.</p> <p>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/m³. All mice at and above 8 mg/m³ had lung inflammation and epithelial hyperplasia.</p> <p>The epididymal spermatozoal motility of males exposed from 8 mg/m³ was significantly decreased. Results on reproductive effects are detailed in section 10.100.</p> <p>NOAEC_{local} = 1 mg/m³ air (males and females) based on increased absolute lung weights and epithelial hyperplasia and inflammation in lungs at 2 mg/m³.</p> <p>NOAEC_{systemic} was 2 mg/m³ in mice based on decreased body weight.</p>	<p>(NTP, 2002) from (ECHA Dissemination, 2017)</p> <p>Study: 006</p> <p>Details in Annex I</p>
<p>Subchronic (26 weeks) inhalation study in monkeys</p> <p>Adult, male cynomolgus monkeys (<i>Macaca fascicularis</i>)</p> <p>8 -9 animals per exposure group</p> <p>The study assessed pulmonary reactivity to V₂O₅ with provocation challenges, and compared V₂O₅ reactivity before and after subchronic V₂O₅</p>	<p>Divanadium pentaoxide, > 99.6 %</p> <p>Whole body inhalation for 6 h/d, 5 d/week for 26 weeks</p> <p>Group 1 (9 animals): 0.1 mg/m³ (Mon, Wed, Fri) and 1.1 mg/m³ (Tue, Thurs)</p> <p>Group 2 (9 animals): 0.5 mg/m³</p> <p>Group 3 (8 animals): control group, exposed against vehicle clean air</p> <p>Provocation challenge before and after</p>	<p>In none of the two exposure groups, pulmonary reactivity to V₂O₅ was increased by subchronic V₂O₅ 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.</p> <p>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.</p> <p>Effects after pre-exposure challenge (acute effects):</p> <p>Pre-exposure challenges with 0.5 and 3 mg V₂O₅/m³ produced a concentration-dependent impairment in pulmonary function, characterized by airway obstructive changes (increased resistance and decreased flow).</p> <p>Analysis of respiratory cells recovered from the lung by</p>	<p>(Knecht <i>et al.</i>, 1992)</p> <p>Also study 010 from (ECHA Dissemination, 2017)</p>

<p>exposure with pulmonary function testing.</p> <p>In addition to pulmonary function testing, bronchial lavage fluid was analysed.</p> <p>GLP: no information</p> <p>Reliability (Klimisch score): 2</p>	<p>subchronic exposure: - 6 h/d with 0.5 mg V₂O₅ and 2 weeks later: - 6 h/d with 3 mg V₂O₅</p>	<p>bronchoalveolar lavage demonstrated that airway obstruction was accompanied by a significant influx of inflammatory cells into the lung.</p> <p>Cytological immunological results test (IgE and IgG analysis) did not indicate allergic sensitization.</p>	
<p>2-year carcinogenicity study in B6C3F1 mice and F344/N rats</p> <p>50 male and 50 female animals per dose group</p> <p>GLP: yes</p> <p>Reliability (Klimisch score): 1</p>	<p>Divanadium pentaoxide, purity: 99% (6 h/d, 5 d/w, 104 weeks)</p> <p>Mice: 0, 1, 2, 4 mg V₂O₅/m³ <i>via</i> inhalation to particulate aerosol</p> <p>Rats: 0, 0.5, 1, 2 mg V₂O₅/m³ <i>via</i> inhalation to particulate aerosol; whole body</p> <p>Diet: NTP-2000</p> <p>MMAD = 1.0-1.3 µm</p>	<p>Rats:</p> <p>Survival of rats in the exposure groups was comparable to animals in control group.</p> <p>Decreased body weight gain was observed at 2 mg/m³.</p> <p>In the respiratory tract (lungs) lesions were observed: inflammation, interstitial fibrosis, histiocytosis and hyperplasia (alveole and bronchiole) mostly in a dose dependent manner starting from 0.5 mg/m³ with squamous metaplasia of the alveoli at 2 mg/m³.</p> <p>In the other part of the respiratory tract, inflammation, fibrosis, degeneration, hyperplasia and squamous metaplasia of the respiratory epithelium of the epiglottis were observed in larynx from 0.5 mg/m³ as well as hyperplasia of the respiratory epithelium of goblet cells in the nose.</p> <p>The incidences (but not severity) of chronic nephropathy were significantly increased in male rats exposed to 1 or 2 mg/m³ (46/50 and 47/50 respectively vs. 37/50 in the control group). Although the NTP doesn't have a formal historical control database for nonneoplastic lesions, a review of recent studies indicates that the incidence in the male control group in the current study is low. Overall, it is not clear if the increased incidences of nephropathy were related to exposure to V₂O₅ or were a reflection of the low incidence in the control group.</p> <p>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.</p> <p>Mice:</p> <p>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 V₂O₅/m³.</p> <p>In the respiratory tract (lungs), lesions were observed: inflammation, interstitial fibrosis, histiocytosis and</p>	<p>(NTP, 2002)</p> <p>Also studies no. 001 and 005 reported in (ECHA Dissemination, 2017)</p> <p>Details in Annex I</p>

		<p>hyperplasia (alveolar and bronchiole) mostly in a dose dependent manner starting from 1 mg/m³.</p> <p>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</p> <p>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.</p> <p>Results on neoplastic lesions are reported in section 10.9.</p>	
--	--	--	--

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
<p>Study on central nervous system effects from subacute exposure of adult rats</p> <p>Reliability (Klimisch score): 3</p>	<p>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.</p> <p>Sodium metavanadate (purity not provided)</p> <p>Histochemical and immunohistochemical staining (brain)</p> <p>Biochemical analysis: lipid peroxidation by thiobarbituric acid reaction</p> <p>Behavioral: Open field tests and Rotarod test were performed the day after the last dose had been administered</p>	<p>Decreased myelin fiber density in different brain areas in exposed rats</p> <p>Lipid peroxidation was significantly elevated in cerebellum and hippocampus in the treated group</p> <p>Significant decrease in locomotor activity and grooming response in the treated group.</p> <p>Similar behaviour (exposed/control) in rotarod test</p>	(García <i>et al.</i> , 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
<p>Cross-sectional case-control study with 63 workers from a V₂O₅-producing company in Finland.</p>	<p>Divanadium pentaoxide</p>	<p>- 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).</p> <p>- 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 V₂O₅/m³</p> <p>- 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 V₂O₅/m³</p> <p>Performed tests:</p> <ul style="list-style-type: none"> - Rhinoscopy - Sputum cells were analysed - Pulmonary ventilation measured - Nasal secretion smear cells were analysed 	<p>Cases self-reported subjective symptoms of respiratory tract irritation</p> <p>Rhinoscopy: no differences between the groups</p> <p>Cytology: Number of neutrophils significantly increased in nasal smears of exposed group.</p> <p>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</p> <p>→ clear signs of inflammation (not related to allergy, since number of eosinophils not significantly changed in exposed group)</p> <p>No results on pulmonary ventilation measurements reported in the publication</p>	<p>(Kiviluoto <i>et al.</i>, 1979)</p> <p>(ECHA Dissemination, 2017) “epidemiological data”</p> <p>Study: 001</p>

		<p>Same collective as indicated above in Kiviluoto <i>et al.</i>, (1979)</p> <p>Performed tests (testing in 1975):</p> <ul style="list-style-type: none"> - Respiratory questionnaire - X-ray analysis of the lung - Pulmonary ventilation measured 	<p>Respiratory symptoms: significantly more wheezing in the exposure group.</p> <p>X-ray analysis: no exposure related differences observed</p> <p>Ventilation measurement: no differences observed</p>	(Kiviluoto, 1980)
		<p>Same collective exposure as indicated above (Kiviluoto <i>et al.</i>, 1979). However the control group consisted of only 22 men. Whether these men were part of the “collective control” mentioned by Kiviluoto <i>et al.</i>(1979) is not described.</p> <p>Performed tests:</p> <ul style="list-style-type: none"> - Hematologic and serum chemical laboratory tests 	<p>No significant differences were observed for the hematologic results of exposed and non-exposed workers.</p> <p>In the serum chemical test, significant differences were observed for serum albumin (↓), chloride (↓), urea (↑), bilirubin (↓) and conjugated bilirubin (↑).</p>	(Kiviluoto <i>et al.</i> , 1981a) (ECHA Dissemination, 2017) “epidemiological data” Study: 003
Case-control study with 12 workers from a vanadium plant in South Africa.	Divanadium pentaoxide	<p>12 workers chronically exposed against < 0.15 - 1.53 mg V₂O₅/m³ with diagnosed bronchial hyperreactivity (by lung function and bronchoprovocation tests). Control subjects (12) worked in the same company but did not show bronchial hyperreactivity.</p> <p>Performed tests/observations:</p> <ul style="list-style-type: none"> - Onset of symptoms - Serum IgE and atopy 	<p>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.</p> <p>Serum IgE and atopy IgE levels between cases and controls were not significantly different.</p> <p>None of the subjects was exposed against toxic levels of SO₂ and NH₃. For 3/12 workers co-exposure against SO₂ and NH₃ could be excluded.</p>	(Irsigler <i>et al.</i> , 1999)

<p>Case-control study with 24 men working in vanadium plants in the USA (13 men from Colorado and 11 men from Ohio)</p>	<p>Divanadium pentaoxide</p>	<p>24 workers were exposed to vanadium (as V₂O₅) <i>via</i> inhalation (at least for 6 months) against the following concentrations:</p> <p>Colorado plant: 0.097 - 0.243 mg V₂O₅ /m³ (mass respirable vanadium: 16.6 % to 51 %; Particle size < 5 µm: 92.5 to 99 %) Ohio plant: 0.018 - 0.925 mg V₂O₅/m³ (mass respirable vanadium: 2 % to 100 %; Particle size < 5 µm: 96.3 to 100 %)</p> <p>45 control subjects matched for age, economic status and job activities, not coming from the vanadium industry.</p> <p>Performed tests/observations:</p> <ul style="list-style-type: none"> - 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 	<p>Symptoms with significant differences increased in exposure vs. control group:</p> <p>Cough, sputum, eye, nose, throat irritation, epistaxia, wheezing, rales, or injected pharynx, green tongue</p> <p>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).</p> <p>No significant differences were found for haematocrit urinalysis and electrocardiogram results</p>	<p>(Lewis, 1959a; b) (ECHA Dissemination, 2017) Study: 004</p>
<p>Experimental study in 24 volunteers (12 workers of a vanadium plant and 12 students)</p>	<p>Divanadium pentaoxide</p>	<p>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.</p> <p>Performed tests:</p> <ul style="list-style-type: none"> - psychological, neuropsychological, psychosomatic and behaviour toxicological performance tests 	<p>No behaviour toxicological changes were observed between the different groups.</p> <p>The variation of exposure to vanadium pentoxide had no influence on eye-hand coordination and on performance in fine motor response</p> <p>→ No influence of variation of exposure to V₂O₅ on neurobehavioural performances was found.</p> <p>→ No correlation between neuropsychological performances and concentration of metabolized vanadium was found.</p>	<p>Hörtnagel <i>et al.</i>, 1994 cited from (ECHA Dissemination, 2017) Study: 005</p>

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/m ³ . In the control areas the concentration range was 0.000 to 0.007 mg/m ³ . Concentrations do not seem to refer to V ₂ O 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 <i>et al.</i> , 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 V₂O₅/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 V₂O₅ for 16 days (6 hours per day) to concentrations of 0, 0.25, 1 and 4 mg/m³. The observed increase in lung weights, alveolar histiocytosis, multifocal subacute alveolitis, multifocal granulocytic infiltration and cell proliferation rate at concentrations of 1 mg/m³ 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/m³ (subchronic studies) and up to 2 and 4 mg/m³ 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/m³ (mice and male rats) and at 16 mg/m³ (female rats). The respiratory effects were more intense with increasing exposure time and started at 2 mg/m³, 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/m³. To be noted that in mice species, hyperplastic 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/m³ or greater as well as lung inflammation. **Lung squamous metaplasia** was observed in one female rat exposed to 16 mg/m³. 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/m³ and greater. NOAEC_{local} is set at 1 mg/m³ air (mice and rat) based on increased absolute lung weights and epithelial hyperplasia and inflammation in lungs at 2 mg/m³ and above associated with lung fibrosis in rats from 2 mg/m³ in mice and rat subchronic studies. NOAEC_{sys} was 2 mg/m³ in mice and 8 mg/m³ 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 V₂O₅ particulate aerosol at 0.5, 1, 2 mg /m³ and at 1, 2, 4 mg/m³ respectively. In rats, decreased body weight gain was noted at 2 mg V₂O₅/m³. In mice, decreased body weight gain was observed from 1 mg V₂O₅/m³ and survival of the highest dosed male mice (4 mg/m³) 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/m³ in rats and from 1 mg/m³ in mice, leading to squamous metaplasia of the alveoli at 2 mg/m³. 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/m³ in rats and from 1 mg/m³ 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/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. The incidence of chronic nephropathy was significantly

increased in male rats exposed from 1 mg/m³. However, it is not clear if the increased incidences in this study were related to exposure to V₂O₅ 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; Rodríguez-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 V₂O₅ (particulate aerosol). These effects occurred, in the 90-day studies, from 2 mg/m³ in both species and in the 2-year studies, from 0.5 mg/m³ in rats and 1 mg/m³ 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 V₂O₅/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 V₂O₅ (particulate aerosol) support the evidence of local respiratory non-neoplastic effects.

Dermal exposure

No relevant repeated dose toxicity study with dermal exposure to divanadium pentoxide 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 V₂O₅ (particulate aerosol). These effects occurred, in the 90-day studies, from 2 mg/m³ in both species and in the 2-year studies, from 0.5 mg/m³ in rats and 1 mg/m³ in mice.

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

Conclusion	CLP criteria
<p><u>Animal data</u> Following inhalation exposure of V₂O₅ (particulate aerosol), the target organ of V₂O₅ 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.</p> <p>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/m³. In rat, lung fibrosis (minimal to moderate) and lung inflammation occurred from 2 mg/m³. Lung squamous metaplasia was observed in one female rat exposed to 16 mg/m³. Nasal inflammation, hyperplasia and metaplasia of the nasal respiratory epithelium were observed from 4 mg/m³ in rats.</p> <p>Systemic toxicity were observed with decreased body weights from 4 mg/m³ (mice) and at 16 mg/m³ (rat) and with lethality at 16 mg/m³ in both species although more important in rat species. Effects on reproductive system were also reported in both species (from 8 mg/m³).</p> <p>NOAEC_{local} was 1 mg/m³ air (mice and rat) based on increased absolute lung weights and lung inflammation in lungs from 2 mg/m³ associated with lung fibrosis in both species in subchronic studies. NOAEC_{sys}t was 2 mg/m³ in mice and 8 mg/m³ in rats.</p> <p>In the 2-year studies, decreased body weight gain was observed from 2 mg V₂O₅/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/m³ in rats and from 1 mg/m³ in mice combined with squamous metaplasia of the alveoli at 2 mg/m³ in rats.</p> <p>Inflammation and degeneration of the respiratory epithelium in larynx were also observed at all tested concentrations - with squamous metaplasia from</p>	<p>STOT RE Category 1 (H372):</p> <p>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.</p> <p>Guidance value to assist on category 1 classification based on 90-day inhalation studies in rat for <i>dust/mist/fume</i> : ≤ 0.02 mg/L/6h/day (= 20 mg/m³)</p>

<p>0.5 mg/m³ in rats and from 1 mg/m³ 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.</p> <p>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.</p> <p><u>Human data</u></p> <p>The respiratory tract was also confirmed as the target organ of V₂O₅ in several occupational studies.</p> <p>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)</p> <p><i>“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.</i></p> <p><i>(d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination.</i></p> <p><i>(e) multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity.</i></p> <p>In conclusion, respiratory effects namely lung fibrosis in 90-day studies occurring from 2 mg/m³ (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.</p> <p>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/m³ (corresponding to 0.0005 mg/L/6h/day) in rats and from 1 mg/m³ (corresponding to 0.001 mg/L/6h/day) in mice with alveoli squamous metaplasia at 2 mg/m³ (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/m³ (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.</p> <p>Human data confirm the respiratory effects of V₂O₅ reported in experimental studies. In this context, V₂O₅ animal and human data allows to conclude that V₂O₅ 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 V₂O₅.</p>	
---	--

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/m³ (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/m³ in rats and from 1 mg/m³ 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).

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

Adachi A, Ogawa K, Tsushi Y, *et al.* 2000b. Balance, excretion and tissue distribution of vanadium in rats after short-term ingestion. *J Health Sci* 46(1):59-62.

AGS, Ausschuss für Gefahrstoffe (2015). Begründung zu Vanadiumverbindungen in TRGS 900. Ausgabe: November 2015. Stand: Mai 2015; online:http://www.baua.de/de/Themen-von-A-Z/Gefahrstoffe/TRGS/Arbeitsplatzgrenzwerte.html_nnn=true

Altamirano-Lozano, M.; Alvarez-Barrera, L.; Basurto-Alcántara, F.; Valverde, M.; Rojas, E. (1996). Reprotoxic and genotoxic studies of vanadium pentoxide in male mice. *Teratogenesis, Carcinogenesis, and Mutagenesis*, 16, 7-17

Altamirano-Lozano, M.; Alvarez-Barrera, L.; Roldán-Reyes, E. (1993). Cytogenetic and teratogenic effects of vanadium pentoxide on mice. *Medical Science Research*, 21, 711-713

Altamirano-Lozano, M.; Valverde, M.; Alvarez-Barrera, L.; Molina, B.; Rojas, E. (1999). Genotoxic studies of vanadium pentoxide (V₂O₅) in male mice. II. Effects in several mouse tissues. *Teratogenesis, Carcinogenesis, and Mutagenesis*, 19, 243-255

Altamirano, M.; Ayala, M.E.; Flores, A.; Morales, L.; Dominguez, R. (1991). Sex differences in the effects of vanadium pentoxide administration to prepubertal rats. *Medical Science Research*, 19, 825-826

Anke, M. (2004). Vanadium - An element both essential and toxic to plants, animals and humans? *Anales de la Real Academia Nacional de Farmacia*, 70, 961-999

Aschner, M.; Levin, E.D.; Sunol, C.; Olopade, J.O.; Helmcke, K.J.; Avila, D.S.; Sledge, D.; Ali, R.H.; Upchurch, L.; Donerly, S.; Linney, E.; Forsby, A.; Ponnuru, P.; Connor, J.R. (2010). Gene-environment interactions: neurodegeneration in non-mammals and mammals. *Neurotoxicology*, 31, 582-588

Assem, F.L.; Levy, L.S. (2009). A review of current toxicological concerns on vanadium pentoxide and other vanadium compounds: gaps in knowledge and directions for future research. *Journal of Toxicology and Environmental Health. Part B*, 12, 289-306

Assem, F.L.; Levy, L.S. (2012). Inhalation toxicity of vanadium. In: Michibata, H., Vanadium: Biochemical and Molecular Biological Approaches Springer Science+Business Media B.V., Dordrecht, 209-224

ATSDR, Agency for Toxic Substances and Disease Registry (2012). Toxicological Profile for Vanadium
U.S. Department of Health and Human Services; Public Health Service

Avila-Costa, M.R.; Colin-Barenque, L.; Zepeda-Rodríguez, A.; Antuna, S.B.; Saldivar, O.L.; Espejel-Maya, G.; Mussali-Galante, P.; del Carmen Avila-Casado, M.; Reyes-Olivera, A.; Anaya-Martínez, V.; Fortoul, T.I. (2005). Ependymal epithelium disruption after vanadium pentoxide inhalation. A mice experimental model. *Neuroscience Letters*, 381, 21-25

Avila-Costa, M.R.; Fortoul, T.I.; Nino-Cabrera, G.; Colín-Barenque, L.; Bizarro-Nevares, P.; Gutiérrez-Valdez, A.L.; Ordóñez-Librado, J.L.; Rodríguez-Lara, V.; Mussali-Galante, P.; Díaz-Bech, P.; Anaya-

Martínez, V. (2006). Hippocampal cell alterations induced by the inhalation of vanadium pentoxide (V₂O₅) promote memory deterioration. *Neurotoxicology*, 27, 1007-1012

Azeez, I.A.; Olopade, F.; Laperchia, C.; Andrioli, A.; Scambi, I.; Onwuka, S.K.; Bentivoglio, M.; Olopade, J.O. (2016). Regional myelin and axon damage and neuroinflammation in the adult mouse brain after long-term postnatal vanadium exposure. *Journal of Neuropathology and Experimental Neurology*, 75, 843-854

Banda, M.; McKim, K.L.; Haber, L.T.; MacGregor, J.A.; Gollapudi, B.B.; Parsons, B.L. (2015). Quantification of *Kras* mutant fraction in the lung DNA of mice exposed to aerosolized particulate vanadium pentoxide by inhalation. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, 789-790, 53-60

Beyersmann, D.; Hartwig, A. (2008). Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. *Archives of Toxicology*, 82, 493-512

Bhattacharya, K.; Cramer, H.; Albrecht, C.; Schins, R.; Rahman, Q.; Zimmermann, U.; Dopp, E. (2008). Vanadium pentoxide-coated ultrafine titanium dioxide particles induce cellular damage and micronucleus formation in V79 cells. *Journal of Toxicology and Environmental Health, Part A*, 71, 976-980

Bizarro-Neves, P.; Rojas-Lemus, M.; Colin-Barenque, L.; Gonzalez-Villalva, A.; Fortoul, T.I. (2016). Inhalation of vanadium modifies gap junctions in mouse seminiferous tubules. *Toxicological & Environmental Chemistry*, 98, 266-278

Black, M.B.; Dodd, D.E.; McMullen, P.D.; Pendse, S.; MacGregor, J.A.; Gollapudi, B.B.; Andersen, M.E. (2015). Using gene expression profiling to evaluate cellular responses in mouse lungs exposed to V₂O₅ and a group of other mouse lung tumorigens and non-tumorigens. *Regulatory Toxicology and Pharmacology*, 73, 339-347

Bogden JD, Higashino H, Lavenhar MA, *et al.* 1982. Balance and tissue distribution of vanadium after short-term ingestion of vanadate. *J Nutr* 112:2279-2285.

Boice, J.D.; Mumma, M.T.; Blot, W.J. (2007). Cancer and noncancer mortality in populations living near uranium and vanadium mining and milling operations in Montrose County, Colorado, 1950–2000. *Radiation Research*, 167, 711-726

Cano-Gutiérrez, G.; Acevedo-Nava, S.; Santamaría, A.; Altamirano-Lozano, M.; Cano-Rodríguez, M.C.; Fortoul, T.I. (2012). Hepatic megalocytosis due to vanadium inhalation: participation of oxidative stress. *Toxicology and Industrial Health*, 28, 353-360

Carlton, B.D.; Beneke, M.B.; Fisher, G.L. (1982). Assessment of the teratogenicity of ammonium vanadate using Syrian golden hamsters. *Environmental Research*, 29, 256-262

Chandra, A.K.; Ghosh, R.; Chatterjee, A.; Sarkar, M. (2007a). Amelioration of vanadium-induced testicular toxicity and adrenocortical hyperactivity by vitamin E acetate in rats. *Molecular and Cellular Biochemistry*, 306, 189-200

Chandra, A.K.; Ghosh, R.; Chatterjee, A.; Sarkar, M. (2007b). Effects of vanadate on male rat reproductive tract histology, oxidative stress markers and androgenic enzyme activities. *Journal of Inorganic Biochemistry*, 101, 944-956

Chandra, A.K.; Ghosh, R.; Chatterjee, A.; Sarkar, M. (2010). Protection against vanadium-induced testicular toxicity by testosterone propionate in rats. *Toxicology Mechanisms and Methods*, 20, 306-315

Clancy, H.A.; Sun, H.; Passantino, L.; Kluz, T.; Munoz, A.; Zavadil, J.; Costa, M. (2012). Gene expression changes in human lung cells exposed to arsenic, chromium, nickel or vanadium indicate the first steps in cancer. *Metallomics*, 4, 784-793

Colín-Barenque, L.; Martínez-Hernández, M.G.; Baiza-Gutman, L.A.; Avila-Costa, M.R.; Ordóñez-Librado, J.L.; Bizarro-Nevarés, P.; Rodríguez-Lara, V.; Pinón-Zarate, G.; Rojas-Lemus, M.; Mussali-Galante, P.; Fortoul, T.I. (2008). Matrix metalloproteinases 2 and 9 in central nervous system and their modification after vanadium inhalation. *Journal of Applied Toxicology*, 28, 718-723

Colín-Barenque, L.; Pedraza-Chaverri, J.; Medina-Campos, O.; Jimenez-Martínez, R.; Bizarro-Nevarés, P.; González-Villalva, A.; Rojas-Lemus, M.; Fortoul, T.I. (2015). Functional and morphological olfactory bulb modifications in mice after vanadium inhalation. *Toxicologic Pathology*, 43, 282-291

Conklin, A.W.; Skinner, C.S.; Felten, T.L.; Sanders, C.L. (1982). Clearance and distribution of intratracheally instilled vanadium - 48 compounds in the rat. *Toxicology Letters*, 11, 199-203

Crans, D.C.; Smee, J.J.; Gaidamauskas, E.; Yang, L. (2004). The chemistry and biochemistry of vanadium and the biological activities exerted by vanadium compounds. *Chemical Reviews*, 104, 849-902

Cuesta, S.; Proietto, R.; García, G.B. (2013). Astrogliosis and HSP 70 activation in neonate rats' brain exposed to sodium metavanadate through lactation. *Neurotoxicology and Teratology*, 37, 57-62

Devereux, T.R.; Holliday, W.; Anna, C.; Ress, N.; Roycroft, J.; Sills, R.C. (2002). Map kinase activation correlates with K-ras mutation and loss of heterozygosity on chromosome 6 in alveolar bronchiolar carcinomas from B6C3F1 mice exposed to vanadium pentoxide for 2 years. *Carcinogenesis*, 23, 1737-1743

Dill, J.A.; Lee, K.M.; Mellinger, K.H.; Bates, D.J.; Burka, L.T.; Roycroft, J.H. (2004). Lung deposition and clearance of inhaled vanadium pentoxide in chronically exposed F344 rats and B6C3F1 mice. *Toxicological Sciences*, 77, 6-18

Domingo, J.L.; Paternain, J.L.; Llobet, J.M.; Corbella, J. (1986). Effects of vanadium in reproduction, gestation, parturition and lactation in rats upon oral administration. *Life Sciences*, 39, 819-824

Drexler, H.; Greim, H. (2006). Biologische Arbeitsstoff-Toleranz-Werte (BAT-Werte), Expositionsäquivalente für krebserzeugende Arbeitsstoffe (EKA) und Biologische Leitwerte (BLW). Arbeitsmedizinisch-toxikologische Begründungen 13. Lfg., DFG Deutsche Forschungsgemeinschaft, WILEY-VCH Weinheim

EC, European Commission (2017). REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL, of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006, including amendments by COMMISSION REGULATION (EU) 2017/776 of 4 May 2017 and before

<http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:02008R1272-20170601&qid=1502288402941&from=EN>

ECHA, European Chemicals Agency (2012). Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.8: Characterisation of dose [concentration]-response for human health. Version 2.1, November 2012

http://echa.europa.eu/documents/10162/17224/information_requirements_r8_en.pdf

ECHA, European Chemicals Agency (2014). Guidance on the preparation of dossiers for harmonised classification and labelling. Version 2.0. August 2014

<http://publications.europa.eu/en/publication-detail/-/publication/3102d5d7-e68b-11e5-8a50-01aa75ed71a1/language-en>

ECHA, European Chemicals Agency (2015). Guidance on the Application of the CLP Criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 4.1. June 2015 https://www.iron-consortium.org/assets/files/ECHA/ECHA_clp_en_1506.pdf

ECHA, European Chemicals Agency (2017). Guidance on the Application of the CLP Criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 5.0 - July 2017. Helsinki, Finland.

https://echa.europa.eu/documents/10162/13562/clp_en.pdf

ECHA Dissemination (2017). Information on Chemicals - Registered Substances. European Chemicals Agency.

Online: <http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances>

Edel, J.; Pietra, R.; Sabbioni, E.; Marafante, E.; Springer, A.; Ubertalli, L. (1984). Disposition of vanadium in rat tissues at different age. *Chemosphere*, 13, 87-93

Edel, J.; Sabbioni, E. (1988). Retention of intratracheally instilled and ingested tetravalent and pentavalent vanadium in the rat. *Journal of Trace Elements and Electrolytes in Health and Disease*, 2, 23-30

Edel, J.; Sabbioni, E. (1989). Vanadium transport across placenta and milk of rats to the fetus and newborn. *Biological Trace Element Research*, 22, 265-275

Ehrlich, V.A.; Nersesyan, A.K.; Atefie, K.; Hoelzl, C.; Ferk, F.; Bichler, J.; Valic, E.; Schaffer, A.; Schulte-Hermann, R.; Fenech, M.; Wagner, K.-H.; Knasmüller, S. (2008). Inhalative exposure to vanadium pentoxide causes DNA damage in workers: results of a multiple end point study. *Environmental Health Perspectives*, 116, 1689-1693

Elfant, M.; Keen, C.L. (1987). Sodium vanadate toxicity in adult and developing rats. *Biological Trace Element Research*, 14, 193

EPA, Environmental Protection Agency (2011). Toxicological Review of Vanadium Pentoxide (V₂O₅). Draft. Do not cite or quote. EPA/635/R-11/004A. U.S. Environmental Protection Agency, Washington, DC

Folarin, O.R.; Adaramoye, O.A.; Akanni, O.O.; Olopade, J.O. (2017a). Changes in the brain antioxidant profile after chronic vanadium administration in mice. *Metabolic Brain Disease*, in press, 9p

Folarin, O.R.; Snyder, A.M.; Peters, D.G.; Olopade, F.; Connor, J.R.; Olopade, J.O. (2017b). Brain metal distribution and neuro-inflammatory profiles after chronic vanadium administration and withdrawal in mice. *Front Neuroanat*, 11, 58

Fortoul, T.I.; Bizarro-Nevarés, P.; Acevedo-Nava, S.; Pinón-Zárate, G.; Rodríguez-Lara, V.; Colín-Barenque, L.; Mussali-Galante, P.; Avila-Casado Mdel, C.; Ávila-Costa, M.R.; Saldivar-Osorio, L. (2007). Ultrastructural findings in murine seminiferous tubules as a consequence of subchronic vanadium pentoxide inhalation. *Reproductive Toxicology*, 23, 588-592

Fortoul, T.I.; Rodríguez-Lara, V.; González-Villalva, A.; Rojas-Lemus, M.; Cano-Gutiérrez, G.; Ustarroz-Cano, M.; Colín-Barenque, L.; Bizarro-Nevarés, P.; García-Pealez, I.; Montaña, L.F.; Jimenez-Martinez, R.S.; Lopez-Valdez, N.; Ruiz-Guerrero, M.L.; Meléndez-García, N.A.; García-Ibarra, F.A.; Martínez-Baez, V.; Alfaro, D.Z.; Muñoz-Rivera-Cambas, A.; López-Zepeda, L.S.; Quezada-Maldonado, E.M.; Cervantes-Yépez, S. (2014). Inhalation of vanadium pentoxide and its toxic effects in a mouse model. *Inorganica Chimica Acta*, 420, 8-15

Fourie, M.H. (2010). Restrospective case-control study of cancer incidence associated with vanadium pentoxide exposure in the mineral processing industry. MSc dissertation, University of Pretoria, Pretoria
<http://upetd.up.ac.za/thesis/available/etd-08032010-181254/>

García-Rodríguez, M.; Hernández-Cortés, L.M.; Altamirano-Lozano, M.A. (2016). In vivo effects of vanadium pentoxide and antioxidants (ascorbic acid and alpha-tocopherol) on apoptotic, cytotoxic, and genotoxic damage in peripheral blood of mice. *Oxididative Medicine and Cellular Longevity*, Article ID 6797851, 11p

García-Rodríguez, M.d.C.; Gordillo-García, A.; Altamirano-Lozano, M. (2017). The Role of Vitamin C in the Protection and Modulation of Genotoxic Damage Induced by Metals Associated with Oxidative Stress. Chapter 5. In: Hamza, A.H., Vitamin C, INTECH, 99-112

García, G.B.; Biancardi, M.E.; Quiroga, A.D. (2005). Vanadium (V)-induced neurotoxicity in the rat central nervous system: a histo-immunohistochemical study. *Drug and Chemical Toxicology*, 28, 329-344

García, G.B.; Quiroga, A.D.; Stürtz, N.; Martinez, A.I.; Biancardi, M.E. (2004). Morphological alterations of central nervous system (CNS) myelin in vanadium (V)-exposed adult rats. *Drug and Chemical Toxicology*, 27, 281-293

Gibson, D.P.; Brauninger, R.; Shaffi, H.S.; Kerckaert, G.A.; LeBoeuf, R.A.; Isfort, R.J.; Aardema, M.J. (1997). Induction of micronuclei in Syrian hamster embryo cells: comparison to results in the SHE cell transformation assay for National Toxicology Program test chemicals. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, 392, 61-70

Gómez, M.; Sánchez, D.J.; Domingo, J.L.; Corbella, J. (1992). Embryotoxic and teratogenic effects of intraperitoneally administered metavanadate in mice. *Journal of Toxicology and Environmental Health*, 37, 47-56

Gorzsás, A.; Andersson, I.; Pettersson, L. (2006). On the fate of vanadate in human blood. *European Journal of Inorganic Chemistry*, 3547-3547

Greim, H. (2006). Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werten, Loseblattsammlung, 41. Lfg DFG Deutsche Forschungsgemeinschaft, WILEY-VCH Verlag Weinheim

HSE, Health & Safety Executive (2002). Vanadium and its inorganic compounds. Risk assessment document. EH72/15 Suffolk, United Kingdom

IARC, International Agency for Research on Cancer (2006). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 86. Cobalt in Hard Metals and Cobalt Sulfate, Gallium Arsenide, Indium Phosphide and Vanadium Pentoxide. WHO, World Health Organization, Lyon, France

IFA, Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung (2017). GESTIS - Internationale Grenzwerte für chemische Substanzen

<http://www.dguv.de/ifa/GESTIS/GESTIS-Internationale-Grenzwerte-für-chemische-Substanzen-limit-values-for-chemical-agents/index.jsp>

Ingram, J.L.; Antao-Menezes, A.; Turpin, E.A.; Wallace, D.G.; Mangum, J.B.; Pluta, L.J.; Thomas, R.S.; Bonner, J.C. (2007). Genomic analysis of human lung fibroblasts exposed to vanadium pentoxide to identify candidate genes for occupational bronchitis. *Respiratory Research*, 8:34, 1-13

Irsigler, G.B.; Visser, P.J.; Spangenberg, P.A. (1999). Asthma and chemical bronchitis in vanadium plant workers. *American Journal of Industrial Medicine*, 35, 366-374

Ivancsits, S.; Pilger, A.; Diem, E.; Schaffer, A.; Rudiger, H.W. (2002). Vanadate induces DNA strand breaks in cultured human fibroblasts at doses relevant to occupational exposure. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, 519, 25-35

Izmerov, N.F.; Sanotsky, I.V.; Sidorov, K.K. (1982). Toxicometric parameters of industrial toxic chemicals under single exposure. Centre of International Projects, GKNT, Moscow

Jiang, M.; Li, Y.; Zhang, B.; Zhou, A.; Zheng, T.; Qian, Z.; Du, X.; Zhou, Y.; Pan, X.; Hu, J.; Wu, C.; Peng, Y.; Liu, W.; Zhang, C.; Xia, W.; Xu, S. (2016). A nested case-control study of prenatal vanadium exposure and low birthweight. *Human Reproduction*, 31, 2135-2141

Katayama, M.; Kaneko, S.; Takamatsu, K.; Tsukimura, T.; Togawa, T. (2013). Determination of trace metals in human seminal plasma using inductively coupled plasma mass spectrometry and multivariate statistical analyses for sperm parameters. *Journal of Molecular Biomarkers & Diagnosis*, 4:3, 7p

Katayose, H.; Shinohara, A.; Chiba, M.; Yamada, H.; Tominaga, K.; Sato, A.; Yanagida, K. (2004). Effects of various elements in seminal plasma on semen profiles. *Journal of Mammalian Ova Research*, 21, 141-148

Kerckaert, G.A.; LeBoeuf, R.A.; Isfort, R.J. (1996). Use of the Syrian hamster embryo cell transformation assay for determining the carcinogenic potential of heavy metal compounds. *Fundamental and Applied Toxicology*, 34, 67-72

Kiviluoto, M. (1980). Observations on the lungs of vanadium workers. *British Journal of Industrial Medicine*, 37, 363-366

Kiviluoto, M.; Pakarinen, A.; Pyy, L. (1981a). Clinical laboratory results of vanadium-exposed workers *Archives of Environmental Health*, 36, 109-113

- Kiviluoto, M.; Pyy, L.; Pakarinen, A. (1981b). Serum and urinary vanadium of workers processing vanadium pentoxide. *International Archives of Occupational and Environmental Health*, 48, 251-256
- Kiviluoto, M.; Rasanen, O.; Rinne, A.; Rissanen, M. (1979). Effects of vanadium on the upper respiratory tract of workers in a vanadium factory. *Scandinavian Journal of Work, Environment and Health*, 5, 50-58
- Kleinsasser, N.; Dirschedl, P.; Staudenmaier, R.; Harréus, U.; Wallner, B. (2003). Genotoxic effects of vanadium pentoxide on human peripheral lymphocytes and mucosal cells of the upper aerodigestive tract. *International Journal of Environmental Health Research*, 13, 373-379
- Knecht, E.A.; Moorman, W.J.; Clark, J.C.; Hull, R.D.; Biagini, R.E.; Lynch, D.W.; Boyle, T.J.; Simon, S.D. (1992). Pulmonary reactivity to vanadium pentoxide following subchronic inhalation exposure in a non-human primate animal model. *Journal of Applied Toxicology*, 12, 427-434
- Leuschner, J.; Haschke, H.; Sturm, G. (1994). New investigations on acute toxicities of vanadium oxides. *Monatshefte fur Chemie*, 125, 623-646.
- Lewis, C.E. (1959a). The biological actions of vanadium. I. Effects upon serum cholesterol levels in man. *AMA Archives of Industrial Health*, 19, 419-425
- Lewis, C.E. (1959b). The biological effects of vanadium. II. The signs and symptoms of occupational vanadium exposure. *AMA Archives of Industrial Health*, 19, 497-503
- Li, H.; Zhou, D.; Zhang, Q.; Feng, C.; Zheng, W.; He, K.; Lan, Y. (2013). Vanadium exposure-induced neurobehavioral alterations among Chinese workers. *Neurotoxicology*, 36, 49-54
- Manjanatha, M.G.; Shelton, S.D.; Haber, L.; Gollapudi, B.; MacGregor, J.A.; Rajendran, N.; Moore, M.M. (2015). Evaluation of *cH* mutations in lung of male Big Blue mice exposed by inhalation to vanadium pentoxide for up to 8 weeks. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, 789-790, 46-52
- Massmann, W. (1956). Experimentelle Untersuchungen über die biologische Wirkung von Vanadinverbindungen. *Archiv fur Toxikologie*, 16, 182-189
- Michibata, H. (2012). Vanadium: Biochemical and Molecular Biological Approaches. Springer Science+Business Media B.V., Dordrecht
- Moore, N.P.; Boogaard, P.J.; Bremer, S.; Buesen, R.; Edwards, J.; Fraysse, B.; Hallmark, N.; Hemming, H.; Langrand-Lerche, C.; McKee, R.H.; Meisters, M.-L.; Parsons, P.; Politano, V.T.; Reader, S.; Ridgway, P.; Hennes, C. (2013). Guidance on classification for reproductive toxicity under the globally harmonized system of classification and labelling of chemicals (GHS). *Critical Reviews in Toxicology*, 43, 850-891
- Morgan, A.M.; El-Tawil, O.S. (2003). Effects of ammonium metavanadate on fertility and reproductive performance of adult male and female rats. *Pharmacological Research*, 47, 75-85
- Mountain, J.J.; Delker, L.L.; Stokinger, H.E. (1953). Studies in vanadium toxicology. Reduction in the cystine content of rat hair. *AMA Archives of Industrial Hygiene an Occupational Medicine*, 8, 406-411

Mussali-Galante, P.; Rodríguez-Lara, V.; Hernández-Tellez, B.; Ávila-Costa, M.R.; Colín-Barenque, L.; Bizarro-Nevarez, P.; Martínez-Levy, G.; Rojas-Lemus, M.; Pinon-Zarate, G.; Saldivar-Osorio, L.; Diaz-Beck, P.; Herrera-Enríquez, M.A.; Tovar-Sánchez, E.; Fortoul, T.I. (2005). Inhaled vanadium pentoxide decrease gamma-tubulin of mouse testes at different exposure times. *Toxicology and Industrial Health*, 21, 215-222

Mustapha, O.; Oke, B.; Offen, N.; Sirén, A.L.; Olopade, J. (2014). Neurobehavioral and cytotoxic effects of vanadium during oligodendrocyte maturation: A protective role for erythropoietin. *Environmental Toxicology and Pharmacology*, 38, 98-111

NTP, National Toxicology Program (2002 a and b). Toxicology and Carcinogenesis Studies of Vanadium Pentoxide in F344/N Rats and B6C3F1 Mice (Inhalation Studies). TR 507. U.S. Department of Health and Human Services; Public Health Service

NTP, National Toxicology Program (2008). Chemical Information Review Document for Oral Exposure to Tetravalent and Pentavalent Vanadium Compounds. U.S. Department of Health and Human Services, Research Triangle Park, NC. https://ntp.niehs.nih.gov/ntp/htdocs/chem_background/exsumpdf/niehs_vanadium_compounds_508.pdf

OECD, Organisation for Economic Co-Operation and Development (2016). QSAR Application Toolbox – Software for grouping chemicals into categories and filling gaps in (eco)toxicity data needed for assessing the hazards of chemicals (Version 3.4)

<http://www.qsartoolbox.org/>

Olopade, J.O.; Fatola, I.O.; Olopade, F.E. (2011). Vertical administration of vanadium through lactation induces behavioural and neuromorphological changes: protective role of vitamin E. *Nigerian Journal of Physiological Sciences*, 26, 55-60

Paramanik, D.; Rajalakshmi, M.G. (2013). In-vivo evaluation of potential toxicity of vanadium pentoxide in male wistar rats. *International Journal of Applied Biology and Pharmaceutical Technology*, 4, 260-271

Paternain, J.L.; Domingo, J.L.; Llobet, J.M.; Corbella, J. (1987). Embryotoxic effects of sodium metavanadate administered to rats during organogenesis. *Revista Espanola de Fisiologia*, 43, 223

Passantino, L.; Munoz, A.B.; Costa, M. (2013). Sodium metavanadate exhibits carcinogenic tendencies *in vitro* in immortalized human bronchial epithelial cells. *Metallomics*, 5, 1357-1367.

Patterson BW, Hansard SL, Ammerman CB, *et al.* 1986. Kinetic model of whole-body vanadium metabolism: Studies in sheep. *Am J Physiol* 251:R325-R332.

Ramanadham S, Heyliger C, Gresser MJ, *et al.* 1991. The distribution and half-life for retention of vanadium in the organs of normal and diabetic rats orally fed vanadium(IV) and vanadium(V). *Biol Trace Elem Res* 30(2):119-124.

Ramírez, P.; Eastmond, D.A.; Laclette, J.P.; Ostrosky-Wegman, P. (1997). Disruption of microtubule assembly and spindle formation as a mechanism for the induction of aneuploid cells by sodium arsenite and vanadium pentoxide. *Mutation Research - Reviews in Mutation Research*, 386, 291-298

Rehder, D. (2013). Vanadium. Its Role for Humans. In: Sigel, A.; Sigel, H.; Sigel, R.K.O., *Interrelations between Essential Metal Ions and Human Diseases*, Springer Netherlands Dordrecht, 139-169

Rodríguez-Lara, V.; Aleman-Muench, G.; Soldevila, G.; García-Zepeda, E.; Fortoul, T.I. (2013). Ultrastructural nuclear changes in mice spleen lymphocytes after vanadium inhalation. *Clinical and Experimental Pharmacology*, S4:003, doi: 10.4172/2161-1459.S4174-4003

Rodríguez-Lara, V.; Morales-Rivero, A.; Rivera-Cambas, A.M.; Fortoul, T.I. (2016). Vanadium inhalation induces actin changes in mice testicular cells. *Toxicology and Industrial Health*, 32, 367-374

Rodríguez-Lara, V.; Muniz-Rivera Cambas, A.; González Villalva, A.; Fortoul, T.I. (2016). Sex-based differences in lymphocyte proliferation in the spleen after vanadium inhalation. *Journal of Immunotoxicology*, 13, 498-508

Rodríguez-Mercado, J.J.; Álvarez-Barrera, L.; Altamirano-Lozano, M.A. (2010). Chromosomal damage induced by vanadium oxides in human peripheral lymphocytes. *Drug and Chemical Toxicology*, 33, 97-102

Rodríguez-Mercado, J.J.; Mateos-Nava, R.A.; Altamirano-Lozano, M.A. (2011). DNA damage induction in human cells exposed to vanadium oxides *in vitro*. *Toxicology In Vitro*, 25, 1996-2002

Rojas-Lemus, M.; Altamirano-Lozano, M.; Fortoul, T.I. (2014). Sex differences in blood genotoxic and cytotoxic effects as a consequence of vanadium inhalation: micronucleus assay evaluation. *Journal of Applied Toxicology*, 34, 258-264

Rojas, E.; Valverde, M.; Herrera, L.A.; Altamirano-Lozano, M.; Ostrosky-Wegman, P. (1996). Genotoxicity of vanadium pentoxide evaluate by the single cell gel electrophoresis assay in human lymphocytes. *Mutation Research - Environmental Mutagenesis*, 359, 77-84

Roldán, R.E.; Altamirano, L.M. (1990). Chromosomal aberrations, sister-chromatid exchanges, cell-cycle kinetics and satellite associations in human lymphocyte cultures exposed to vanadium pentoxide. *Mutation Research - Letters*, 245, 61-65

Rondini, E.A.; Walters, D.M.; Bauer, A.K. (2010). Vanadium pentoxide induces pulmonary inflammation and tumor promotion in a strain-dependent manner. *Particle and Fibre Toxicology*, 7:9, 1-13

Sabbioni, E.; Maroni, M. (1984). A Study on Vanadium in Workers from Oil Fired Power Plants. Report EUR 9005 EN. Published by the COMMISSION OF THE EUROPEAN COMMUNITIES. Directorate-General. Information Market and Innovation. Luxembourg.

Sax, N.I.; Lewis, R.J. (1989). Dangerous Properties of Industrial Materials. 7th ed., Van Nostrand Reinhold New York

Schaller, K.H. (2007). Vanadium und seine anorganischen Verbindungen. (Addendum zur Begründung Vanadiumpentoxid, 8. Lieferung 1996). In: Drexler, H.; Greim, H., Biologische Arbeitsstoff-Toleranz-Werte (BAT-Werte), Expositionsäquivalente für krebserzeugende Arbeitsstoffe (EKA) und Biologische Leitwerte (BLW). Arbeitsmedizinisch-toxikologische Begründungen, 14. Lfg., DFG Deutsche Forschungsgemeinschaft, WILEY-VCH Weinheim.
<http://onlinelibrary.wiley.com/doi/10.1002/3527600418.bb131462d0014/pdf>,

Schuler, D.; Chevalier, H.-J.; Merker, M.; Morgenthal, K.; Ravanat, J.-L.; Sagelsdorff, P.; Walter, M.K.; Weber, K.; McGregor, D. (2011). First steps towards an understanding of a mode of carcinogenic action for vanadium pentoxide. *Journal of Toxicologic Pathology*, 24, 149-162

Sjöberg, S.G. (1950). Vanadium pentoxide dust: A clinical and experimental investigation on its effect after inhalation. *Acta Medica Scandinavica*, 138, Suppl. 238, 6-188

Soazo, M.; Garcia, G.B. (2007). Vanadium exposure through lactation produces behavioral alterations and CNS myelin deficit in neonatal rats. *Neurotoxicology and Teratology*, 29, 503-510

Starr, T.B.; MacGregor, J.A. (2014). Vanadium pentoxide: Risk assessment implications of a treatment- but not dose-related tumorigenic response in B6C3F1 mice. *Regulatory Toxicology and Pharmacology*, 69, 333-337

Starr, T.B.; MacGregor, J.A.; Ehman, K.D.; Nikiforov, A.I. (2012). Vanadium pentoxide: use of relevant historical control data shows no evidence for a carcinogenic response in F344/N rats. *Regulatory Toxicology and Pharmacology*, 64, 155-160

Sun, L.; Wang, K.; Li, Y.; Fan, Q.; Zheng, W.; Li, H. (2017). Vanadium exposure-induced striatal learning and memory alterations in rats. *Neurotoxicology*, 62, 124-129

Sun, M. (1987). Toxicity of Vanadium and its Environmental Health Standard. Chengdu, West China University of Medical Science, zitiert nach WHO, 1988

Thomas, R.S.; Bao, W.; Chu, T.M.; Bessarabova, M.; Nikolskaya, T.; Nikolsky, Y.; Andersen, M.E.; Wolfinger, R.D. (2009). Use of short-term transcriptional profiles to assess the long-term cancer-related safety of environmental and industrial chemicals. *Toxicological Sciences*, 112, 311-321

Todorich, B.; Olopade, J.O.; Surguladze, N.; Zhang, X.; Neely, E.; Connor, J.R. (2011). The mechanism of vanadium-mediated developmental hypomyelination is related to destruction of oligodendrocyte progenitors through a relationship with ferritin and iron. *Neurotoxicity Research*, 19, 361-373

Toya, T.; Fukuda, K.; Takaya, M.; Arito, H. (2001). Lung lesions induced by intratracheal instillation of vanadium pentoxide powder in rats. *Industrial Health*, 39, 8-15

Uche, F.; Obianime, A.; Gogo-Abite, M. (2008). Effects of vanadium pentoxide on the histological and sperm parameters of male guinea pigs. *Journal of Applied Sciences and Environmental Management*, 12,

Usende, I.L.; Leitner, D.F.; Neely, E.; Connor, J.R.; Olopade, J.O. (2016). The deterioration seen in myelin related morphophysiology in vanadium exposed rats is partially protected by concurrent iron deficiency. *Nigerian Journal of Physiological Sciences*, 31, 11-22

Ustarroz-Cano, M.; Garcia-Pelaez, I.; Cervantes-Yeppez, S.; Lopez-Valdez, N.; Fortoul, T.I. (2017). Thymic cytoarchitecture changes in mice exposed to vanadium. *Journal of Immunology*, 14, 9-14

Vintinner, F.J.; Vallenias, R.; Carlin, C.E.; Weiss, R.; Macher, C.; Ochoa, R. (1955). Study of the health of workers employed in mining and processing of vanadium ore. *AMA Archives of Industrial Health*, 12, 635-642

VPRA, Vanadium Producers & Reclaimers Association (2010). Data submission for REACh. Vanitec HSE Meeting. London, 2010-12-13

[https://yosemite.epa.gov/oppts/epatscat8.nsf/ALLIDS/CB9A28337B8EACF6852578B900508034/\\$FILE/88110000116.pdf?OpenElement](https://yosemite.epa.gov/oppts/epatscat8.nsf/ALLIDS/CB9A28337B8EACF6852578B900508034/$FILE/88110000116.pdf?OpenElement)

VPRA, Vanadium Producers & Reclaimers Association (2011). Comments of the Vanadium Producers & Reclaimers Association on U.S. Environmental Protection Agency's (EPA) draft "Toxicological Review of Vanadium (V₂O₅) - In Support of Information on the Integrated Risk Information System (IRIS)" (External Review Draft). 76 Fed. Reg. 60825. EPA/635/R-11/004A; Docket ID No. EPA-HQ-ORD-2011-0738

Wang, D.-C.; Lin, Y.-Y.; Lin, H.-T. (2015). Recovery of motor coordination after exercise is correlated to enhancement of brain-derived neurotrophic factor in lactational vanadium-exposed rats. *Neuroscience Letters*, 600, 232-237

WHO, World Health Organization (1988). Environmental Health Criteria 81, Vanadium. IPCS International Programme on Chemical Safety; World Health Organization Geneva

WHO, World Health Organization (2001). Concise International Chemical Assessment Document No. 29. Vanadium Pentoxide and other Inorganic Vanadium Compounds. Geneva

Wide, M. (1984). Effect of short-term exposure to five industrial metals on the embryonic and fetal development of the mouse. *Environmental Research*, 33, 47-53

Yang, H.; Yao, D.; Feng, S.; Qin, J.; Chen, Y. (1986). [A study of the teratogenicity of vanadium pentoxide] Dukou Sanitary and Anti-Epidemic Station, pp. 35–43 (internal report), cited in (WHO, 2001)

Yao, D.; Li, S.; *et al.* (1986). A long-term study on the chronic toxicity and carcinogenicity of the inhalation of vanadium pentoxide dust on mice. Dukou Sanitary and Anti-Epidemic Station, cited in WHO, 2001

Zafar, A.; Eqani, S.A.; Bostan, N.; Cincinelli, A.; Tahir, F.; Shah, S.T.; Hussain, A.; Alamdar, A.; Huang, Q.; Peng, S.; Shen, H. (2015). Toxic metals signature in the human seminal plasma of Pakistani population and their potential role in male infertility. *Environmental Geochemistry and Health*, 37, 515-527

Zhang, T.; Gou, X.; Yang, Z. (1991). [A study on developmental toxicity of vanadium pentoxide in NIH mice]. *Hua Xi Yi Ke Da Xue Xue Bao*, 22, 192-195

Zhang, T.; Gou, X.; Yang, Z. (1993a). [Study of teratogenicity and sensitive period of vanadium pentoxide in Wistar rats]. *Hua Xi Yi Ke Da Xue Xue Bao*, 24, 202-205

Zhang, T.; Yang, Z.; Zeng, C.; Gou, X. (1993b). [A study on developmental toxicity of vanadium pentoxide in Wistar rats]. *Hua Xi Yi Ke Da Xue Xue Bao*, 24, 92-96

Zhong, B.Z.; Gu, Z.W.; Wallace, W.E.; Whong, W.Z.; Ong, T. (1994). Genotoxicity of vanadium pentoxide in Chinese hamster V79 cells. *Mutation Research - Genetic Toxicology*, 321, 35-42

Zwolak, I. (2014). Vanadium carcinogenic, immunotoxic and neurotoxic effects: a review of *in vitro* studies. *Toxicology Mechanisms and Methods*, 24, 1-12

