# Annex II to the CLH report

**READ – ACROSS JUSTIFICATION DOCUMENT**

# Proposal for Harmonised Classification and Labelling

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

# International Chemical Identification:

## Divanadium pentaoxide

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

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

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

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# 1 INTENTION FOR READ ACROSS

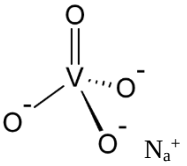
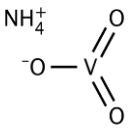
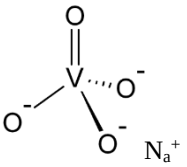
In the present CLH report for classification of divanadium pentaoxide, read across using other pentavalent vanadium compounds: sodium metavanadate (CAS-No.: 13718-26-8) and ammonium metavanadate (CAS-No.: 7803-55-6) has been applied:

- as supporting evidence regarding adverse effects on male sexual function and fertility (source substance: divanadium pentaoxide), and,
- as key information for adverse effects on development (source substance: ammonium metavanadate and sodium metavanadate)
- as key information for adverse effects on or *via* lactation (source substance: sodium metavanadate).

Limiting read across to these toxicological endpoints and to these vanadates is reasonable because:

- data on other vanadates (specifically sodium orthovanadate) corroborate the conclusions provided below and documentation would not provide further insight into any aspect of read across justification (based on a careful analysis from VANITEC not documented in this annex),
- there are sufficient information to conclude germ cell mutagenicity and carcinogenicity based on data with divanadium pentaoxide,
- read across data on germ cell mutagenicity with either ammonium metavanadate, sodium metavanadate or sodium orthovanadate corroborate the classification as assessed based on divanadium pentaoxide and documentation would not provide further evidence to justification (based on a careful analysis from VANITEC not documented in this annex),
- there are too few, not representative and less qualified data on carcinogenicity from other vanadates, but those existing data would not provide further insight into justification for carcinogenicity classification of divanadium pentaoxide, and
- a potential extension of read across to tetravalent vanadium compounds like vanadyl sulfate (as performed and accepted by REACH registrants) is not intended and is considered questionable.

**Table AII - 1: Source substances and toxicological endpoints for read across for classification of divanadium pentaoxide**

| Endpoint | Source substance | Study type and reference | Reference |
|---|---|---|---|
| Adverse effects on development | Sodium metavanadate (CAS 13721-39-6)  | Study on fertility and prenatal developmental toxicity in rats, by intra-gastric administration. | (Domingo *et al*., 1986) |
| | | Study on prenatal developmental toxicity in rats by intra-gastric administration. | (Paternain *et al*., 1987) |
| | | Developmental toxicity study in Swiss mice by intraperitoneal injection. | (Gómez *et al*., 1992) |
| | | Behavioural test and mechanistic study on early postnatal sensitivity for neurological impairments in rat by intraperitoneal injection. | Todorich *et al*., 2011) |
| | Ammonium metavanadate (CAS 7803-55-6)  | Study on fertility and prenatal developmental toxicity in rats, drinking water exposure | (Morgan and El-Tawil, 2003) |
| | | Study on prenatal developmental toxicity in hamster by intraperitoneal route of exposure. | (Carlton *et al*., 1982) |
| Adverse effects *via* lactation | Sodium metavanadate (CAS 13721-39-6)  | Postnatal developmental study (neurobehavioural tests; immunohistochemistry analysis) of sucklings; nursing mice (i.p. application of substance to dams postnatally). | (Mustapha *et al*., 2014) |
| | | Neurobehavioral toxicity study in weaned rats after lactational exposure in rat intraperitoneal injection. | (Wang *et al*., 2015) |
| | | Postnatal developmental study (neurobehavioural tests; immunohistochemistry analysis) of sucklings; nursing mice (i.p. application of substance to dams postnatally). | (Olopade *et al*., 2011) |
| | | Postnatal developmental study (neurobehavioural tests; microscopic analysis) of sucklings; nursing rat (i.p. application of substance to dams postnatally). | (Soazo and Garcia, 2007) |

| | | | |
|---|---|---|---|
| Adverse effects *via* lactation | Sodium metavanadate (CAS 13721-39-6) | Postnatal developmental study (enzyme activity analysis in brain of sucklings); nursing rat (i.p. application of substance to dams postnatally). | (Cuesta *et al*., 2013) |
| | Ammonium metavanadate (CAS 7803-55-6) | Study on fertility and prenatal developmental toxicity in rats, drinking water exposure | Morgan and El-Tawil, 2003) |
| Adverse effects on fertility | Sodium metavanadate (CAS 13721-39-6) | Study on fertility and prenatal developmental toxicity in rats, by intra-gastric administration. | (Domingo *et al*., 1986) |
| | | Study on fertility and reproductive toxicity in exposed male mice | (Llobet *et al*., 1993) |
| | | Study on fertility and reproductive toxicity in exposed male rats | (Chandra *et al*., 2007b) |
| | | Study on fertility and reproductive toxicity in exposed male rats | (Chandra *et al*., 2007a) |
| | Ammonium metavanadate (CAS 7803-55-6) | Study on fertility and prenatal developmental toxicity in rats, drinking water exposure | (Morgan and El-Tawil, 2003) |

# 2 DATA DOCUMENTATION

## 2.1 Chemistry and biochemistry of pentavalent compounds

Vanadium exists in different oxidation states (0, +2, +3, +4, +5). In geological sites and seawater, it exists usually as pentavalent vanadium. Incineration plants send out vanadium in the form of divanadium pentaoxide ($V_2O_5$), the stable form of vanadium in the presence of environmental oxygen. Elementary vanadium is not stable in the presence of oxygen and is oxidised spontaneously when heated to form $V_2O_5$ (Greim, 2006). Also, the oxidation states +2 and +3 are easily oxidised by atmospheric oxygen (MAK, 2005). Different from metallic vanadium, vanadium (V) compounds are “moderately soluble” or “soluble” in water with some specific data provided below.

**Table AII - 2: Identification of vanadium and vanadium compounds and main physico-chemical characteristics:**

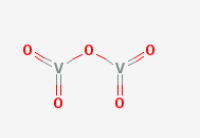
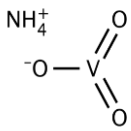
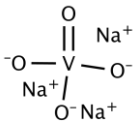
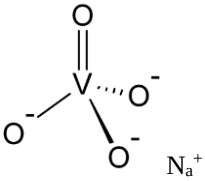
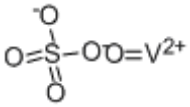
| Identification of test substance | Molecular Formula | Structure | Valency | Water solubility (Ref) | |
|---|---|---|---|---|---|
| Vanadium<br>CAS N°7440-62-2 | V | V | **0** | 0.15 mg/L (20°C; pH 5.8, dissolved after 72h) | (ECHA, 2017)<br>VPRA (2010) |
| Divanadium pentaoxide:<br>CAS N°1314-62-1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

**Table AII - 3: Water solubility of selected vanadium compounds**

| Vanadium compound | Water solubility | Source: |
|---|---|---|
| V(0) | 0.15 mg/L (20°C; pH 5.8, dissolved after 72h) | (ECHA, 2017)<br>VPRA (2010) |
| $V_2O_5$ | 920 mg/L (20°C; pH 2.7; duration not provided) | (ECHA, 2017)<br>VPRA (2010) |
| $NaVO_3$ | 89 g/L (20°C; pH 9.5; duration not provided) | (ECHA, 2017) |
| $NH_4VO_3$ | 7.8 g/L (20°C; pH 6; duration not provided) | (ECHA, 2017) |

**Note: further solubility data are provided in AGS, 2015.**

**In aqueous solution, vanadium chemistry is governed by pH, temperature and concentration, which affect the equilibrium of these reactions. Biochemical reactions of the various oxidation states are very complex.**

$V_2O_5$ is a poorly soluble oxide which, in water or body fluids, releases some vanadium ions either in cationic ($VO^{2+}$) or anionic ($HVO_4^{2-}$) forms [at physiological pH: $H_2VO_4^{-}$] (IARC, 2006). Toya *et al*. (2001) showed that $V_2O_5$ powder (geometric mean diameter, 0.31 µm) was eight times more soluble in an artificial biological fluid (Gamble's solution) than in water. Other vanadium species like vanadium (III) only occur under physiological conditions in the form of coordination complexes. $V_2O_5$ may also be present as decavanadate ion in physiological media (Assem and Levy, 2009). The vanadyl cation and the vanadate anion are readily and interchangeably transformed under physiological conditions by redox reactions (Rehder 1995). The dissolved vanadate probably enters the cell *via* an anion transporter, which was suggested from data for sodium metavanadate and decavanadate (Yang *et al*., 2003; Aureliano and Gandara, 2005). The biochemical reactions of vanadium compounds are very complex as a result of the different oxidation states and compound forms [cationic as vanadyl (IV) cation ($VO^{2+}$), anionic as vanadate (V) anions ($VO_3^{1-}$ or $VO_4^{3-}$)]. Inside the cell, the vanadate anion is reduced to $VO^{2+}$ (tetravalent vanadyl cation), the predominant intracellular species (NTP, 2008). On the one hand, vanadium as vanadate anion interacts competitively with phosphate in the metabolism; on the other hand, as vanadyl cation, it forms complexes with biomolecules such as proteins (IARC, 2006). Toxicologically relevant are the reactions of pentavalent vanadium with thiols to form thiyl radicals (Shi *et al*. 1990), of pentavalent vanadium with NADH to form the hydroxyl radical (Shi and Dalal 1992) and the production of reactive oxygen species by tetravalent vanadium (Shi *et al*. 1996). There is insufficient information to explain organ-specific ligand-binding or tendencies of monomeric vanadate to oligomerise or complexate under various physiological conditions in mammalian tissues.

Bioaccessibility differs depending on the respective physiological media and is compared in Table AII - 4 for pentavalent divanadium pentaoxide and sodium metavanadate.

**Table AII - 4: Bioaccessibility and speciation after 2 and 24 hrs in physiological media for pentavalent vanadium compounds and tetravalent vanadyl sulfate in comparison**

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

Note:
- Loading of 100 mg/L
- Solubility and speciation after 2 and 24h

- Five synthetic body fluids were selected:
  - Phosphate-buffered saline (PBS, pH 7.4), a standard physiological fluid that mimics the ionic strength of human blood serum.
  - Gamble's solution (GMB, pH 7.4), mimics the interstitial fluid within the deep lung under normal health conditions (Stopford *et al*., 2003).
  - Artificial sweat (ASW, pH 6.5), simulates the hypoosmolar fluid, linked to hyponatraemia (loss of Na+ from blood), which is excreted from the body upon sweating.
  - Artificial lysosomal fluid (ALF, pH 4.5), simulates intracellular conditions in lung cells occurring in conjunction with phagocytosis and represents relatively harsh conditions - Artificial gastric fluid (GST, pH 1.5), mimics the very harsh digestion setting of high acidity in the stomach (quoted from Mörsdorf *et al.*, 2015).

Divanadium pentaoxide can be present in water or body fluids partially in ionized form as cation or anion (see MAK, 2015). From these bioaccessibility data, VPRA (2010) concludes that (total) solubility is similar ( ~100%) in all physiological media for divanadium pentaoxide and sodium metavanadate and that vanadium transforms to the pentavalent form in all media except in artificial lysosomal fluid **(**ALF). The authors find that bioaccessibility is an adequate indicator for bioavailability: "*The readily soluble vanadium substance such as $NaVO_3$ and $V_2O_5$ dissolve practically completely in all physiological media already after only a short period of time, rendering them to be expected of similar and extensive bioavailability".* In ALF even pentavalent forms are converted to tetravalent species. However, relevant quantitative differences in artificial gastric fluid and artificial sweat should be noted for vanadyl sulfate compared to the two pentavalent compounds. However, despite good solubility in physiological media, divanadium pentaoxide is retained and accumulates in the lung of experimental animals after dust inhalation (Cohen *et al*., 2007; Dill *et al*., 2004; NTP, 2002).

## 2.2 Divanadium pentaoxide forms and characteristics:

Classification and labelling process usually does not discriminate the hazard effects pending on the forms of the tested compound and classification and labelling proposal is assigned to the chemical compound "as such". However, in particular cases such as for silica, very different toxicological characteristics may be due to a particular form (amorphous *versus* crystalline silica forms) and will therefore influence the classification proposal. In the case of divanadium pentaoxide, it may be available in environmental media in different forms. Therefore it is discussed below, whether different forms namely crystalline or fused forms of divanadium pentaoxide are associated with highly different toxicity or whether it is justified not to discriminate forms and assign a classification to the chemical compound "as such".

It has been shown in Section 2.1 of this Annex that divanadium pentaoxide is dissolved in water and in physiological media at pH 7 (see also IARC, 2006). Its solubility characteristics may potentially be relevant specifically for inhalation exposure to either the particulate substance or to the (partially or completely) dissolved compound.

Divanadium pentaoxide is an inorganic substance with several appearances: yellow to rust brown (crystals), yellow-orange (powder), dark grey (flakes). The compound is produced by roasting. The usual commercial 'flake' form is obtained from the solidified melt (IARC, 2006). IARC describes that "primary industrial compounds…are principally 98% (by weight) fused pentoxide, air-dried (technical-grade) pentoxide and technical-grade ammonium metavanadate".

The major use of divanadium pentaoxide is in the production of metal alloys, which are produced preferably from fused flakes with the "high density of the molten oxide". In residual oils from combustion, most of the vanadium is released into the environmental atmosphere in the form of divanadium pentaoxide as part of fly ash particulates (IARC, 2006).

Generally, identification as "powder" refers to an ill-defined term with regard to the particle size range, but for divanadium pentaoxide the physical state of powders is *crystalline*. Granulations may vary typically in the range 10 mesh [~ 1600 µm] and 325 mesh [~ 35 µm] (IARC, 2006). However, exposure may be also due to microsized or nanosized divanadium pentaoxide powder. For example, in a study by Toya *et al*., 2001 the particle size was reported to be 0.31 µm in geometric mean diameter with geometric standard deviation of 2.19. The terms *granules* and *powder* are not strictly discriminated.

The term "fused" largely refers to amorphous divanadium pentaoxide. Flaking of the fused vanadium pentoxide is elicited by deposition on a cooled surface. The resultant flakes provide a very desirable high density product in fine form for ease of distribution in a metallurgical bath. However, different degrees of recrystallization may occur.

Duffus (2007) criticised the NTP (2002) study since no sufficient description of the form of divanadium pentaoxide applied was reported. According to Duffus (2007), "orthorhombic crystals or otherwise" may influence divanadium pentaoxide solubility and thus its biological effects. Leuschner *et al*., showed that two $V_2O_5$ molecules crystallise in orthorhombic needles in each unit cell (Leuschner *et al*., 1994). However, as evident from the IARC description above, exposure may also be to fused divanadium pentaoxide. The various experimental studies, particle size and form (fused or crystalline), submicron-sized particles (< 1 µm) divanadium pentaoxide may differ pending on the various experimental studies. Lastly, the form of divanadium pentaoxide was not documented in a few study publication.

For inhalation, Table AII - 5 provides data on acute "no effect levels" (NOEL) and lethal concentrations ($LC_{50}$) for different forms (powder, fused) and different purities of divanadium pentaoxide in Sprague-Dawley rats.

Table AII - 5: No observed effect level (NOEC) and lethal concentration (LC50) in Sprague-Dawley rats for various forms of divanadium pentaoxide (fused or powder; analytical or technical grade) after a single administration by inhalation

| **Test substance** | **NOEL (male; female) inhalation [mg/m³]** | **$LC_{50}$ (male; female) inhalation [mg/m³]** |
|---|---|---|
| $V_2O_5$, analytical grade, powder | (2420; 900)<br>MMAD: >2.01-2.44 µm<br>Dust | (11090; 4300)<br>MMAD: >2.01-2.44 µm<br>Dust |
| $V_2O_5$, technical grade, fused | (970; 970)<br>MMAD: >1.31-4.04 µm<br>Dust | (16200; 4000)<br>MMAD: >1.31-4.04 µm<br>Dust |
| $V_2O_5$, technical grade, powder | (1620; 1100)<br>MMAD: >2.72-4.15 µm<br>Dust | (4400; 2200)<br>MMAD: >2.72-4.15 µm<br>Dust |
| Source: (Leuschner *et al*., 1994), MMAD: mass medium aerodynamic diameter | | |

For subacute inhalation exposure, the NTP study (NTP, 2002) and a study by Schuler *et al*. (2011) possibly used different test material. But the results are similar:

- NTP (2002) exposed female $B6C3F_1$ mice to particulate aerosols (MMAD 1-1.3 µm) at concentrations of 0, 2, or 4 mg $V_2O_5$/m³ for 16 days (6 h/d). The colour of the particles is described as "light orange" (NTP, 2002). However, Duffus reported that an analytical aliquot was later described as "dark gray" (Duffus, 2007) pointing to a different form. Moreover, apart from the MMAD and the geometric standard deviation, the full size distribution is not provided by NTP and may, according to Duffus, include a substantial amount of submicron particles because of the milling procedure of the test material in this study. Particle form and size in the NTP study was only determined at one point in time and may have changed due to moisture.
- Schuler *et al*. (2011) exposed $B6C3F_1$ mice to either 0, 0.25, 1 or 4 mg/m³ divanadium pentaoxide (MMAD: 1.22-1.43 µm, "yellow-orange solid") by inhalation for 16 days (6 h/d) and documented effects in the lung after terminating exposure. The orange colour indicates a granular form of $V_2O_5$.

Both experiments resulted in increased lung weight, inflammatory lesions and an increased cell proliferation rate at or above 1 mg/m³ with increased dose-dependent severity.

Therefore, it is concluded that the different forms do not significantly influence respiratory (local) toxicity of divanadium pentaoxide. In discussions with the EU "Technical Committee on Classification and Labelling" in 2007 this conclusions has been confirmed by the manufacturing industry (IND): "*IND stated that they did not support any differentiation between classifications of different species of vanadium pentoxide, why the earlier request from Member States on information on different species tested and marketed, would no longer be relevant*." (Follow-up III of the meeting of the Technical Committee on Classification and Labelling in Arona, 26-28 September 2007). This statement relates to "vanadium species", but is applicable to the different vanadium "forms" with the terminology used in this document.

However, all such inhalation experiments have been performed with vanadium as a particle. This particle form may influence local retention, local toxicity and transformation, and may be different, if exposure is to the liquid aerosol with (partially) dissolved vanadium in a vehicle suspension. Long retention of divanadium pentaoxide dust in the lung was demonstrated in rodents, e.g., by Dill *et al*. (2004) with an associated chronic respiratory effects (AGS, 2015). This retention may either result from slow dissolution of the dust in the lung (Toya *et al*., 2001), or from local binding reactions, e.g., to proteins within the respiratory tract.

In several studies, $V_2O_5$ was dissolved or suspended in a vehicle before the administration of the compound. In a tumour promotion study by Rondini *et al*. (2010) the substance was suspended in phosphate buffered saline (PBS) before application by oropharyngeal aspiration to different mice strains. Suspension in PBS leads to dissolution and transformation to pentavalent vanadate (Table AII - 4). A number of experimental studies from the Mexico University with inhalation exposure to $V_2O_5$ (e.g., Fortoul *et al*., 2014) exposed experimental animals to an aqueous aerosol of $V_2O_5$ with a concentration of 1.4 mg/m³ $V_2O_5$ (1 h/twice per week; 4 to 12 weeks, depending on the specific single study). In those studies, the compound was dissolved in a vehicle (deionised water, saline, or Tween80 - detergence). Fortoul *et al*. (2011) or Colín-Barenque *et al*. (2015) refer to saline and identical experimental conditions as applied by Avila-Costa *et al*. (2005) or Mussali-Galante *et al*. (2005). However, the latter two studies reported the use of deionised water as vehicle. It may be expected that better dissolution conditions prior to application of $V_2O_5$ may contribute, to some extent, to a better bioaccessibility of $V_2O_5$ to potential target tissues. When classification and labelling process did not taken into consideration cut-off levels as for mutagenicity, carcinogenicity or reproductive toxicity, this expected higher bioaccessibility will not influence identification of the intrinsic hazard properties of $V_2O_5$. However, when cut-off levels are involved (see acute toxicity classification or repeated-dose toxicity study), such influence will be taken into consideration.

For oral toxicity studies, $V_2O_5$ is usually applied in water or other vehicles leading to a dissolution of divanadium pentaoxide in the vehicle prior to reaching the stomach and will be further dissolved in the gastrointestinal tract to vanadium compounds. Since no information indicates that systemic toxicity of $V_2O_5$ is due to the undissolved compound, studies where $V_2O_5$ was administered orally with a vehicle (aqueous suspensions or solutions) are regarded suitable for systemic toxicity assessment.

For inhalation exposure, all forms of $V_2O_5$ (crystalline, powder, or fused), are regarded sufficiently similar, to be regarded as one entity for classification purposes. Typical exposure to $V_2O_5$ is due to the particulate compound *via* inhalation. However, if available, toxicity data from liquid aerosol (partially dissolved $V_2O_5$) will be considered for classification purposes.

To justify read across, analogous substances and divanadium pentaoxide should be associated with similar a) toxicokinetics, b) toxicological endpoints, and c) mode of action.

**Toxicokinetic considerations:**

This section addresses the toxicokinetics of the pentavalent vanadium compounds.

a) Absorption

No quantitative data are available for systemic absorption from inhalation studies of the different pentavalent vanadium compounds. However, 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 intra-tracheal exposure in female rat, 40% of $^{48}V_2O_5$ was recovered in urine by day 3 post-exposure while the skeleton accounted for 30% by day 7 leading to a systemic absorption rate of at least 70% with uptake being much greater *via* the intra-tracheal route than by the oral route (Conklin *et al*., 1982). Intratracheal studies in animals indicate that vanadium, from either divanadium pentaoxide or other pentavalent vanadium compounds, is systemically available with an absorption rate of at least 70% (Conklin *et al*., 1982) with uptake being much greater than by the oral route (approximately 3% of the administered vanadium dose according to WHO, 2001).

However, an analysis of further toxicokinetic studies shows more heterogeneous data on oral absorption: Nielsen (1995) report a range from 1 to more than 10% for the absorption of vanadium compounds (not further specified). Feed composition, fasting, concentration, duration of exposure, age of the tested species and method for quantification, all may influence significantly absorption. Based on a kinetic study with sodium metavanadate, Bogden *et al*. (1982) showed that 39.7 ± 18.5% of ingested vanadium was retained and that 59.1 ± 18.8% of ingested vanadium was excreted in the feces. These values indicate greater absorption and retention of ingested vanadium than found in previously reported investigations. These authors conclude: "*caution in assuming that ingested vanadium will always be poorly absorbed from the gastrointestinal tract, is suggested* A higher absorption of vanadium in young animals due to a greater nonselective permeability of the undeveloped intestinal barrier, is assumed (EPA, 2011).

The overall percentage of the bioavailable substance will also depend on the amount retained in the bones. For example, Adachi *et al*. report an oral absorption of 16.5% of sodium metavanadate (calculated as "total intake" minus "faecal excretion"), but elimination from urine only was 0.86%, whereas 15.7% were retained in the skeleton after 7 days of exposure (Adachi *et al*., 2000). Therefore, because accumulation in bone depends on exposure duration and figures on absorption sometimes are just derived from renal elimination, heterogeneity in terminology (bioavailability not necessarily being identical to oral absorption) may explain some discrepant presented figures on absorption.

In conclusion, even though in respective studies and reviews similarly low oral absorption is usually claimed for pentavalent vanadium compounds in general, there is some uncertainty on the relative oral absorption rate for substances in question. There are few and insufficient qualified data, specifically on oral absorption of divanadium pentaoxide. Moreover, there may be a significant variability for each of the vanadium compounds due to exposure conditions. Therefore, a higher oral absorption of metavanadates compared to divanadium pentaoxide cannot be excluded. This conclusion and the associated uncertainty are important for further read-across justification.

b) Distribution

Only few studies analyse systemic distribution of divanadium pentaoxide or pentavalent vanadates in direct comparison. No comparable data on tissue distribution pattern are available for inhalation or oral exposure of the source substances. However, tissue distribution data after oral gavage with $^{48}V_2O_5$ or $^{48}V$-pentavanadate ions (not further specified) reveal similar distributions (Conklin *et al*., 1982; Edel and Sabbioni, 1988). Also, after repeated oral exposure of rats to divanadium pentaoxide in drinking water or to sodium metavanadate in diet (Adachi *et al*., 2000; Kučera *et al*., 1990), the highest concentrations of either substance were detected in bone, kidney and spleen, again indicating similar distribution patterns. After intratracheal instillation of rats to $^{48}$Vanadium-labelled pentavalent and tetravalent vanadium (not further specified), 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 (Edel and Sabbioni, 1988). They concluded, that "…*the tissue distributions of $^{48}V$ in rats per os administrated V(IV) or V(V) ions support previous observations on the similarities of the metabolic pathways of dissimilar vanadium species intravenously injected in the rat*". The authors referred to a publication by Sabbioni *et al*. (1978), presenting those previous observations. Moreover, Edel and Sabbioni (1988) also found similar intracellular distributions (nuclear, cytosol, mitochondrial, lysosomal, microsomal fractions) for the various vanadate (and vanadyl) compounds.

Studies with intravenous injection of ammonium metavanadate (≈0.01mg $^{48}$V/rat to male albino COBS rats) by Sabbioni and Marafante (1978) or with intratracheally instilled divanadium pentaoxide (≈0.2 mg $^{48}$V/rat to male Sprague-Dawley rats) by Sharma *et al*. (1987) again demonstrated relatively highest concentrations of both compounds in kidney and spleen. In addition relative liver concentrations in both studies were similarly high. No comparative data were available for bones (only measured and most relevant in the study by Sharma *et al*.). Concentrations in lungs greatly differed, but this was due to different pathways of application (intravenous *versus* intratracheal instillation) and therefore is not regarded as a substance specific difference between ammonium metavanadate and divanadium pentaoxide, respectively. Divanadium pentaoxide was shown to cross the placenta barrier (Greim, 2006; NTP, 2002). Correspondingly, fetotoxic effects observed after exposure of pregnant rats to ammonium metavanadate (Morgan and El-Tawil, 2003) suggest transplacental transfer of these pentavalent vanadium compounds. Moreover, Edel and Sabbioni (1989) demonstrated that $^{48}$V-vanadate reaches the foetus.

In conclusion, there is evidence that divanadium pentaoxide and vanadates have similar systemic distribution patterns. This is probably not true for local compartments at the port of entry (i.e., respiratory tract).

c) Metabolism and speciation

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, undergo ligand-interactions, and may be oligomerised or monomerised.

Solubility greatly depends on local pH (organ specific or specific to the subcellular environment), concentration, and particle size, where the site of transformation (e.g., from vanadate to vanadyl or *vice versa*) may differ from the site of interaction with biological tissues. Solubility data for pentavalent vanadium compounds in the various physiological fluids are provided in Table AII - 4.

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, considering port of entry effects (e.g., respiratory tract), divanadium pentaoxide dissolves slowly and may be present undissolved in significant quantities. In the lung, divanadium pentaoxide can therefore be discriminated from other pentavalent vanadium compounds with higher solubility. Elimination half-time differs and speciation may be different depending on the solubility.

NTP (2008) provides a review with a compilation of studies related to speciation of vanadium compounds in the various compartments of the mammalian organism after oral exposure. Generally, the authors do not distinguish between single pentavalent vanadium compounds, but assume similar behaviour. In blood, transferrin is predicted to be the major carrier of pentavalent vanadium (Gorzsás *et al*., 2006). Inside the cell, vanadate is reduced to $VO^{2+}$ (tetravalent vanadium), considered to be the predominant intracellular species. Some of the toxic activities ascribed to vanadate monomer may be due to certain oligomers (NTP, 2008), but a more specific discrimination of the critical speciation and oligomerisation for different organs and associated toxic effects usually is not available.

d) Elimination

After inhalation exposure, pentavalent vanadium compounds are primarily excreted *via* the urine (Greim, 2006). From animal data it is shown that the more soluble sodium orthovanadate was eliminated more rapidly than divanadium pentaoxide after intratracheal instillation (NTP, 2002; Sharma *et al*., 1987).

After sodium metavanadate ingestion the percentage of the compound excreted in the urine and faeces were 0.86 and 83.5%, respectively, of the intake (Adachi *et al*., 2000), indicating low bioavailability with relevant amounts retained in the bones. After oral application of ammonium metavanadate within 6 days more than 80% of the dose was recovered in the faeces (ATSDR, 2012; Greim, 2006), indicating a similar excretion pattern as for other pentavalent vanadium compounds.

In summary, from the comparative presentation of toxicokinetic data above, it is concluded that different pentavalent vanadium compounds have similar toxicokinetic properties. This is specifically true for the source substances sodium metavanadate and ammonium metavanadate, if compared with divanadium

pentaoxide. However, quantitative differences in absorption of divanadium pentaoxide and ammonium metavanadate cannot be excluded and indicate subsequently possible differences in effect potency due to different oral absorption for the pentavalent vanadium compounds.

IARC (2006) summarised accordingly and emphasised the possibility of variations: "*In general, the absorption, distribution and elimination of divanadium pentaoxide and other vanadium compounds are similar. There are, however, variations depending on the solubility of the administered compound, the route of exposure and the form of vanadium administered*."

However, for systemic effects, similarities in toxicokinetics from oral uptake are regarded sufficient for read-across and are supported by toxicological effect data (Section 2.3).

## 2.3 Mammalian toxicological data

For three toxicological endpoints, read-across considerations were applied,

a) to support classification for adverse effects on sexual function and fertility in males and females,

b) to justify classification for adverse effects on development, and

c) to justify classification for adverse effects on or *via* lactation.

The data background to support similarity from the mammalian toxicological data and the common mode of action for these endpoints are presented in this Section 2.3.

### 2.3.1 Adverse effects on sexual function and fertility

**Male fertility**:

The available data indicate that all three vanadium compounds (divanadium pentaoxide, ammonium and sodium metavanadates) negatively affect sperm parameters.

Assuming a low oral absorption (e.g., 5 %, for the purpose of this plausibility check), the corresponding systemic effect dose for ammonium metavanadate, based on the study by Morgan and El- Tawil (2003), is 0.2 mg V/kg bw/d. This dose is therefore in good agreement with the results by Chandra *et al*. (2007b; a; 2010) for sodium metavanadate after intraperitoneal application, reporting effect concentration of ≥ 0.2 mg V/kg bw/d.

In the inhalation study with divanadium pentaoxide (NTP, 2002) a dose of 0.14 mg V/kg bw/d (100% absorption assumed) induced only marginal effects on sperm parameters (impaired motility). Again, considering potential differences and uncertainties due to route-to-route extrapolation, species differences

and different study design, the effects observed with $V_2O_5$ *via* inhalation fit well to the effects obtained with sodium and ammonium metavanadates by oral or intraperitoneal application. However, potency differences between divanadium pentaoxide and sodium and ammonium metavanadates cannot be excluded as no comparable set of data are available.

The LOAEC obtained with $V_2O_5$ *via* inhalation (e.g., Fortoul *et al*., 2007) was not transformed into an internal dose, because of relevant uncertainties in their quantitative interpretation. The studies from the Mexico University with divanadium pentaoxide provide a possible mechanistic background to the observed testes effects. However, similar studies have not been performed with the other vanadates. Altamirano-Lozano *et al*. (2014) state that "exact mechanisms by which vanadium compounds immobilize sperm are unknown", but the authors speculate on influence of reactive oxygen species (ROS) being relevant, with no specific discussion on single vanadium compounds. Therefore similarity in mode of action of the three pentavalent vanadium compounds with respect to adverse effects on sexual function and fertility (male) is not explicitly confirmed but is highly plausible.

**Female fertility:**

The available data indicate that divanadium pentaoxide and ammonium metavanadate negatively affect female fertility (NTP, 2002; Altamirano *et al*., 1991; Morgan and El-Tawil (2003))Based on the reduced number of cycling female F344 rats at 16 mg/m$^3$ and a significant longer estrous cycle at 8 mg $V_2O_5$/m$^3$, atrophy of the secondary reproductive organ at 16 mg/m$^3$, NOAEC for female fertility was set at 4 mg/m³ (2.25 mg V /m$^3$) in the 90-day NTP, 2002 study by inhalation. No such effect was observed in mice (NTP, 2002). In another study conducted with high i.p. dose (12.5 mg/kg) of $V_2O_5$, ovulation rate in prepubertal female CIIZ rats was reduced with no other reproductive effects observed in females (Altamirano *et al*., 1991).

The reproductive toxicity study from Morgan and El-Tawil (2003), a drinking-water study with ammonium metavanadate, induced a reduction of the number of female rats with regular estrous cycle. When the treated females were mated to untreated males, the index for mating and fertility were both decreased, the total number of corpora lutea in females was reduced, the number of delayed birth date was increased as well as an increased number of dams showing signs of dystocia. Overall, consistent adverse effects were observed on female rat sexual function after inhalation of $V_2O_5$ (NTP, 2002) or after oral exposure with ammonium metavanadate (Morgan and El-Tawil, 2003). The NOAEC for female fertility was set at 4 mg/m³ (2.25 mg V /m$^3$) in NTP, 2002 and a LOAEC at 11.4 mg $NH_4VO_3$/kg bw/d (4.97 mg V/kg bw/d) in Morgan and El-Tawil (2003). Considering potential differences and uncertainties due to route-to-route extrapolation, species differences and different study design, the effects observed with$V_2O_5$ *via* inhalation fit well to the effects obtained ammonium metavanadate (oral application). However, potency differences between divanadium pentaoxide and ammonium metavanadate cannot not be excluded as no comparable set of data are available. In conclusion, due to similarity in toxicokinetic behaviour, type of reported effects and

assumed mode of action, for divanadium pentaoxide, ammonium metavanadate and sodium metavanadate, read-across is justified for effects on sexual function and fertility.

### 2.3.2 Adverse effects on development

Data for sodium metavanadate, ammonium metavanadate and divanadium pentaoxide point to similar types of developmental effects at a similar dose range. All three substances show retarded foetal growth, foetolethality, delayed ossification and malformations, partly at doses, which do not cause maternal toxicity.

Developmental effects (including decreased foetal body weight, embryofetal mortality and malformations) were reported with $V_2O_5$ in studies of low reliability. Observations from the experimental drinking water study with ammonium metavanadate ($NH_4V0_3$) (Morgan and El-Tawil, 2003) also demonstrate adverse developmental effects (e.g., number of dead foetuses/dam, significantly reduced foetal body weight, increased incidences of visceral and skeletal anomalies in the foetus, partly to be classified as malformations). Observations from the oral reproductive toxicity study (Domingo *et al*., 1986) with sodium metavanadate ($N_aVO_3$) showed in animals allowed to birth, a significant developmental decrease (decreases in body weight, body length, tail length) of the young from birth and during all the lactation period. Additionally, developmental effects were also observed with sodium metavanadate by Gómez *et al*., (1992) with reduced foetal weight, increased embryo/foetolethality and statistically significant increased incidence of cleft palate. Those effects are considered not to be secondary to other toxic effects. Similarity of these observed developmental effects with ammonium and sodium metavanadate but also with divanadium pentaoxide strengthen the use of read across for developmental effects even though possible differences in the systemic absorption from the gastrointestinal tract may interplay.

### 2.3.3 Adverse effects on or *via* lactation

Relevant studies to assess adverse effects *via* lactation have been performed mainly with sodium metavanadate. This includes behavioural effect studies, analyses for myelin damage or astrocytosis and further mechanistic studies with exposure of newborn mice by other pathways than lactation. Respective studies are reported in the main document. No indications were found that divanadium pentaoxide or the vanadates would act in the brain through different mode of action. The only study performed with ammonium metavanadate (Morgan and El-Tawil (2003)) mention deficits in behavioural response and foetal growth retardation and associate those effects in the newborns with the lactation pathway and oral parental exposure to ammonium metavanadate. Nevertheless, it should be noticed that these indication were insufficiently backed by reported experimental data.

There is uncertainty, how much vanadium will be transferred to the milk for the different pentavalent vanadium compounds. However, as discussed in the toxicokinetics section, generally high percentages of

vanadium are excreted to the milk (Elfant and Keen, 1987). Suckling rats showed a significant absorption of vanadium taken up by the milk, …[as] $^{48}V$ is easily absorbed in form of low molecular weight components (Edel and Sabbioni, 1989). A higher absorption of vanadium in young animals due to a greater nonselective permeability of the undeveloped intestinal barrier, is assumed (EPA, 2011). The lower absorption rate, as derived from feed or drinking water studies, will therefore not be adequate to estimate oral absorption of pentavalent vanadium compounds by the suckling *via* the lactation route.

## 3 CONCLUSIONS FOR C&L

Existing toxicological assessments on divanadium pentaoxide usually do not discriminate data for $V_2O_5$ from information received on other pentavalent vanadium compounds like sodium metavanadate or ammonium metavanadate. For example, the REACH registration dossier for divanadium pentaoxide uses information on sodium orthovanadate by Sanchez *et al*. (1991) to derive long-term DNELs (endpoint developmental toxicity) (ECHA, 2017). From data compiled for REACH by the Vanadium Consortium, read across is also suggested by the authors (VPRA, 2010). The approach taken by the Vanadium Consortium is supported by the dossier submitter for the considered endpoints based on the overall evidence.

Therefore, reading across data from pentavalent vanadium compounds, such as ammonium and sodium metavanadate, for classification of divanadium pentaoxide as a mutagen, carcinogen and reprotoxic appears an appropriate approach. It remains that chemistry and biochemistry of vanadium is very complex which may lead to subtle differences. Despite the mentioned uncertainties, effects observed from sodium and ammonium metavanadate as source substances are regarded as similarly relevant, as if they were observed with divanadium pentaoxide. This is mainly due to the direct observation of adverse effects in the offspring, the unambiguous link of the adverse effects to vanadium exposure and the consistent mechanistic understanding of this effect, which all give no indication of a substance specific effect of sodium or ammonium metavanadate, but rather a relevance for the other pentavalent vanadium compounds.

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