Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

Divanadium pentaoxide

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

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

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

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SUPPORT ON HOW TO COMPILE ANNEX I TO THE CLH REPORT

Annex I to the CLH report may be compiled from DARs, CARs and/or other sources. Non-confidential DAR/CAR can be annexed as such provided that it has sufficient level of details on the studies. The DS is encouraged to remove any irrelevant parts of the DAR/CAR. The DS must ensure that Annex I can be published during PC, i.e. it does not contain any confidential information.

For support, below is an example on how each study could be presented individually under its own subchapter including the study reference, detailed study summary and results. The format of the detailed study summary of an individual study is flexible as long as the summary is clearly reported and under a correct hazard class. Detailed support can be found below under each subchapter. If DAR/CAR is annexed to the CLH report as Annex I, it must be indicated clearly in the evaluation part of the report where in Annex I the relevant study can be found. If read-across to structurally or mechanistically similar substance is used please provide a justification for using data from this substance and, if known, present the calculations to convert dose/concentration levels from the test substance to the substance for which CLH is proposed. Please provide also a justification for providing non-testing data by any other approaches such as quantitative structure-activity relationships (QSARs) or grouping methods. Support on grouping of substances and read-across can be found in the following links:

http://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf

http://echa.europa.eu/documents/10162/13655/pg_report_qsars_en.pdf

http://echa.europa.eu/documents/10162/13655/pg_report_readacross_en.pdf

http://www.qsartoolbox.org/

http://www.oecd.org/chemicalsafety/risk-assessment/groupingofchemicalschemicalcategoriesandread-across.htm

http://echa.europa.eu/en/view-article/-/journal_content/title/assessing-read-across-how-echa-does-it

1 HEALTH HAZARDS

1.1 Acute toxicity – oral route

1.1.1 Animal data

1.1.1.1 [Leuschner et al., 1994]

Study reference:

Study reports, 1991 [confidential]

Leuschner, J. et al., New investigations on acute toxicities of vanadium oxides, Monatshefte für Chemie, 125, 623-646, 1994 (condensed report) (Leuschner et al., 1994)

Detailed study summary and results:

Test type

OECD Guideline 401 (Acute Oral Toxicity, standard acute method, no limit test, adopted 24 February 1987) without restrictions, but minor deviations:

- According to the guideline, the relative humidity should be between 30 - 70 %. In this study the relative humidity was slightly higher (60 +/- 20 %).
- According to the guideline, a stomach tube or a suitable intubation cannula should be used when the test substance is administered by gavage. The study report does not state what was used in order to administer the test substance by gavage.
- According to the guideline, after the substance has been administered, food may be withheld for a further 3 - 4 hours. In the study report it was not stated if food was withheld after the administration of the test substance.
- According to the guideline, the individual weights should be determined before the application of the test substance, which was done in this study. The individual weights were not provided in the report but only the average weight of the males and females per dose.
- According to the guideline, the 95 per cent confidence interval for the LD50 should be stated in the test report. In this study the confidence level was not stated for the technical grade pulverised test material.

GLP compliance is given (including certificate).

Test substance

In the study report three test materials were tested and analysed, which were individually reported in the disseminated database-file and were jointly reported in this annex, although, if needed, individual data were given for each test material.

- Name of test material (as cited in study report): Divanadium pentaoxide analytical grade pulverised (V_2O_5). Test material used in the study is equivalent to the substance identified in the CLH dossier.
- Degree of analytical purity: Vanadium: 56.05 % (Calculated V_2O_5: 97.86 %)
- Impurities: no information given
- Batch number: no information given
- Physical state: beige, solid (powder)
- Stability under test conditions: Stable during the course of the study
- Storage condition of test material: Dry, in closed container at room temperature
- Median particle size 3.0 - 3.9 µm

- Name of test material (as cited in study report): Divanadium pentaoxide technical grade fused (V_2O_5). Test material used in the study is equivalent to the substance identified in the CLH dossier.
- Degree of analytical purity: Vanadium: 56.25 % (Calculated V_2O_5: 100.4 % (approximately 1 % V2O4))
- Impurities: no information given
- Batch number: no information given
- Physical state: beige, solid (powder)
- Stability under test conditions: Stable during the course of the study
- Storage condition of test material: To be kept dry in a well closed container at room temperature
- Median particle size 10.5 µm

- Name of test material (as cited in study report): Vanadium pentaoxide technical grade pulverised (V_2O_5). Test material used in the study is equivalent to the substance identified in the CLH dossier.
- Degree of analytical purity: Vanadium: 55.6 % (Calculated V_2O_5: 99.3%)
- Impurities: no information given
- Batch number: MM 84097413 (given in disseminated database-file), however, also given as batch number of vehicle. Therefore unclear if this information is correct.)
- Physical state: beige, solid (powder)
- Stability under test conditions: Stable during the course of the study
- Storage condition of test material: To be kept dry in a well closed container at room temperature
- Median particle size 2.9 µm

Test animals

- Rat/Sprague-Dawley, male and female animals
- 5 animals per sex per dose
- Age at the study initiation: approx. 40 – 60 days
- Weight at the study initiation:
    - analytical grade pulverised V_2O_5: 150 – 200 g
    - technical grade fused V_2O_5: 159 – 200 g
    - technical grade pulverised V_2O_5 159 – 196 g

Administration/exposure

- Mode of administration: gavage
- Duration of test/exposure period: single
- Doses (no data on dose selection given):
    - analytical grade pulverised V_2O_5: 215, 316, 464, 681, and 1000 mg/kg b.w.
    - technical grade fused V_2O_5: 316, 464, 681, 1000, and 1470 mg/kg b.w.
    - technical grade pulverised V_2O_5: 147, 215, 316, and 681 mg/kg b.w. (males were also exposed against 464 mg/kg b.w.)
- Post exposure observation period: 14 d
- Control group and treatment: not specified
- Vehicle: Test materials were suspended in 0.8 % aqueous hydroxypropyl-methylcellulose gel (Methocel E 4 M), Batch no. (Vehicle): MM 84097413, volume of application was 20 mL/kg b.w. for all groups. The dose interval factor was 1.47 and additionally 2.15 for V_2O_5 technical grade pulverised.
- Statistical methods: For analytical grade pulverised and technical grade fused V_2O_5, LD50 was calculated according to FINNEY (probit analysis) and for technical grade pulverised V_2O_5 by regression analysis.

Results

In Table AI - 1 the acute oral toxicity of analytical grade pulverised divanadium pentaoxide in male and female rats is shown. Under the present test conditions first intolerance reactions (death) occurred at 316 mg V_2O_5 analytical grade pulverised/kg b.w. p.o. The lowest lethal dose for males and females was 316 mg/kg b.w. p.o. Animals died between 48 hours and 7 days unobserved. Reduced motility, ataxia and dyspnoea were observed from a dose-level of 464 mg/kg b.w. onwards. Animals showed symptoms between 24 h and days 2 and varied between slight and moderate. There was no inhibition of body weight gain seen in males or females. There were no pathological findings in males or females of deceased or alive animals.

Table AI - 1: Acute oral toxicity of analytical grade pulverised divanadium pentaoxide in male and female rats

Doses (mg/kg b.w.)	Mortality		Symptoms, pathology and histology
	male	female	
316	1/5	1/5	No symptoms observed.
464	1/5	2/5	reduced motility (4/5 males; 3/5 females); ataxia (5/5 males, 3/5 females); dyspnoea (5/5 males; 3/5 females)
681	5/5	5/5	reduced motility (5/5 males; 3/5 females); ataxia (4/5 males, 3/5 females); dyspnoea (4/5 males; 3/5 females)
1000	5/5	5/5	reduced motility (5/5 males; 5/5 females); ataxia (5/5 males, 5/5 females); dyspnoea (5/5 males; 5/5 females)

Results taken from disseminated database-file and registration dossier.

The calculated oral LD50 of analytical grade pulverised divanadium pentaoxide was 474.2 mg/kg b.w. (95 % CL: >379.01 ≤ 593.35, slope: 7.82) in males and 466.93 mg/kg b.w. in females (LD50 was calculated by regression analysis; no estimation of the confidence limits possible, due to the low number of animals employed and the steepness of the mortality curve (slope: 29.94)).

Acute oral toxicity of technical grade fused divanadium pentaoxide in male and female rats is shown in Table AI - 2. Under the present conditions first intolerance reactions (death) occurred at 464 mg V_2O_5 technical grade fused/kg b.w. p.o.. Lowest lethal dose for males was 681 mg/kg b.w. p.o. and for females 464 mg/kg b.w. p.o. Animals died between 24 hours and 7.5 days unobserved. Reduced motility (4/5 males; 5/5 females), ataxia (4/5 males; 5/5 females) and dyspnoea (4/5 males; 4/5 females) were observed at the highest tested dose-level of 1470 mg/kg b.w. Animals showed slight symptoms between 24 hours and day 3. At 1000 mg/kg b.w. the surviving animals showed a slightly or severely reduced body weight gain. There were no pathological findings observed in autopsy of surviving animals.

Table AI - 2: Acute oral toxicity of technical grade fused divanadium pentaoxide in male and female rats

Doses (mg/kg b.w.)	Mortality		Symptoms, pathology and histology
	male	female	
316	0/5	0/5	No symptoms observed.
464	0/5	1/5	No symptoms observed.
681	3/5	3/5	No symptoms observed.
1000	4/5	4/5	Reduced body weight gain
1470	5/5	5/5	Reduced motility (4/5 males; 5/5 females), ataxia (4/5 males; 5/5 females) and dyspnoea (4/5 males; 4/5 females), enlarged stomach (1/5 males; 5/5 females)

Results taken from disseminated database-file and registration dossier.

The calculated oral LD50 of technical grade fused divanadium pentaoxide was 715.7 mg/kg b.w. (95 % CL: >567.73 ≤ 888.06, slope: 8.21) in males and 658.4 mg/kg b.w. (95 % CL: >514.38 ≤ 842.64, slope: 6.25) in females.

Acute oral toxicity of technical grade fused divanadium pentaoxide in male and female rats is shown in Table AI - 3. Under the present test conditions the lowest lethal dose-level for female rats was 215 mg/kg b.w. p.o. and for male rats 316 mg V_2O_5 technical grade pulverised/kg b.w. by oral administration. Animals died within 24 hours and 6 days. None of the deceased or surviving animals showed abnormal behaviour or external appearance. There were no pathological findings observed in autopsy of surviving animals.

Table AI - 3: Acute oral toxicity of technical grade pulverised divanadium pentaoxide in male and female rats

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

Results taken from disseminated database-file and registration dossier.

The calculated oral LD50 of technical grade pulverised divanadium pentaoxide was 313.8 mg/kg b.w. (slope: 29.88) in males and 221.1 mg/kg b.w. (slope: 29.53) in females. The calculation of confidence limits was not possible, due to the low number of animals employed and the steepness of the mortality curves.

1.1.1.2 [Massmann et al., 1956]

Study reference:

Massmann, W., Experimentelle Untersuchungen über die biologische Wirkung von Vanadinverbindungen [Experimental studies on the biological effects of vanadium compounds], Archiv für Toxikologie, 16, 182-189, 1956, in German

Detailed study summary and results:

Test type

Acute toxicity of divanadium pentaoxide after peroral administration was tested in rats and LD50 values were determined. The study was not conducted according to a guideline and no information on GLP compliance is given (though study published before GLP was introduced).

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: divanadium pentaoxide
- Degree of purity: analytical controls ensured degree of purity (no further information given)
- Impurities: no information given
- Batch number: no information given

Test animals

- rat (no further information)
- 7 animals per dose
- Age and weight at the study initiation: age not given, weight was between 160 and 180 g

Administration/exposure

- Mode of administration: peroral
- Duration of test/exposure period: single
- Doses/concentration levels, rationale for dose level selection: not given
- Post exposure observation period: 14 d
- Control group and treatment: not specified
- Vehicle: dissolved in 1/6 m sodium carbonate, m/100 solutions were used
- Statistical methods: LD50 calculation according to Kärber

Results

The number of deaths at each dose level is not specified. A LD50 value of 10.4 mg/kg bw for divanadium pentoxide and a surviving time of 1 to 7 days is given. Confidence limits are not stated. Except of a premortal coma, no further clinical signs or findings are reported. Histological examination of deceased animals revealed venous hyperemia of pia (likely: pia mater), lung, heart, and liver. The adrenal cortex showed a significant decrease of lipoid, only in the zona glomerulosa a few substances stained with Sudan were detectable.

1.2 Acute toxicity – dermal route

1.2.1 Animal data

1.2.1.1 [Leuschner et al., 1994]

Study reference:

Study reports, 1991 [confidential]

Leuschner, J. et al., New investigations on acute toxicities of vanadium oxides, Monatshefte für Chemie, 125, 623-646, (condensed report) (Leuschner et al., 1994)

Detailed study summary and results:

Test type

OECD Guideline 402 (Acute Dermal Toxicity, standard acute method, limit test, adopted 24 February 1987) without restrictions, but minor deviations:

- According to the guideline, a limit test of at least 2000 mg/kg bw can be carried out. In this study one additional concentration above the limit dose was also tested.
- According to the guideline, the individual weights should be determined before the application of the test substance. The individual weights were not provided in the report but only the average weight of the males and females per dose.
- The guideline suggested that the rats should be in the weight range of 200 to 300 g at the beginning of the study but in this study the rats were in the weight range of 162 - 196 g.
- According to the guideline, the rats should be housed individually. In this study they were housed in groups of two or three rats.
- According to the guideline the relative humidity should be between 30 - 70 %. In this study the relative humidity was slightly higher (60 +/- 20 %).
- According to the guideline, the test substance should be applied to the skin. In this study the test substance was applied first to the patch and the patch was placed onto the skin.

GLP compliance is given (including certificate).

Test substance

In the study report three test materials were tested and analysed. Individual data were given for each test material and also reported in the disseminated database-file and in this annex.

- Name of test material (as cited in study report): Divanadium pentaoxide analytical grade pulverised (V_2O_5). Test material used in the study is equivalent to the substance identified in the CLH dossier.
- Degree of analytical purity: Vanadium: 56.05 % (Calculated V_2O_5: 97.86%)
- Impurities: no information given
- Batch number: no information given
- Physical state: beige, solid (powder)
- Stability under test conditions: Stable during the course of the study
- Storage condition of test material: Dry, in closed container at room temperature
- Median particle size 3.0 - 3.9 µm

- Name of test material (as cited in study report): Divanadium pentaoxide technical grade fused (V_2O_5). Test material used in the study is equivalent to the substance identified in the CLH dossier.
- Degree of analytical purity: Vanadium: 56.25 % (Calculated V_2O_5: 100.4 % (approximately 1 % V2O4))
- Impurities: no information given
- Batch number: no information given
- Physical state: beige, solid (powder)
- Stability under test conditions: Stable during the course of the study
- Storage condition of test material: Dry, in closed container at room temperature
- Median particle size 10.5 µm

- Name of test material (as cited in study report): Vanadium pentaoxide technical grade pulverised (V2O5). Test material used in the study is equivalent to the substance identified in the CLH dossier.
- Degree of analytical purity: Vanadium: 55.6 % (Calculated V_2O_5: 99.3 %)
- Impurities: no information given
- Physical state: beige, solid (powder)
- Stability under test conditions: Stable during the course of the study
- Storage condition of test material: Dry, in closed container at room temperature
- Median particle size 2.9 µm

Test animals

- Rat/Sprague-Dawley/ male and female
- 5 animals per sex per dose
- Age at the study initiation: approx. 40 – 60 days
- Weight at the study initiation:
    - analytical grade pulverised V_2O_5: 166 – 181 g

- technical grade fused V_2O_5: 168 – 196 g
- technical grade pulverised V_2O_5: 162 – 181 g

Administration/exposure

- Mode of administration: The hair on the site of application was clipped with hair-clippers without causing injury approximately 24 hours before application. The site was situated on the animal's back between the fore and hind extremities and had an area of approx. 5 x 6 cm. The test substance was applied to 8 layers of gauze and then to the application site. The patch was covered with a plastic sheet and secured with adhesive plaster.
- Duration of test/exposure period: 24 h
- Doses: 2000 and 2500 mg/kg b.w. (no data on dose selection given)
- Post exposure observation period: 14 d
- Control group and treatment: not specified
- Vehicle: 0.8 % aqueous hydroxypropyl-methylcellulose gel (Methocel E 4 M), Batch No. (Vehicle): MM 84097413 (no further information)
- Area covered (e.g. x % of body surface): 5 x 6 cm, 1/10 of body surface
- Occlusion: occlusive
- Total volume applied: The volume of application was 20 mL/kg b.w.. The dose interval factor was 1.25.
- Removal of test substance: At the end of the exposure period, residual substance was removed with tepid tap water.
- Statistical methods: The LD50 could not be calculated because none of the animals died prematurely.

But the skin was observed for the development of erythema and oedema and was rated as follows:

Erythema

0 = no erythema

1 = very slight erythema (barely perceptible)

2 = well-defined erythema

3 = moderate to severe erythema

4 = severe erythemy (beet redness) to slight eschar formation (injuries in depth)

Oedema

0 = no oedema

1 = very slight oedema (barely perceptible)

2 = slight oedema (edges of area well defined by definite raising)

3 = moderate oedema (raised approx. 1 mm)

4 = severe oedema (raised more than 1 mm and extending beyond the area of exposure)

Results

None of the animals died prematurely by dermal administration of three divanadium pentaoxide compounds. Therefore LD50 ≥ 2500 mg/kg b.w. were determined in male and female rats for the three divanadium pentaoxide compounds. Up to the highest tested dose-level of 2500 mg/kg b.w. no local or systemic intolerance reactions were observed by dermal administration of three divanadium pentaoxide compounds in rats. There was no inhibition of body weight gain or pathological findings seen in male or female rats dosed at 2000 or 2500 mg/kg b.w.

1.3 Acute toxicity – inhalation route

1.3.1 Animal data

1.3.1.1 [Leuschner et al., 1994]

Study reports, 1991, [confidential]

Leuschner, J. et al., New investigations on acute toxicities of vanadium oxides, Monatshefte für Chemie, 125, 623-646, (condensed report) (Leuschner et al., 1994)

Detailed study summary and results:

Test type

OECD Guideline 403 (Acute Inhalation Toxicity, standard acute method, no limit test, adopted 12 May 1981) without restrictions, but minor deviations:

- According to the guideline, the weight variation in animals or between groups used in a test should not exceed +/- 20 % of the mean weight. The males of the group with the 2.42 mg/l air concentration were slightly outside this weight range.
- According to the guideline, the relative humidity should be between 30 - 70 % in the animal room. In this study the relative humidity was slightly higher (60 +/- 20 %).
- According to the guideline, animals should be tested with inhalation equipment designed to sustain a dynamic air flow of 12 to 15 air changes per hour. In the high concentration group of analytical and technical grade pulverised V_2O_5 the air flow was 29.4 and 18.8 changes per hour. In the low and medium concentration groups of technical grade fused V_2O_5 the air flow was 9.6 changes per hour.
- According to the guideline, the duration of exposure should be at least 4 hours after equilibration of the chamber concentration. It was not stated if the 4 hours started after equilibration of the chamber concentration.

- According to the guideline, weight changes should be calculated and recorded when survival exceeds one days. This is missing in this study.
-The GSD for the MMAD is missing.
- The 95 % confidence level is missing for females (analytical grade pulverised V_2O_5) and males and females (technical grade pulverised V_2O_5).
GLP compliance is given (including certificate).

Test substance

In the study report three test materials were tested and analysed, which were individually reported in the disseminated database-file and were jointly reported in this annex, although, if needed individual data were given for each test material.

- Name of test material (as cited in study report): Divanadium pentaoxide analytical grade pulverised (V_2O_5). Test material used in the study is equivalent to the substance identified in the CLH dossier.
- Degree of analytical purity: Vanadium: 56.05 % (Calculated V_2O_5: 97.86%)
- Impurities: no information given
- Batch number: no information given
- Physical state: beige, solid (powder)
- Stability under test conditions: Stable during the course of the study
- Storage condition of test material: Dry, in closed container at room temperature
- Median particle size 3.0 - 3.9 µm

- Name of test material (as cited in study report): Divanadium pentaoxide technical grade fused (V_2O_5). Test material used in the study is equivalent to the substance identified in the CLH dossier.
- Degree of analytical purity: Vanadium: 56.25 % (Calculated V_2O_5: 100.4 % (approximately 1 % V2O4))
- Impurities: no information given
- Batch number: no information given
- Physical state: beige, solid (powder)
- Stability under test conditions: Stable during the course of the study
- Storage condition of test material: Dry, in closed container at room temperature
- Median particle size 10.5 µm

- Name of test material (as cited in study report): Vanadium pentaoxide technical grade pulverised (V_2O_5). Test material used in the study is equivalent to the substance identified in the CLH dossier.
- Degree of analytical purity: Vanadium: 55.6 % (Calculated V_2O_5: 99.3%)
- Impurities: no information given

- Physical state: beige, solid (powder)
- Stability under test conditions: Stable during the course of the study
- Storage condition of test material: Dry, in closed container at room temperature
- Median particle size 2.9 µm
- Type or preparation of particles: The dust was generated with a dust generator and dosing apparatus (BURGHART, D-2000 Wedel/Holstein). The generator was fed with compressed air from a compressor.

Test animals

- Rat/Sprague-Dawley/ male and female
- 5 animals per sex per dose
- Age at the study initiation:
    - analytical grade pulverised V_2O_5: 52 – 69 d
    - technical grade fused V_2O_5: 51 – 67 d
    - technical grade pulverised V_2O_5: 48 – 64 d
- Weight at the study initiation:
    - analytical grade pulverised V_2O_5: 188 – 270 g (males); 164 – 206 (females)
    - technical grade fused V_2O_5: 185 – 249 g (males); 174 – 212 (females)
    - technical grade pulverised V_2O_5: 188 – 254 g (males); 170 – 208 (females)

Administration/exposure

- Type of inhalation exposure and test conditions: The study was carried out using a dynamic inhalation apparatus with a nose only exposure of the animals according to KIMMERLE & TREPPER (Rhema- Labortechnik, D-6238 Hofheim/Taunus). The apparatus consists of a cylindrical exposure chamber (volume 40 l) which holds a maximum of 20 animals in pyrex tubes at the edge of the chamber in a radial position. The method of exposure was nose only.
- Source and rate of air: At the bottom of the exposure chamber the air was sucked off at the similar rate as created by the dust generator in order to produce a homogenous distribution in the exposure chamber. Air-flow meters (Rotameter, ROTA Apparate- und Maschinenbau, D-7867 Wehr 2/Baden) were used to control the constant supply of compressed air and vacuum. Flow rates were checked at least once/hour and corrected if necessary.
    - analytical grade pulverised V_2O_5: Air flow was 480 l/h (low and medium concentration); 1175 l/h (high concentration). Air change was 12.0 changes per hour (low and medium concentration); 29.4 changes per hour (high concentration)

  - technical grade fused V_2O_5: Air flow was 383 l/h (low and medium concentration); 480 l/h (high concentration). Air change was 9.6 changes per hour (low and medium concentration); 12.0 changes per hour (high concentration)
  - technical grade pulverised V_2O_5: Air flow was 480 l/h (low and medium concentration); 750 l/h (high concentration). Air change was 12.0 changes per hour (low and medium concentration); 18.8 changes per hour (high concentration)
- Duration of test/exposure period: 4 h
- Doses:
  - analytical grade pulverised V_2O_5: Mean actual concentration 0.90 +/- 0.39 (nominal concentration: 1.9 mg/l air), 2.42 +/- 0.38 (nominal concentration: 7.4 mg/l air), 4.72 +/- 1.45 (nominal concentration: 8.6 mg/l air) mg V_2O_5analytical grade pulverised/l air. 4.72 mg/l air was the highest concentration that could be generated in the inhalation chamber.
  - technical grade fused V_2O_5: Mean actual concentration 0.97 +/- 0.62 mg/l air (nominal concentration 6.1 mg/l air), 2.71 +/- 1.94 mg/l air(nominal concentration 16.9 mg/l air) and 6.0 +/- 0.57 mg/l air (nominal concentration 37.8 mg/l air). 6.0 mg/l air was the highest concentration that could be generated in the inhalation chamber.
  - technical grade pulverised V_2O_5: Actual concentration: 1.11 +/- 0.08 mg/l air (nominal concentration: 7.0 mg/l air), 1.62 +/- 0.27 mg/l air (nominal concentration: 9.8 mg/l air) and 5.2 +/- 1.52 mg/l air nominal concentration: 23.1 mg/l air). 5.2 mg/l air was the highest concentration that could be generated in the inhalation chamber.
- Vehicle: Air
- Analytical verification of test atmosphere concentrations:

  Method of particle size determination: An analysis of the particle size distribution was carried out twice during the exposure period using a cascade impactor according to MAY (1975).

  The impactor is a device that classifies particles present in a sample of air or gas into known size ranges. It does this by drawing the air sample through a cascade of progressively finer nozzles. The air jets from these impact on plane sampling surfaces (slides) covered with adhesive tape. Each stage represents an aerodynamic size range. The dust from the exposure chamber was sucked through the cascade impactor for 1 to 6 minutes at a constant flow rate of 5 l/min. The slides were removed from the impactor and were weighed on an analytical balance (SARTORIUS, type 1601004, precision 10 µg).

  Brief description of analytical method used: The dust concentration in the inhalation chamber was measured with an air sample filter (Minisart SM 17598) and pump (water jet air pump controlled by a rotameter). Dust samples were taken during the first half and during the second half of the exposure. The Minisart SM 17598 filters were placed close to the animals' nose and sucked through

with a constant flow of air of 300 l/h for 1 to 3 minutes. The filters were weighed before and after sampling (accuracy 0.01 mg).

- analytical grade pulverised V_2O_5:

  Respirable amount (particle size <= 4 µm)

  Dose level 0.90 mg/l air: 0.61 mg/l air

  Dose level 2.42 mg/l air: 2.01 mg/l air

  Dose level 4.72 mg/l air: 3.52 mg/l air

  The sample supplied had a particle size distribution of 55.9 % in the particle size range of 2.4 to 5.0 µm.

  TEST ATMOSPHERE

  Particle size distribution: The sample supplied had a particle size distribution of 55.9 % in the particle size range of 2.4 to 5.0 µm measured with a Malvern particle sizer.

  Detailed particle size and mean are given in Table AI - 4.

Table AI - 4: Particle size and mean of analytical grade pulverised divanadium pentaoxide

Particle size (µm) and mean (concentration 0.90 mg/l air):	**Particle size (µm) and mean (concentration 2.42 mg/l air):**	**Particle size (µm) and mean (concentration 4.72 mg/l air):**
<0.5 µm: 1.33 %	<0.5 µm: 0.0 %	<0.5 µm: 1.87 %
0.5 µm: 12.80 %	0.5 µm: 5.18 %	0.5 µm: 8.85 %
1 µm: 22.25 %	1 µm: 17.19 %	1 µm: 31.02 %
2 µm: 52.36 %	2 µm: 52.89 %	2 µm: 69.52 %
4 µm: 68.25 %	4 µm: 83.10 %	4 µm: 74.59 %
8 µm: 75.84 %	8 µm: 87.50 %	8 µm: 78.18 %
16 µm: 90.17 %	16 µm: 93.73 %	16 µm: 91.77 %
>= 32 µm: 99.90 %	>= 32 µm: 100.01 %	>= 32 µm: 100.0 %

  MMAD:

  Dose level 0.90 mg/l air: 2.44 µm

  Dose level 2.42 mg/l air: 2.32 µm

  Dose level 4.72 mg/l air: 2.01µm

- technical grade fused V_2O_5:

  Respirable amount (particle size <= 4 µm)

Dose level 0.97 mg/l air: 0.73 mg/l air

Dose level 2.71 mg/l air: 2.12 mg/l air

Dose level 6.0 mg/l air: 3.55 mg/l air

TEST ATMOSPHERE

Detailed particle size and mean are given in Table AI - 5.

Table AI - 5: Particle size and mean of technical grade fused divanadium pentaoxide

Particle size (µm) and mean (concentration 0.97 mg/l air):	**Particle size (µm) and mean (concentration 2.71 mg/l air):**	**Particle size (µm) and mean (concentration 6.0 mg/l air):**
<0.5 µm: 10.63 %	<0.5 µm: 1.30 %	<0.5 µm: 0.86 %
0.5 µm: 20.68 %	0.5 µm: 7.66 %	0.5 µm: 2.46 %
1 µm: 49.01 %	1 µm: 22.57 %	1 µm: 10.96 %
2 µm:70.86 %	2 µm: 62.81 %	2 µm: 44.96 %
4 µm: 75.16 %	4 µm: 78.21 %	4 µm: 59.16 %
8 µm: 80.41 %	8 µm: 80.62 %	8 µm: 61.00 %
16 µm 94.83 %	16 µm: 97.06 %	16 µm: 85.40 %
>= 32 µm: 99.99 %	>= 32 µm: 100.0 %	>= 32 µm: 99.97 %

MMAD:

Dose level 0.97 mg/l air: 1.31 µm

Dose level 2.71 mg/l air: 2.08 µm

Dose level 6.0 mg/l air: 4.04 µm

– technical grade pulverised V_2O_5:

Respirable amount (particle size <= 4 µm)

Dose level 1.11 mg/l air: 0.66 mg/l air

Dose level 1.62 mg/l air: 0.97 mg/l air

Dose level 5.2 mg/l air: 2.99 mg/l air

TEST ATMOSPHERE

Detailed particle size and mean are given in Table AI - 6.

Table AI - 6: Particle size and mean of technical grade pulverised divanadium pentaoxide

Particle size (µm) and mean (concentration 1.11 mg/l air):	**Particle size (µm) and mean (concentration 1.62 mg/l air):**	**Particle size (µm) and mean (concentration 5.2 mg/l air):**
<0.5 µm: 2.46 %	<0.5 µm: 0.46 %	<0.5 µm: 1.43 %
0.5 µm: 10.95 %	0.5 µm: 2.90 %	0.5 µm: 9.16 %
1 µm: 29.85 %	1 µm: 9.81 %	1 µm: 29.26 %
2 µm: 53.68 %	2 µm: 23.69 %	2 µm: 48.30 %
4 µm: 59.21 %	4 µm: 59.80 %	4 µm: 57.57 %
8 µm: 67.90 %	8 µm: 62.55 %	8 µm: 63.22 %
16 µm: 86.63 %	16 µm: 88.48 %	16 µm: 88.88 %
>= 32 µm: 100.0 %	>= 32 µm: 99.99 %	>= 32 µm: 99.99 %

MMAD:

Dose level 1.11 mg/l air: 2.72 µm

Dose level 1.62 mg/l air: 4.25 µm

Dose level 5.2 mg/l air: 2.96 µm

- Post exposure observation period: 14 d
- Control group and treatment: not specified
- Statistical methods: The mass median aerodynamic diameter (MMAD) was estimated by means of nonlinear regression analysis (LITCHFIELD & WILCOXON). The 32 µm particle size range was not included in the determination of the MMAD in order not to give undue weight to this value. The LC50 (24 hours (only analytical grade pulverised V_2O_5) and 14 days) was calculated by regression analysis (if possible, according to FINNEY).

Results

In Table AI - 7 the acute inhalative toxicity of analytical grade pulverised divanadium pentaoxide in male and female rats is shown. At the dose-level of 2.42 mg/L, 1/5 males died 4 days after start of exposure. At the dose level of 4.72 mg/L, 1/5 males and 4/5 females died 2 days after start of exposure. At the lowest dose, no signs of systemic toxicity were observed. The body weight gain in males and females was not affected by analytical grade pulverised divanadium pentaoxide.

Table AI - 7: Acute inhalative toxicity of analytical grade pulverised divanadium pentaoxide in male and female rats

Doses (mg/L air)	Mortality		Symptoms, pathology and histology
	male	female	
0.90	0/5	0/5	no signs of systemic intolerance (males and females)
2.42	1/5	0/5	except for mortality no signs of systemic intolerance (males and females) surviving animals: lungs haemorrhagic (2/4 males; 1/5 females) histopathology in lung: vascular congestion (2/2 males); oedema (1/2 males) and (initial) haemorrhagic bronchopneumonia (2/2 males)
4.72	1/5	4/5	except for mortality no signs of systemic intolerance (males and females) deceased animals: lungs haemorrhagic (1/1 males; 3/4 females) surviving animals: lungs haemorrhagic and light dark coloured (1/4 males) histopathology in lung: vascular congestion (2/2 males; 3/3 females); oedema (1/2 males; 2/3 females) and (initial) haemorrhagic bronchopneumonia (2/2 males; 3/3 females)

Results taken from disseminated database-file and registration dossier.

The calculated LC50 of analytical grade pulverised divanadium pentaoxide was 11.09 mg/L air (95 % CL: >0.68 ≤ 180.68, slope: 1.783) in males and 4.29 mg/L air in females (LD50 was calculated by regression analysis; no estimation of the confidence limits possible, due to the low number of animals employed and the steepness of the mortality curve (slope: 20.14)).

The acute inhalative toxicity of technical grade fused divanadium pentaoxide in male and female rats is shown in Table AI - 8. At the dose-level of 2.71 mg/L prematurely animals (1/5 males; 1/5 females) died 3 or 4 days after start of exposure. At the dose-level of 6.0 mg/L prematurely animals (1/5 males; 4/5 females) died 2 to 6 days after start of exposure. At the lowest dose, no signs of systemic toxicity were observed. The body weight gain in males and females was not affected by technical grade fused divanadium pentaoxide.

Table AI - 8: Acute inhalative toxicity of technical grade fused divanadium pentaoxide in male and female rats

Doses (mg/L air)	**Mortality**		**Symptoms, pathology and histology**
	male	**female**	
0.97	0/5	0/5	no signs of systemic intolerance (males and females) surviving animals: lungs haemorrhagic (1/5 males)
2.71	1/5	1/5	except for mortality, no signs of systemic intolerance deceased animals: lungs haemorrhagic (1/1 males; 1/1 females) surviving animals: lungs haemorrhagic (1/4 males; 2/4 females) histopathology in lung: (focal) vascular congestion (2/2 males; 2/2 females), oedema (1/2 males; 2/2 females), (initial) haemorrhagic bronchopneumonia (2/2 males; 2/2 females)
6.00	1/5	4/5	except for mortality, no signs of systemic intolerance deceased animals: lungs haemorrhagic (1/1 male; 2/4 females); kidneys light coloured (1/1 male; 2/4 females) surviving animals: lungs haemorrhagic (2/4 males) histopathology in lung: (focal) vascular congestion (2/2 males; 2/2 females), oedema (2/2 females), (initial) haemorrhagic bronchopneumonia (2/2 males; 2/2 females) and kidney: vascular congestion (1/1 females)

Results taken from disseminated database-file and registration dossier.

The calculated LC50 of technical grade fused divanadium pentaoxide was 16.19 mg/L air (95 % CL: >0.61 ≤ 431.06, slope: 1.54) in males and 4.04 mg/L air (95 % CL: >2.68 ≤ 6.09, slope: 4.95) in females.

The acute inhalative toxicity of technical grade pulverised divanadium pentaoxide in male and female rats is shown in Table AI - 9. At the dose-level of 1.62 mg/l, 1/5 females died 3 or 4 days after start of exposure. At the dose-level of 5.2 mg/l, 4/5 males and 5/5 females died between 4 hours and 3 days after start of exposure. At the lowest dose, no signs of systemic toxicity were observed. The body weight gain in males and females was not affected by technical grade pulverised divanadium pentaoxide.

Table AI - 9: Acute inhalative toxicity of technical grade pulverised divanadium pentaoxide in male and female rats

Doses (mg/L air)	**Mortality**		**Symptoms, pathology and histology**
	male	**female**	
1.11	0/5	0/5	no signs of systemic intolerance (males and females)
1.62	0/5	1/5	except for mortality, no signs of systemic intolerance surviving animals: lungs haemorrhagic (3/5 males; 3/4 females) histopathology in lung: vascular congestion (3/3 males; 3/3 females), oedema (1/3 males; 2/3 females), (haemorrhagic) bronchopneumonia (1/3 males; 1/3 females)
5.20	4/5	5/5	except for mortality, no signs of systemic intolerance deceased animals: lungs haemorrhagic (1/4 males; 4/5 females) surviving animals: lungs haemorrhagic (1/1 male)

Results taken from disseminated database-file and registration dossier.

The calculated LC50 of technical grade pulverised divanadium pentaoxide was 4.40 mg/L air (slope: 11.53) in males and 2.21 mg/L air (slope: 14.09) in females. LD50 was calculated by regression analysis; no confidence limits were given, perhaps due to the low number of animals employed and the steepness of the mortality curve.

1.3.1.2 [Anonymous, 2011]

Study report, 2011 [confidential]

Note: this study is included in the registrants' IUCLID file in section 7.9.3 Specific investigations: mechanistic studies

Detailed study summary and results:

Test type

According to: OECD 436 "Acute Inhalation Toxicity - Acute Toxic Class Method" (adopted 2009-09-07)

The limit test for this study (2 mg/L) was conducted in compliance with the requirements of the following test guidelines with the exception that body weights were not obtained 1 and 3 days after exposure: 1) US EPA Health Effects Testing Guideline, OPTTS 870.1300, entitled Acute Inhalation Toxicity, 1998 and 2) OECD test guideline 403 (1981). In order to reduce animal use and to provide an acute inhalation toxicity estimate and information for the Globally Harmonized System (GHS) of classification and labelling for the test substance, all subsequent exposures were conducted in compliance with OECD test guideline 436 (2009) (with the exception that body weights were not obtained 1 and 3 days after exposure). The initial subsequent

exposure was determined by the study director in conjunction with the sponsor and any subsequent exposures were determined according to the scheme in Annex 3b of the OECD 436 test guideline.

- GLP compliance: yes
- Study period: 2010-03-10 to 2010-11-17

Test substance

- Name of test material (as stated in study report): Granular vanadium pentoxide
- Physical state: Yellow-orange granular powder
- Storage condition of test material: Dry, in closed container at room temperature
- Purity: [confidential information]
- Batch No: [confidential information]

Test animals

- Rat/Fischer 344/ male and female
- 5 (high concentration) or 3 (lower concentrations) animals per sex per dose
- Source: Charles River Laboratories (Portage, MI)
- Age at receipt: 55 and 59 days
- Weight at receipt: 109 to 176 g
- Housing: animal were double-housed and then after randomization, single-housed, in stainless steel cages suspended over absorbent paper cage boards
- Diet (ad libitum, except during inhalation exposure): Certified Rodent Chow 5002 meal (PMI Nutrition International, Inc., Brentwood, MO)
- Water (ad libitum, except during inhalation exposure): City of Chicago water
- Quarantine period: approx. one week; during quarantine the rats were observed daily for signs of disease and general unthriftiness.
- To condition the animals to placement and restraint in the nose-only holding tubes (CH Technologies, U.S.A., Westwood, NJ) and to reduce stress during the exposure phase, the animals were placed in the holding tubes over three days according to the following schedule: one hour on Day 1, two hours on Day 2 and three hours on Day 3 prior to their exposure.
- ENVIRONMENTAL CONDITIONS:- Temperature: 19.0 to 25.3°C
  - Relative humidity: 30 to 68%
  - Air changes: minimum of 10 air changes per hour
  - Photoperiod (hrs dark / hrs light): 12/12

Administration/exposure

- An aliquot of the test substance was milled. The milled test substance was aerosolised as a powder for the inhalation exposures.
- GENERATION OF TEST ATMOSPHERE / CHAMBER DESCRIPTION

  - Exposure apparatus/Method of holding animals in test chamber: a 52-port, flow-past nose-only inhalation exposure chamber (Lab Products Inc., Seaford, DE) was used. The animals were restrained in nose-only exposure animal holding tubes (CH Technologies, U.S.A., Westwood, NJ). Each tube was placed in a pre-designated port of the inhalation exposure chamber.

  - System of generating particulates/aerosols: the test atmosphere was created by generating an aerosol (with filtered air) of the test substance using a compressed air-operated Wright Dust Aerosol Generation System (BGI Incorporated, Waltham, MA) positioned over the chamber. The test substance was weighed and packed into a dust reservoir forming a cake. A constant speed rotating scraper separated a thin film of the test substance at the surface of the cake and delivered it into a dispersing unit, drawn in by aspiration and dispersed by a high velocity air jet. The resulting test atmosphere entered a mixing plenum where it, when necessary, was diluted with breathable quality compressed air to achieve target concentration prior to introduction to the exposure chamber. The exhaust from the exposure chamber was moved through a high efficiency particulate air (HEPA) filter by a ring compressor and exhausted outside the building. Inlet and exhaust flows to and from the chamber were controlled and continuously monitored by rotometers. T90 time was assessed to be less than 30 seconds.

  - Temperature, humidity, oxygen percentage in air chamber: oxygen percentage was measured once during the exposure with an Altair Oxygen Sensor and Detector (MSA Instrument Division, Pittsburgh, PA).

  Inhalation exposure chamber temperature and relative humidity were monitored with a hand-held thermohygrometer (model # 8721, Control Company, Fisher Scientific, Friendswood, TX) and recorded at approximately half-hour intervals during exposure.

  Temperature (mean ± SD):

  2.00 mg/L: 21.3°C ± 0.49

  0.50 mg/L: 21.2°C ± 0.13

  0.056 mg/L: 21.3°C ± 0.21

  Relative humidity (mean ± SD):

  2.00 mg/L: 14.3% ± 2.57%

  0.50 mg/L: 43.2% ± 1.60%

  0.056 mg/L: 29.3% ± 1.88%

Oxygen (%):

2.00 mg/L: no data to report; the oxygen sensor was inoperable during the exposure

0.50 mg/L: 20.8%

0.056 mg/L: 20.8%

- Method of particle size determination: aerosol particle size was monitored at least once per two hours during the exposure with a quartz crystal microbalance (QCM) cascade impactor (California Measurements Inc., Sierra Madre, CA). The mass median aerosol diameter (MMAD) and geometric standard deviation (GSD) were calculated from the mass accumulated on each stage of the QCM.

- TEST ATMOSPHERE:

  - Brief description of analytical method used: the concentration of the test substance in the test atmosphere was monitored gravimetrically by filter-collected samples. One sample was taken from the breathing zone of the animals every hour of exposure. The gravimetric sampling train consisted of a pre-weighed filter in a series with a dry-gas meter connected to a constant flow vacuum pump. The dry-gas meter measured the corresponding volume of chamber air sampled and the weight to volume ratio was determined to obtain the aerosol mass concentration. Filter-collected samples were weighed, and one randomly-selected filter was analysed chemically by ICP-MS for determination of vanadium content and to confirm the gravimetric weight measurement.

  Aerosol concentration was monitored with a real-time aerosol sensor (model #pDR-1000AN, MIE, Inc. Bedford, MA). The sensors were employed as a real-time indicator of short term changes in aerosol concentration.

  - Samples taken from breathing zone: yes

- Particle Size

  - MMAD (Mass median aerodynamic diameter) / GSD (Geometric st. dev.):

  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)

- Analytical verification of doses or concentrations: yes

- Duration of treatment / exposure: 4 hours

- Frequency of treatment: once

- Post exposure period: 14 days

- Concentrations:

  0.056, 0.50 and 2.00 mg/L (analytical conc.)

0.05, 0.5 and 2.0 mg/L (nominal conc.)

- No. of animals per sex per dose:

  2.00 mg/L: 5 males / 5 females
  0.50 mg/L: 3 males / 3 females
  0.056 mg/L: 3 males / 3 females

  no control animals

- Study design:

  A group of five male and five female rats was exposed to an aerosol of granular vanadium pentoxide in a nose-only inhalation exposure chamber for 4 hours at a concentration of 2.0 mg/L. Due to the mortality observed at the 2.0 mg/L exposure concentration an additional group of three male and three female rats was exposed to an aerosol of granular vanadium pentoxide in a nose-only inhalation exposure chamber for 4 hours at a concentration of 0.5 mg/L. Due to the mortality observed at the 0.5 mg/L exposure concentration a third group of three male and three female rats was exposed to an aerosol of granular vanadium pentoxide in a nose-only inhalation exposure chamber for 4 hours at a concentration of 0.05 mg/L. The animals were then observed for a 14-day post-exposure period.

- Examinations:

  Mortality and clinical observation: the animals were observed for signs of toxicity during the exposure, immediately after the exposure and at least once daily during the 14-day post-exposure observation period. Cage side observations included, but were not limited to, changes in skin, fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects such as salivation, effects on the central nervous system, and somatomotor activity and behaviour pattern. Particular attention was devoted to observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep, and coma.
  Body weights and body weight gains: body weights were determined one day after animal receipt, prior to randomization, on Day 1 prior to exposure, and one week after exposure. All animals were weighed on the day of their scheduled necropsy, prior to euthanasia. Body weights of animals found dead were also collected prior to necropsy. Body weight changes were calculated for rats surviving more than 24 hours.
  Necropsy: at the end of the observation period, all surviving animals were euthanized by an overdose of sodium pentobarbital (intraperitoneal injection) and subjected to necropsy. The necropsy included examination of all body surfaces and openings, and examining the external appearance of the brain, heart, liver, kidneys, lungs (especially any changes in the immediately associated and exposed respiratory tract), gastrointestinal tract, spleen, gonads and the urinary

bladder. The gastrointestinal tract and the urinary bladder were opened and examined if lesions were present. Respiratory tracts were saved and fixed in 10% neutral buffered formalin, abnormal tissue were also retained for possible pathologic evaluation, when considered needed. All decedents were also subject to the necropsy procedures described above.

Results:

LC50 (4 hours, male and female rats) = 0.25 mg/L

Mortality and clinical observations: all ten rats in the 2.0 mg/L exposure group died within four days of exposure (one male on study Day 2, two males and five females on study Day 3, and two males on study Day 4). Following the 0.5 mg/L exposure, five of the six rats died during the study (one female on study Day 3, one female on study Day 4, two males on study Day 6, and one male on study Day 11). One 0.5 mg/L group female survived to scheduled termination on Day 15. No animals died after exposure to the test substance at a concentration of 0.05 mg/L. Skin/fur discolouration, redness around eyes/nose fur, wet inguinal fur and hypoactivity were seen in most animals following exposure. The clinical signs exhibited were either an indication of toxicity (rough coat, scant faeces, diarrhoea, hypoactivity, hunched posture), were considered to have resulted from being in the nose-only exposure tubes (wet inguinal fur), or were due to exposure to the test article (skin/fur discolouration and yellow material around the nose). -Body weights and body weight gains: all surviving rats gained weight during the study although transient weight loss was observed in one female at 0.5 mg/L and two females at 0.05 mg/L. Necropsy: at necropsy, mottled lungs were observed in all animals in the 2.0 mg/L exposure group, with the exception of one female that had spongy lungs. In the 0.5 mg/L exposure group, mottled lungs were observed in all animals, with the exception of one female that had no gross lesions. Foci were also observed on the lungs of one female. No gross lesions were observed at necropsy in any animal in the 0.05 mg/L exposure group.

1.3.1.3 [Anonymous, 2011]

Study report, 2011 [confidential]

Note: this study is included in the registrants' IUCLID file in section 7.9.3 Specific investigations: mechanistic studies

Detailed study summary and results:

Test type

According to: OECD 436 "Acute Inhalation Toxicity - Acute Toxic Class Method" (adopted 2009-09-07)

The limit test for this study (2 mg/L) was conducted in compliance with the requirements of the following test guidelines with the exception that body weights were not obtained 1 and 3 days after exposure: 1) US EPA Health Effects Testing Guideline, OPTTS 870.1300, entitled Acute Inhalation Toxicity, 1998 and 2) OECD test guideline 403 (1981). In order to reduce animal use and to provide an acute inhalation toxicity estimate and information for the Globally Harmonized System (GHS) of classification and labelling for the test substance, all subsequent exposures were conducted in compliance with OECD test guideline 436 (2009) (with the exception that body weights were not obtained 1 and 3 days after exposure). The initial subsequent exposure was determined by the study director in conjunction with the sponsor and any subsequent exposures were determined according to the scheme in Annex 3b of the OECD 436 test guideline.

- GLP compliance: yes
- Study period: 2010-03-10 to 2010-11-17

Test substance

- Name of test material (as stated in study report): Granular vanadium pentoxide
- Physical state: Yellow-orange granular powder
- Storage condition of test material: Dry, in closed container at room temperature
- Purity: [confidential information]
- Batch No: [confidential information]

Test animals

- Mice B6C3F1, male and female
- 5 (high concentration) or 3 (lower concentrations) animals per sex per dose
- Source: Charles River Laboratories (Raleigh, NC)
- Age at receipt: males: 51 and 56 days; females: 56 and 63 days
- Weight at receipt: 15 to 28 g
- Housing: animals were double-housed and then after randomization, single-housed, in stainless steel cages suspended over absorbent paper cage boards
- Diet (ad libitum, except during inhalation exposure): Certified Rodent Chow 5002 meal (PMI Nutrition International, Inc., Brentwood, MO)
- Water (ad libitum, except during inhalation exposure): City of Chicago water
- Quarantine period: 7 days:

  During quarantine the mice were observed daily for signs of disease and general unthriftiness. To condition the animals to placement and restraint in the nose-only holding tubes (CH Technologies, U.S.A., Westwood, NJ) and to reduce stress during the exposure phase, the animals

were placed in the holding tubes over three days according to the following schedule: one hour on Day 1, two hours on Day 2 and three hours on Day 3 prior to their exposure

- ENVIRONMENTAL CONDITIONS:
  - Temperature: 19.0 to 25.3°C
  - Relative humidity: 30 to 68%
  - Air changes: minimum of 10 air changes per hour
  - Photoperiod (hrs dark / hrs light): 12/12

Administration/exposure

- An aliquot of the test substance was milled. The milled test substance was aerosolised as a powder for the inhalation exposures.
- GENERATION OF TEST ATMOSPHERE / CHAMBER DESCRIPTION
  - Exposure apparatus/Method of holding animals in test chamber: a 52-port, flow-past nose-only inhalation exposure chamber (Lab Products Inc., Seaford, DE) was used. The animals were restrained in nose-only exposure animal holding tubes (CH Technologies, U.S.A., Westwood, NJ). Each tube was placed in a pre-designated port of the inhalation exposure chamber.
  - System of generating particulates/aerosols: the test atmosphere was created by generating an aerosol (with filtered air) of the test substance using a compressed air-operated Wright Dust Aerosol Generation System (BGI Incorporated, Waltham, MA) positioned over the chamber. The test substance was weighed and packed into a dust reservoir forming a cake. A constant speed rotating scraper separated a thin film of the test substance at the surface of the cake and delivered it into a dispersing unit, drawn in by aspiration and dispersed by a high velocity air jet. The resulting test atmosphere entered a mixing plenum where it, when necessary, was diluted with breathable quality compressed air to achieve target concentration prior to introduction to the exposure chamber. The exhaust from the exposure chamber was moved through a high efficiency particulate air (HEPA) filter by a ring compressor and exhausted outside the building. Inlet and exhaust flows to and from the chamber were controlled and continuously monitored by rotometers.

    T90 time was assessed to be less than 30 seconds.
  - Temperature, humidity, oxygen percentage in air chamber: oxygen percentage was measured once during the exposure with an Altair Oxygen Sensor and Detector (MSA Instrument Division, Pittsburgh, PA).

    Inhalation exposure chamber temperature and relative humidity were monitored with a hand-held thermohygrometer (model # 8721, Control Company, Fisher Scientific, Friendswood, TX) and recorded at approximately half-hour intervals during exposure.

    Temperature (mean ± SD):

2.00 mg/L: 21.3°C ± 0.49
0.50 mg/L: 21.2°C ± 0.13
0.056 mg/L: 21.3°C ± 0.21

Relative humidity (mean ± SD):
2.00 mg/L: 14.3% ± 2.57%
0.50 mg/L: 43.2% ± 1.60%
0.056 mg/L: 29.3% ± 1.88%
Oxygen (%):
2.00 mg/L: no data to report; the oxygen sensor was inoperable during the exposure
0.50 mg/L: 20.8%
0.056 mg/L: 20.8%
- Method of particle size determination: aerosol particle size was monitored at least once per two hours during the exposure with a quartz crystal microbalance (QCM) cascade impactor (California Measurements Inc., Sierra Madre, CA). The mass median aerosol diameter (MMAD) and geometric standard deviation (GSD) were calculated from the mass accumulated on each stage of the QCM.

- TEST ATMOSPHERE:

- Brief description of analytical method used: the concentration of the test substance in the test atmosphere was monitored gravimetrically by filter-collected samples. One sample was taken from the breathing zone of the animals every hour of exposure. The gravimetric sampling train consisted of a pre-weighed filter in a series with a dry-gas meter connected to a constant flow vacuum pump. The dry-gas meter measured the corresponding volume of chamber air sampled and the weight to volume ratio was determined to obtain the aerosol mass concentration. Filter-collected samples were weighed, and one randomly-selected filter was analysed chemically by ICP-MS for determination of vanadium content and to confirm the gravimetric weight measurement. Aerosol concentration was monitored with a real-time aerosol sensor (model #pDR-1000AN, MIE, Inc. Bedford, MA). The sensors were employed as a real-time indicator of short term changes in aerosol concentration.
- Samples taken from breathing zone: yes

- Particle Size:

- MMAD (Mass median aerodynamic diameter) / GSD (Geometric st. dev.):
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)

- Analytical verification of doses or concentrations: yes

- Duration of treatment / exposure: 4 hours
- Frequency of treatment: once
- Post exposure period: 14 days
- Concentrations:

  0.056, 0.50 and 2.00 mg/L (analytical conc.)

  0.05, 0.5 and 2.0 mg/L (nominal conc.)
- No. of animals per sex per dose:

  2.00 mg/L: 5 males / 5 females
  0.50 mg/L: 3 males / 3 females
  0.056 mg/L: 3 males / 3 females

  no control animals
- Study design:

  A group of five male and five female mice was exposed to an aerosol of granular vanadium pentoxide in a nose-only inhalation exposure chamber for 4 hours at a concentration of 2.0 mg/L. Due to the mortality observed at the 2.0 mg/L exposure concentration an additional group of three male and three female mice was exposed to an aerosol of granular vanadium pentoxide in a nose-only inhalation exposure chamber for 4 hours at a concentration of 0.5 mg/L. Due to the mortality observed at the 0.5 mg/L exposure concentration a third group of three male and three female mice was exposed to an aerosol of granular vanadium pentoxide in a nose-only inhalation exposure chamber for 4 hours at a concentration of 0.05 mg/L. Surviving animals were then observed for a 14-day post-exposure period.

Examinations

Mortality and clinical observation: the animals were observed for signs of toxicity during the exposure, immediately after the exposure and at least once daily during the 14-day post-exposure observation period. Cage side observations included, but were not limited to, changes in skin, fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects such as salivation, effects on the central nervous system, and somatomotor activity and behaviour pattern. Particular attention was devoted to observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep, and coma.
Body weights and body weight gains: body weights were determined one day after animal receipt, prior to randomization, on Day 1 prior to exposure, and one week after exposure. All surviving animals were weighed on the day of their scheduled necropsy, prior to euthanasia. Body weights of animals found dead were also collected prior to necropsy. Body weight changes were calculated for mice surviving more than 24 hours.

Necropsy: at the end of the observation period, all surviving animals were euthanized by an overdose of sodium pentobarbital (intraperitoneal injection) and subjected to necropsy. The necropsy included examination of all body surfaces and openings, and examining the external appearance of the brain, heart, liver, kidneys, lungs (especially any changes in the immediately associated and exposed respiratory tract), gastrointestinal tract, spleen, gonads and the urinary bladder. The gastrointestinal tract and the urinary bladder were opened and examined if lesions were present. Respiratory tracts were saved and fixed in 10% neutral buffered formalin, abnormal tissue were also retained for possible pathologic evaluation, when considered needed. All decedents were also subject to the necropsy procedures described above.

Results :

LC50 (4 hours, male mice) > 0.5 mg/L

LC50 (4 hours, female mice) < 0.05 mg/l

Mortality and clinical observations: skin/fur discolouration, wet inguinal fur and hypoactivity were seen in most animals during the exposure period.

All ten mice in the 2.0 mg/L exposure group died within four days of being exposed to the test substance (two males and five females on study Day 3, and three males on study Day 4). Following the 0.5 mg/L exposure, one male and all three females died within five days of being exposed to the test substance (one male on study Day 3, two females on study Day 4 and one female on study Day 5). Two females died within five days (study Days 4 and 5) of being exposed to the test substance at a concentration of 0.05 mg/L.

Following the 2.0 mg/L exposure, mice displayed the following clinical signs: skin/fur discolouration, wet inguinal fur and/or hypoactivity. Following the 0.5 mg/L exposure, mice were observed to have skin/fur discolouration, wet inguinal fur and/or hypoactivity. Following the 0.05 mg/l exposure, mice displayed the following clinical signs: wet inguinal fur, yellow material around the nose and/or hypoactivity (females only). The clinical signs exhibited were either an indication of toxicity (hypoactivity), were considered to have resulted from being in the nose-only exposure tubes (wet inguinal fur), or were due to exposure to the tes substance (skin/fur discolouration and yellow material around the nose).

Body weights and body weight gains: all surviving mice gained weight during the study. Necropsy: at necropsy, mottled and/or bright or dark red lungs were observed in all animals in the 2.0 mg/L exposure group, with the exception of one female. In addition, two females had yellow material in the stomach. In the 0.5 mg/L exposure group, no gross lesions were observed

in males and bright red lungs were observed in one female. No gross lesions were observed at necropsy in any animal in the 0.05 mg/L exposure group

1.4 Germ cell mutagenicity

1.4.1 Animal data

1.4.1.1 [Altamirano-Lozano et al., 1996]

Study reference:

Altamirano-Lozano et al., Reprotoxic and genotoxic studies of vanadium pentoxide in male mice, Teratogenesis, Carcinogenesis, and Mutagenesis, 16, 7-17, 1996 (Altamirano-Lozano et al., 1996)

Detailed study summary and results:

Test type

Study of reproductive function and testicular DNA damage in male mice (Dominant lethal test). No information on GLP compliance is given.

In the publication a separate experiment (Comet assay) is listed which is not described in this annex.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: divanadium pentaoxide
- Degree of purity: 99.6%
- Impurities: no information given
- Batch number: no information given

Test animals

- CD-1 mice
- 15-30 male per group
- All animals mated with unexposed females (1:2) after exposure
- 45 days old and 26-29 g (males) at study initiation. Information not available for females

Administration/exposure

- 0 or 8.5 µg/g bw in saline (1/2 of LD_{50} determined in the laboratory for subchronic treatments)
- Vehicle: saline (no further information available)

- Male animals were intraperitoneally injected every 3rd day for 60 days.On day 61, animals were subjected to a fertility assessment test (mating with unexposed females) and sacrificed 5 days later.
- In a separate study design 30 animals received V_2O_5 every 3rd day for 60 days and groups of 5 animals were sacrificed every 10 days after the beginning of the treatment.
- Control animals: 20 control animals received vehicle (saline) every 3rd day for 60 days and were mated with unexposed females
- No positive controls
- Statistical methods: results on fertility, sperm motility, abnormal sperm were analysed with the z-test. Frequency of implants, resorptions, live and dead foetuses, body weight, testis weight, sperm count analysed with Student´s t-test

Results and discussion

In the following Table AI - 10 the effects of divanadium pentaoxide exposure on the reproductive function on male mice are shown. It can be seen, that V_2O_5 treatment resulted in decrease in fertility rate. Implantation sites, live foetuses, and foetal weight were significantly decreased. The number of resorptions/dam and dead foetuses was increased.

Table AI - 10: Effects of divanadium pentaoxide exposure on the reproductive function on male mice

	Control	**V_2O_5 (8.5 µg/g bw)**
Males (n)	20	15
Mated females (n)	40	30
Pregnant (n)	34	10
Fertility (%)	85	33
Implantation sites	10.88 ± 1.60	5.80 ± 1.33**
Resorptions	0.24 ± 0.42	2.00 ± 1.67 *
Live foetuses	10.53 ± 1.42	3.40 ± 0.49**
Dead foetuses	0.12 ± 0.32	0.40 ± 0.49
Foetal weight (mg)	145 ± 4.0	121 ± 7.0*

* $p < 0.05$, **$p < 0.001$, results taken from (Altamirano-Lozano et al., 1996)

The following Table AI - 11 shows the effects of divanadium pentaoxide on several parameters of male fertility. Results for 20 days, 30 days, 40 and 50 days are not presented, but available in the original literature.

Sperm count, motility, and morphology are impaired after V_2O_5 treatment. The effects were getting more severe with longer exposure times. After 10 days of exposure, sperm motility was already significantly decreased. After 60 days of exposure significant effects were observed on testis weight, sperm count, motility and abnormal sperm.

Table AI - 11: Effects of divanadium pentaoxide on several parameters of male fertility.

		V_2O_5 (8.5 µg/g bw)	
	Control	**10 days**	**60 days**
Males (n)	20	5	20
Initial weight (g)	28.04 ± 0.61	26.74 ± 0.71	27.44 ± 0.49
Final weight (g)	31.20 ± 1.09	27.14 ± 0.71	24.64 ± 1.34*
Testis weight (mg)	135.90 ± 16.80	131.80 ± 24.30	118.72 ± 22.33**
Sperm count ($x10^6$/mL)	29.19 ± 2.43	23.50 ± 7.53	7.27 ± 2.31**
Motility (%)	73.10 ± 19.40	40.60 ± 10.10**	4.01 ± 2.91**
Abnormal sperm (%)	6.40 ± 1.80	5.50 ± 3.90	10.83 ± 3.70**

* $p < 0.05$, **$p < 0.001$, results taken from (Altamirano-Lozano et al., 1996)

1.4.1.2 [Schuler et al., 2011]

Study reference:

Schuler, D et al., First steps towards an understanding of a mode of carcinogenic action for vanadium pentoxide, Journal of Toxicologic Pathology, 24, 149-162, 2011 (Schuler et al., 2011)

Detailed study summary and results:

Test type

Study in mice: DNA damage and/or repair after V_2O_5 exposure. This study was conducted according to the procedures indicated by the following recommendations: Tice, R.R.; et al. (2003): Single Cell Gel/Comet Assay: Guidelines for in vitro and in vivo genetic toxicology testing. Environmental and Molecular Mutagenesis and Hartmann, A. et al. (2003) Recommendations for conducting the in vivo alkaline Comet assay. Mutagenesis 18(1), 45-51. GLP compliance is given.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: divanadium pentaoxide
- Degree of purity: 99.8%
- Impurities: 0.13% vanadium tetraoxide
- Batch number: H060391 from Stractor Technology Center, USA

Test animals

- Female B6C3F1 mice
- 4 groups of 48 mice (group 1 (control group) and groups 2-4; 1 group of 6 mice (positive control: group 5)
- Age at study initiation: 8 - 9 weeks, weight: 16.9 – 23.2 g

Administration/exposure

- 0, 0.25, 1.0, 4.0 mg/m³, the target aerosol concentrations were proposed by the Sponsor and are based on the results obtained within the previous National Toxicology Program (Toxicology and Carcinogenesis Studies of Vanadium Pentoxide (CAS No. 1314-62-1) in F344/N Rats and B6C3F1 Mice (inhalation studies)).
- Vehicle: air
- Inhalation exposure was performed using a system similar to that originally described by Sachsse et al. The mice are confined separately in restraint tubes which are positioned radially around the flow-past, nose-only exposure chamber described by Cannon et al. The design of this chamber is based upon the fluid dynamic modeling of the test aerosol flow. The exposure system ensured a uniform distribution and provided a constant flow of test material to each exposure tube.
- Brief description of analytical method used: The aerosol concentrations of the test item determined gravimetrically and chemically, relative humidity, temperature and oxygen concentration were measured on test aerosol samples collected directly from the delivery tube in the breathing zone of the mice, at an empty port of the exposure chamber. Airflow rates were measured for the collection of samples for the determination of test item concentration and particle size using a dry-test meter (‘Schlumberger Industries SA’, City of Geneva) and/or a pressure gauge (Timeus & Co., Zürich), calibrated with a reference dry-test meter.Duration of study: Animals were exposed daily 6 h/d for 7 or 16 days.
- Observations and measurements made following nose-only exposure to a vanadium pentoxide aerosol 6h/day for 16 days included concentrations of V in lungs and blood, lung weight, histopathology of the airways, cell proliferation in lungs, concentrations in lungs of α-tocopherol, glutathione (reduced and total), 8-isoprostane F2α, as a representative of the isoprostanes, and 9 specific DNA-oxy-adducts and DNA damage (comet assays) in lung and bronchiolar-alveolar lavage (BAL) fluid cells.
- At the end of the exposure time animals were sacrificed and lung samples were taken. In detail, the following procedure was done: As soon as feasible after the last exposure (groups 1-4) and 4 hours after the treatment of the positive controls (group 5), the following procedures were performed:
    - Perfusion of lung tissue: After anaesthetizing the mice with 46% Ketamin, 23% Xylazin and 31% Midazolan (approx. 2 ml/kg body weight) the lung were perfused through the right ventricle with saline. Afterwards the lungs were intubated via the trachea and lavaged with approx. 20 ml mincing buffer (20 mM EDTA, 10% DMSO in HBSS pH 7.4-7.6) for collection of BAL cells (Bronchio Alveolar Lavage cells). The lungs were then further perfused under resuscitation. Excised lung lobes were then minced in 1 ml ice-cold mincing buffer using fine scissors to obtain a single cell suspension containing minced lung tissue cells.

- Preparation of BAL cells: The cells isolated from lung lavage were centrifuged and resuspended in 0.7% agarose and afterwards brought onto slides.
- Preparation of minced lung tissue: The minced cells were filtered through a 40 µm cell strainer. The cell suspension was centrifuged and resuspended in 0.7% agarose and brought onto slides.

For the study of DNA oxo-lesion:

DNA was extracted from thawed lung samples followed by a digestion step with nuclease, DNAse II and phosphodiesterase II. After digestion, samples were analysed by HPLC-MS/MS. Detection of the different DNA lesions was performed in multiple reactions monitoring (mrm) mode.

- Control animals: yes, treated the same as exposed animals, but without V_2O_5
- Positive control: methylmethanesulfonate, 99% (MMS, Sigma-Aldrich Chemie GmbH)
  - Justification for choice of positive control: The oral administration of MMS resulted in significantly increased percentage tail DNA intensities of the analysed lung cells in the lungs as demonstrated in Harlan CCR validation study 1048302 (group 5)
  - Route of administration: oral (gavage)
  - Doses / concentrations: 200 mg/kg b.w. (single dose)
- Methods of slide preparation: 3 slides per minced lung tissue cells and one slide per BAL cells per animal were prepared with 10% cell suspension and 90% of a 0.7% (w/v) agarose (low melting point agarose) solution. 100 µl were applied per slide. The slides were cooled before being submerged in lysis buffer. The following steps of protocol were performed with the slides:
  - Lysis: 1 hour up to 7 days in Lysis buffer pH 10 at 2-8°C in the dark.
  - Alkaline treatment: 20 minutes in electrophoresis buffer, pH >13 at 2-8°C in the dark.
  - Electrophoresis: 30 minutes in electrophoresis buffer 25 V, 300 mA, at 2-8°C in the dark.
  - Neutralisation: about 11 minutes in neutralisation buffer.
  - Dehydration: approximately 2 minutes in 99% ethanol.

  After dehydration the slides were air-dried and stored protected from dust and light until evaluation.
- Criteria for scoring and number of cells analysed per animal: The DNA of the cells was stained with the fluorescence dye ethidium bromide (20 µg/mL; 40 µl per slide), immediately before evaluation. Where possible all mice per test group, 100 cells per preparation and per animal (BAL: 100 cells from one slide, minced lung tissue cells: 50 cells per slide) were evaluated on coded slides with a fluorescence microscope. The damage of each nucleus was measured and recorded by an image analysis programme (Comet Assay IV, Perceptive Instruments).

  An increasing extent of DNA migration detected with the Comet Assay results in an increase of the mean of tail % intensity of one test group compared to the vehicle control. Tail % intensity is expressed as a percentage of the Comet's total intensity. Additionally, the number of nuclei from apoptotic or necrotic cells per 500 total nuclei was determined.

  The following criteria are used for analysis of slides:

- Only clearly defined non-overlapping cells are scored.
- Nuclei from dead/apoptotic cells are not scored (% tail intensity above 80%).
- Cells with unusual staining artefacts are not scored.
- All other normal cells, 100/animal, are scored where possible.

- Statistical methods: The experimental unit of exposure for in vivo studies is the animal, and all analysis was based on individual animal response. Values obtained from each parameter were treated as follows:
  - The median is calculated for each slide.
  - For each animal the mean of the medians are calculated, if possible.
  - The mean of the medians and standard error of the means is calculated for each group. Statistical methods were used as an aid in evaluating the results. Normally distributed data were analysed using a one-tailed Student's t-test. An F-test was first used to test for homogeneity of variances for pairwise analysis. However, the primary point of consideration was the biological relevance of the results.

Results and discussion

In the following Table AI - 12 the results from the Comet assay (mean percentage tail intensities in BAL and lung cells) are shown. In addition results from the positive control substance (methylmethanesulfonate MMS) are shown. It can be seen, that the Comet assay showed negative results for the two cell types tested as well as for all exposure concentrations.

Table AI - 12: Results from the Comet assay (mean percentage tail intensities in BAL and lung cells)

Cell type scored	**V_2O_5 concentration in air[mg/m³]**				**MMS**
	0	**0.25**	**1**	**4**	**200 mg/kg**
BAL cells	0.97 ± 0.94	0.31 ± 0.32	2.34 ± 2.23	0.28 ± 0.12	65.10 ± 9.95
Lung cells	0.53 ± 0.43	0.62 ± 1.00	0.21 ± 0.14	0.43 ± 0.40	48.34 ± 11.88

Results taken from (Schuler et al., 2011)

The analysis of cells obtained from bronchioalveolar lavage (BAL cells) or mincing of the lung tissue showed that neither the treatment of the animals with the vehicle nor the treatment of the animals with the indicated doses of V_2O_5 induced DNA damage. The obtained values for the treated animals were near to those treated with air control. The mean % tail intensities of the animals treated with 0.25, 1.0 and 4.0 mg/m³ air were 0.307, 2.343 and 0.288 for BAL cells and 0.615, 0.210 and 0.428 for minced lung tissue cells, respectively. The variations in the values between the dose groups were not significant. All obtained values were within the historical control group.

The number of nuclei from apoptotic or necrotic cells per 500 total nuclei was determined for each sample to indicate the quality of the slide preparation. Neither the slides prepared from animals treated with the negative control nor from those treated with any dose level of V_2O_5 showed a remarkable increase in number

of dead cells indicating a good slide preparation. However, the amount of dead cells could not be determined for the positive control groups. There, the cells showed a structure very close to dead cells complicating the macroscopic differentiation. Nevertheless, the validity of the study was not jeopardised, because the comet results where dead cells (% tail intensity above 80%) could be excluded showed a clear and statistically significant increase in DNA damage in cells treated with the positive control.

Study of DNA oxo-lesions:

The quantities of some DNA lesions were below the limit of detection (8-oxodAdo, 5-OH-dCyd, ThdGly, 5-hmdUrd, 5-FodUrd, EdGuo and EdAdo, data not presented here). According to the authors it cannot be concluded that treatment was without effect only that the natural and any induced occurrence were below the limit of detection. Levels of 8-oxo Guo and dCyd 341 were high enough for quantification. As it can be seen in the following Table AI - 13, a statistically significant concentration–related increase in 8-oxo Guo was observed at 1 and 4 mg/m³.

Table AI - 13: Number of lesions/10^6 nucleosides

Nucleoside oxidation product	**V_2O_5 concentration in air[mg/m³]**			
	0	**0.25**	**1**	**4**
8-oxo Guo mean ± SD	0.24 ± 0.04	0.26 ± 0.07	0.43± 0.14[a]	0.49± 0.11[b]
dCyd 341 mean ± SD	0.067 ± 0.007	0.061 ± 0.028	0.075 ± 0.022	0.068 ± 0.010

[a] $p < 0.01$, [b] $p < 0.001$, results taken from (Schuler et al., 2011)

Pulmonary effects:

Increased lung weight, inflammatory lesions and increased cell proliferation rate were noted at the two higher exposure levels (1 and 4 mg/ m³), with increasing dose-dependent severity.

Lung weight:

Dose-related increases in lung weight which occurred in the 1 and 4 mg/m³ groups were not assumed by Schuler et al., as the simple result of vanadium pentoxide deposition: the quantities deposited over 16 days were considered insufficient to account for the observed lung weight increases. A much more likely explanation is that the weight increases were due to the recruitment of inflammatory cells and an accumulation of fluid within the pulmonary tissues.

Cell proliferation:

The mean cell proliferation rate grades, as indicated by immunohistochemical staining for Ki-67 protein after 7 and 16 days, were time-dependently and dose-relatedly increased in the 1 and 4 mg/ m³ groups (see Table AI – 14). There were also dose-related increases in immunohistochemical staining for proliferating cell nuclear antigen (PCNA) in the 1 and 4 mg/ m³ groups after 7 days, but only in the 4 mg/ m³ group after 16 days, the score for the 1 mg/ m³ group being similar to the controls. With neither method was there evidence for cell proliferation following exposure to 0.25 mg/ m³.

Table AI - 14: Cell proliferation in lungs of mice exposed to Vanadium pentoxide for 7 or 16 days

Cell Proliferation in Lungs of Mice Exposed to Vanadium Pentoxide for 7 or 16 Days				
Indicator and sample time	**V_2O_5 concentration in air[mg/m³]**			
	0	**0.25**	**1**	**4**
Ki-67-positive cells after 7 days	10.1 (1)	11.4 (1)	18.1 (2)	24.7 (2)
Ki-67-positive cells after 16 days	10.0 (1)	9.2 (1)	27.1 (2)	87.8 (5)
PCNA-positive cells after 7 days	25.1 (2)	25.0 (2)	41.4 (3)	47.8 (4)
PCNA-positive cells after 16 days	33.0 (3)	26.3 (2)	34.9 (3)	68.3 (5)

Figures in parentheses indicate the severity grades (quoted from Schuler et al., 2011)

Histopathological observations in the mice killed after 16 days exposure:

Multifocal/diffuse alveolar histiocytosis that was to some degree dose-dependent was found in all 6 mice of the 1 and 4 mg/ m³ groups.

Multifocal subacute alveolitis that was clearly dose-dependent in 5 mice each of the 1 and 4 mg/ m^3 groups; and multifocal granulocytic infiltration at similar severity in 4 mice at 1 mg/ m^3 and 5 mice at 4 mg/ m^3. Deposition of vanadium pentoxide particles in the lungs was considered to result in histopathological changes in the 1 and 4 mg/ m^3 groups that may have been the result of incomplete particle clearance from the lung as well as a direct toxic action on the pulmonary epithelium induced by the oxide.

1.4.1.3 [Anonymous, 2011]

Study reference:

Study report, 2011 [confidential]

Detailed study summary and results:

Test type

OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test) with minor deviations from the guideline without effecting the general outcome (no further information available). GLP compliance is given (including certificate).

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: divanadium pentaoxide
- Degree of purity: [confidential]
- Impurities: no information available
- Batch number: [confidential]

Test animals

- Out-bred young adult male Sprague Dawley rats
- 6 animals/dose group, Satellite animals per group: 3
- Age at study initiation: 7-8 weeks
- Weight at study initiation: 219.3 - 273.7 g

Administration/exposure

- Dose levels-range-finder: 90, 120, 170, 240, 300 mg/kg bw
  Dose levels - MN study: 0, 30, 60, 120 mg/kg bw/day
  Maximum dose: Maximum tolerated dose based on Range-finder data.
- Vehicle: corn (no further information available)
- Dose volume: 10 mL/kg, applied via gavage
- Bone marrow sampled: 24 or 48 hour post final administration

- Tissue sampled (satellite animals): 24 or 48 hour post final administration
- Control groups and treatment
  - Cyclophosphamide (clastogenic positive control) 20 mg/kg bw, single oral administration
  - Vinblastine (aneugenic positive control) 0.5 mg/kg bw/day, two administrations via intraperitoneal injection
- Methods of slide preparation
  - Bone marrow smears for micronucleus assessment:

    - marrow from two femurs from each animal was flushed from the cavity with foetal bovine serum

    - samples were filtered and centrifuged

    - cell pellet was resuspended and smears were made

    - two slides per animal were assigned to MN assessment and two to antikinetochore assessment
  - Tissue samples for bioanalysis:

    - Bone marrow samples (femur and humerus) were scraped from the bone and samples pooled per animal.

    - Both testes were sampled.

    - Tissues were flash frozen in liquid nitrogen and held on dry ice prior to transfer to ≤ -50°C for long term storage.

- Criteria for scoring and number of cells analysed per animal
  - Slide analysis:

    - Slides from all groups were independently coded and analysed blindly in a random design.

    - Initially the relative proportions of PCE, seen as bright orange enucleate cells, and normochromatic erythrocytes (NCE), seen as smaller dark green enucleate cells, were determined until a total of at least 1000 cells (PCE plus NCE) had been analysed. Then at least 2000 PCE per animal were examined for the presence of micronuclei (MN).
  - Calculation:

    1. % PCE for each animal and the mean for each group. The group mean % PCE values were examined to see if there was any decrease in groups of treated animals that could be taken as evidence of bone marrow toxicity.

    2. Frequency of MN PCE (i.e. MN per 2000 PCE) and % MN PCE for each animal and the group mean % MN PCE (± standard deviation). The MN PCE data from the vehicle control group(s) were compared with the laboratory's historical vehicle control ranges (individual animal distribution data and calculated group mean 95% confidence interval) to determine whether the assay was acceptable.

- Acceptance criteria

  The assay was considered valid if all the following criteria were met:
  - The vehicle control MN PCE data were comparable with the laboratory's historical vehicle control ranges
  - At least five animals out of each group were available for analysis, and
  - The positive control chemical (CPA and VIN) induced a statistically significant increase in the frequency of MN PCE.

- Evaluation criteria

  For valid data, the test article was considered to induce clastogenic / aneugenic damage if:
  - A statistically significant increase in the frequency of MN PCE occurred at one or more dose levels
  - The incidence and distribution of MN PCE in individual animals at such a point exceeded the laboratory's historical vehicle control data
  - The group mean MN PCE value at such a point exceeds the 95% calculated confidence interval for the mean historical vehicle control data
  - A dose-response trend in the proportion of MN PCE was observed (where more than two dose levels were analysed).

  The test article was considered positive in this assay if all of the above criteria were met. The test article was considered negative in this assay if none of the above criteria were met.

  Results which only partially satisfied the above criteria were dealt with on a case-by-case basis. Evidence of a dose-related effect was considered useful but not essential in the evaluation of a positive result (Scott et al, 1990). Biological relevance was taken into account, for example consistency of response within and between dose levels.

- Statistics

  For each group, inter-individual variation in the numbers of MN PCE was estimated by means of a heterogeneity chi-square test (Lovell et al, 1989). The numbers of MN PCE in each treated group were compared with the numbers in vehicle control groups by using a 2 x 2 contingency table to determine chi-square (Lovell et al, 1989). Probability values of $p \leq 0.05$ were accepted as significant. A further statistical test (for linear trend) was used to evaluate possible dose-response relationships.

Results and discussion

- Genotoxic effects: negative
- Vehicle control: valid, negative controls: valid, positive controls: valid

- The genetic toxicity of divanadium pentaoxide was assessed by testing the ability of V_2O_5 to induce an increase in the frequency of micronucleated erythrocytes in bone marrow of male rats. Vanadium tissue levels increased in all sampled tissues with increased V_2O_5 dosage levels. Thus, vanadium reached the target tissues bone marrow and testes in a dose-dependent manner. Divanadium pentaoxide, administered orally to male rats by gavage up to the maximum tolerated dose of 120 mg/kg bw/day, did not induce micronuclei in polychromatic erythrocytes of bone marrow. No further information or data available.

1.4.1.4 [NTP, 2002]

Study reference:

NTP, Technical report on the toxicology and carcinogenesis - studies of vanadium pentoxide (CAS NO. 1314-62-1) in F344/N rat and B6C3F1 mice (inhalation studies), TR 507, 2002 (NTP, 2002).

Detailed study summary and results:

Test type

Equivalent to OECD Guideline 475 (Mammalian Bone Marrow Chromosome Aberration Test), GLP compliance is given.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: divanadium pentaoxide
- Degree of purity: approximately 99%
- Impurities: no information available
- Batch number: 1210490

Test animals

- B6C3F1 male and female mice
- 10 male and 10 female mice per dose group
- Age at study initiation: 6-7 weeks
- Weight at study initiation: 25-26 g (males) and 20-21 g (females)

Administration/exposure

- 0, 1, 2, 3, 8, 16 mg/m³, based on results of a dose range finding study doses were selected
- Vehicle: air
- Inhalation exposure (whole body inhalation, 6 h/d plus T90 (15 minutes), 5d/w for 3 months)
- Control animals treated without V_2O_5

- GENERATION OF TEST ATMOSPHERE / CHAMBER DESCRIPTION
    - For the 3-month studies, divanadium pentaoxide aerosol generation was based on the principle of pneumatic dispersion and consisted of two major components: a screw feeder (Model 310, Accurate, White Water, WI) that metered divanadium pentaoxide powder at a constant rate and a Jet-O-Mizer jetmill (Fluid Energy Corp., Harfield, PA) that used compressed air to disperse the metered powder and form the aerosol.
    - Aerosol leaving the jetmill passed through a one-stage impactor and a vertical elutriator to eliminate or deagglomerate the large particles before entering a plenum and manifold distribution system. The aerosol delivery system consisted of three holding chambers that diluted the aerosol in three stages. A metered amount of diluted aerosol was removed and mixed with conditioned air at the inlet to each exposure chamber to achieve the appropriate exposure concentration. The electrical charge buildup on the aerosol particles was neutralised by mixing the aerosol with high concentrations of bipolar ions, which were generated using a Pulse Gun (Static Control Services, Palm Springs, CA) air nozzle. A transvector air pump was installed at the aerosol inlet to each exposure chamber to provide additional control of the aerosol flow rate and improve stability of the chamber concentration.
    - The stainless-steel inhalation exposure chambers (Lab Products, Inc., Maywood NJ), were designed so that uniform aerosol concentrations could be maintained throughout the chambers when catch pans were in place. The total active mixing volume of each chamber was 1.7 m^3.

- CHAMBER ATMOSPHERE CHARACTERISATION
    - The particle size distribution in each chamber was determined prior to the start of all studies, during the first week and monthly thereafter.
    - A 10-stage Quartz Crystal Microbalance-based cascade impactor was used to separate the aerosol particles into sequential size ranges; the mass median aerodynamic diameter was calculated from the corresponding mass fraction of particles at each stage.

- OTHER
    - The uniformity of aerosol concentration in the inhalation exposure chambers without animals was evaluated before each of the studies began; concentration uniformity with animals present in the chambers was also measured. Minor excursions in chamber uniformity values were observed in one or more exposure chambers, but these excursions had no impact on the studies.

- The stability of divanadium pentaoxide in the exposure system was tested with XRD analysis. XRD analyses indicated no detectable build-up of degradation products at a detection limit of approximately 1%.

- DETAILS OF SLIDE PREPARATION: Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded.

- METHOD OF ANALYSIS: Slides were scanned to determine the frequency of micronuclei in 2000 normochromatic erythrocytes (NCEs) in each of nine or ten animals per exposure group.

  OTHER: In addition, the ratio of polychromatic erythrocytes (PCEs) to NCEs among 1000 total erythrocytes was determined as a measure of bone marrow toxicity.

- Evaluation criteria:
  Statistical as well as biological factors are considered. For an individual assay, the statistical procedure for data analysis has been described in the preceding protocol. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay.

- Statistics:
  The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analysed by a statistical software package that tested for increasing trend over exposure groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposed group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single exposed group is less than or equal to 0.025 divided by the number of exposed groups.

Results and discussion

- RESULTS OF RANGE-FINDING STUDY
  Dose range: Based on decreased survival in the 32 mg/m^3 males and body weight decreases in 32 mg/m^3 males and females, an exposure concentration of 32 mg/m^3 was considered too high for use in

a 3-month study. Therefore, the exposure concentrations selected for the 3-month inhalation study in rats were 0, 1, 2, 4, 8, and 16 mg/m^3.

RESULTS OF DEFINITIVE STUDY

Induction of micronuclei (for Micronucleus assay): No increase in the frequency of micronucleated NCEs was seen in peripheral blood samples from male or female mice exposed to divanadium pentaoxide for 3 months by inhalation.

Ratio of PCE/NCE (for Micronucleus assay): Chemical exposure had no effect on the ratio of PCEs to NCEs in peripheral blood, indicating no toxicity to the bone marrow by divanadium pentaoxide.

The results are listed in the following Table AI - 14.

Table AI - 14: Results of Micronucleus test

Compound	**Dose [mg/m^3]**	**Number of mice with erythrocytes scored**	**Micronucleated NCEs/1000 NCEs[a]**	**P-value[b]**
Male				
Air (chamber control)		10	1.00 ± 0.15	
Divanadium pentaoxide	1	10	1.10 ± 0.16	0.3787
	2	10	0.6 ± 0.15	0.9214
	3	10	0.95 ± 0.24	0.5636
	8	10	0.95 ± 0.16	0.5636
	16	9	1.00 ± 0.22	0.5000
			P = 0.812[c]	
Female				
Air (chamber control)		10	0.50 ± 0.11	
Divanadium pentaoxide	1	10	0.45 ± 0.16	0.5907
	2	10	0.70 ± 0.11	0.2070
	3	10	0.40 ± 0.16	0.6814
	8	10	0.35 ± 0.11	0.7666
	16	10	0.40 ± 0.12	0.6814
			P = 0.402	

[a] mean ± standard error, [b] comparison with the chamber control, significant at P ≤ 0.005, [c] Significance of micronucleated NCEs/1000 NCEs tested by the one-tailed trend test, significant at P ≤ 0.025, results taken from (NTP, 2002).

1.4.2 Human data

1.4.2.1 [Ehrlich, 2008]

Study reference:

Ehrlich V.A. et al., Inhalative Exposure to Vanadium Pentoxide Causes DNA Damage in Workers: Results of a multiple End point Study, Environmental Health Perspectives, 116, 1689-1693, 2008 (Ehrlich et al., 2008)

Detailed study summary and results:

Test type

Case control study with workers from divanadium pentaoxide factory and matching controls. 53 exposed workers were matched with 52 control persons working as jail wardens (matching for age, BMI and cigarettes/day). No information is available concerning the air concentration in the divanadium pentaoxide factory or the time, the workers already worked there. Workers are exposed to vanadium durst during the entire shift (8 h) and should wear protective masks and gloves. No information about workers compliance is given.

Blood samples from exposed workers and controls were taken and plasma was obtained.

1) Exposure assessment
   Concentrations of vanadium in plasma: exposed workers 7-times higher than in controls (0.3 µg/L, range 0.24 – 0.39 versus 2.2 µg/L, range 1.54 – 3.89)
2) Measurement of vitamin B6 and B12 status: slightly higher in exposed workers
3) Comet assay with leucocytes
   a) With standard conditions (detecting single and double strand breaks)→ negative results
   b) With formamidopyrimidine glycosylase (FPG) and endonuclease III (ENDO III) to detect endogenous formation of oxidized DNA bases→ positive results (Formation of oxidised purine bases: elevated by 7% (FPG), Formation of oxidised pyrimidine bases elevated by 33% (ENDO III))
   c) With Bleomycin (BLEO) to study BLEO-induced DNA damage and DNA repair capacity → positive results (Sensitivity of cells towards Bleomycin –induced DNA damage was higher (25%), and DNA repair was only very slightly observed)

   For details on results see the following Table AI - 15.

Table AI - 15: DNA migration in leucocytes of exposed workers and controls (results of different modifications of the Comet assay, median values (25th -75th percentiles) shown)

Test conditions	Measure	Controls (n=52)	Exposed (n=52)	Δ° (%)	p-value
Standard conditions	TL	4.3 (4.07-4.64)	4.1 (3.89-4.44)	-5	>0.05
	TM	2.4 (2.27-2.79)	2.3 (2.16-2.59)	-4	>0.05
FPG	TL	4.5 (4.21-4.80)	4.8 (4.14-5.39)	+7	0.0236
	TM	2.5 (2.34-2-62)	2.7 (2.27-3.24)	+8	0.0007
ENDO III	TL	7.3 (6.71-8.17)	9.7 (7.84-12-08)	+33	0.0019
	TM	3.5 (3.24-3.89)	3.8 (3.18-6.03)	+9	0.0023
BLEO	TL	13.3 (11.93-16.58)	16.5 (13.55-23.56)	+25	<<0.0001
	TM	8.7 (7.55-11.72)	12.2 (10.07-15.98)	+40	<<0.0001
BLEO + DNA repair	TL	6.6 (6.02-7.20)	17.8 (13.62-19.65)	+172	<<0.0001
	TM	3.7 (3.41-4.20)	8.3 (7.10-11.39)	+124	<<0.0001

TL = tail length in µm, TM = tail moment, Δ°= difference between exposed subjects and controls. Results taken from (Ehrlich et al., 2008)

4) Cytokinesis-block micronucleus cytome assay (CBMN Cyt) with isolated lymphocytes

   Detection of MN, nucleoplasmic bridges (NPBs), nuclear buds (Nbuds)and apoptotic and nectrotic cells.

   → 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%

   For details on results see the following Table AI - 16.

Table AI - 16: Frequencies of micronucleated lymphocytes, total number of MNs, NPBs, Nbuds, apoptotic and necrotic cells per 2000 binucleated cells (median values (25th -75th percentiles)

End point	Controls (n=23)	Exposed (n=24)	Δ° (%)	p-value
Total no. of MNs	2.0 (1.00-4.00)	5.0 (2.50-9.00)	+150	0.0132
Micronucleated cells	2.0 (1.00-4.00)	5.0 (2.50-9.00)	+150	0.0132
NPBs	1.0 (0.00-4.00)	7.0 (5.00-9.00)	+600	<<0.0001
Nbuds	1.0 (0.00-4.00)	3.0 (2.00-5.00)	+200	<<0.0001
Necrotic cells (%)	13.5 (10.7-16.5)	20.9 (19.4-25.9)	+55	<<0.0001
Apoptotic cells (%)	3.0 (1.1-10.7)	4.5 (3.6-5.2)	+50	>0.05
Nuclear division index (NDI)	1.9 (1.81-1.94)	1.9 (1.86-1.96)	0	>0.05
Nuclear division cytotoxicity index (NDCI)	1.7 (1.64-1.76)	1.7 (1.61-1.70)	0	>0.05

Δ°= difference between exposed subjects and controls. Results taken from (Ehrlich et al., 2008)

1.5 Carcinogenicity

1.5.1 Animal data

1.5.1.1 [NTP, 2002]

Study reference:

NTP, Toxicology and Carcinogenesis Studies of Vanadium Pentoxide in F344/N Rats and B6C3F1 Mice (Inhalation Studies). TR 507, 2002 (NTP, 2002).

Detailed study summary and results:

Test type

2-year carcinogenicity study in rats and mice, no guideline followed, GLP compliance is given.

Male and female rats and mice (N=50/sex/dose) were exposed to various concentrations of V_2O_5 6 h/d, for 5 d/w and for a period of 2 years. Clinical signs, body weights, blood and urine were observed during the study. Surviving animals were sacrificed at study end and necropsy was performed. Appearance of neoplasms in exposed animals compared to controls was analysed. Male and female F344 rats were exposed to 0, 0.5, 1 and 2 mg/m^3 V_2O_5, mice were exposed to 0, 1, 2 and 4 mg/m^3 V_2O_5.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: divanadium pentaoxide
- Degree of purity: approximately 99%
- Impurities: less than 1%
- Batch number: 1210140

Test animals

- Fischer 344 male and female rats and B6C3F1 male and female mice
- 50 male and female animals /dose group
- Rats:
    - Age at study initiation: 6 or 7 weeks on the first day of the study
    - Weight at study initiation for week 1: 134-136 g (males) and 130-105 g (females)
- Mice:
    - Age at study initiation: 6 or 7 weeks on the first day of the study
    - Weight at study initiation for week 1: 24.0-24.4 g (males) and 19.6-19.9 g (females)

Administration/exposure

- Route of administration – inhalation (aerosol)
- 6 h/d, 5 d/w for 104 weeks
- Rats: 0, 0.5, 1 and 2 mg/m^3 V_2O_5, mice: 0, 1, 2 and 4 mg/m^3 V_2O_5

  Rats: Dose selection rationale - based on the incidences and severities of respiratory lesions and increased lung weights in male and female rats in the 90-day study, concentrations of 4 mg/m^3 or greater were considered to be too high for use in a 2-year study.

  Mice: Dose selection rationale - the highest exposure concentration (4 mg/m^3) selected for mice was based on reduced body weight gain of 8 and 16 mg/m^3 males and females, increased lung weights of 4 mg/m^3 or greater in males and females, increased incidences of epithelial hyperplasia, and increased incidences and severities of inflammatory lesions of the lungs in mice exposed to 8 mg/m^3 versus those exposed to 4 mg/m^3 in the 90-day study.
- Control group animals were chamber exposed
- Historical control data is available for NTP studies
- No post exposure time
- Vehicle: air
- MMAD = 1.0-1.3 µm, GSD = 2.3-2.8
- The generation and delivery system consisted of a linear dust feeder, a particle attrition chamber, and an aerosol distribution system. The linear dust feeder, a slide-bar dust-metering device, was composed of a shuttle bar, body, outlet port, and hopper. As the compressed-air-driven shuttle bar slid back and forth during generation, the metering port aligned with the hopper, which served as a reservoir for the bulk chemical, and was filled with a small amount of divanadium pentaoxide powder. As the shuttle bar slid to the dispersing position, the metering port aligned with a compressed-air port in the body and a puff of air from this port dispersed the divanadium pentaoxide into the particle attrition chamber. Generator output was regulated by adjusting the cadence of the shuttle bar. The particle attrition chamber used low fluid energy from an air jet tangential to the chamber to deagglomerate the divanadium pentaoxide particles. After deagglomeration, the particles were swept into a classification zone where smaller particles exited to the distribution line; larger particles were thrown to the perimeter of the classifier by centrifugal force and were reentrained into the impacting air jet, and the process was repeated until the particles were sufficiently deagglomerated. The aerosol passed through the distribution lines to the exposure chambers. A pneumatic pump designed by the study laboratory was located at each chamber inlet and drew aerosol from the distribution line into the chamber inlet, where it was diluted with conditioned air to the appropriate concentration. Flow through the distribution line was controlled by Air-Vac pumps (Air-Vac Engineering, Milford, CT), and pressure was monitored by photohelic differential pressure gauges (Dwyer Instruments, Inc., Michigan City, IN).

- The Stainless steel chambers (Lab Products, Inc., Harford Systems Division, Aberdeen, MD), were designed so that uniform aerosol concentrations could be maintained throughout the chambers when catch pans were in place. The total active mixing volume of each chamber was 1.7 m^3.
- The particle size distribution in each chamber was determined prior to the start of the study, during the first 2 weeks of the 2-year study, and monthly.
- A Mercer-style seven-stage impactor was used. The stages (glass coverslips lightly sprayed with silicon) were analyzed by ICP/AES, and the relative mass collected on each stage was analyzed by probit analysis.
- The uniformity of aerosol concentration in the inhalation exposure chambers without animals was evaluated before each of the studies began; concentration uniformity with animals present in the chambers was also measured. Chamber concentration uniformity was acceptable throughout the 2-year studies.
- The stability of divanadium pentaoxide in the exposure system was tested with XRD analysis. XRD analyses indicated no detectable build-up of degradation products at a detection limit of approximately 1%.
- During the studies, chamber aerosol concentrations were monitored with real-time aerosol monitors (RAMs) that used a pulsed-light-emitting diode in combination with a silicon detector to sense light scattered over a forward angular range of 45° to 95° by particles traversing the sensing volume. The instruments respond to particles 0.1 to 20 µm in diameter.
- The sampling system consisted of a valve that multiplexed each RAM to two or three exposure chambers and to a HEPA filter and/or the control chamber or room; selection of sampling streams and data acquisition from each RAM was remotely controlled by a computer. Equations for calibration curves were stored in the computers and were used to convert the measured voltages to exposure concentrations.
- Each RAM was calibrated two times per week by ICP/AES or ICP/mass spectrometry analysis of Pallflex® TX40H120WW glass fiber filters.

Results and discussion

Rats:

- Clinical signs: no effects
- Body weight gain: Body weights of the animals were similar to the controls except body weight of the 2 mg/m^3–exposed females which was less
- Food/water consumption: do data
- Ophthalmoscopic examination: no data
- Clinical chemistry: no data
- Haematology: no data

- Urinalysis: no data
- Organ weights: no data
- Mortality and time to death (see Table AI - 17 for details)

Table AI - 17: Survival data for male and female rats

	Chamber control	0.5 mg/m³	1 mg/m³	2 mg/m³
MALE rats				
Animals initially in the study	50	50	50	50
Moribund	25	17	18	13
Natural deaths	5	4	6	10
Animals surviving to study termination	20	29	26	27
Mean survival (days)	668	680	692	671
FEMALE rats				
Animals initially in the study	50	50	50	50
Moribund	14	20	17	15
Natural deaths	3	6	4	5
Animals surviving to study termination	33	24	29	30
Mean survival (days)	688	678	679	683

Results taken from (NTP, 2002)

HISTOPATHOLOGY: NON-NEOPLASTIC

Non-neoplastic lesions occurred in respiratory system of males and females (lung, larynx, and nose) and the severities of these lesions generally increased with increasing exposure concentration.

LUNGS:

Effects in males: alveolar epithelium, hyperplasia (7/50, 24/49, 34/48, 49/50); bronchiole, epithelium hyperplasia (3/50, 17/49, 31/48, 49/50); alveolar epithelium, metaplasia, squamous (1/50, 0/49, 0/48, 21/50); bronchiole, metaplasia, squamous (0/50, 0/49, 0/48, 7/50); inflammation, chronic active (5/50, 8/49, 24/48, 42/50); interstitial, fibrosis (7/50, 7/49, 16/48, 38/50); alveolus, infiltration cellular, histiocyte (22/50, 40/49,45/48, 50/50).

Effects in females: alveolar epithelium, hyperplasia (4/49, 8/49, 21/50, 50/50); bronchiole, epithelium hyperplasia (6/49, 5/49, 14/50, 48/50); alveolar epithelium, metaplasia, squamous (0/49, 0/49, 0/50, 6/50); inflammation, chronic active (10/49, 10/49, 14/50, 40/50); interstitial, fibrosis (19/49, 7/49, 12/50, 32/50); alveolus, infiltration cellular, histiocyte (26/49, 35/49, 44/50, 50/50).

There were increased incidences of alveolar epithelial hyperplasia and bronchiole hyperplasia in the lungs of males exposed to 0.5 mg/m^3 or greater and females exposed to 1 or 2 mg/m^3. The severities of these lesions

were increased in 2 mg/m^3 males and females. In affected animals, this was essentially a diffuse change with proliferation of epithelium in the distal terminal bronchioles and immediately associated alveolar ducts and alveoli. Normally flattened epithelium was replaced with cuboidal epithelium.

Increased incidences of squamous metaplasia of the alveoli occurred in male and, to a lesser extent, in female rats exposed to 2 mg/m^3. There was a spectrum of changes ranging from minimal to severe. Minimal lesions were characterized by a single alveolus with the thin type I cells which normally line alveoli replaced by one to several layers of squamous epithelium. Severe lesions were much larger, often involving an area approximately 1 cm in diameter. Many alveoli were involved and there was apparent coalescence of the metaplasia. There were also lesions of intermediate severity. Keratin production was a prominent feature of the squamous metaplasia observed in this study. Keratin often filled the affected alveoli, and in some of the lesions, cyst-like structures filled with keratinous material were formed. In a few animals (predominantly males), the squamous metaplasia extended into the distal airways and was diagnosed as bronchiole squamous metaplasia. Commonly dispersed within the squamous lesions were areas of respiratory epithelial metaplasia in which the alveolar epithelium was replaced by tall cuboidal to columnar epithelium with cilia often present and with mucous material filling the alveolar lumen.

Incidences of minimal to mild chronic active inflammation and interstitial fibrosis in the lungs were significantly increased in males exposed to 1 or 2 mg/m^3 and females exposed to 2 mg/m^3, and the incidences of histiocytic cellular infiltrate of the alveolus were increased in all exposed groups of males and females. The inflammatory lesions were primarily minimal to mild and consisted of interstitial and perivascular infiltrates of mostly mononuclear inflammatory cells that were occasionally within alveoli. Alveolar septa were occasionally thickened by thin strands of eosinophilic fibrillar material (fibrosis). The histiocytic infiltrate was also minimal to mild, consisting of scattered intra-alveolar macrophages that contained large amounts of foamy intracytoplasmic material, interpreted as pulmonary surfactant. Additionally, scant amounts of eosinophilic material (surfactant) similar to that observed within alveolar macrophages was also free within alveoli; however, a separate diagnosis was not made. A brownish pigment (pigmentation) was visible in alveolar macrophages in some males and females exposed to 2 mg/m^3 and in females exposed to 1 mg/m^3; it was a mild change considered of little biological significance and was not further characterized.

LARYNX:

Effects in males: inflammation, chronic (3/49, 20/50, 17/50, 28/49); respiratory epithelium, epiglottis degeneration (0/49, 22/50, 23/50, 33/49); respiratory epithelium, epiglottis, hyperplasia (0/49, 18/50, 34/50, 32/49); respiratory epithelium, epiglottis, metaplasia, squamous (0/49, 9/50, 16/50, 19/49).

Effects in females: inflammation, chronic (8/50, 26/49, 27/49, 37/50); respiratory epithelium, epiglottis degeneration (2/50, 33/49, 26/49, 40/50); respiratory epithelium, epiglottis, hyperplasia (0/50, 25/49, 26/49, 33/50); respiratory epithelium, epiglottis, metaplasia, squamous (2/50, 7/49, 7/49, 16/50).

There were increased incidences of minimal to mild lesions of the larynx in exposed males and females. The incidences generally increased with increasing exposure concentration and included chronic inflammation of the larynx and degeneration, hyperplasia, and squamous metaplasia of the respiratory epithelium of the epiglottis. The inflammation consisted of a mixture of mononuclear and granulocytic inflammatory cells in the submucosa beneath the epithelium lining the base of the epiglottis, ventral pouch, and caudal larynx. The degeneration of the respiratory epithelium was characterized by a loss or decrease in the height of cilia and shortening of the normally columnar to cuboidal surface epithelial cells lining the laryngeal surface of the base of the epiglottis. Squamous metaplasia was diagnosed when the ciliated cells were replaced by one or more layers of flattened squamous epithelium. In the same area, the respiratory epithelium was mildly thickened in many animals; this change was diagnosed as hyperplasia. These changes are relatively minimal, commonly occur in rats in NTP inhalation studies, and represent a common response to laryngeal injury.

NOSE:

Effects in males: goblet cell, respiratory epithelium, hyperplasia (4/49, 15/50, 12/49, 17/48)

Effects in females: goblet cell, respiratory epithelium, hyperplasia (13/50, 18/50, 16/50, 30/50)

There were increased incidences of mild goblet cell hyperplasia of the nasal respiratory epithelium in all groups of exposed male rats and in females exposed to 2 mg/m^3. Increased numbers of goblet cells were most notable in the respiratory epithelium lining the median septum adjacent to the area of the vomeronasal organ.

KIDNEY:

The incidences of nephropathy were significantly increased in male rats exposed to 1 or 2 mg/m^3 (37/50, 42/50, 46/49, 47/50). Nephropathy is a common lesion in aged rats, particularly males, and has been diagnosed in virtually all males in NTP 2-year studies that used the NIH-07 diet. In those studies, chemical exacerbation of nephropathy was identified by increased severity. With the NTP-2000 diet, the severity of spontaneous nephropathy has been reduced. In this study, the severity of nephropathy was not increased in exposed groups of males. Also, exposed females were not affected (24/50, 21/50, 20/49, 32/50).

HISTOPATHOLOGY: NEOPLASTIC (if applicable):

LUNG:

Effects in males: 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)

Effects in females: alveolar/bronchiolar adenoma (0/49, 3/49, 1/50, 0/50); alveolar/bronchiolar adenoma or carcinoma (0/49, 3/49, 1/50, 1/50)

Alveolar/bronchiolar carcinomas were present in exposed groups of males and one 2 mg/m^3 female. Alveolar/bronchiolar adenomas were present in 0.5 and 1 mg/m^3 females.

Although there were no statistically significant increases in the incidences of lung neoplasms in rats, the incidences of alveolar/ bronchiolar adenoma in 0.5 mg/m^3 males and of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) in 0.5 and 2 mg/m^3 males exceeded the historical ranges in controls (all routes) given NTP-2000 diet and in inhalation chamber controls given NIH-07 diet (NTP, report 2002). The incidence of alveolar /bronchiolar adenoma in 0.5 mg/m^3 females was at the upper end of the historical control range for studies using NTP-2000 diet and exceeded the range in the larger database for inhalation studies using NIH-07 diet.

UTERUS:

The incidences of stromal polyp occurred with a positive trend in female rats (chamber control, 6/50; 0.5 mg/m^3, 3/50; 1 mg/m^3, 7/50; 2 mg/m^3, 13/50). However, the incidence in the 2 mg/m^3 group was within the historical range in controls. Endometrial stromal polyps are common neoplasms in the F344/N rat in NTP studies. They are benign neoplasms and generally do not progress to malignancy; however, they occasionally do progress to stromal sarcoma. In this study, when the incidences of stromal polyp were combined with the single incidence of stromal sarcoma, the combined incidence in 2 mg/m^3 females was significantly increased.

OTHER FINDINGS

LUNG BURDEN STUDIES:

Histopathology: the left lung lobe from each animal was infused with 10% neutral buffered formalin, and sections were examined microscopically. The purpose was to follow progression of the lung lesions. Following day 1 of exposure, there was an infiltrate of alveolar macrophages in the lungs. With continued exposure, increased numbers of alveolar macrophages, interstitial mononuclear inflammatory cell infiltrates, and hyperplasia of alveolar and bronchiolar epithelium were observed. In rats exposed to 2 mg/m^3, there was an increase in severity of the hyperplasia between days 54 and 173. An increase in severity was not obvious between days 173 and 360, but hyperplasia appeared more severe on day 542. Hyperplasia was observed in only a few animals exposed to 1 mg/m^3 and only on day 542. The minimal fibrosis observed in the 2-year study was not readily apparent on day 542 or earlier.

Lung weights from exposed female rats increased throughout the study. Although there appeared to be an exposure concentration-related increase in lung weights after day 26 of the study, it was primarily due to increases in lung weights of female rats exposed to 2 mg/m^3. In general, lung weights of 0.5 or 1 mg/m^3 females were similar.

Though deposition patterns were similar between rats and mice, the maximum lung burdens occurred at day 173 in rats. The lung burdens appeared to reach steady state at the lowest exposure concentrations (0.5 mg/m^3). A decline in lung burdens was observed. The retention of vanadium in the lungs at 18 months was ca. 13% to 15% in rats. The total lung doses for rats exposed to 0.5, 1, or 2 mg/m^3 were estimated to be 130, 175, and 308 µg vanadium, respectively.

Lung clearance half-times were considerably longer than those observed in the 16-day special studies.

BLOOD:

Vanadium was detected in the blood at concentrations several orders of magnitude lower than those measured in the lungs of exposed rats, and blood vanadium concentrations in exposed groups were only marginally increased over that of the chamber control group. Overall, blood vanadium concentrations appeared to increase with increasing exposure concentration; however, this proportionality was less clear when the 0.5 and 1 mg/m^3 groups were compared.

Blood vanadium concentrations in all exposed groups appeared to peak on days 26 or 54 after which there was a decline throughout the rest of the study. This response was similar to that seen in lung burdens. However, these changes in concentrations were small, making it difficult to determine if there was an increase in elimination of vanadium from the blood or a decreased absorption from the lung due to reduced deposition, especially at the higher exposure concentrations.

Mice:

- Survival: survival of males exposed to 4 mg/m^3 was significantly less than that of the chamber controls.
- Clinical signs: Abnormal breathing was observed particularly in those exposed to 2 or 4 mg/m^3. Many mice exposed to divanadium pentaoxide were thin
- Body weight gain: Mean body weight of all females and highest dosed males was below the controls; and mean body weights of males exposed to 2 mg/m^3 were less from week 85 to the end of the study
- Food/water consumption: do data
- Ophthalmoscopic examination: no data
- Clinical chemistry: no data
- Haematology: no data
- Urinalysis: no data
- Organ weights: no data
- Mortality and time to death (see Table AI - 18 below)

Table AI - 18: Survival data for male and female mice

	Chamber control	0.5 mg/m³	1 mg/m³	2 mg/m³
MALE mice				
Animals initially in this study	50	50	50	50
Moribund	7	11	13	20
Natural deaths	4	6	1	3
Animals surviving to study termination	39	33	36	27
Mean survival (days)	710	692	704	668
FEMALE mice				
Animals initially in th study	50	50	50	50
Moribund	8	10	14	16
Natural deaths	4	5	4	2
Animals surviving to study termination	38	32	30	32
Mean survival (days)	692	655	653	688

Results taken from (NTP, 2002)

HISTOPATHOLOGY: NON-NEOPLASTIC

Non-neoplastic lesions occurred in respiratory system (lung, larynx, and nose) of males and females, and the severities of these lesions generally increased with increasing exposure concentration.

Bronchial lymph node hyperplasia was present in most of the exposed females.

LUNG:

There were significantly increased incidences of alveolar epithelial hyperplasia and bronchiolar epithelial hyperplasia in the lungs of exposed male and female mice. Incidences of chronic inflammation and histiocytic cellular infiltrate were significantly increased in exposed groups of mice, and the incidences of interstitial fibrosis were increased in mice exposed to 2 or 4 mg/m^3.

Males: alveolar epithelium, hyperplasia (3/50, 41/50, 49/50, 50/50); bronchiole, epithelium, hyperplasia (0/50, 15/50, 37/50, 46/50); inflammation chronic (6/50, 42/50, 45/50, 47/50); alveolus, infiltration cellular, histiocyte (10/50, 36/50, 45/50, 49/50); interstitial fibrosis (1/50, 6/50, 9/50, 12/50)

Females: alveolar epithelium, hyperplasia (0/50, 31/50, 38/50, 50/50); bronchiole, epithelium, hyperplasia (0/50, 12/50, 34/50, 48/50); inflammation chronic (4/50, 37/50, 39/50, 49/50); alveolus, infiltration cellular, histiocyte (0/50, 34/50, 35/50, 45/50); interstitial fibrosis (0/50, 1/50, 4/50, 8/50)

The hyperplasia was essentially a diffuse change with proliferation of epithelium in the distal terminal bronchioles and immediately associated alveolar ducts and alveoli. Normally flattened epithelium was replaced with cuboidal epithelium. The hyperplasia of the alveolar epithelium was pronounced and increased in severity with increasing exposure concentration, while the hyperplasia of the distal bronchioles was

minimal to mild with slight increases in severity in mice exposed to 4 mg/m^3. The changes in mice were similar to those observed in rats but were not as pronounced.

The inflammatory lesions were primarily minimal to mild and consisted of interstitial, perivascular, and peribronchiolar infiltrates of mostly mononuclear inflammatory cells (mostly lymphocytes) that were occasionally present within alveoli. The most prominent histiocytic infiltrate occurred within alveoli in close proximity to alveolar/bronchiolar neoplasms, particularly carcinomas.

LARYNX:

There were significantly increased incidences of minimal squamous metaplasia of the respiratory epithelium of the epiglottis in exposed groups.

Males: respiratory epithelium, epiglottis, metaplasia, squamous (2/49, 45/50, 41/48, 41/50)

Females: respiratory epithelium, epiglottis, metaplasia, squamous (0/50, 39/50, 45/49, 44/50)

Squamous metaplasia was diagnosed when the ciliated cells were replaced by one to three layers of flattened squamous epithelium. This change commonly occurs in mice in NTP inhalation studies and represents a common response to laryngeal injury.

NOSE:

There were increased incidences of minimal to mild suppurative inflammation of the nose in males and females exposed to 2 or 4 mg/m^3.

Males: inflammation suppurative (16/50, 11/50, 32/50, 23/50); olfactory epithelium, degeneration, hyaline (1/50, 7/50, 23/50, 30/50); respiratory epithelium, degeneration, hyaline (8/50, 22/50, 38/50, 41/50); respiratory epithelium, metaplasia, squamous (0/50, 6/50, 6/50, 2/50)

Females: inflammation suppurative (19/50, 14/50, 32/50, 30/50); olfactory epithelium, atrophy (2/50, 8/50, 5/50, 14/50); olfactory epithelium, degeneration, hyaline (11/50, 23/50, 34/50, 48/50); respiratory epithelium, degeneration, hyaline (35/50, 39/50, 46/50, 50/50); respiratory epithelium, metaplasia, squamous (0/50, 3/50, 7/50, 8/50); respiratory epithelium, necrosis (0/50, 0/50, 1/50, 7/50)

The inflammation consisted of focal aggregates of few to moderate numbers of neutrophils generally subjacent to the epithelium of the turbinates, septum, or lateral wall of the anterior nose. In the more severe cases (predominantly in females exposed to 4 mg/m^3), a short segment of the overlying epithelium was ulcerated (necrosis). Similarly, in some males and females, the overlying respiratory epithelium was replaced by one or more layers of flattened epithelium (squamous metaplasia), and the incidences were marginally increased in some exposed groups of mice.

The majority of the olfactory epithelium covers the turbinates in the distal portion of the nose. There were marginal but significant increases in the incidences of atrophy of this epithelium in females exposed to 1 or 4 mg/m^3.

BRONCHIAL LYMPH NODE

There were significant increases in the incidences of hyperplasia of the bronchial lymph node in exposed groups of females, and while not significant, a positive trend in the incidences of this lesion also occurred in males.

Females: hyperplasia (3/39, 13/40, 14/45, 20/41)

HISTOPATHOLOGY: NEOPLASTIC (if applicable)

LUNG:

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 compared to controls

Males: alveolar/bronchiolar adenoma (13/50, 16/50, 26/50, 15/50); alveolar/ bronchiolar carcinoma (12/50, 29/50, 30/50, 35/50); alveolar/ bronchiolar adenoma or carcinoma (22/50, 42/50, 43/50, 43/50)

Females: alveolar/bronchiolar adenoma (1/50, 17/50, 23/50, 19/50); alveolar/ bronchiolar carcinoma (0/50, 23/50, 18/50, 22/50); alveolar/ bronchiolar adenoma or carcinoma (1/50, 32/50, 35/50, 32/50)

The incidences of alveolar/bronchiolar adenoma were significantly increased in males exposed to 2 mg/m^3 and in all groups of exposed females. 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. These increased incidences exceeded the historical ranges for controls.

SPLEEN:

There was a positive trend in the incidences of hemangiosarcoma of the spleen in male mice (chamber control, 0/50; 1 mg/m^3, 0/50; 2 mg/m^3, 0/50; 4 mg/m^3, 3/50). However, the incidence in the 4 mg/m^3 group was within the historical range in controls. The incidences of hemangioma or hemangiosarcoma (combined) in all organs were not significant.

OTHER FINDINGS

TISSUE BURDEN STUDIES:

Histopathology: lung lesions were identified early, and were more severe in females exposed to higher concentrations and progressed with time. Minimal bronchiolar epithelial hyperplasia and interstitial inflammation were initially observed in the 4 mg/m^3 group on day 5. Bronchiolar epithelial hyperplasia was characterized by slight piling up of cells, some nuclear pleomorphism, and occasional karyorrhectic cells. On day 12, exposure-related lesions were evident in 2 mg/m^3 females, while alveolar epithelial hyperplasia was more prominent in the 4 mg/m^3 group. Lesions were first observed in the 1 mg/m^3 group on day 54, and lesion severities in the 2 and 4 mg/m^3 groups appeared to be increased on day 54. On day 362, lesion severity in lungs of females exposed to 4 mg/m^3 was noticeably increased, and bronchiolar hyperplasia included more nuclear pleomorphism. On day 535, mice exposed to 2 mg/m^3 had hyperplastic alveolar epithelial lesions and, similar to females exposed to 4 mg/m^3, the hyperplasia was oriented around the terminal bronchiolar/alveolar duct. Also on day 535, lung nodules/masses were observed. While some were

confirmed as neoplasms, many were in the right lung lobe and thus were not confirmed histopathologically. Similar lesions, although more severe, were observed in the core group of animals at 2 years.

Lung burden data appeared proportional to exposure concentration in mice. Rats removed vanadium from the lungs much slower than mice, with clearance half-times of vanadium lung burdens approximately six to nine fold longer in rats than in mice at comparable exposure concentrations. In rats and mice, lung clearance half-times were considerably longer than those observed in the 16-day special studies.

The maximum lung burdens occurred between days 26-54 in mice. The lung burdens appeared to reach steady state at the lowest exposure concentrations (1 mg/m^3). A decline in lung burdens was observed. The retention of vanadium in the lungs at 18 months was 2% to 3% in mice. The total lung doses for mice exposed to 1, 2, or 4 mg/m^3 were estimated to be 153, 162, and 225 µg vanadium, respectively.

1.5.1.2 [Rondini et al., 2010]

Study reference:

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

Detailed study summary and results:

Test type

Tumor promotion study in male A/J, BALB/cJ and C57BL/6J mice. After injection of methylcholanthren (MCA) animals were exposed for 5 weeks to V_2O_5. After 20 weeks the tumor rate was determined. No information on GLP compliance is available.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: divanadium pentaoxide
- Degree of purity: approximately >99.9%
- Impurities: less than 1%
- No batch number available

Test animals

- Male A/J, BALB/cJ (BALB), C57BL/6J (B6) mice
- Number of mice per treatment group:
  - A/J: Oil/PBS and Oil/V_2O_5 (n = 3), MCA/PBS (n = 4), MCA/V_2O_5 (n = 15);
  - BALB: Oil/PBS and Oil/V_2O_5 (n = 3-5), MCA/PBS (n = 8), MCA/V_2O_5 (n = 13); B6, Oil/PBS and Oil/V_2O_5 (n = 3-8), MCA/PBS (n = 7), MCA/ V_2O_5 (n = 12).
- Animals were 5-6 weeks of age and were allowed one week to acclimatize prior to treatment.

Administration/exposure

- Route of administration – pulmonary exposure *via* oropharyngeal aspiration
- After injection of methylcholanthren (MCA) animals were exposed for once per week for 5 weeks to V_2O_5. After 20 weeks the tumor rate was determined.
- Animals were exposed against 4 mg/kg bw. V_2O_5 was suspended in sterile-filtered Dulbecco's phosphate buffered saline (10 mM PBS, pH 7.4), sonicated for 20 minutes and diluted to a working concentration of 2 mg/mL. Prior to aspirations, mice were anesthetized.
- Dose selection rationale: preliminary dose response studies
- Frequency of treatment: once a week for 5 weeks
- Historical control data: not available
- V_2O_5 was suspended in sterile-filtered Dulbecco´s phosphate buffered saline (10 mM PBS, pH 7.4)
- control animals were treated with corn oil and/or PBS and without V_2O_5 or without the initiator substance MCA but with V_2O_5
- Post exposure observation period: Animals were exposed against V_2O_5 for 5 weeks and sacrificed in week 21

For inhalation studies:

- Type of inhalation exposure and test conditions: oropharyngeal aspiration
- Mice were anesthetized with 3% isoflurane in 1-2 L/min oxygen prior to application of V_2O_5

Results and discussion

- Mortality: no data
- Clinical signs: no data
- Body weight gain: no data
- Food/water consumption: no data
- Ophthalmoscopic examination: no data
- Clinical chemistry: special tests for determination of inflammation. Cellularity of BALF and protein content were analysed up to 21 days after the last V_2O_5 exposure. BALF protein content increased significantly in all strains at 6 h after the application and returned to baseline by 21 days. A comparable effect was observed for the number of total cells /mL BALF, macrophages/mL BALF, lymphocytes/mL BALF, polymorphonuclear leukocytes/mL BALF. Strain differences were observed. The susceptibility of animals to pulmonary inflammation proceeded in the order of A/J mice > BALB mice > B6 mice.

  Expression of V_2O_5-induced inflammatory chemokines was examined up to three days after the last V_2O_5 treatment. The chemokines KC (keratinocyte Chemoatractant), MIP-2 (macrophage

inflammatory protein-2) and MCP-1 (monocyte chemoattractant protein-1) increased significantly and fell back to normal levels 3 days after exposure.

- Haematology: no data
- Urinalysis: no data
- Organ weights: no data
- Necropsy findings: no data
- Histopathological findings: tumor incidence in A/J and BALB mice was significantly increased in animals exposed to MCA and V_2O_5. with a majority of solid adenomas (80%) and the remaining papillary (20%)
- Lung tumour incidence: see the following Table AI - 19

Table AI - 19: Lung tumour incidences in the different mouse strains

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

Results taken from (Rondini et al., 2010)

No tumors were found in B6 mice.

- Mode of action: tumor promotor

1.5.1.3 [Yao et al., 1986]

Study reference:

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. Yao, D.; Li, S.; et al. (1986)

Detailed study summary and results:

Test type

Carcinogenicity study in male and female mice (1 year exposure).

Male and female mice (N= 62 - 84 male and female mice /dose) were exposed to various concentrations of V_2O_5 dust, 0.5, 2, or 8 mg V_2O_5 dust/m^3 for 4 h/day for 1 year. No further information is available on the strain of mice used, the purity of V_2O_5 or on the particle size.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: divanadium pentaoxide

- Degree of purity: not available
- Impurities: not available
- No batch number available

Test animals

- Male and female mice
- Number of mice per treatment group: 62 - 84 male and female mice /dose
- No further information available.

Administration/exposure

- Route of administration: inhalation
- No further information available.

For inhalation studies:

- Type of inhalation exposure and test conditions: not available

Results and discussion

- Mortality: no data
- Clinical signs: no data
- Body weight gain: no data
- Food/water consumption: no data
- Ophthalmoscopic examination: no data
- Haematology: no data
- Urinalysis: no data
- Organ weights: no data
- Necropsy findings: no data
- Histopathological findings:

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 were reported in control animals and at 0.5 mg/m^3.

1.6 Reproductive toxicity

1.6.1 Animal data

1.6.1.1 [NTP, 2002]

Study reference:

NTP, National Toxicology Program, 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, 2002 (NTP, 2002)

Detailed study summary and results:

Test type

Groups of 10 male and 10 female rats were exposed to particulate aerosols of divanadium pentaoxide at concentrations of 0, 1, 2, 4, 8, or 16 mg/m^3 by inhalation, 6 hours per day, 5 days per week for 3 months. Effects on fertility and sexual function of male and female rats were evaluated after 90 days of inhalation exposure. GLP compliance is given.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: divanadium pentaoxide
- Degree of purity: approximately 99%
- Impurities: less than 1%
- Batch number: 1210490

Test animals

- Fischer 344 male and female rats, 10 male and female animals /dose group
- Age at study initiation: average age: 6 or 7 weeks on the first day of the study
  Weight at study initiation: 133-137 g (males) and 108-111 g (females)

Administration/exposure

- Route of administration – inhalation (aerosol)
- 6 hours per day + T_{90} (15 min), 5 days per week for 3 months
- 0, 1, and 2, 4, 8, 16 mg/m^3 V_2O_5

- Dose selection rationale - based on decreased survival in the 32 mg/m^3 males and body weight decreases in 32 mg/m^3 males and females in a 16-day study, an exposure concentration of 32 mg/m^3 was considered too high for use in a 3-month study.
- Historical control data is available for NTP studies
- No post exposure time
- Vehicle: air
- Generation of test atmosphere / chamber description: For the 3-month studies, divanadium pentaoxide aerosol generation was based on the principle of pneumatic dispersion and consisted of two major components: a screw feeder (Model 310, Accurate, White Water, WI) that metered divanadium pentaoxide powder at a constant rate and a Jet-O-Mizer jetmill (Fluid Energy Corp., Harfield, PA) that used compressed air to disperse the metered powder and form the aerosol.

  Aerosol leaving the jetmill passed through a one-stage impactor and a vertical elutriator to eliminate or deagglomerate the large particles before entering a plenum and manifold distribution system. The aerosol delivery system consisted of three holding chambers that diluted the aerosol in three stages. A metered amount of diluted aerosol was removed and mixed with conditioned air at the inlet to each exposure chamber to achieve the appropriate exposure concentration. The electrical charge buildup on the aerosol particles was neutralized by mixing the aerosol with high concentrations of bipolar ions, which were generated using a Pulse Gun (Static Control Services, Palm Springs, CA) air nozzle. For the 3-month studies, a transvector air pump was installed at the aerosol inlet to each exposure chamber to provide additional control of the aerosol flow rate and improve stability of the chamber concentration.

  The stainless-steel inhalation exposure chambers (Lab Products, Inc., Maywood NJ), were designed so that uniform aerosol concentrations could be maintained throughout the chambers when catch pans were in place. The total active mixing volume of each chamber was 1.7 m³.
- CHAMBER ATMOSPHERE CHARACTERIZATION

  The particle size distribution in each chamber was determined prior to the start of all studies, during the first week of the 3-month studies, and monthly during the 3-month study. For the 3-month study, a 10-stage Quartz Crystal Microbalance-based cascade impactor was used to separate the aerosol particles into sequential size ranges; the mass median aerodynamic diameter was calculated from the corresponding mass fraction of particles at each stage.
- OTHER

  The uniformity of aerosol concentration in the inhalation exposure chambers without animals was evaluated before each of the studies began; concentration uniformity with animals present in the chambers was also measured. During the 3-month studies, minor excursions in chamber uniformity values were observed in one or more exposure chambers, but these excursions had no impact on the studies.

The stability of divanadium pentaoxide in the exposure system was tested with XRD analysis. XRD analyses indicated no detectable build-up of degradation products at a detection limit of approximately 1%.

- Details on analytical verification of doses or concentrations:

  Chamber aerosol concentrations were monitored with real-time aerosol monitors (RAMs) that used a pulsed-light-emitting diode in combination with a silicon detector to sense light scattered over a forward angular range of 45° to 95° by particles traversing the sensing volume. The instruments respond to particles 0.1 to 20 µm in diameter.

  During the 16-day study, an individual monitor was used for each exposure chamber. The voltage output of the online monitors was read and recorded, and the calibration curve was applied to the voltages measured by the RAM to convert the measured voltages to exposure chamber concentrations.

  Each RAM was calibrated daily during the 16-day study by correlating the measured voltage with divanadium pentaoxide concentrations determined by gravimetric analysis of glass fiber filters.

Description of test design:

- Animals were not mated
- parameters assessed:
  - estrous cyclicity

    At the end of the 3-month study, samples were collected for vaginal cytology evaluations from core study rats exposed to 0, 4, 8, or 16 mg/m^3. For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus).
  - sperm parameters

    At the end of the 3-month study, samples were collected for sperm motility evaluations from core study rats exposed to 0, 2, 4, 8 mg/m^3.

    Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers.

    Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the

saline solution and then heat fixed at 65°C. Sperm density was then determined microscopically with the aid of a hemacytometer.

To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenising the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenisation-resistant spermatid nuclei were counted with a hemacytometer.

- Clinical observations performed: yes, weekly
- Gross necroscopy/organ weights:
    - Necropsies were performed on all core study animals.
    - The right testis was weighed.
    - Histopathology:

      -Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin.

      A complete histopathologic examination was performed on 0, 8, and 16 mg/m^3 rats.

Results and discussion

- Statistical treatment of results:

  Analysis of Continuous Variables

  Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, urinalysis, urine concentrating ability, cardiopulmonary, immunotoxicologic, cell proliferation, tissue concentrations, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973). Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across exposure concentrations (for more information see publication).

- Time of death during the study and whether animals survived to termination:

Seven males and three females exposed to 16 mg/m^3 died during the study (time of death: females week 9, 12, 13; males week 8, 8, 8, 9, 11, 11, 11).

Abnormal breathing, thinness, lethargy, abnormal posture, and ruffled fur were observed in rats exposed to 16 mg/m^3. Abnormal breathing, marked by shallow, rapid respiration, was first observed during and immediately following exposure periods; this was observed in all 16 mg/m^3 rats by week 2 and in all 8 mg/m^3 rats by week 4. By week 9, abnormal breathing was also observed in 16 mg/m^3 rats during non-exposure periods. Some rats in the 16 mg/m^3 groups had diarrhoea and nasal/eye discharge. Abnormal posture was observed in two males exposed to 8 mg/m^3, and one of these rats was thin with ruffled fur and nasal/eye discharge.

- Body weight data:

  Final mean body weights and body weight gains of males exposed to 4 mg/m^3 or greater and of females exposed to 16 mg/m^3 were significantly less than those of the chamber controls.

- Reproductive function: estrous cycle

  The estrous cycle of females exposed to 8 mg/m^3 was significantly longer than that of the chamber control group.

  The number of cycling females in the 16 mg/m^3 group was reduced. These data can be seen in the following Table AI - 20.

Table AI - 20: Reproductive effects on female animals

	Chamber control	**4 mg/m^3**	**8 mg/m^3**	**16 mg/m^3**
N (females)	10	10	10	8
Estrous cycle length (days)	5.00 ± 0.00	5.00 ± 0.08	5.50 ± 0.14**[a]	5.25 ± 0.25[b]
Estrous stage				
Diestrus	39.2	40.8	49.2	71.9
Proestrus	18.3	16.7	15.8	10.4
Estrus	20.8	19.2	17.5	10.4
Metestrus	21.7	22.5	17.5	7.3
Uncertain diagnosis	0.0	0.8	0.0	0.0

**Significantly different from chamber control group (P≤0.01), [a] Estrous cycle was longer than 12 days or was unclear in 1/10 animals, [b] Estrous cycle was longer than 12 days or was unclear in 6/8 animals. Results taken from (NTP, 2002).

- Reproductive function: sperm measures

  No effect.

- Organ weights

  Organ weight differences were considered to be related to body weight decreases.

- Gross pathology and histopathology

Atrophy of the secondary reproductive organs was observed in 16 mg/m^3 males and females. Hypospermia of the testis and atypical cells of the epididymis were observed in 16 mg/m^3 males. These lesions may have been associated with the marked body weight loss and general debilitation of these rats. No other effect on reproductive organs was reported.

- Other effects

Local effects were reported in both males and females with a NOAEClocal of 1 mg/m^3 air based on increased lung weights and epithelial hyperplasia, inflammation and fibrosis in lungs at 2 mg/m^3 and above (see section on STOT RE for further details).

1.6.1.2 [NTP, 2002]

Study reference:

NTP, National Toxicology Program, 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, 2002 (NTP, 2002)

Detailed study summary and results:

Test type

Groups of 10 male and 10 female mice were exposed to particulate aerosols of divanadium pentaoxide at concentrations of 0, 1, 2, 4, 8, or 16 mg/m^3 by inhalation, 6 hours per day, 5 days per week for 3 months. Effects on fertility and sexual function of male and female rats were evaluated after 90 days of inhalation exposure. GLP compliance is given.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: divanadium pentaoxide
- Degree of purity: approximately 99%
- Impurities: less than 1%
- Batch number: 1210490

Test animals

- B6C3F1 male and female mice, 10 male and female animals /dose group
- Age at study initiation: average age: 6 or 7 weeks
- Weight at study initiation (mean): 25-26 g (males) and 20-21 g (females)

Administration/exposure

- Route of administration – inhalation (aerosol)
- 6 hours per day + T_{90} (15 min), 5 days per week for 3 months
- 0, 1, and 2, 4, 8, 16 mg/m^3 V_2O_5
- Dose selection rationale - based on decreased survival in the 32 mg/m^3 males and body weight decreases in 32 mg/m^3 males and females in a 16-day study, an exposure concentration of 32 mg/m^3 was considered too high for use in a 3-month study.
- Historical control data is available for NTP studies
- No post exposure time
- Vehicle: air
- Generation of test atmosphere / chamber description: For the 3-month studies, divanadium pentaoxide aerosol generation was based on the principle of pneumatic dispersion and consisted of two major components: a screw feeder (Model 310, Accurate, White Water, WI) that metered divanadium pentaoxide powder at a constant rate and a Jet-O-Mizer jetmill (Fluid Energy Corp., Harfield, PA) that used compressed air to disperse the metered powder and form the aerosol.

  Aerosol leaving the jetmill passed through a one-stage impactor and a vertical elutriator to eliminate or deagglomerate the large particles before entering a plenum and manifold distribution system. The aerosol delivery system consisted of three holding chambers that diluted the aerosol in three stages. A metered amount of diluted aerosol was removed and mixed with conditioned air at the inlet to each exposure chamber to achieve the appropriate exposure concentration. The electrical charge buildup on the aerosol particles was neutralized by mixing the aerosol with high concentrations of bipolar ions, which were generated using a Pulse Gun (Static Control Services, Palm Springs, CA) air nozzle. For the 3-month studies, a transvector air pump was installed at the aerosol inlet to each exposure chamber to provide additional control of the aerosol flow rate and improve stability of the chamber concentration.

  The stainless-steel inhalation exposure chambers (Lab Products, Inc., Maywood NJ), were designed so that uniform aerosol concentrations could be maintained throughout the chambers when catch pans were in place. The total active mixing volume of each chamber was 1.7 m³.

- CHAMBER ATMOSPHERE CHARACTERIZATION

  The particle size distribution in each chamber was determined prior to the start of all studies, during the first week of the 3-month studies, and monthly during the 3-month study. For the 3-month study, a 10-stage Quartz Crystal Microbalance-based cascade impactor was used to separate the aerosol particles into sequential size ranges; the mass median aerodynamic diameter was calculated from the corresponding mass fraction of particles at each stage.

- OTHER

  The uniformity of aerosol concentration in the inhalation exposure chambers without animals was evaluated before each of the studies began; concentration uniformity with animals present in the chambers was also measured. During the 3-month studies, minor excursions in chamber uniformity values were observed in one or more exposure chambers, but these excursions had no impact on the studies.

  The stability of divanadium pentaoxide in the exposure system was tested with XRD analysis. XRD analyses indicated no detectable build-up of degradation products at a detection limit of approximately 1%.

  Details on analytical verification of doses or concentrations:

  Chamber aerosol concentrations were monitored with real-time aerosol monitors (RAMs) that used a pulsed-light-emitting diode in combination with a silicon detector to sense light scattered over a forward angular range of 45° to 95° by particles traversing the sensing volume. The instruments respond to particles 0.1 to 20 µm in diameter.

  During the 16-day study, an individual monitor was used for each exposure chamber. The voltage output of the online monitors was read and recorded, and the calibration curve was applied to the voltages measured by the RAM to convert the measured voltages to exposure chamber concentrations.

  Each RAM was calibrated daily during the 16-day study by correlating the measured voltage with divanadium pentaoxide concentrations determined by gravimetric analysis of glass fiber filters.

Description of test design:

- Animals were not mated
- Parameters assessed:
  - Estrous cyclicity

    At the end of the 3-month study, samples were collected for vaginal cytology evaluations from core study mice exposed to 0, 4, 8, or 16 mg/m^3. For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus).
  - Sperm parameters

    At the end of the 3-month study, samples were collected for sperm motility evaluations from core study rats exposed to 0, 4, 8 or 16 mg/m^3.

    Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was

then removed from the epididymal body (corpus epididymis) and weighed. Test yolk was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers.

Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer.

To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenising the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenisation-resistant spermatid nuclei were counted with a hemacytometer.

- Clinical observations performed: yes, weekly
- Gross necroscopy/organ weights:
    - Necropsies were performed on all core study animals.
    - The right testis were weighed.
    - Histopathology:

      -Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin.

      A complete histopathologic examination was performed on 0, 8, and 16 mg/m^3 rats.

Results and discussion

- Statistical treatment of results:

  Analysis of Continuous Variables - Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, urinalysis, urine concentrating ability, cardiopulmonary, immunotoxicologic, cell proliferation, tissue concentrations, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's

test). Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973). Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across exposure concentrations. (for more information see publication).

- Time of death during the study and whether animals survived to termination:
  One male exposed to16 mg/m^3 died before the end of the study (week 11). The mouse that died early appeared thin.
  There were no other clinical findings related to divanadium pentaoxide exposure.

- Body weight data:
  Mean body weights of 8 and 16 mg/m^3 males and 4 mg/m^3 or greater females were significantly less than those of the chamber controls.

- Reproductive function: estrous cycle
  No significant differences were noted in estrous cycle parameters between exposed and chamber control females.

- Reproductive function: sperm measures
  The epididymal spermatozoal motility of males exposed to 8 or 16 mg/m^3 was significantly decreased. This can be seen in the following Table AI - 21.

Table AI - 21: Effects on male reproductive function

	Chamber control	**4 mg/m^3**	**8 mg/m^3**	**16 mg/m^3**
N (males)	9	10	9	8
Epididymal spermatozoal measurements: motility (%)	88.63 ± 0.90	86.23 ± 1.64	77.10 ± 3.15**	83.11 ± 2.48*

* Significantly different from chamber control group (P ≤ 0.05), **Significantly different from chamber control group (P ≤ 0.01). Results taken from (NTP, 2002).

- Organ weights
  Organ weight changes were considered related to body weight decreases.

- Gross pathology and histopathology
  No effects on reproductive organs reported.

- Other effects: local effects were reported in both males and females with a NOAEClocal of 1 mg/m³ air (males and females) based on increased absolute lung weights and epithelial hyperplasia and inflammation in lungs at 2 mg/ m^3 (see section on STOT RE for further details).

1.6.1.3 [Uche et al., 2008]

Study reference:

Uche, F.; Obianime, A.; Gogo-Abite, M., Effects of vanadium pentoxide on the histological and sperm parameters of male guinea pigs, Journal of Applied Sciences and Environmental Management, 12, 200 (Uche et al., 2008)

Detailed study summary and results:

Test type

Study of histological parameters and sperm parameters after exposure of male guinea pigs to divanadium pentaoxide. No information on GLP compliance is given.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: divanadium pentaoxide
- Degree of purity: analytical grade
- Impurities: no information available
- Batch number: no information available

Test animals

- Male guinea pigs (no strain indicated)
- 5 male animals/dose group
- Weight at the study initiation: 300 – 500 g (age not indicated)

Administration/exposure

- Intraperitoneally injected
- Experiment 1: 0, 4.5, 6.5, 8.5, 10.5, 12.5 mg/kg bw single injection
- Experiment 2: 0, 8.5 mg/kg bw/d injected. Tissue of 5 animals evaluated after 24, 48, 72 and 96 h.
- No rationale for dose selection given
- Control animals: treated with normal saline (0.5 mL/animal), same treatment as exposed animals
- No historical control data available

- Vehicle: normal saline. A stock solution of divanadium pentaoxide was prepared in distilled water (10 mg/mL), no further information available.

Description of test design:

- Animals were not mated
- Parameters assessed:
  - sperm parameters (sperm count, motility, shape)
  - histopathology of the testes
- No information on clinical observations available

Results and discussion

- Analysis of variance (ANOVA) was performed for the sperm parameters
- No deaths reported
- Body weight at sacrifice and absolute and relative organ weight data for the parental animals: no data available
- Effects on sperm:
  - Experiment 1: Statistically significant increase in percentage basal cell death (from 5% in control to 75% ± 1% at 12.5 mg/kg bw/d), reduction in sperm motility (from 90% in control to 31.0% ± 3.9% at 12.5 mg/kg bw/d), reduction in sperm count (from 80 x 10^6 cells/mL in control to 25.0 ± 4.0 x 10^6 cells/mL at 12.5 mg/kg bw/d) and alteration in the spermatic cell morphology (i.e. causing a change in the cellular structure of sperm cells and an increase in the number of dead cells and abnormal sperm count). No further information available.

    Please note, that results for the other doses are not given in numbers but only shown in a graph. In addition numbers given do not always completely match with the shown graph. However, the tendency is the same.
  - In addition, at a fixed time interval of exposure different doses of divanadium pentaoxide (4.5 -10.5 mg/kg bw) caused significant dose dependant reduction in spermatogonia, formation of hyperplastic seminiferous tubules and epidydimis, vacuolar dilatation, severe bleeding of numerous blood vessels and mild necrosis of testicular tissue. No further information available. Only microscopic pictures are shown in the publication.
  - Experiment 2: Testicular cells showed different degrees of response in a time-dependant way: Significant decrease in spermatogonia, alterations or destruction of semniferous tubules of testicular cells, severe bleeding of vessels and vacuolar dilatation. No further information available. Only microscopic pictures are shown in the publication.
- Necropsy findings: not evaluated

1.6.1.4 [Morgan and El-Tawil, 2003]

Study reference:

Morgan, A.M.; El-Tawil, O.S., Effects of ammonium metavanadate on fertility and reproductive performance of adult male and female rats, Pharmacological Research, 47, 75-85, 2003 (Morgan und El-Tawil, 2003)

Detailed study summary and results:

Test type

In two experiments, male rats (10 animals/dose groups) were mated with female rats (20 animals/dose group). Either the male animals were exposed to 200 ppm Ammonium metavanadate in the drinking water or the female animals prior to mating. In both experiments, fertility and reproductive performance were monitored. No information on GLP compliance is given.

Test substance

- Test material used in the study is not equivalent to the substance identified in the CLH dossier. Test material is Ammonium metavanadate
- EC number: 232-261-3
- CAS number: 7803-55-6
- Degree of purity: no information available
- Impurities: no information available
- Batch number: no information available

Test animals

- Male and female Sprague Dawley rats
- Male rats: n=30 (10 control; 10 group 1, 10 group 2)
- Female rats: n=60 (20 control; 20 group 1, 20 group 2)
- No. of animals per sex per dose
- Age and weight at the study initiation

Administration/exposure

- Route of administration – oral (drinking water)
- Male rats: 70 day exposure
- Female rats: 61 days exposure (14 days premating, during mating, till weaning of pups (21 days of age)), daily treatment

- 200 ppm in drinking water
    - calculated with factors according to (ECHA, 2012):
      male rat: 10 mg NH_4VO_3/kg bw/d, 4,35 mg V/kg bw/d
      female rat: 11.43 mg NH_4VO_3/kg bw/d, 4,97 mg V/kg bw/d
      In disseminated database a dose of 20 mg NH_4VO_3/kg body weight/day is given
      No rationale for dose level selection given
- Control group: yes, no positive controls
- Historical control data: no
- Vehicle: water
- The test substance was dissolved in drinking water at concentration of 200 ppm, no information on analytical verification of doses available
- Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test

- **Description of test design:**
- Details on mating procedure:
    - Fertility of males was investigated by mating of 10 exposed males and 10 control males with virgin untreated females (ratio 1M:2F).
    - Female fertility was investigated by mating of 20 exposed females and 20 control females with untreated males (ratio 1M:2F).
    - Length of cohabitation: 5 days
    - Proof of pregnancy: Presence of sperm in the vaginal smear
- Premating exposure period for males: 70 day exposure, for females: 61 days exposure (14 days premating, during mating, till weaning of pups (21 days of age))
- Daily application in drinking water
- Standardization of litters: no
- Parameters assessed for P:
    - BODY WEIGHT: Yes
    - WATER CONSUMPTION AND COMPOUND INTAKE (if drinking water study): No data
    - Estrous cyclicity:
      The control and ammonium metavanadate exposed females mated with untreated males were examined for estrous cycle regularity during the premating period.
    - Sperm parameters:
      After sacrifice of treated and control males, which were mated with untreated females, the following measurements were recorded: body weight and weight of testes, epididymis, prostate, and seminal vesicles.
    - SACRIFICE

Male animals: exposed males were removed after the mating period and killed by cervical dislocation under light ether anesthesia.

Maternal animals: untreated females, which have been paired with treated males, were investigated to evaluate the effects of ammonium metavanadate exposure on fertility. Half of these untreated females were sacrificed with their foetuses on the 20th day of gestation, while the other half was sacrificed with their pups on day 21 of lactation to record the fertility endpoints.

- GROSS NECROPSY

  - Number of corpora lutea, implantation sites, resorptions, dead and live foetuses.

  - Body weight at the end of gestation period and gravid uterine and placental weights.

- Parameters assessed for F1:
  - Dead and live foetuses, foetal body weight (at birth and at days 4, 7, 14 and 21 after birth), foetal survival and viability indices during lactation period.
  - Pups were examined for the presence of any behavioral defects (especially in the offspring obtained during lactation).
  - SACRIFICE

    F1 offspring were sacrificed on day 20 of gestation and on day 21 of lactation.
  - GROSS NECROPSY

    Offspring were subjected to macroscopic postmortem examinations for the presence of any gross malformations.
  - HISTOPATHOLOGY / ORGAN WEIGTHS

    1/3 of the foetuses were preserved in Bouin's solution and examined for the presence of any visceral anormalies using the Wilson free hand technique.

    The remaining 2/3 of the foetuses were preserved in 95% ethanol and examined for the presence of any skeletal anormalies.

- Reproductive indices:
  - The most indicative fertility endpoints were recorded:

    Mating and fertility indices, number of dams showing delayed birth date, pre- and postimplantation losses.
  - Offspring viability indices:

    No details are reported about the viability indices that were calculated from lactation records of litters.

Results and discussion

- Statistical treatment of results:

The data of treated male and female groups were compared to the control group. The data presented as percentage were analysed using Chi-square, however, other data were analysed using either one-way ANOVA or Student's t-test. The differences in the data were considered statistically significant at probability of P<0.05.

For P and F1 adults (per dose):

- Number of animals at the start of the test and matings:
  - Experiment 1. Male fertility: 10 males in exposure group, 10 males in control group
  - Experiment 2: female fertility: 20 females in exposure group, 20 females in control group
- Time of death during the study and whether animals survived to termination: all animals survived until termination.
- Body weight data for P animals selected for mating: Body weight of male animals from experiment I not impaired. Body weight/dam at termination in both experiments significantly reduced
- Organ weight data for male animals: see the following Table AI - 22

Table AI - 22: Body weight and organ weights of adult male rats

Parameters	Control males	Treated males
Number of males	10	10
Body weight (mean ± SD)	200 ± 1.25 g	196 ± 1.95 g
Testes weight (mean ± SD)	3.88 ± 0.19 g	2.75 ± 0.35 g[a]
Epididymis weight (mean ± SD)	0.61 ± 0.06 g	0.42 ± 0.08 g[a]
Prostate gland weight (mean ± SD)	0.54 ± 0.01 g	0.44 ± 0.03 g[a]
Seminal Vesicles weight (mean ± SD)	1.18 ± 0.25	0.86 ± 0.29[a]

[a] Significant differences from control based on Student's t-test at P < 0.05, results taken from (Morgan und El-Tawil, 2003)

Table AI - 23: Effect of exposure on fertility and reproductive performance on male and female rats

Parameters	Control group (10 male and 20 females)	Treated male group (10 treated males + 20 untreated females)	Treated female group (10 untreated males + 20 treated females)
Number of females showing regular estrous cycle	20	20	12
Number of females with confirmed matings	20	13	14
Number of pregnant females	19	6	10
Mating index (%)	100	65	70

Fertility index (%)	95	46.15	71.43
Number of dams showed delayed birth date	0	3	5
Number of dams showed signs of dystocia	0	1	4
Total number of corpora lutea	220	54	94
Total number of implantations	218	38	65
Number of implants/dam[a]	11.45 ± 0.55	6.15 ± 0.65[b]	6.32 ± 0.74[b]
Number of resorptions/dam[a]	0	1.21 ± 0.08 [b]	1.71 ± 0.13[b,c]
Number of dead foetuses/dam[a]	0.15 ± 0.05	1.12 ± 0.07 [b]	1.16 ± 0.12 [b]
Number of live festuses/dam[a]	11.32 ± 0.60	3.92 ± 0.35 [b]	3.38 ± 0.25 [b]
Preimplantation loss (%)	0.90 ± 0.04	29.63 ± 2.07 [b]	30.85 ± 2.16 [b]
Postimplantation loss (%)	0.92 ± 0.05	47.36 ± 3.31 [b]	46.15 ± 3.25 [b]
Body weight/dam at termination (g) [a]	252.1 ± 9.50	215.1 ± 10.5 [b]	209.5 ± 11.57 [b]
Gravid uterine weight/dam (g) [a]	67.50 ± 2.57	35.50 ± 3.20 [b]	30.35 ± 1.49 [b]
Placental weight/foetus (g)[a]	0.45 ± 0.01	0.31 ± 0.03 [b]	0.27 ± 0.02 [b]
Mean foetal body weight (g) [a]			
At birth	5.43 ± 0.12 (n = 216)	5.21 ± 0.23 (n = 20)	4.32 ± 0.2 [b,c] (n = 35)
At 4th day after birth	7.92 ± 0.50 (n = 216)	5.86 ± 0.40 [b] (n = 18)	5.15 ± 0.76 [b] (n = 30)
At 7th day after birth	8.95 ± 0.72 (n = 214)	6.21 ± 0.66 [b] (n = 17)	5.95 ± 0.76 [b] (n = 26)
At 14th day after birth	11.88 ± 0.83 (n = 214)	8.06 ± 0.86 [b] (n = 16)	7.02 ± 0.96 [b] (n = 24)
At 21th day after birth	22.51 ± 1.36 (n = 213)	15.02 ± 1.58 [b] (n = 16)	10.34 ± 1.56 [b,c] (n = 23)
Live/birth index (%)	100	100	100
Survival index (%)	100	90	85.71
Viability index (%)	99.07	85	74.28

[a] Values are expressed as mean ± S.E., [b] Significant difference between control and exposure group by one-way ANOVA at P < 0.05, [c] Significant difference between the two exposure groups by one-way ANOVA at P < 0.05, results taken from (Morgan und El-Tawil, 2003)

Table AI - 24: Indices of gross anomalies in the foetuses

Parameters	**Foetuses of control group**	**Foetuses of exposed male group**	**Foetuses of exposed female group**
Total foetuses examined	216	20	35
Stunted growth	0	40 [a]	48.57 [a]
Subcutaneous hemorrhages	0	35 [a]	85.71 [a,b]
Micrognathia	0	25 [a]	40 [a]

Values represent percentage of gross deformed foetuses in relation to total number of examined foetuses, [a] Significant difference between control and exposure group based on Chi-square test at $P < 0.05$, [b] Significant difference between the two exposure groups based on Chi-square test at $P < 0.05$, results taken from (Morgan und El-Tawil, 2003)

Table AI - 25: Indices of visceral anomalies in the foetuses

Parameters	**Foetuses of control group**	**Foetuses of exposed male group**	**Foetuses of exposed female group**
Total foetuses examined	72	7	12
Total foetuses with visceral anomalies	3	4	9
Total foetuses with head anomalies	0	2	3
- Dilated brain ventricles (hydrocephaly)	0	14.28[a]	25.0 [a]
- Dilated nares	0	28.57 [a]	25.0 [a]
- Olfactory pulp hypoplasia	0	14.28 [a]	16.66 [a]
- Cerebral hemisphere hypoplasia	0	28.57 [a]	25.0 [a]
- Micro- and anopthalmia	0	14.28 [a]	25 [a]
Total foetuses with thorax anomalies	1	3	5
- Heart hypertrophy	0	42.86 [a]	25.0 [a]
- Lung hypoplasia	0	28.57 [a]	33.33 [a]
- Intrathoracic hemorrhages	1.39	14.28 [a]	41.66 [a,b]
Total foetuses with pelvic anomalies	2	3	6
- Dilated renal pelvis (hydronephrosis)	0	42.86 [a]	41.67 [a]

- Hydroureter	2.78	28.57 [a]	33.34 [a]
- Renal hypoplasia	0	14.28 [a]	25.00 [a]

Values represent percentage of visceral deformed foetuses in relation to total number of examined foetuses, [a] Significant difference between control and exposure group based on Chi-square test at P < 0.05, [b] Significant difference between the two exposure groups based on Chi-square test at P < 0.05, results taken from (Morgan und El-Tawil, 2003)

Table AI - 26: Indices of skeletal anomalies in the foetuses

Parameters	**Foetuses of control group**	**Foetuses of exposed male group**	**Foetuses of exposed female group**
Total foetuses examined	144	13	23
Total foetuses with skeletal anomalies	1	8	15
Total foetuses with skull anomalies	0	4	9
- Wide separation of parietal bones	0	15.38 [a]	26.08 [a]
- Incomplete ossification of parietal bones	0	23.07 [a]	34.78 [a]
- Incomplete ossification of interparietal bones	0	30.77 [a]	30.43 [a]
Total foetuses with sternbrae anomalies	0	5	11
- Incomplete ossification of sternbrae	0	15.38 [a]	21.74 [a]
- Reduced sternbrae number	0	30.77 [a]	43.48 [a]
- Deformed ribs (wavy ribs)	0	23.07 [a]	26.08 [a]
- Extra ribs	0	7.69 [a]	17.39 [a]
Total foetuses with limb anomalies	1	6	13
- Absence of carpal and/or metacarpal bones	0	38.46 [a]	43.48 [a]
- Absence of tarsal and/or metatarsal bones	0	30.77 [a]	39.13 [a]
- Absence of phalanges	0.69	15.38 [a]	95.65 [a,b]
- Absence of caudal bones	0	23.07 [a]	30.43 [a]

Values represent percentage of skeletal deformed foetuses in relation to total number of examined foetuses, [a] Significant difference between control and exposure group based on Chi-square test at P < 0.05, [b] Significant

difference between the two exposure groups based on Chi-square test at $P < 0.05$, results taken from (Morgan und El-Tawil, 2003)

1.6.1.5 [Soazo & Garcia, 2007]

Study reference:

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

Detailed study summary and results:

Test type

Study on postnatal developmental toxicity in suckling rats, no information on GLP compliance is given.

Test substance

- Test material used in the study is not equivalent to the substance identified in the CLH dossier. Test material is Sodium metavanadate
- EC number: 13718-26-8
- CAS number: 13718-26-8
- Degree of purity: no information available
- Impurities: no information available
- Batch number: no information available

Test animals

- Wistar rats grouped in litters of 8 pups/dam (4 male and 4 female pups)
- Pups were 1 day old at the start of the study, no information on weight available

Administration/exposure

- Route of administration – daily intraperitoneal injection to dams from PND 10 – 21
- Dose levels: 0, 3 mg $NaVO_3$/kg bw (1.25 mg V/kg bw/d), dose was dictated by previous results (see (García et al., 2004)) which indicated changes in locomotor activity, specific myelin staining and lipid peroxidation in some brain areas in adult rats at comparable doses.
- Offspring of 4 dams in exposure group and offspring of 4 dams in control group. Control group dams were injected with saline solution.
- Historical control data: no information available
- Vehicle: distilled water, no further information available

- Test substance formulation: no information available

Description of test design:

- Animals were not mated
- Dams were treated daily from PND 10 – 21,
- Standardization of litters (yes on day 1 after birth, 4 male and 4 female animals per dams selected if possible)
- Offspring behavioural studies were performed every 48 h always at the same time of day (exception: Open field test which was only performed in 21st PND):
    - Surface righting reflex
    - Negative geotaxis
    - Forelimb field
    - Open field
- Histological analysis of brains (animals sacrificed on PND 22) with detection of myelin and myelin basic protein via histochemical staining
- Dams were weighted at the end of the study and observed during the study.

Results and discussion

- Statistical treatment of results: All data presented are litter mean ± SEM, evaluation was performed by ANOVA. Student's t-test was used when no interaction between sexes was observed. Some data were tested by Mann-Whitney test when differences between SDs were significant. Optical density data were statistically evaluated by an unpaired t-test. Significance level was set to $p < 0.05$.

Data on maternal animals:

- At the end of treatment dams showed lethargy and piloerection without any other signs of illness.
- Body weight was less in exposed animals (249 ± 23 g) compared to controls (259 ±23 g)

Offspring studies:

- No newborn mortality was observed. Body weight gain was not different between control and exposed pups and no signs of vanadium toxicosis (pallor, asphyxia, lethargy, diarrhoea) were observed.
- No difference in brain weight was observed
- Eye opening delayed in exposed pups: mean day in exposed pups was 16.3 ± 0.2 (control: 15.3 ± 0.3)

- Surface righting reflex:

- Progressive decrease in righting time was less marked in exposed pups (not statistically significant) (no numbers available, only figure in publication).

- Negative geotaxis
  - Similar effects observed for exposed and control animals (no numbers available, only figure in publication).

- Forelimb support
  - A decreased forelimb support latency on day 20 (significant effect) was observed in exposed animals. No significant differences observed at earlier time points (no numbers available, only figure in publication)

- Open field:
  - No difference between treated animals and controls observed for the number of rearing, grooming and fecal pellets. However, exposed pups showed a significant decrease in locomotor activity (expressed as number of crosses) (no numbers available, only figure in publication).

- Microscopic studies
  - Immunohistochemical stainings showed a decreased myelin fiber density in different brain areas exposed pups.
  - Computerized image analysis (CIA) confirmed the observations: Relative optical density (ROD, mean ± SEM), was 0.33 ± 0.005 in exposed and 0.41 ± 0.006 in control animals. In the corpus callosum ROD was 0.17 ± 0.007 in exposed pups (control group: 0.25 ± 0.008).
  - Immunostainig against Myelin-binding protein showed a decrease in staining in cerebellum and cerebrum of exposed pups.

1.6.1.6 [Cuesta et al., 2013]

Study reference:

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

Detailed study summary and results:

Test type

Study on postnatal developmental toxicity in suckling rats, No information on GLP compliance is given.

Test substance

- Test material used in the study is not equivalent to the substance identified in the CLH dossier. Test material is Sodium metavanadate
- EC number: 13718-26-8
- CAS number: 13718-26-8
- Degree of purity: no information available
- Impurities: no information available
- Batch number: no information available

Test animals

- Wistar rats grouped in litters of 8 pups/dam (4 male and 4 female pups)
- Pups were 1 day old at the start of the study, no information on weight available

Administration/exposure

- Route of administration – daily intraperitoneal injection to dams from PND 10 – 21
- Dose levels: 0, 3 mg $NaVO_3$/kg bw (1.25 mg V/kg bw/d), dose was dictated by previous results (see (García et al., 2004) and (Soazo und Garcia, 2007)) which indicated changes in locomotor activity, specific myelin staining and lipid peroxidation in some brain areas in adult rats at comparable doses.
- Offspring of 4 dams in exposure group and offspring of 4 dams in control group. Control group dams were injected with saline solution (equal amount as exposed animals: 400 -500 µL).
- Historical control data: no information available
- Vehicle: distilled water, no further information available
- Test substance formulation: no information available

Description of test design:

- Animals were not mated
- Dams were treated daily from PND 10 – 21,
- Standardization of litters (yes on day 1 after birth, 4 male and 4 female animals per dams selected if possible)
- Body weight and brain weight were measured in pubs at the end of the exposure period (PND 22)
- Determination of malondialdehyde (MDA) in different brain tissue
- Microscopic studies with brain tissue:
    - NADPHd histochemistry
    - HSP70 immunohistochemistry

  - GFAP immunohistochemistry
  - S100 immunohistochemistry
- Dams were weighted at the end of the study and observed during the study.

Results and discussion

- Statistical treatment of results: All data presented are litter mean ± SEM (tested with Student's t-test). All data for each litter were previously evaluated by ANOVA (looking for interaction between sexes). In all cases the significance level was $p < 0.05$.

Data on maternal animals:

  - Body weight gain was significantly reduced in exposed animals (-6.25 ± 2.87 g) compared to controls (2.00 ± 5.75 g).

Offspring studies:

- No newborn mortality was observed. Body weight gain was not different between control and exposed pups and no signs of vanadium toxicosis (pallor, asphyxia, lethargy, diarrhoea) were observed.
- No significant differences in body and brain weight were observed.

- MDA detection:
  - No significant differences were observed.

- Microscopic studies
  - NADPHd histochemistry
    No significant differences observed.

  - HSP70 immunohistochemistry
    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)

  - GFAP immunohistochemistry
    Astrogliosis: Glial fibrillary acidic protein (GFAP)-positive astrocytes were larger in exposure group. This was confirmed by computerized image analysis
    Cerebellum: GFAP-IR astrocytic surface in control pups 30.50 ± 2.8 µm², in exposed pups 36.30 ± 3.0 µm².)
    Hippocampal hilus: control pups 25.53 ± 1.39 µm², in exposed pups 33.79 ± 2.6 µm².). No differences in hippocampal subiculum

- S100 immunohistochemistry
  No significant differences observed.

1.6.1.7 [Mustapha et al., 2014]

Study reference:

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

Detailed study summary and results:

Test type

Postnatal developmental toxicity study with nursing mice, No information on GLP compliance is given.

Test substance

- Test material used in the study is not equivalent to the substance identified in the CLH dossier. Test material is Sodium metavanadate
- EC number: 13718-26-8
- CAS number: 13718-26-8
- Degree of purity: no information available
- Impurities: no information available
- Batch number: no information available

Test animals

- Nursing CD-1 mice
- 2 nursing females and their pubs /group
- No information on age and weight at the study initiation available

Administration/exposure

- Intraperitoneal injection in dams
- Dams were injected daily with 0 or 3 mg sodium metavanadate/kg bw.
- No rationale for dose level selection given
- Control group: treated intraperitoneally with sterile water
- Historical control data: no information given
- Vehicle: no information given

Description of test design:

- Dams were intraperitoneally injected for 14 days or 21 days starting on PND1of the pups. At the end of the exposure time behavioural test were performed and animals were sacrificed.
- standardization of litters: no
- Dams were not examined.
- For pups the following parameters were monitored:
    - Daily body weight
    - Behavioural tests (line crossing, centre square entries, centre square duration, rearing, freezing, stretch attend posture, grooming, negative geotaxis)
    - Immunohistochemistry (GFAP staining, MBP staining)
- In addition cell culture experiments with oligodendrocytes were performed, results are not presented here.

Results and discussion

- Statistical treatment of results: All data for animals are mean ± SEM. Results from behavioural tests were evaluated with the Student's t-test and Mann-Whitney U test. Results from cell cultures are presented as mean ± SD. P-values < 0.05 were associated with statistical significance. Statistical significance was assessed by Student´s two-tailed test for independent samples and with Mann-Whitney U-test for not-normally distributed data.

- Significant difference was observed in mean body weight between exposed and control animals between PND 15-21. (No numbers available, only figures shown in the publication).

- Differences between exposed and control animals were observed in behavioural test. Significant decreased effects were observed for "centre square duration" (exposure time of 22 days) and "stretch attend posture" (exposure time of 22 days). (No numbers available, only figures shown in the publication).

- GFAP-staining showed an upregulation of GFAP in the exposed groups. Examination on PND 15: moderate reactive astrogliosis in exposed animals, hypertrophy of soma. Examination on PND 22: severe diffuse reactive astrogliosis (no numbers available, only figures shown in the publication).

1.7 Specific target organ toxicity – repeated exposure

1.7.1 Animal data

1.7.1.1 [NTP, 2002]

Study reference:

NTP, Toxicology and Carcinogenesis Studies of Vanadium Pentoxide in F344/N Rats and B6C3F1 Mice (Inhalation Studies). TR 507, 2002 (NTP, 2002).

Detailed study summary and results:

Test type

2-year carcinogenicity study in rats and mice, no guideline followed, GLP compliance is given.

Male and female rats and mice (N = 50/sex/dose) were exposed to various concentrations of V_2O_5 6 h/d, for 5 d/w and for a period of 2 years. Clinical signs, body weights, blood and urine were observed during the study. Surviving animals were sacrificed at study end and necropsy was performed. Male and female F344 rats were exposed to 0, 0.5, 1 and 2 mg/m^3 V_2O_5, mice were exposed to 0, 1, 2 and 4 mg/m^3 V_2O_5.

A reliability of 2 is given for this study in the registration dossier.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: divanadium pentaoxide
- Degree of purity: approximately 99%
- Impurities: less than 1%
- Batch number: 1210140

Test animals

- Fischer 344 male and female rats and B6C3F1 male and female mice
- 50 male and female animals /dose group
- Diet: NTP-2000
- Rats:
    - Age at study initiation: 6 or 7 weeks on the first day of the study
    - Weight at study initiation for week 1: 134-136 g (males) and 130-105 g (females)
- Mice:
    - Age at study initiation: 6 or 7 weeks on the first day of the study
    - Weight at study initiation for week 1: 24.0-24.4 g (males) and 19.6-19.9 g (females)

Administration/exposure

- Route of administration – inhalation (aerosol)
- 6 h/d, 5 d/w for 104 weeks
- Rats: 0, 0.5, 1 and 2 mg/m^3 V_2O_5, mice: 0, 1, 2 and 4 mg/m^3 V_2O_5

  Rats: Dose selection rationale - based on the incidences and severities of respiratory lesions and increased lung weights in male and female rats in the 90-day study, concentrations of 4 mg/m^3 or greater were considered to be too high for use in a 2-year study.

  Mice: Dose selection rationale - the highest exposure concentration (4 mg/m^3) selected for mice was based on reduced body weight gain of 8 and 16 mg/m^3 males and females, increased lung weights of 4 mg/m^3 or greater in males and females, increased incidences of epithelial hyperplasia, and increased incidences and severities of inflammatory lesions of the lungs in mice exposed to 8 mg/m^3 versus those exposed to 4 mg/m^3 in the 90-day study.
- Control group animals were chamber exposed
- Historical control data is available for NTP studies
- No post exposure time
- Vehicle: air
- MMAD = 1.0-1.3 µm, GSD = 2.3-2.8
- The generation and delivery system consisted of a linear dust feeder, a particle attrition chamber, and an aerosol distribution system. The linear dust feeder, a slide-bar dust-metering device, was composed of a shuttle bar, body, outlet port, and hopper. As the compressed-air-driven shuttle bar slid back and forth during generation, the metering port aligned with the hopper, which served as a reservoir for the bulk chemical, and was filled with a small amount of divanadium pentaoxide powder. As the shuttle bar slid to the dispersing position, the metering port aligned with a compressed-air port in the body and a puff of air from this port dispersed the divanadium pentaoxide into the particle attrition chamber. Generator output was regulated by adjusting the cadence of the shuttle bar. The particle attrition chamber used low fluid energy from an air jet tangential to the chamber to deagglomerate the divanadium pentaoxide particles. After deagglomeration, the particles were swept into a classification zone where smaller particles exited to the distribution line; larger particles were thrown to the perimeter of the classifier by centrifugal force and were reentrained into the impacting air jet, and the process was repeated until the particles were sufficiently deagglomerated. The aerosol passed through the distribution lines to the exposure chambers. A pneumatic pump designed by the study laboratory was located at each chamber inlet and drew aerosol from the distribution line into the chamber inlet, where it was diluted with conditioned air to the appropriate concentration. Flow through the distribution line was controlled by Air-Vac pumps

(Air-Vac Engineering, Milford, CT), and pressure was monitored by photohelic differential pressure gauges (Dwyer Instruments, Inc., Michigan City, IN).

- The Stainless steel chambers (Lab Products, Inc., Harford Systems Division, Aberdeen, MD), were designed so that uniform aerosol concentrations could be maintained throughout the chambers when catch pans were in place. The total active mixing volume of each chamber was 1.7 m^3.
- The particle size distribution in each chamber was determined prior to the start of the study, during the first 2 weeks of the 2-year study, and monthly.
- A Mercer-style seven-stage impactor was used. The stages (glass coverslips lightly sprayed with silicon) were analyzed by ICP/AES, and the relative mass collected on each stage was analyzed by probit analysis.
- The uniformity of aerosol concentration in the inhalation exposure chambers without animals was evaluated before each of the studies began; concentration uniformity with animals present in the chambers was also measured. Chamber concentration uniformity was acceptable throughout the 2-year studies.
- The stability of divanadium pentaoxide in the exposure system was tested with XRD analysis. XRD analyses indicated no detectable build-up of degradation products at a detection limit of approximately 1%.
- During the studies, chamber aerosol concentrations were monitored with real-time aerosol monitors (RAMs) that used a pulsed-light-emitting diode in combination with a silicon detector to sense light scattered over a forward angular range of 45° to 95° by particles traversing the sensing volume. The instruments respond to particles 0.1 to 20 µm in diameter.
- The sampling system consisted of a valve that multiplexed each RAM to two or three exposure chambers and to a HEPA filter and/or the control chamber or room; selection of sampling streams and data acquisition from each RAM was remotely controlled by a computer. Equations for calibration curves were stored in the computers and were used to convert the measured voltages to exposure concentrations.
- Each RAM was calibrated two times per week by ICP/AES or ICP/mass spectrometry analysis of Pallflex® TX40H120WW glass fiber filters.

Results and discussion

<u>Rats:</u>

- Clinical signs: no effects
- Body weight gain: Body weights of the animals were similar to the controls except body weight of the 2 mg/m^3–exposed females which was less
- Food/water consumption: do data
- Ophthalmoscopic examination: no data

- Clinical chemistry: no data
- Haematology: no data
- Urinalysis: no data
- Organ weights: no data
- Mortality and time to death (see Table AI - 17 for details)

Table AI - 27: Survival data for male and female rats

	Chamber control	0.5 mg/m³	1 mg/m³	2 mg/m³
MALE rats				
Animals initially in the study	50	50	50	50
Moribund	25	17	18	13
Natural deaths	5	4	6	10
Animals surviving to study termination	20	29	26	27
Mean survival (days)	668	680	692	671
FEMALE rats				
Animals initially in the study	50	50	50	50
Moribund	14	20	17	15
Natural deaths	3	6	4	5
Animals surviving to study termination	33	24	29	30
Mean survival (days)	688	678	679	683

Results taken from (NTP, 2002)

HISTOPATHOLOGY: NON-NEOPLASTIC

Non-neoplastic lesions occurred in respiratory system of males and females (lung, larynx, and nose and the severities of these lesions generally increased with increasing exposure concentration.

LUNGS:

Effects in males: alveolar epithelium, hyperplasia (7/50, 24/49, 34/48, 49/50); bronchiole, epithelium hyperplasia (3/50, 17/49, 31/48, 49/50); alveolar epithelium, metaplasia, squamous (1/50, 0/49, 0/48, 21/50); bronchiole, metaplasia, squamous (0/50, 0/49, 0/48, 7/50); inflammation, chronic active (5/50, 8/49, 24/48, 42/50); interstitial, fibrosis (7/50, 7/49, 16/48, 38/50); alveolus, infiltration cellular, histiocyte (22/50, 40/49,45/48, 50/50).

Effects in females: alveolar epithelium, hyperplasia (4/49, 8/49, 21/50, 50/50); bronchiole, epithelium hyperplasia (6/49, 5/49, 14/50, 48/50); alveolar epithelium, metaplasia, squamous (0/49, 0/49, 0/50, 6/50); inflammation, chronic active (10/49, 10/49, 14/50, 40/50); interstitial, fibrosis (19/49, 7/49, 12/50, 32/50); alveolus, infiltration cellular, histiocyte (26/49, 35/49, 44/50, 50/50).

incidences of minimal to mild chronic active inflammation and interstitial fibrosis in the lungs were significantly increased in males exposed to 1 or 2 mg/m^3 and females exposed to 2 mg/m^3, and the incidences of histiocytic cellular infiltrate of the alveolus were increased in all exposed groups of males and females. The inflammatory lesions were primarily minimal to mild and consisted of interstitial and perivascular infiltrates of mostly mononuclear inflammatory cells that were occasionally within alveoli. Alveolar septa were occasionally thickened by thin strands of eosinophilic fibrillar material (fibrosis). The histiocytic infiltrate was also minimal to mild, consisting of scattered intraalveolar macrophages that contained large amounts of foamy intracytoplasmic material, interpreted as pulmonary surfactant. Additionally, scant amounts of eosinophilic material (surfactant) similar to that observed within alveolar macrophages was also free within alveoli; however, a separate diagnosis was not made. A brownish pigment (pigmentation) was visible in alveolar macrophages in some males and females exposed to 2 mg/m^3 and in females exposed to 1 mg/ m^3; it was a mild change considered of little biological significance and was not further characterized.

LARYNX:

Effects in males: inflammation, chronic (3/49, 20/50, 17/50, 28/49); respiratory epithelium, epiglottis degeneration (0/49, 22/50, 23/50, 33/49); respiratory epithelium, epiglottis, hyperplasia (0/49, 18/50, 34/50, 32/49); respiratory epithelium, epiglottis, metaplasia, squamous (0/49, 9/50, 16/50, 19/49).

Effects in females: inflammation, chronic (8/50, 26/49, 27/49, 37/50); respiratory epithelium, epiglottis degeneration (2/50, 33/49, 26/49, 40/50); respiratory epithelium, epiglottis, hyperplasia (0/50, 25/49, 26/49, 33/50); respiratory epithelium, epiglottis, metaplasia, squamous (2/50, 7/49, 7/49, 16/50).

There were increased incidences of minimal to mild lesions of the larynx in exposed males and females. The incidences generally increased with increasing exposure concentration and included chronic inflammation of the larynx and degeneration, hyperplasia, and squamous metaplasia of the respiratory epithelium of the epiglottis. The inflammation consisted of a mixture of mononuclear and granulocytic inflammatory cells in the submucosa beneath the epithelium lining the base of the epiglottis, ventral pouch, and caudal larynx. The degeneration of the respiratory epithelium was characterized by a loss or decrease in the height of cilia and shortening of the normally columnar to cuboidal surface epithelial cells lining the laryngeal surface of the base of the epiglottis. Squamous metaplasia was diagnosed when the ciliated cells were replaced by one or more layers of flattened squamous epithelium. In the same area, the respiratory epithelium was mildly thickened in many animals; this change was diagnosed as hyperplasia. These changes are relatively minimal, commonly occur in rats in NTP inhalation studies, and represent a common response to laryngeal injury.

NOSE:

Effects in males: goblet cell, respiratory epithelium, hyperplasia (4/49, 15/50, 12/49, 17/48)

Effects in females: goblet cell, respiratory epithelium, hyperplasia (13/50, 18/50, 16/50, 30/50)

There were increased incidences of mild goblet cell hyperplasia of the nasal respiratory epithelium in all groups of exposed male rats and in females exposed to 2 mg/m^3. Increased numbers of goblet cells were most notable in the respiratory epithelium lining the median septum adjacent to the area of the vomeronasal organ.

KIDNEY:

The incidences of nephropathy were significantly increased in male rats exposed to 1 or 2 mg/m^3 (37/50, 42/50, 46/49, 47/50). Nephropathy is a common lesion in aged rats, particularly males, and has been diagnosed in virtually all males in NTP 2-year studies that used the NIH-07 diet. In those studies, chemical exacerbation of nephropathy was identified by increased severity. With the NTP-2000 diet, the severity of spontaneous nephropathy has been reduced. In this study, the severity of nephropathy was not increased in exposed groups of males. Also, exposed females were not affected (24/50, 21/50, 20/49, 32/50). Although the NTP doesn't have a formal historical control database for non-neoplastic lesions, a review of recent studies indicates that the incidence in the male chamber control group in the current study is low. It is not clear if the increased incidences in this study were related to exposure to vanadium or were a reflection of the low incidence in the control group. Regardless, nephropathy was a relatively weak response and was likely of marginal biological significance.

For results on neoplastic lesions see section **Error! Reference source not found.**.

OTHER FINDINGS

LUNG BURDEN STUDIES:

Histopathology: the left lung lobe from each animal was infused with 10% neutral buffered formalin, and sections were examined microscopically. The purpose was to follow progression of the lung lesions. Following day 1 of exposure, there was an infiltrate of alveolar macrophages in the lungs. With continued exposure, increased numbers of alveolar macrophages, interstitial mononuclear inflammatory cell infiltrates, and hyperplasia of alveolar and bronchiolar epithelium were observed. In rats exposed to 2 mg/m^3, there was an increase in severity of the hyperplasia between days 54 and 173. An increase in severity was not obvious between days 173 and 360, but hyperplasia appeared more severe on day 542. Hyperplasia was observed in only a few animals exposed to 1 mg/ m^3 and only on day 542. The minimal fibrosis observed in the 2-year study was not readily apparent on day 542 or earlier.

Lung weights from exposed female rats increased throughout the study. Although there appeared to be an exposure concentration-related increase in lung weights after day 26 of the study, it was primarily due to increases in lung weights of female rats exposed to 2 mg/m^3. In general, lung weights of 0.5 or 1 mg/m^3 females were similar.

Though deposition patterns were similar between rats and mice, the maximum lung burdens occurred at day 173 in rats. The lung burdens appeared to reach steady state at the lowest exposure concentrations (0.5

mg/m^3). A decline in lung burdens was observed. The retention of vanadium in the lungs at 18 months was ca. 13% to 15% in rats. The total lung doses for rats exposed to 0.5, 1, or 2 mg/m^3 were estimated to be 130, 175, and 308 µg vanadium, respectively.
Lung clearance half-times were considerably longer than those observed in the 16-day special studies.

BLOOD:
Vanadium was detected in the blood at concentrations several orders of magnitude lower than those measured in the lungs of exposed rats, and blood vanadium concentrations in exposed groups were only marginally increased over that of the chamber control group. Overall, blood vanadium concentrations appeared to increase with increasing exposure concentration; however, this proportionality was less clear when the 0.5 and 1 mg/m^3 groups were compared.
Blood vanadium concentrations in all exposed groups appeared to peak on days 26 or 54 after which there was a decline throughout the rest of the study. This response was similar to that seen in lung burdens. However, these changes in concentrations were small, making it difficult to determine if there was an increase in elimination of vanadium from the blood or a decreased absorption from the lung due to reduced deposition, especially at the higher exposure concentrations.

Effect level (according to registration dossier):
$LOAEC_{local}$: 0.5 mg/m³ air (males and females) based on non-neoplastic changes (epithelial hyperplasia, squamous metaplasia, chronic inflammation, degeneration) in the respiratory system (lung, larynx, and nose) of male and female rats.

<u>Mice:</u>

- Clinical signs: Survival of the highest dosed males (4 mg/m³) was significantly less than controls. Abnormal breathing was observed particularly those exposed to 2 or 4 mg/m³. Many mice exposed to divanadium pentaoxide were thin
- Body weight gain: Mean body weight of all females and highest dosed males was below the controls; and mean body weights of males exposed to 2 mg/ m^3 were less from week 85 to the end of the study
- Food/water consumption: do data
- Ophthalmoscopic examination: no data
- Clinical chemistry: no data
- Haematology: no data
- Urinalysis: no data
- Organ weights: no data
- Mortality and time to death (see Table AI - 28 below)

Table AI - 28: Survival data for male and female mice

	Chamber control	0.5 mg/m³	1 mg/m³	2 mg/m³
MALE mice				
Animals initially in the study	50	50	50	50
Moribund	7	11	13	20
Natural deaths	4	6	1	3
Animals surviving to study termination	39	33	36	27
Mean survival (days)	710	692	704	668
FEMALE mice				
Animals initially in th study	50	50	50	50
Moribund	8	10	14	16
Natural deaths	4	5	4	2
Animals surviving to study termination	38	32	30	32
Mean survival (days)	692	655	653	688

Results taken from (NTP, 2002)

HISTOPATHOLOGY: NON-NEOPLASTIC

Non-neoplastic lesions occurred in respiratory system (lung, larynx, and nose) of males and females, and the severities of these lesions generally increased with increasing exposure concentration.

Bronchial lymph node hyperplasia was present in most of the exposed females.

LUNG:

There were significantly increased incidences of alveolar epithelial hyperplasia and bronchiolar epithelial hyperplasia in the lungs of exposed male and female mice. Incidences of chronic inflammation and histiocytic cellular infiltrate were significantly increased in exposed groups of mice, and the incidences of interstitial fibrosis were increased in mice exposed to 2 or 4 mg/m^3.

Males: alveolar epithelium, hyperplasia (3/50, 41/50, 49/50, 50/50); bronchiole, epithelium, hyperplasia (0/50, 15/50, 37/50, 46/50); inflammation chronic (6/50, 42/50, 45/50, 47/50); alveolus, infiltration cellular, histiocyte (10/50, 36/50, 45/50, 49/50); interstitial fibrosis (1/50, 6/50, 9/50, 12/50)

Females: alveolar epithelium, hyperplasia (0/50, 31/50, 38/50, 50/50); bronchiole, epithelium, hyperplasia (0/50, 12/50, 34/50, 48/50); inflammation chronic (4/50, 37/50, 39/50, 49/50); alveolus, infiltration cellular, histiocyte (0/50, 34/50, 35/50, 45/50); interstitial fibrosis (0/50, 1/50, 4/50,
8/50)

The hyperplasia was essentially a diffuse change with proliferation of epithelium in the distal terminal bronchioles and immediately associated alveolar ducts and alveoli. Normally flattened epithelium was replaced with cuboidal epithelium. The hyperplasia of the alveolar epithelium was pronounced and increased

in severity with increasing exposure concentration, while the hyperplasia of the distal bronchioles was minimal to mild with slight increases in severity in mice exposed to 4 mg/m^3. The changes in mice were similar to those observed in rats but were not as pronounced.

The inflammatory lesions were primarily minimal to mild and consisted of interstitial, perivascular, and peribronchiolar infiltrates of mostly mononuclear inflammatory cells (mostly lymphocytes) that were occasionally present within alveoli. The most prominent histiocytic infiltrate occurred within alveoli in close proximity to alveolar/bronchiolar neoplasms, particularly carcinomas.

LARYNX:

There were significantly increased incidences of minimal squamous metaplasia of the respiratory epithelium of the epiglottis in exposed groups.

Males: respiratory epithelium, epiglottis, metaplasia, squamous (2/49, 45/50, 41/48, 41/50)

Females: respiratory epithelium, epiglottis, metaplasia, squamous (0/50, 39/50, 45/49, 44/50)

Squamous metaplasia was diagnosed when the ciliated cells were replaced by one to three layers of flattened squamous epithelium. This change commonly occurs in mice in NTP inhalation studies and represents a common response to laryngeal injury.

NOSE:

There were increased incidences of minimal to mild suppurative inflammation of the nose in males and females exposed to 2 or 4 mg/m^3.

Males: inflammation suppurative (16/50, 11/50, 32/50, 23/50); olfactory epithelium, degeneration, hyaline (1/50, 7/50, 23/50, 30/50); respiratoryepithelium, degeneration, hyaline (8/50, 22/50, 38/50, 41/50); respiratory epithelium, metaplasia, squamous (0/50, 6/50, 6/50, 2/50)

Females: inflammation suppurative (19/50, 14/50, 32/50, 30/50); olfactory epithelium, atrophy (2/50, 8/50, 5/50, 14/50); olfactory epithelium, degeneration, hyaline (11/50, 23/50, 34/50, 48/50); respiratory epithelium, degeneration, hyaline (35/50, 39/50, 46/50, 50/50); respiratory epithelium, metaplasia, squamous (0/50, 3/50, 7/50, 8/50); respiratory epithelium, necrosis (0/50, 0/50, 1/50, 7/50)

The inflammation consisted of focal aggregates of few to moderate numbers of neutrophils generally subjacent to the epithelium of the turbinates, septum, or lateral wall of the anterior nose. In the more severe cases (predominantly in females exposed to 4 mg/m^3), a short segment of the overlying epithelium was ulcerated (necrosis). Similarly, in some males and females, the overlying respiratory epithelium was replaced by one or more layers of flattened epithelium (squamous metaplasia), and the incidences were marginally increased in some exposed groups of mice.

The majority of the olfactory epithelium covers the turbinates in the distal portion of the nose. There were marginal but significant increases in the incidences of atrophy of this epithelium in females exposed to 1 or 4 mg/m^3.

BRONCIAL LYMPH NODE

There were significant increases in the incidences of hyperplasia of the bronchial lymph node in exposed groups of females, and while not significant, a positive trend in the incidences of this lesion also occurred in males.

Females: hyperplasia (3/39, 13/40, 14/45, 20/41)

For results on neoplastic lesions see section **Error! Reference source not found.**.

OTHER FINDINGS

TISSUE BURDEN STUDIES:

Histopathology: lung lesions were identified early, and were more severe in females exposed to higher concentrations and progressed with time. Minimal bronchiolar epithelial hyperplasia and interstitial inflammation were initially observed in the 4 mg/m^3 group on day 5. Bronchiolar epithelial hyperplasia was characterized by slight piling up of cells, some nuclear pleomorphism, and occasional karyorrhectic cells. On day 12, exposure-related lesions were evident in 2 mg/m^3 females, while alveolar epithelial hyperplasia was more prominent in the 4 mg/m^3 group. Lesions were first observed in the 1 mg/m^3 group on day 54, and lesion severities in the 2 and 4 mg/m^3 groups appeared to be increased on day 54. On day 362, lesion severity in lungs of females exposed to 4 mg/m3 was noticeably increased, and bronchiolar hyperplasia included more nuclear pleomorphism. On day 535, mice exposed to 2 mg/m^3 had hyperplastic alveolar epithelial lesions and, similar to females exposed to 4 mg/m^3, the hyperplasia was oriented around the terminal bronchiolar/alveolar duct. Also on day 535, lung nodules/masses were observed. While some were confirmed as neoplasms, many were in the right lung lobe and thus were not confirmed histopathologically. Similar lesions, although more severe, were observed in the core group of animals at 2 years.

Lung burden data appeared proportional to exposure concentration in mice. Rats removed vanadium from the lungs much slower than mice, with clearance half-times of vanadium lung burdens approximately six to nine fold longer in rats than in mice at comparable exposure concentrations. In rats and mice, lung clearance half-times were considerably longer than those observed in the 16-day special studies.

The maximum lung burdens occurred between days 26-54 in mice. The lung burdens appeared to reach steady state at the lowest exposure concentrations (1 mg/m^3). A decline in lung burdens was observed. The retention of vanadium in the lungs at 18 months was 2% to 3% in mice. The total lung doses for mice exposed to 1, 2, or 4 mg/m^3 were estimated to be 153, 162, and 225 µg vanadium, respectively.

Effect level (according to registration dossier):

$NOAEL_{local}$: 1 mg/m³ air (males and females) based on non-neoplastic changes (epithelial hyperplasia, squamous metaplasia, chronic inflammation, degeneration) in the respiratory system (lung, larynx, and nose) of male and female mice. (In the conclusion however, a $LOAEC_{local}$ of 1 mg/m³ is reported. With regard to the data, the description “LOAEC” seems to be correct.)

1.7.1.2 [NTP, 2002]

Study reference:

NTP, National Toxicology Program, 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, 2002 (NTP, 2002)

Detailed study summary and results:

Test type

Groups of 10 male and 10 female rats were exposed to particulate aerosols of divanadium pentaoxide at concentrations of 0, 1, 2, 4, 8, or 16 mg/m^3 by inhalation, 6 hours per day, 5 days per week for 3 months. Effects on fertility and sexual function of male and female rats were evaluated after 90 days of inhalation exposure. GLP compliance is given.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: divanadium pentaoxide
- Degree of purity: approximately 99%
- Impurities: less than 1%
- Batch number: 1210490

Test animals

- Fischer 344 male and female rats, 10 male and female animals /dose group
- Age at study initiation: average age: 6 or 7 weeks on the first day of the study
- Weight at study initiation: 133-137 g (males) and 108-111 g (females)
- Diet: NIH-07

Administration/exposure

- Route of administration – inhalation (aerosol)
- 6 hours per day + T_{90} (15 min), 5 days per week for 3 months
- 0, 1, and 2, 4, 8, 16 mg/m^3 V_2O_5
- Dose selection rationale - based on decreased survival in the 32 mg/m^3 males and body weight decreases in 32 mg/m^3 males and females in a 16-day study, an exposure concentration of 32 mg/m^3 was considered too high for use in a 3-month study.

- Historical control data is available for NTP studies
- No post exposure time
- Vehicle: air
- Generation of test atmosphere / chamber description: For the 3-month studies, divanadium pentaoxide aerosol generation was based on the principle of pneumatic dispersion and consisted of two major components: a screw feeder (Model 310, Accurate, White Water, WI) that metered divanadium pentaoxide powder at a constant rate and a Jet-O-Mizer jetmill (Fluid Energy Corp., Harfield, PA) that used compressed air to disperse the metered powder and form the aerosol.

  Aerosol leaving the jetmill passed through a one-stage impactor and a vertical elutriator to eliminate or deagglomerate the large particles before entering a plenum and manifold distribution system. The aerosol delivery system consisted of three holding chambers that diluted the aerosol in three stages. A metered amount of diluted aerosol was removed and mixed with conditioned air at the inlet to each exposure chamber to achieve the appropriate exposure concentration. The electrical charge buildup on the aerosol particles was neutralized by mixing the aerosol with high concentrations of bipolar ions, which were generated using a Pulse Gun (Static Control Services, Palm Springs, CA) air nozzle. For the 3-month studies, a transvector air pump was installed at the aerosol inlet to each exposure chamber to provide additional control of the aerosol flow rate and improve stability of the chamber concentration.

  The stainless-steel inhalation exposure chambers (Lab Products, Inc., Maywood NJ), were designed so that uniform aerosol concentrations could be maintained throughout the chambers when catch pans were in place. The total active mixing volume of each chamber was 1.7 m^3.
- CHAMBER ATMOSPHERE CHARACTERIZATION

  The particle size distribution in each chamber was determined prior to the start of all studies, during the first week of the 3-month studies, and monthly during the 3-month study. For the 3-month study, a 10-stage Quartz Crystal Microbalance-based cascade impactor was used to separate the aerosol particles into sequential size ranges; the mass median aerodynamic diameter was calculated from the corresponding mass fraction of particles at each stage.
- OTHER

  The uniformity of aerosol concentration in the inhalation exposure chambers without animals was evaluated before each of the studies began; concentration uniformity with animals present in the chambers was also measured. During the 3-month studies, minor excursions in chamber uniformity values were observed in one or more exposure chambers, but these excursions had no impact on the studies.

  The stability of divanadium pentaoxide in the exposure system was tested with XRD analysis. XRD analyses indicated no detectable build-up of degradation products at a detection limit of approximately 1%.

- Details on analytical verification of doses or concentrations:

  Chamber aerosol concentrations were monitored with real-time aerosol monitors (RAMs) that used a pulsed-light-emitting diode in combination with a silicon detector to sense light scattered over a forward angular range of 45° to 95° by particles traversing the sensing volume. The instruments respond to particles 0.1 to 20 µm in diameter.

  During the 16-day study, an individual monitor was used for each exposure chamber. The voltage output of the online monitors was read and recorded, and the calibration curve was applied to the voltages measured by the RAM to convert the measured voltages to exposure chamber concentrations.

  Each RAM was calibrated daily during the 16-day study by correlating the measured voltage with divanadium pentaoxide concentrations determined by gravimetric analysis of glass fiber filters.

Results and discussion

- CLINICAL SIGNS AND MORTALITY

  - 7 males and 3 females exposed to 16 mg/m^3 died during the study

  - In the highest dose groups (16 mg/m^3) abnormal breathing, thinness, lethargy, abnormal posture and ruffled fur were observed

  - Diarrhea and nasal/eye discharge were observed in some rats in the 16 mg/m^3 groups.

- BODY WEIGHT AND WEIGHT GAIN

  - Final mean body weights and body weight gains of males exposed to 4 mg/m^3 or greater and of females exposed to 16 mg/m^3 were significantly less than those of the chamber controls

- HAEMATOLOGY

  - Microcytic erytrocytosis was observed in males and females

  - Exposure of rats to vanadium pentoxide affected the circulating red cell mass

  - In general, the erythrocyte counts reflected similar and proportional, but not significant, decreases.

  - There were exposure concentration-related decreases in the mean cell volumes and mean cell hemoglobin values on day 23, which are consistent with a developing erythrocyte microcytosis.

  - The mean cell hemoglobin concentration was minimally decreased in 16 mg/m^3 females

  - Microscopic evaluation of the red blood cell morphology detected increased polychromasia, anisochromia, and hypochromia in the 16 mg/m^3 groups

  - At week 13, there was evidence of an exposure-related leukopenia, demonstrated by decreases in leukocyte counts in 16 mg/m^3 males and females; leukopenia appeared to be related to decreased lymphocyte counts. This alteration in lymphocyte counts suggests a physiological response and would be consistent with a stress-related corticosteroid- induced lymphopenia. Rats are considered a steroid-sensitive species and corticosteroid-induced lymphopenia may be related to lympholysis in blood and altered distribution (Jain, 1986).

  - The 16 mg/m^3 males and females demonstrated microscopic evidence of lymphoid depletion in

numerous lymphoid organs. Since there was an exceptional effect on body weights of 16 mg/m^3 rats, it is possible that these animals were under significant stress that then lead to increased endogenous corticosteroid concentrations and subsequent decreases in tissue and blood lymphocytes.

- CLINICAL CHEMISTRY

  - At all time points, evidence of a hepatocellular effect was demonstrated by increases in serum alanine aminotransferase activities in 8 and 16 mg/m^3 males and females. However, sorbitol dehydrogenase activities, another marker of hepatocellular injury or leakage, were not affected, and there were no histopathologic liver changes consistent with hepatocellular injury. This suggests that the increased alanine aminotransferase activities were not related to hepatocellular injury. Studies have shown that corticosteroids can induce increases in liver alanine animotransferase activities (Rosen, 1959; Rosen et al., 1959). Thus, stress-related corticosteroid-induced increases in liver alanine aminotransferase activities may, in part, help explain increased serum alanine aminotransferase activities.

  - At all time points, there were generally exposure concentration-related decreases in serum alkaline phosphatase activities and total protein and albumin concentrations in males and females. At week 13, urea nitrogen concentrations were increased in 16 mg/m^3 males and females, suggesting a possible effect on renal clearance. However, creatinine concentrations, another marker of renal clearance, were minimally decreased on day 23 in 8 mg/m^3 males and at week 13 in 16 mg/m^3 males and females; these decreases would be consistent with the decreased body weights observed in these groups. Since alkaline phosphatase activity and total protein, albumin, and urea nitrogen concentrations can be affected by altered nutritional status, the changes in these variables may have been related secondarily to body weight decreases and altered food intake.

  - Significant increases and decreases in bile acid concentrations and creatine kinase activities in various exposure groups at various time points were not considered to be toxicologically relevant.

- URINALYSIS

  - After 12 (females) or 13 (males) weeks of exposure, the baseline (water-replete) overnight urine collection demonstrated decreased urine volumes and increased urine specific gravities in the 8 mg/m^3 male and 16 mg/m^3 female groups, suggesting these animals were able to concentrate their urine; the 16 mg/m^3 male group was not tested. These findings also suggest that these exposure groups may have been in a partially dehydrated state prior to the water deprivation studies.

  - The 4-hour urine collection following a 16-hour water deprivation period demonstrated no differences in urine volume or specific gravity. This suggests that animals in all exposed groups maintained their ability to concentrate urine.

  - For baseline and water deprivation test samples, microscopic evaluation of the urine demonstrated slight increases in formed elements in 8 mg/m3 males (baseline: casts, epithelial cells, erythrocytes, and leukocytes; water-deprived: casts) and 16 mg/m^3 females (baseline and water deprived: leukocytes) (data not presented). While changes in formed urine elements can be indicative of

various renal effects, the alterations in this study were not excessive and possibly reflected the hydration status of the animals; they were not considered to be toxicologically relevant.

- ORGAN WEIGHTS

  -Absolute and relative lung weights were significantly higher at and above 4 mg/m^3 in males and females than for the chamber controls as were the relative lung weights of 2 mg/m^3 males

  - Other organ weight differences were considered to be related to body weight decreases

- GROSS PATHOLOGY

  - The carcasses of males and females exposed to 16 mg/m^3 were very thin, and the spleens and thymuses appeared disproportionately small

  - Lungs of 4 mg/m^3 or greater males and 8 and 16 mg/m^3 females varied from red to pale or mottled.

- HISTOPATHOLOGY: NON-NEOPLASTIC

  Lungs:

  - Incidence if several nonneoplastic lesions of the lung and nose were increased in animals exposed to 2 mg/m^3 or greater

  - There were significant increases in the incidences of epithelial hyperplasia of the lung in males and females exposed to 2 mg/m^3 or greater. The epithelial hyperplasia occurred in the distal airways and associated alveolar ducts and alveoli. In the distal airways, the epithelium was composed of ciliated cuboidal to columnar cells crowded together in multiple layers, sometimes forming folds and small papillary projections. More distal and including the alveolar ducts and alveoli, the epithelium tended to be a single layer. The epithelial cells were larger and rounded and appeared morphologically similar to bronchioles. Goblet cells sometimes occurred within the epithelium of some of the smaller airways. Alveoli often contained one or two cells that were very large and occasionally binucleate.

  - The incidences of inflammation or fibrosis were significantly increased in males exposed to 2 mg/m^3 or greater and females exposed to 4 mg/m^3 or greater.

  - In one female exposed to 16 mg/m^3, a single focus of squamous metaplasia was observed within an area of hyperplasia.

  - Exudate of the bronchiole was diagnosed in many males and females exposed to 8 or 16 mg/m^3 and was characterized by a mucoid material with admixed inflammatory cells within the lumens of some of the hyperplastic airways. The inflammation was characterized by accumulations of alveolar macrophages with abundant foamy cytoplasm in the alveoli adjacent to airways lined by hyperplastic epithelium. Mononuclear cells were also observed within the interstitium.

  - Foci of fibrosis were found in the same areas and sometimes extended into surrounding alveoli. These foci were irregularly shaped and consisted of loosely arranged, delicate collagen fibers, fibroblasts, and mononuclear cells.

  Nose:

  - The incidences of hyperplasia and metaplasia of the nasal respiratory epithelium were significantly

increased in males exposed to 8 or 16 mg/m^3 and in females exposed to 4 mg/ m^3 or greater. There were significantly increased incidences of inflammation of the nose in males and females exposed to 16 mg/m^3. The hyperplasia and metaplasia involved the respiratory epithelium covering 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. There were cellular crowding and epithelium thickening (hyperplasia) in some areas with loss of goblet cells and one or more layers of flattened nonkeratinizing squamous epithelium (metaplasia) in others.

- Inflammation was characterized by an infiltrate primarily of lymphocytes and lesser numbers of neutrophils within the affected mucosa and subjacent submucosa. Occasionally, neutrophils predominated, and an exudate was present.

Other:

- 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 in 16 mg/m^3 males and females and hypospermia of the testis and atypical cells of the epididymis were observed in 16 mg/m^3 males. These lesions may have been associated with the marked body weight loss and general debilitation of these rats.

OTHER FINDINGS

- Significant exposure-related changes in pulmonary function were observed in male and female rats exposed to 4, 8, or 16 mg/m^3; these results indicate that more restrictive lesions were present in groups exposed to 4 mg/m^3 or greater, evidenced by reduced lung compliance, changes in breathing measurements, impaired capacity to diffuse carbon monoxide, reduced static and dynamic lung volumes, and exaggerated flows.
- Pulmonary function changes indicate an obstructive disease in the 16 mg/m^3 groups, evidenced by changes in breathing mechanics, static lung volumes, and forced expiratory maneuvers. Expiratory resistance, an indicator of bronchoconstriction, and end expiratory and residual volume were increased, while dynamic lung volume was decreased.
- Together, the pulmonary function changes indicate that a restrictive disease was present in male and female rats exposed to 4 mg/m^3 or greater, while an obstructive lung disease may have been present only in the 16 mg/m^3 groups.
- The pulmonary lavage data indicate an inflammatory response in the lungs of exposed rats. Exposure concentration-related increases were observed in the total numbers of cells, lymphocytes, neutrophils, and protein recovered in pulmonary lavage fluid from rats exposed to vanadium pentoxide at concentrations up to 8 mg/m^3.

1.7.1.3 [NTP, 2002]

Study reference:

NTP, National Toxicology Program, 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, 2002 (NTP, 2002)

Detailed study summary and results:

Test type

Groups of 10 male and 10 female mice were exposed to particulate aerosols of divanadium pentaoxide at concentrations of 0, 1, 2, 4, 8, or 16 mg/m^3 by inhalation, 6 hours per day, 5 days per week for 3 months. Effects on fertility and sexual function of male and female rats were evaluated after 90 days of inhalation exposure. GLP compliance is given.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: divanadium pentaoxide
- Degree of purity: approximately 99%
- Impurities: less than 1%
- Batch number: 1210490

Test animals

- B6C3F1 male and female mice, 10 male and female animals /dose group
- Age at study initiation: average age: 6 or 7 weeks
- Diet: NIH-07
- Weight at study initiation (mean): 25-26 g (males) and 20-21 g (females)

Administration/exposure

- Route of administration – inhalation (aerosol)
- 6 hours per day + T_{90} (15 min), 5 days per week for 3 months
- 0, 1, and 2, 4, 8, 16 mg/m^3 V_2O_5
- Dose selection rationale - based on decreased survival in the 32 mg/m^3 males and body weight decreases in 32 mg/m^3 males and females in a 16-day study, an exposure concentration of 32 mg/m^3 was considered too high for use in a 3-month study.
- Historical control data is available for NTP studies
- No post exposure time
- Vehicle: air

- Generation of test atmosphere / chamber description: For the 3-month studies, divanadium pentaoxide aerosol generation was based on the principle of pneumatic dispersion and consisted of two major components: a screw feeder (Model 310, Accurate, White Water, WI) that metered divanadium pentaoxide powder at a constant rate and a Jet-O-Mizer jetmill (Fluid Energy Corp., Harfield, PA) that used compressed air to disperse the metered powder and form the aerosol.

  Aerosol leaving the jetmill passed through a one-stage impactor and a vertical elutriator to eliminate or deagglomerate the large particles before entering a plenum and manifold distribution system. The aerosol delivery system consisted of three holding chambers that diluted the aerosol in three stages. A metered amount of diluted aerosol was removed and mixed with conditioned air at the inlet to each exposure chamber to achieve the appropriate exposure concentration. The electrical charge buildup on the aerosol particles was neutralized by mixing the aerosol with high concentrations of bipolar ions, which were generated using a Pulse Gun (Static Control Services, Palm Springs, CA) air nozzle. For the 3-month studies, a transvector air pump was installed at the aerosol inlet to each exposure chamber to provide additional control of the aerosol flow rate and improve stability of the chamber concentration.

  The stainless-steel inhalation exposure chambers (Lab Products, Inc., Maywood NJ), were designed so that uniform aerosol concentrations could be maintained throughout the chambers when catch pans were in place. The total active mixing volume of each chamber was 1.7 m^3.

- CHAMBER ATMOSPHERE CHARACTERIZATION

  The particle size distribution in each chamber was determined prior to the start of all studies, during the first week of the 3-month studies, and monthly during the 3-month study. For the 3-month study, a 10-stage Quartz Crystal Microbalance-based cascade impactor was used to separate the aerosol particles into sequential size ranges; the mass median aerodynamic diameter was calculated from the corresponding mass fraction of particles at each stage.

- OTHER

  The uniformity of aerosol concentration in the inhalation exposure chambers without animals was evaluated before each of the studies began; concentration uniformity with animals present in the chambers was also measured. During the 3-month studies, minor excursions in chamber uniformity values were observed in one or more exposure chambers, but these excursions had no impact on the studies.

  The stability of divanadium pentaoxide in the exposure system was tested with XRD analysis. XRD analyses indicated no detectable build-up of degradation products at a detection limit of approximately 1%.

Details on analytical verification of doses or concentrations:

Chamber aerosol concentrations were monitored with real-time aerosol monitors (RAMs) that used a pulsed-light-emitting diode in combination with a silicon detector to sense light scattered over a forward angular range of 45° to 95° by particles traversing the sensing volume. The instruments respond to particles 0.1 to 20 µm in diameter.

During the 16-day study, an individual monitor was used for each exposure chamber. The voltage output of the online monitors was read and recorded, and the calibration curve was applied to the voltages measured by the RAM to convert the measured voltages to exposure chamber concentrations.

Each RAM was calibrated daily during the 16-day study by correlating the measured voltage with divanadium pentaoxide concentrations determined by gravimetric analysis of glass fiber filters.

Results and discussion

- CLINICAL SIGNS AND MORTALITY

  - One male exposed to 16 mg/m³ died before the end of the study
- BODY WEIGHT AND WEIGHT GAIN

  - Mean body weights of 8 and 16 mg/m³ males and 4 mg/m³ or greater females were significantly less than those of the chamber controls
- ORGAN WEIGHTS

  - 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

  - The absolute lung weight was also significantly increased in males exposed to 2 mg/m^3 and above

  - Other organ weight changes were considered related to body weight decreases
- GROSS PATHOLOGY

  - Gross findings were observed in males and females exposed to 8 or 16 mg/m^3 and included lungs that were pale or contained white or red (females) foci; the lungs of males in these groups were sometimes gray or mottled.
- HISTOPATHOLOGY: NON-NEOPLASTIC

  - Some mice exposed to 2 or 4 mg/m³ and all mice at and above 8 mg/m^3 had inflammation and epithelial hyperplasia of the lung. The severities of these lesions generally increased with increasing exposure concentration.

1.7.1.4 [Moutain et al., 1953]

Study reference:

Mountain, J.T. et al., Studies in vanadium toxicology, AMA Arch. Ind. Hyg. Occup. Med. 8, 406-411, 1953. (Mountain et al., 1953)

Detailed study summary and results:

Test type

In a subchronic mechanistic study, cysteine content of the hair of rats fed diets containing vanadium pentoxide was measured to determine if there were changes in cysteine content indicative of abnormal metabolism.

Groups of 5 male rats were given diets supplemented with vanadium at both low and high levels. In a separate experiment, methionine was also included in the diet of vanadium fed rats to determine whether this sulfur amino acid would counteract the effects of vanadium.

The study was not conducted according to a guideline and GLP compliance was not met (though study was published before GLP was introduced).

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: Divanadium pentaoxide
- No further information on test substance was stated.

Test animals

- Rat/Wistar/male
- 5 animals per dose, except 7 animals in the high level vanadium pentoxide with added methionine exposure group
- weight at the study initiation: 200 to 350 g (details for low level vanadium pentoxide exposure group)
- No further information on test animals was stated.

Administration/exposure

- route of administration – oral (feed)
- duration and frequency of test/exposure period: daily treatment
    - low level vanadium exposure: 103 days
    - high level vanadium exposure: 75 days
    - high level vanadium with added methionine exposure: 63 days
- doses/concentration levels, rationale for dose level selection:
    - low level vanadium group: 25 ppm and 50 ppm (first 35 days); 100 ppm and 150 ppm (36 - 103 days)
    - high level vanadium group: 500 and 1000 ppm; a fourth group of five rats which served as group-paired fed control for those receiving 500 ppm was added.

    - high level vanadium with added methionine group: 500 ppm with or without methionine
- post exposure observation period: not specified
- vehicle: Purina Dog Chow Checkers (diet, no further information stated)
- control group and treatment:
    - low level vanadium group: one control group of five rats
    - high level vanadium group: one control group of five rats (food ad libitum) and one group of 5 rats as group-paired fed control for those receiving 500 ppm
    - high level vanadium with added methionine group: group of six rats was fed a diet containing 500 ppm V only and six rats in the control-fed group
- test substance formulation/diet preparation, achieved concentration by sex and dose level, stability and homogeneity of the preparation:
    - high level vanadium with added methionine group: dl-methionine in amounts of 1.6 % was added to diet (containing 500 ppm V as pentoxide) of one group of rats
- analytical verification of doses: metallic composition of the stock and vanadium-supplemented diets were determined by spectrographic analysis. Stock diet of the rats contained an average of 0.7 γ of vanadium per gram
- actual dose (mg/kg bw/day) and conversion factor from diet test substance concentration (ppm) to the actual dose: conversion given for LOEL (ppm in diet) to LOAEL (mg/kg bw/d) in section result and discussion
- statistical methods: analysis of variance was used
- OTHER: Prior to the introduction of vanadium into the experimental diets, samples of hair were clipped from all the animals. Other hair samples were taken at 60 and 103 days (low level vanadium) or 36 and 75 days (high level vanadium). The hair samples were obtained by first skirting off and discarding about 1 to 1.5 cm of the hair back from the tips. An electric clipper was then used to cut of the hair close to the skin over an area from the shoulder to the base of the tail on the back of the animal. Samples were defatted by placing in evaporating dishes containing benzene for 30 minutes, rinsing with fresh benzene, draining, and air drying (Sullivan, M.X.; Hess, W.C., and Howe, P. E.: A Comparison of the Wool and Skins of Full-Fed and Maintenance-Fed Lambs, J. Agric. Res. 61: 877, 1940). Hair samples were stored in a vacuum desiccator over Drierite for 24 hours or until analysed. Samples were analysed for cysteine content.

Results and discussion

- low level vanadium exposure group:
  BODY WEIGHT AND WEIGHT GAIN/FOOD CONSUMPTION AND COMPOUND INTAKE

- food intake of all groups was nearly the same (total food intake: control: 1,996 gm/rat; 100 ppm group: 2,130 gm/rat; 150 ppm group: 2,019 gm/rat)
- vanadium test groups gained more weight than controls, not statistically significant (average body weight gain: control: 101 g/rat; 100 ppm group: 156 g/rat; 150 ppm group: 146 g/rat)

HAEMATOLOGY
- at 100 and 150 ppm, lower erythrocyte counts were obtained (at study termination: control: 7.7; 100 ppm group: 6.8; 150 ppm group: 6.3)
- Haemoglobin level were decreased, not statistically significant (at study termination: control: 15.0; 100 ppm group: 14.5; 150 ppm group: 13.7)

OTHER FINDINGS:
- 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 the 150 ppm level, a decrease in the average hair cysteine values occurred
- The hair cysteine of all the individual rats in the control group increased, the smallest increase amounting to 1.15 %
- Of three rats in the vanadium groups which showed higher hair cysteine, the greatest single increase (0.76 %) occurred in those receiving the 100 ppm V, but is still a fraction of the gain shown by lowest control animal
- decrease in cysteine are considered significant on a relative basis, i.e., comparing the vanadium-fed groups with their corresponding controls
- average change in cysteine was statistically significant different from the 100 ppm group and 150 ppm group (at the 0.5 % level).

- high level vanadium exposure group:

CLINICAL SIGNS AND MORTALITY
- One rat died in the third week.

BODY WEIGHT AND WEIGHT GAIN
- A decreased rate of body-weight gain occurred in both high level vanadium treated groups, compared with controls
- The decreased weight gain was greater in both vanadium groups than in the group-paired fed controls, indicating that food restriction was not the sole factor of growth impairment
- Average body weight gains: control: 125 g/rat; 500 ppm group: 42 g/rat; food control group: 90 g/rat; 1000 ppm group: - 2 g/rat; this was not statistically significant.

OTHER FINDINGS

- The cystine content of hair of rats fed the higher levels of vanadium, 500 and 1000 ppm, was depressed in comparison with that of the controls
- Average change in cystine: control: 0.44 +/- 0.20 %, 500 ppm group: -0.19 +/- 0.48 %; food control group: -0.26 +/- 0.25 %; 1000 ppm group: -0.76 +/- 0.13 %; statistically significant at the 5 % level).
- This finding corresponded to that at the lower levels, with the difference that the percentage decrease in cystine at the 1000 ppm level was greater (0.76%) and occurred sooner (75 days) than at the 150 ppm level which required 103 days to produce a 0.41 % average decrease in cystine content.
- The 0.76 % decrease in hair cystine content at the 1000 ppm level of vanadium feeding is highly significant on an absolute basis, i.e., comparing final and original cystine percentages within the same group.

- high level vanadium with added methionine exposure group:

BODY WEIGHT AND WEIGHT GAIN

- The weight gain per 100 gm of food consumed by the rats on the methionine-vanadium diets somewhat improved, as compared with those on vanadium diets (5.66 m. vs. 6.62 m, not statistically significant).

ORGAN WEIGHTS

- Methionine did not significantly affect either growth or relative liver weight in vanadium-fed animals.
- However, average values for these suggest a definite tendency toward improvement as a result of the administration of methionine.
- The methionine supplement, however, did not significantly change the liver-weight to body weight rations.

OTHER FINDINGS

- The methionine supplement, however, did not significantly improve the growth of the hair.
- It was noted that the hair growth of the methionine-fed animals was markedly inhibited (10 - 90 %).

COMBINED FINDINGS:

- Substantiating evidence for chemical changes found in the hair of vanadium-treated animals were structural changes, both gross and microscopic.
- The regrowth of hair was sparser, coarser, and stiffer compared with the more fleece-like hair of the controls.

It was concluded by the authors of the study that hair cysteine level appears to be a sensitive measure of vanadium intoxication. Reduced erythrocyte counts and levels of hair cysteine were observed dose-dependently at dose levels of 100 and 150 ppm in the diet.

1.7.1.5 [Schuler et al., 2011]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
16-day subacute study in mice Female B6C3F1 mice (5 groups of 48 mice) GLP: yes Reliability according to disseminated database: 1 Reliability (this assessment): 1	Divanadium pentaoxide, purity: 99.8 % 0, 0.25, 1, 4 mg V_2O_5/m³ (nose-only inhalation, 6 h/d for 16 days) Lung samples were analysed for DNA strand breaks using the comet assay and analysis of 9 specific DNA-oxo-adducts in lung tissue	In the lowest exposure group no histological alterations were observed. At 1 and 4 mg/m³ lung weights were increased dose-dependently, multifocal /diffuse alveolar histiocytosis and multifocal sub-acute alveolitis was observed dose-dependently in addition to multifocal granulocytic infiltration. Also, see Annex I, where the study is described in detail under section “germ cell mutagenicity”.	(Schuler et al., 2011)

1.7.1.6 [Knecht et al., 1992]

Subchronic (26 weeks) inhalation study in monkeys The study assessed pulmonary reactivity to V_2O_5 with provocation challenges, and compared V_2O_5 reactivity before and after subchronic V_2O_5 exposure with pulmonary function	Divanadium pentaoxide, > 99.6 % Whole body inhalation for 6 h / d, 5 d/week for 26 weeks Group 1 (9 animals): 0.1 mg/m^3 (Mon, Wed, Fri) and 1.1 mg/m^3 (Tue, Thurs) Group 2 (9 animals): 0.5 mg/m^3 Group 3 (8 animals): control goup, exposed against vehicle clean air	In none of the two exposure groups pulmonary reactivity to V_2O_5 was increased by subchronic V_2O_5 exposure in comparison to control group. Instead, a decrease was found in both exposure groups. This result indicates that the subchronic exposure may induce tolerance under the exposure conditions used in this study. One animal in the control group was removed from the study because of a	(Knecht et al., 1992) Also study 010 from (ECHA Dissemination, 2017)

testing. In addition to pulmonary function testing, bronchial lavage fluid was analysed. Adult, male cynomolgus monkeys (*Macaca fascicularis*) 8 -9 animals per exposure group GLP: no information Reliability according to disseminated database: 2 Reliability (this assessment): 2	Provocation challenge before and after subchronic exposure: - 6 h/d with 0.5 mg V_2O_5 and 2 weeks later: - 6 h/d with 3 mg V_2O_5	parasitic infestation. One animal in the peak exposure group died unexpectedly of an effect unrelated to the exposure. **Effects after pre-exposure challenge (acute effects):** Pre-exposure challenges with 0.5 and 3 mg V_2O_5/m^3 produced a concentration-dependent impairment in pulmonary function, characterized by airway obstructive changes (increased resistance and decreased flow). Analysis of respiratory cells recovered from the lung by bronchoalveolar lavage demonstrated that airway obstruction was accompanied by a significant influx of inflammatory cells into the lung.	

1.7.2 Human data

1.7.2.1 [Kiviluoto et al., 1979 and 1980]

Study reference:

Kiviluoto, M. et al., Effects of vanadium on the upper respiratory tract of workers in a vanadium factory, Scand. J. Work Environ. & Health 5: 50 – 58, 1979. (Kiviluoto et al., 1979)

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

Detailed study summary and results:

Test type

Publication 1 (Kiviluoto, M. et al. (1979):

An epidemiological cross-sectional case-control study (retrospective) on the injurious effects of vanadium was carried out among the workers of a V_2O_5 factory. The upper respiratory tract of 63 male workers exposed to V_2O_5 dust was examined macroscopically and microscopically, and the findings were compared with those of a reference group of workers who were exposed to inert dust only. The groups compared were of similar ages and had similar smoking habits. Nasal smears and sputum cells were studied microscopically, and biopsies for histological study were taken from the nasal mucosa.

Publication 2 (Kiviluoto, M. (1980):

This case-control study examines the possible effects of V_2O_5 exposure on ventilatory function, findings in chest radiographs and respiratory symptoms in the workers of the V_2O_5 factory. Out of a total of 79 employees at the factory making V_2O_5 from magnetite ore 63 were investigated by respiratory questionnaire, chest radiography, and tests of ventilatory function. The findings were compared with a reference group of 63 men, matched for age (to within two years) and for smoking habit (to within five cigarettes daily) selected from workers at a magnetite ore mine.

The studies were not conducted according to a guideline and GLP compliance is not specified. Ethical approval is not provided for Publication 1, but confirmed for publication 2 (no further information available). A reliability of 2 is given for these studies in the registration dossier.

TEST MATERIAL

The test material used in both studies is divanadium pentaoxide.

PLEASE NOTE: In both publications, the same setting and subjects were investigated. For this reason, the information on setting and subjects were stated together for both publications.

SETTING: V_2O_5 factory of the Otanmäki Mine of Rautaruukki Company, Finland, which has been manufacturing vanadium pentaoxide since 1956.

STUDY POPULATION

- Total number of subjects participating in study: The exposed population consisted of 63 men, who were process workers, repairmen, foremen, and laboratory workers in the V_2O_5 factory.
- Selection criteria: Every man who had worked in the V_2O_5 factory for at least four months was selected for examination, but those on holiday or sick leave at that time were not seen.
- Age: 19 - 56 years
- Average height: 170.8 cm (19-39 years of age); 170.0 cm (40 -56 years of age)
- Smoker/non-smoker: The average smoking time of the smokers within the exposed group was 15.8 years. The average amount in grams of tobacco smoked was 14 g a day.

COMPARISON POPULATION

- Type: Reference group
- Details: The reference group of 63 men was chosen from men living in the same area and thus exposed to similar air pollution. The referents worked in the concentrating plant, the mine, the repair shop (with possible

exposure to inert dusts), and the office, and they were matched for age within two years and for the number of cigarettes smoked daily within five cigarettes.

- Smoker/non-smoker: The average smoking time of smokers within the reference group 16.0 years. The average amount in grams of tobacco smoked was 12.5 g a day.
- Age: 19 - 56 years
- Average height: 175.6 cm (19-39 years of age); 171.7 cm (40 -56 years of age) (From the whole sample, the height of the referents was significantly greater ($p< 0.01$), but there was no difference in the weights of the two groups.)

PUBLICATION 1:

HYPOTHESIS TESTED: No studies on simultaneous macroscopic, cytological, and histological changes have been made on humans exposed to V_2O_5. The purpose of the present study was to explain these changes.

METHOD OF DATA COLLECTION

The upper respiratory tract of the workers in both groups was examined before they returned to work after their 2- to 4-week summer vacation in June-August 1975; the exposed group was also examined in March-June 1976 after several months of exposure. (The measurements of dust concentration were also made at this time). At the time of the former examination the cytological smears of the nasal mucosa and mucosal biopsies were taken from the middle part of the nasal inferior turbinates for microscopic examination. There was no evidence of epidemic upper respiratory infection in the examined subjects at the time of the study. The subjects took sputum cell specimens for microscopical examinations. Also, measurements of pulmonary ventilation and some laboratory tests were made.

Nasal secretion smears were made and studied according to the methods of Holopainen (Holopainen, E. Nasal mucous membrane in atrophic rhinitis with reference to symptom free nasal mucosa: Histology, histochemistry and exfoliative cytology. Acta oto laryngologica suppl. 227 (1967) 53 p.).

The histological biopsies were fixed in 10 % neutral formalin and embedded in paraffin blocks. They were cut into sections which were 5 µm thick. Different stainings were used to demonstrate overall morphology, ordinary findings, tissue plasma cells, eosinophils, mast cells, and neutrophils.

The metaplastic cells and eosinophils were examined from the sputum.

In smears taken from the nasal inferior turbinates, the columnar epithelial cells, goblet cells, metaplastic cells, basal cells squamous epithelial cells, cilia cytophoria cells, neutrophils, eosinophils, mast cells, lymphocytes, plasma cells, and bacteria were examined.

Columnar epithelial cells with or without cilia, possible degeneration, goblet cells, metaplastic cells, inflammatory cells (the plasma cells included), eosinophils, and mast cells were examined in the biopsies taken from the nasal inferior turbinates. In addition, the thickness of the connective tissue membrane, changes in the mucous glands and in the vessels, and the stromal fibrosis were examined and a histopathological diagnosis considering a possible acute, subchronic or chronic inflammation was also made.

STUDY PERIOD: A periodic examination in 1973.

PUBLICATION 2:

HYPOTHESIS TESTED: This case-control study examines the possible effects of V_2O_5 exposure on ventilatory function, findings in chest radiographs and respiratory symptoms in the workers of the V_2O_5 factory.

METHOD OF DATA COLLECTION

Forced vital capacity (FVC) and forced expiratory volume (FEV1) were determined with a dry bellows spirometer (Vitalograph) and expressed at BTPS. The highest value out of five attempts was used. Investigation of the exposed men took place at the end of their summer holidays in 1975, and at the same time the assisting nurse filled in a standardized questionnaire on respiratory symptoms (Medical Research Council. Committee on Research into Chronic Bronchitis. Questionnaire on respiratory symptoms. London: MRC. 1966). Lung function tests of the reference group were also undertaken in summer 1975. Full-size chest radiographs were taken in April 1976 and read, mixed and blinded by an experienced radiologist at the Institute of Occupational Health in Finland.

PUBLICATION 1:

EXPOSURE LEVELS: Determinations of the concentration of V_2O_5 dust in the factory air have been carried out since 1970. According to these measurements, 0.2 - 0.5 mg of vanadium per cubic meter (corresponds to 0.357 – 0.892 mg V_2O_5 /m³) can be regarded as the average level of previous exposure (determined from the total dust gathered on a paper filter with the atomic absorption spectrophotometric method, a total of 64 samples between 1970 and 1975).

At the beginning of 1976 the management intensified the flushing of the floors in order to stop the dust from rising. Thereafter the exposure level was about 0.01 - 0.04 mg/m^3 (194 determinations of dust concentration in the spring of 1976, 114 samples of which were taken in the breathing zones of individual workers and the rest at stationary sites, corresponds to 0.0178 – 0.0714 mg V_2O_5 /m³). The dust was collected by suction on a paper filter. The concentration of vanadium was determined by an atomic absorption spectrophotometer with the graphite furnace method. The total dust concentrations were very small in the factory. In addition to vanadium, the air contained inert magnetite dust. In ordinary workplaces near sintering furnaces, the concentration of sulphur dioxide was also determined with indicator tubes, the determing limit of which, 0.5 cm^3/m^3, was not exceeded. The samples were taken by methods commonly used by the Institute of Occupational Health in Finland (Suomen Standardisoimisliitto. Measurement of dust concentration in workplace air with filter method (SFS 3860). Helsinki 1976. 6 p.).

Since the subjects had worked an average of 11 years at the factory, attention had to be paid to the previous levels of dust before a reliable picture of the possible chronic effects of V_2O_5 could be formed.

PUBLICATION 2:

TYPE OF EXPOSURE: In the process magnetite is roasted with sodium carbonate. The roasted product is leached, and vanadates are precipitated from the leach liquor. The "red cake" produced is filtered, washed, and smelted to vanadium pentaoxide.

TYPE OF EXPOSURE MEASUREMENT:

The concentration of V_2O_5 dust at various sites in the factory were determined on eight days in March-May 1976. The determination covered two whole shifts. An atomic absorption spectrophotometer using flameless graphite atomiser (Perkin-Elmer 300, HGA 74) was used for these determinations. This could detect vanadium down to 0.002 mg/m^3. The coefficient of variation within run was 6 % at the concentration level of 0.05 mg/m^3. The particle size of the dust samples was determined by a modified Andreasen method from five dust samples, which were taken separately with a high volume sampler (Öhman H. Ödelycke P. Provtagnings-ochanalysförfaranden för silikosfarligt damm (Al-rapport No 3). Stockholm: Arbetsmedicinska Institutet. 1968:20.)

EXPOSURE LEVELS: Workers were previous exposed to 0.1 to 3.9 mg V/m^3 measured as total dust for 11 years.

STATISTICAL METHODS:

PUBLICATION 1:

The results were statistically analyzed by means of matched pairs according to the McNemar chi-square test. The changes noticed within the V_2O_5 group after exposure were also examined according to McNemar's chi-square test. The pathologic anatomic changes were also analyzed by these means after the 10 persons with the shortest periods of employment at the factory were eliminated from the group.

PUBLICATION 2:

Because the exposed and reference populations were matched for age and smoking habit, the use of Cole's (Cole TJ. Linear and proportional regression models of ventilatory function. Journal of the Royal Statistical Society, Series A 1975: 138:297 - 337) finding that FEV1 = height2 X(c+dX age) simplified the analysis to a paired t-test of the differences FEV1/Height2 (exposed) - FEV1/Height2 (referent). The distribution of respiratory symptoms between the exposed and referents was analyzed by McNemar's test for paired observations.

RESULTS

EXPOSURE (Publication 2)

The respirable fraction of the dust (particle size 5 µm or less) was 20 %. Concentrations exceeding the TLV of 0.5 mg/m^3 were found only during the grinding process of the laboratory samples. High concentrations of vanadium (V_2O_5) were also found at the packing of the smelt, but elsewhere in the factory the vanadium concentration was constantly below 0.1 mg/m^3.

FINDINGS OF PUBLICATION 1 AND PUBLICATION 2:

No differences were observed in the anterior and posterior rhinoscopy in the exposed groups after 11 years of exposure to average levels of 0.2 - 0.5 mg V/m^3. Furthermore, there was no difference in the number of blood vessels between the exposed and non-exposed groups. However, the number of neutrophils in the nasal smears and the number of plasma cells in the nasal mucosa were increased indicative of a protective mechanism in the mucosa. Other examined factors of the biopsies and cell findings did not differ between the exposed workers and the controls. Chest radiographs and lung function tests did not reveal any differences. After further 7 - 11 months of V_2O_5 exposure to 0.01 to 0.04 mg V/m^3 measured as total dust, a subsequent re-examination revealed that the cell findings did not indicate any further significant changes between the studied exposed groups and that there were no significant changes in the number of eosinophils of cytological and histological samples.

Altogether, no pneumoconiosis and no other signs indicative of allergic inflammation, including nasal catarrh, cough, or phlegm, were observed in the exposed subjects working for 11 years under these occupational conditions.

Publication 3:

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

Cross-sectional case-control study with 63 workers from a V_2O_5-producing company in Finland. Reliability according to disseminated database: 2 Reliability (this assessment): 2	Divanadium pentaoxide			
		Same exposure collective as indicated above (Kiviluoto et al., 1979) However the control group consisted of only 22 men. Whether these men were part of the "control collective" mentioned by Kiviluoto et al.(1979) is not described. **Performed tests:** - Hematologic and serum chemical laboratory tests	No significant differences were observed for the hematologic results of exposed and non-exposed workers. In the serum chemical test significant differences were observed for serum albumin(↓), chloride (↓), urea (↑), bilirubin (↓) and conjugated bilirubin (↑).	(Kiviluoto et al., 1981a) Also study 003 from (ECHA Dissemination, 2017) "epidemiological data"

Case-control study with 12 workers from a vanadium plant in South Africa. Reliability (this assessment): 3	Divanadium pentaoxide	12 workers chronically exposed against < 0.15 - 1.53 mg V_2O_5/m^3 with diagnosed bronchial hyperreactivity (by lung function and bronchoprovocation tests). Control subjects (12) worked in the same company but did not show bronchial hyperreactivity. **Performed tests/observations:** - Onset of symptoms - Serum IgE and atopy	**Onset of symptoms** in 7/12 subjects symptoms of cough and breathing difficulties developed within 6 months after start of the work in the factory. In the control group only 2/12 experienced the same symptoms within this time period. **Serum IgE and atopy** IgE levels between cases and controls were not significantly different. None of the subjects was exposed against toxic levels of SO_2 and NH_3. For 3/12 workers co-exposure against SO_2 and NH_3 could be excluded.	(Irsigler et al., 1999)

Case-control study with 24 men working in vanadium plants in the USA (13 men from Colorado and 11 men from Ohio) Reliability according to disseminated database: 2 Reliability (this assessment): 2	Divanadium pentaoxide	24 workers were exposed to vanadium (as V_2O_5) via inhalation (at least for 6 months) against the following concentrations: Colorado plant: 0.097 - 0.243 mg V_2O_5 /m³ (mass respirable vanadium: 16.6 % to 51 %; Particle size < 5 µm: 92.5 to 99 %) Ohio plant: 0.018 - 0.925 mg V_2O_5/m³ (mass respirable vanadium: 2 % to 100 %; Particle size < 5 µm: 96.3 to 100 %) 45 control subjects matched for age, economic status and job activities, not coming from the vanadium industry. **Performed tests/observations:** - physical examination, - history (incl. detailed occupational history and a subjective evaluation of alcohol and fat intake), - electrocardiogram, urinalysis, hematocrit, serum cholesterol, and analysis of urine for its content of vanadium	**Symptoms with significant differences increased in exposure versus control group:** Cough, sputum, eye, nose, throat irritation, epistaxia, wheezing Wheezes, rales, or ronchi, injected pharynx, green tongue After an analysis of variance and the geographical effects were removed, the cholesterol levels of the exposed subjects are found to be significantly lower than those of the controls ($p < 0.05$). No significant differences were found for haematocrit urinalysis and electrocardiogram results	(Lewis, 1959a; b) (ECHA Dissemination, 2017) Study: 004

Experimental study in 24 volunteers (12 workers of a vanadium plant and 12 students) Reliability according to disseminated database: 2 Reliability (this assessment): 2	Divanadium pentaoxide	8 subjects (4 workers and 4 students) were attributed to each of three different exposure zones (high: 0.028 - 0.062 mg V/m³; medium: 0.004 - 0.019 mg V/m³; low: 0.008 - 0.019 mg V/m³). The subjects had to be present during the 8 hours lasting working day for 5 days. **Performed tests:** - psychological, neuropsychological, psychosomatic and behaviour toxicological performance tests	No behaviour toxicological changes were observed between the different groups. The variation of exposure to vanadium pentoxide had no influence on eye-hand coordination and on performance in fine motor response → No influence of variation of exposure to V_2O_5 on neurobehavioural performances was found. → No correlation between neuropsychological perfromances and concentration of metabolized vanadium was found.	Hörtnagel et al., 1994 cited from (ECHA Dissemination, 2017) Study: 005

Retrospective cohort study with 78 workers engaged in the processing of vanadium-bearing ore, and 37 controls in Peru Reliability according to disseminated database: 2 Reliability (this assessment): 2	Divanadium pentaoxide	Vanadium concentrations in air varied from 0.01 - 58.80 mg/m³. In the control areas the concentration range was 0.000 to 0.007 mg/m³. Concentrations do not seem to refer to V_2O_5. All dust particles were below 5 µm in diameter. The concentration of sulphur dioxide in air in various work places ranged between 0.0 and 2.0 ppm.	Abnormally high prevalence of signs and symptoms indicative of irritation to the upper respiratory tract and to the eyes among workers exposed to vanadium-bearing dusts as compared with workers not exposed to such dust. Vital capacity, circulation, neurological findings, muscular strength: No significant differences among the three groups of workers were observed.	(Vintinner et al., 1955) (ECHA Dissemination, 2017) Study: 006

A series of studies from a group of investigators of the University of Mexico, which investigated effects of divanadium pentaoxide on various organ systems is not further discussed in detail here (Avila-Costa et al., 2006; Cano-Gutiérrez et al., 2012; Colín-Barenque et al., 2015; Rodríguez-Lara et al., 2013; Rodríguez-Lara et al., 2016; Rodriguez-Lara et al., 2016; Ustarroz-Cano et al., 2017). These studies are considered to be of limited reliability, as only one exposure concentration was used in each study and divanadium pentaoxide exposure was not to dust, but to an aerosol produced from divanadium pentaoxide by suspension in various aqueous solutions (see also the discussion on limitations of these studies in chapter 10.10.2).

2 REFERENCES TO ANNEX I

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

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

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

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

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. (1980)

Observations on the lungs of vanadium workers

British Journal of Industrial Medicine, 37, 363-366

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

Leuschner, J.; Haschke, H.; Sturm, G. (1994)

New investigations on acute toxicities of vanadium oxides

Monatshefte fur Chemie, 125, 623-646

Manjanatha, M.G.; Shelton, S.D.; Haber, L.; Gollapudi, B.; MacGregor, J.A.; Rajendran, N.; Moore, M.M. (2015)

Evaluation of *cII* 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

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

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)

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

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

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

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

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,

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