Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

trinickel disulfide; nickel subsulfide; [1] heazlewoodite [2]

EC Number: 234-829-6 [1] – [2]
CAS Number: 12035-72-2 [1] 12035-71-1 [2]

CLH-O-0000001412-86-272/F

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

Adopted

15 March 2019

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

Trinickel Disulphide

EC Number: **234-829-6**

CAS Number: **12035-72-2**

Index Number: **028-007-00-4**

Registrant's Identity: Johnson Matthey Chemicals GmbH

Lead Registrant of JS_Nickel_subsulfide

Date: December 2017

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON TRINICKEL DISULFIDE; NICKEL SUBSULFIDE; [1] HEAZLEWOODITE [2]

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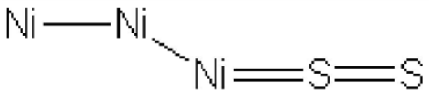
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON TRINICKEL DISULFIDE; NICKEL SUBSULFIDE; [1] HEAZLEWOODITE [2]

1. IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Trinickel disulphide, Trinickel disulfide, Nickel subsulphide, Nickel subsulfide, HEAZLEWOODITE
Other names (usual name, trade name, abbreviation)	Nickel subsulphide
ISO common name (if available and appropriate)	not applicable
EC number (if available and appropriate)	234-829-6*
EC name (if available and appropriate)	Trinickel disulphide
CAS number (if available)	12035-72-2*
Other identity code (if available)	CLP Annex VI Index number, 028-007-00-4
Molecular formula	Ni_3S_2
Structural formula	
SMILES notation (if available)	not applicable
Molecular weight or molecular weight range	240.21 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	100.0 % (w/w)

* This CLH dossier for trinickel disulphide is intended to cover all substances identified by CLP index number 028-007-00-4, which includes trinickel disulfide, nickel subsulfide (EC# 234-829-6, CAS# 12035-72-2) and heazlewoodite (EC# 234-829-6, CAS# 12035-71-1).

1.2 Composition of the substance

Name: Trinickel disulphide

Degree of purity: 100.0 % (w/w)

Table 2: Trinickel disulphide constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Trinickel disulphide* EC no.: 234-829-6	100.0 % (w/w)	Carc. 1A ; H350i Muta. 2 ; H341 STOT RE 1 ; H372** Skin Sens. 1; H317 Aquatic Acute 1: H400 Aquatic Chronic 1; H410	Acute Tox. 4; H332

* This CLH dossier for trinickel disulphide is intended to cover all substances identified by CLP index number 028-007-00-4, which includes trinickel disulfide, nickel subsulfide (EC# 234-829-6, CAS# 12035-72-2) and heazlewoodite (EC# 234-829-6, CAS# 12035-71-1).

** The classification under 67/548/EEC indicating the route of exposure has been translated into the corresponding class and category according to this Regulation, but with a general hazard statement not specifying the route of exposure as the necessary information is not available.

Table 3: Trinickel disulphide impurities (non-confidential information)

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Not applicable				

Table 4: Trinickel disulphide additives (non-confidential information)

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Not applicable					

Table 5: Test substances (non-confidential information)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Sections/studies where this test substance was used	Other information
Trinickel disulphide* EC no.: 234-829-6	100.0 %	Not applicable	Acute inhalation toxicity: EPSL (2010)	Information as provided by the supplier

* This CLH dossier for trinickel disulphide is intended to cover all substances identified by CLP index number 028-007-00-4, which includes trinickel disulfide, nickel subsulfide (EC# 234-829-6, CAS# 12035-72-2) and heazlewoodite (EC# 234-829-6, CAS# 12035-71-1).

2. PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6: C&L table

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard Statement Code(s)	Pictogram, Signal Word Code(s)	Hazard Statement Code(s)	Suppl. Hazard Statement Code(s)		
Current Annex VI entry	028-007-00-4	Trinickel disulfide nickel subsulfide; [1] heazlewoodite [2]	234-829-6 [1] - [2]	12035-72-2 [1] 12035-71-1 [2]	Carc. 1A Muta. 2 STOT RE 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H350i H341 H372** H317 H400 H410	GHS08 GHS07 GHS09 Dgr	H350i H341 H372** H317 H410			
Dossier submitters proposal					**Acute Tox.4 (ADD)**	**H332 (ADD)**		**H332 (ADD)**			
Resulting Annex VI entry if agreed by RAC and COM	028-007-00-4	Trinickel disulfide nickel subsulfide; [1] heazlewoodite [2]	234-829-6 [1] - [2]	12035-72-2 [1] 12035-71-1 [2]	**Acute Tox. 4** Carc. 1A Muta. 2 STOT RE 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	**H332** H350i H341 H372** H317 H400 H410	GHS08 GHS07 GHS09 Dgr	**H332** H350i H341 H372** H317 H410			

** Route of exposure cannot be excluded - For certain hazard classes, e.g. STOT, the route of exposure should be indicated in the hazard statement only if it is conclusively proven that no other route of exposure can cause the hazard in accordance to the criteria in Annex I. Under Directive 67/548/EEC the route of exposure is indicated for classifications with R48 when there was data justifying the classification for this route of exposure. The classification under 67/548/EEC indicating the route of exposure has been translated into the corresponding class and category according to this Regulation, but with a general hazard statement not specifying the route of exposure as the necessary information is not available. Under Directive 67/548/EEC, this substance was classified as R48 T; R48/23 Toxic; Toxic: danger of serious damage to health by prolonged exposure through inhalation.

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

Hazard class	Reason for no classification	Should the hazard class be open for commenting during the public consultation?
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	hazard class not assessed in this dossier	No
Acute toxicity via dermal route	hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	not applicable (harmonised classification proposed)	Yes
Skin corrosion/ irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	hazard class not assessed in this dossier	No
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	hazard class not assessed in this dossier	No
Germ cell mutagenicity	hazard class not assessed in this dossier	No

Carcinogenicity	hazard class not assessed in this dossier	No
Reproductive toxicity	hazard class not assessed in this dossier	No
Specific target organ toxicity-single exposure	hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	hazard class not assessed in this dossier	No
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	hazard class not assessed in this dossier	No
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3. HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The original proposal to include trinickel disulphide (EC No. 234-829-6; CAS No. 12035-72-2) as a new entry in Annex I of Council Directive 67/548/EEC was reviewed and agreed for inclusion in the 31st ATP (EC, 2009). Classification of trinickel disulphide for some endpoints (carcinogenicity, chronic inhalation toxicity, sensitization, and toxicity to the environment) was based on grouping of insoluble nickel compounds. However, classification for acute toxicity was not included in this approach. Therefore, it is assumed that the lack of classification for acute toxicity of trinickel disulphide was due to a lack of substance-specific oral or inhalation data at the time of entry.

Trinickel disulphide's lack of classification for acute toxicity was carried forward unchanged into ATP 1 to the CLP Regulation corresponding to ATP 30 and 31 to the 67/548/EEC Directive, and as such currently carries no classification for acute toxicity classified in accordance with criteria set up in Annex VI to Directive 67/548/EEC.

RAC general comment
This proposal was limited to acute inhalation exposure.

4. JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Justification that action is needed at Community level is required.

Reason for a need for action at Community level: ***Change due to new data***

Further detail on need of action at Community level

Consumers do not come into contact with trinickel disulphide. Therefore, the need for action at the community level is focused on occupational exposure in the workplace. The current lack of classification for this endpoint creates the false perception that trinickel disulphide is not of concern for acute inhalation toxicity, although available data suggest trinickel disulphide warrants classification for this endpoint. Newly available animal data (Henderson et al., 2012b; EPSL, 2010) support no classification for acute toxicity via the oral route (as listed in Annex VI of the CLP), while indicating that the minimum classification (Acute Tox. 4; H332) should be implemented for acute toxicity via the inhalation route.

5. DATA SOURCES

- Chemical Safety Report (CSR) for Nickel Subsulphide (2017 update), including unpublished laboratory reports referenced within the CSR
- Comprehensive scientific literature search related to toxicokinetics and acute inhalation toxicity of trinickel disulphide
- Searching of the ECHA website and general internet searching related to the history of the previous classification and labelling
- Searching of the ECHA database and the CLP (including ATPs) for registration dossiers of impurities related to classifications and self-classifications

6. PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid, powder	Harlan Labs, 2010	Physical state: solid, powder Colour: grey Odour: not reported
Melting/freezing point (°C)	> 359.85 (633 K)	Harlan Labs, 2010	Tested with differential scanning calorimetry, using ASTM E537-86, Method A1 Melting/Freezing Temperature of Commission Regulation (EC) No 440/2008 of 30 May 2008 and Method 102 of the OECD Guidelines for Testing of Chemicals, 27 July 1995.
Boiling point (°C)	Not applicable	Not applicable	The study does not need to be conducted because the substance is a solid which melts above 300ºC. Column 2 of REACH Annex VII provides the following exemption: A study does not need to be conducted for solids which either melt above 300°C or decompose before boiling. The test material was confirmed not to melt below 573 K (300°C).
Relative density	5.98 at 23 ± 0.5 °C	Harlan Labs, 2010	Tested with a gas comparison pycnoter, Method A3 Relative Density of Commission Regulation (EC) No 440/2008 of 30 May 2008 and Method 109 of the OECD Guidelines for Testing of Chemicals, 27 July 1995.
Vapour pressure (Pa)	Not applicable	Not applicable	The study does not need to be conducted because the melting point is above 300ºC. Column 2 of REACH Annex VII provides the following exemption: A study does not need to be conducted for solids which either melt above 300°C or decompose before boiling. The melting temperature of the test material was greater than 300°C.

Surface tension (N/m)	Not applicable	Not applicable	The study does not need to be conducted because based on structure, surface activity is not expected or can't be predicted. Column 2 of REACH Annex VII states that the surface tension study needs only be conducted if: - based on structure, surface activity is expected or can be predicted; or - surface activity is a desired property of the material. Accordingly, surface tension does not need to be determined because the test material is not designed or anticipated to have surfactant properties.
Water solubility (mg/l)	≥0.00735 - ≤0.0116 g/L Note: A definitive water solubility result could not be obtained for the test material. No saturation plateau was observed even on extension of the initial saturation period beyond that required by the method guidelines.	Harlan Labs, 2010	On applying the saturation periods of 24, 48 and 72 hours, the mean dissolved nickel concentration increased from 2.27 x 10^{-3} to 6.62 x 10^{-3} g/L of solution at 20.0 ± 0.5°C (equivalent to 3.09 x 10^{-3} to 9.03 x 10^{-3} g/L of dissolved nickel subsulphide). Therefore, extended saturation periods of 96, 120 and 144 hours were applied to a further three samples. Again no saturation plateau was evident on analysis, with the mean dissolved nickel concentration increasing from 7.35 x 10^{-3} to 1.16 x 10^{-2} g/L of solution 20.0 ± 0.5°C across these three samples (equivalent to 1.00 x 10^{-2} to 1.58 x 10^{-2} g/L of dissolved nickel subsulphide). Monitoring the total dissolved nickel concentration of the sample solutions, the test material failed to achieve a saturation plateau using the saturation and equilibration periods stated in EC Method A6 and OECD Method 105. In the absence of a saturation plateau even after adaptation of the method guidelines and applying an extended saturation period of 144 hours, it was not considered that a definitive water solubility result could be obtained for the test material. However, it is equally important to note that as both ICP and AAS are non-substance specific elemental methods only, the generation of the dissolved nickel content in the sample solutions may also be as a product of transformation as opposed to true dissolution of the parent subsulphide salt.

Partition coefficient n-octanol/water	Not applicable	Not applicable	The study does not need to be conducted because the substance is inorganic. Column 2 of REACH Annex VII provides the following exemption: A study does not need to be conducted for inorganic substances.
Particle size distribution (granulometry)	Proportion of test material having an inhalable particle size less than 100 µm: 28.5% Proportion of test material having a thoracic particle size less than 10.0 µm: ≤0.43% Proportion of test material having a respirable particle size less than 5.5 µm: ≤0.18%	Harlan Labs, 2010	Granulometry data was acquired using a procedure designed to comply with the European Commission technical guidance document 'Particle Size Distribution, Fibre Length and Diameter Distribution' (June 1996), which satisfies the requirents of OECD Guideline 110.
Flash point (°C)	Not applicable	Not applicable	The study does not need to be conducted because the substance is inorganic. Column 2 of REACH Annex VII states that the study does not need to be conducted if the substance is inorganic. Accordingly, the flash point does not need to be determined because the test material is inorganic.
Auto flammability	>400°C	Harlan Labs, 2009	Testing was conducted using Method A16 Relative Self-Ignition Temperature for Solids of Commission Regulation (EC) No 440/2008 of 30 May 2008.
Flammability	Non-flammable	Harlan Labs, 2009	The flammability (solids) of the test material was determined using Method A 10 Flammability (Solids) of Commission Regulation (EC) No 440/2008 of 30 May 2008.
Explosiveness	Not applicable	Not applicable	According to Column 2 of REACH Annex VII, determining the explosiveness of nickel subsulphide is not necessary because it does not contain chemical groups associated with explosivity.
Oxidising properties	Not applicable	Not applicable	According to Column 2 of REACH Annex VII, determining the oxidising properties of nickel subsulphide is not necessary because it does not contain chemical groups associated with oxidising properties.

Stability in organic solvents and identity of relevant degradation products (if relevant)	Not applicable	Not applicable	The study does not need to be conducted because the substance is inorganic. Stability in organic solvents and identity of relevant degradation products is not an applicable endpoint for inorganic substances such as trinickel disulphide, according to Column 2 of Annex IX of REACH regulations.
Dissociation constant	Not applicable	Not applicable	The study does not need to be conducted because the substance is insoluble.
Viscosity **dynamic viscosity** (Pas) **kinematic viscosity** (mm^2/s)	Not applicable	Not applicable	The study does not need to be conducted because the substance is a solid. According to Column 2 of REACH Annex IX, viscosity data are not required the test material is a solid substance.

7. TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 9: Summary table of toxicokinetic studies via inhalation

Method	Results	Remarks	Reference
in vitro study In situations where the bioavailability of a metal substance is not known or not feasible to determine experimentally, the amount of metal ion "available for absorption" may be measured using *in vitro* methods. In this application, the dissolution (*e.g.*, elution or extraction) of metal ion from various nickel compounds in surrogate (synthetic) tissue fluids is measured. The resultant value is termed bioaccessibility and is defined as the amount of a substance (*e.g.*, metal ion) available for absorption.	For sample N18 of nickel subsulphide, the nickel release was: • 22.65% of Ni content after 2 hours in gastric fluid • 0.25% of Ni content after 24 hours in intestinal fluid • 4.8% of Ni content and 3.35% of sample weight after 72 hours in lung interstitial fluid • 37.4% of Ni content and 26.2% of sample weight after 72 hours in lysosomal fluid • 5.35% of Ni content after 24 hours in sweat fluid	Klimisch score: 2 (reliable with restrictions) supporting study experimental result **Test material (EC name): trinickel disulphide**	KMHC (2010) Henderson, RG, Cappellini D, Seilkop SK, Bates HK and Oller AR (2012a) [gastric and intestinal fluid results only]
rat (F344/N) male/female inhalation: aerosol Exposure regime: 6 hr/day, 5 day/wk, 12 days Doses/conc.: 0, 0.6, 1.2, 2.5,	Transfer (atmosphere to lungs): distinct transfer (Test No.: #1) Transfer (atmosphere to turbinates): slight transfer (nickel was detected but below quantitation limit) (Test No.: #2) Transfer (lungs to lung-associated lymph nodes): slight transfer (nickel was detected but	Klimisch score: 2 (reliable with restrictions) supporting study experimental	Benson JM, Carpenter RL, Hahn FF, Haley PJ, Hanson RL, Hobbs CH, Pickrell (1987)

5.0, 10 mg Ni_3S_2/m^3 (MMAD = 2.8 µm, GSD = 2.0) Rats were exposed to Ni_3S_2 for 6 hr/d, 5 d/wk, for 12 exposure days (0.6-10 mg/m^3). Tissue burden was examined in select tissues.	below quantitation limit) (Test No.: #3) Transfer (lungs to kidney): distinct transfer (Test No.: #4) Transfer (lungs to blood): slight transfer (nickel was detected but below quantitation limit) (Test No.: #5) Transfer (lungs to testes): slight transfer (Test No.: #6) Details on metabolites: only Ni content was measured in tissues Evaluation of results: bioaccumulation potential cannot be judged based on study results	result **Test material (EC name): trinickel disulphide**	Dunnick JK, Benson JM, Hobbs CH, Hahn FF, Cheng YS, Eidson AF (1988) NTP (1996)
mouse (B6C3F1) male/female inhalation: aerosol Exposure regime: 6 hr/day, 5 day/wk, 12 days Doses/conc.: 0, 0.6, 1.2, 2.5, 5.0, 10 mg Ni_3S_2/m^3 (MMAD = 2.8 µm, GSD = 2.0) Mice were exposed to Ni_3S_2 for 6 hr/d, 5 d/wk, for 12 exposure days (0.6-10 mg/m^3). Tissue burden was examined in select tissues.	Transfer (atmosphere to lungs): distinct transfer (Test No.: #1) Transfer (atmosphere to kidneys): distinct transfer (Test No.: #2) Transfer (atmosphere to turbinates): slight transfer (nickel was detected but below quantitation limit) (Test No.: #3) Details on metabolites: only Ni content was measured in tissues Evaluation of results: bioaccumulation potential cannot be judged based on study results	Klimisch score: 2 (reliable with restrictions) supporting study experimental result **Test material (EC name): trinickel disulphide**	Benson JM, Carpenter RL, Hahn FF, Haley PJ, Hanson RL, Hobbs CH, Pickrell (1987) Dunnick JK, Benson JM, Hobbs CH, Hahn FF, Cheng YS, Eidson AF (1988) NTP (1996)
rat (F344/N) male inhalation: aerosol Exposure regime: 120 min, single exposure Doses/conc.: 5.7 +/- 1.1 mg $^{63}Ni_3S_2/m^3$ (MMAD = 1.3 µm, GSD = 1.5) equivalent or similar to OECD Guideline 417 (Toxicokinetics)	Transfer (atmosphere to lungs): distinct transfer (Test No.: #1) Transfer (atmosphere to turbinates): distinct transfer (Test No.: #2) Transfer (atmosphere to skull): distinct transfer (Test No.: #3) Transfer (atmosphere to trachea/larynx): distinct transfer (Test No.: #4) Transfer (lungs to lung-associated lymph nodes): slight transfer (delayed transfer, observed after 2 days post exposure) (Test No.: #5) Transfer (lungs to kidneys): distinct transfer (Test No.: #7) Transfer (atmosphere/lungs to GI tract): distinct transfer (Test No.: #8) Toxicokinetic parameters: fraction of inhaled Ni_3S_2 deposited in total respiratory tract = 0.14 +/- 0.01 (Test No.: #1) fraction of inhaled Ni_3S_2 deposited in upper respiratory tract = 0.09 +/- 0.01 (Test No.: #1) fraction of inhaled Ni_3S_2 deposited in lower respiratory tract = 0.05 +/- 0.002 (Test No.: #1) Half-life 1st: lung clearance = 4.6 days (Test No.: #1)	Klimisch score: 1 (reliable without restriction) supporting study experimental result **Test material (EC name): trinickel disulphide**	Benson JM, Barr EB, Bechtold WE, Cheng YS, Dunnick JK, Eastin WE, (1994)

	Details on metabolites: Compound tracked as ^{63}Ni, *i.e.* moiety not identified. Evaluation of results: no bioaccumulation potential based on study results (However, the exposure duration was acute.)		
rat (F344/N) male/female inhalation: aerosol Exposure regime: 6 hr/day, 5 days/wk, for 22 days Doses/conc.: 0.6 or 2.5 mg Ni_3S_2/m^3 (0.6 mg dose, MMAD=2.07 μm, 2.5 mg dose, MMAD=1.98 μm) Nickel lung burden and clearance were examined throughout the duration of exposure.	Transfer (atmosphere to lung): distinct transfer (Test No.: #1) Toxicokinetic parameters: Half-life 1st: for accumulation and clearance: 3.5-8 days (Test No.: #1) accumulation rate (dose: 0.62 mg/m^3): 0.09 μg Ni/g lung/day (Test No.: #1) accumulation rate (dose: 2.5 mg/m^3): 0.2 μg Ni/g lung/day (Test No.: #1) Details on metabolites: Only nickel content was measured. Evaluation of results: low bioaccumulation potential based on study results	Klimisch score: 2 (reliable with restrictions) supporting study experimental result **Test material (EC name): trinickel disulphide**	Benson JM, Cheng YS, Eidson AF, Hahn FF, Henderson RF, Pickrell JA (1995)
rat (F344/N) male/female inhalation: aerosol Exposure regime: 6 hr/day; 5 d/wk for 2 years Doses/conc.: 0, 0.15, 1.0 mg Ni_3S_2/m^3; 0, 0.11, 0.73 mg Ni/m^3. (MMAD = 2.0-2.2 μm, GSD = 2.0) equivalent or similar to OECD #543	Transfer (atmosphere to lungs): distinct transfer (Test No.: #1) Details on metabolites: only measured for Ni Evaluation of results: no bioaccumulation potential based on study results	Klimisch score: 2 (reliable with restrictions) supporting study experimental result **Test material (EC name): trinickel disulphide**	Dunnick JK, Elwell MR, Radovsky AE, Benson JM, Hahn FF, Nikula KJ (1995) NTP (1996)
mouse (B6C3F1) male/female inhalation: aerosol Exposure regime: 6 hr/day; 5 d/wk for 2 years Doses/conc.: 0, 0.6, 1.2 mg Ni_3S_2/m^3; 0, 0.44, 0.9 mg Ni/m^3. (MMAD = 2.0-2.2 μm, GSD = 2.0) equivalent or similar to OECD #543	Transfer (atmosphere to lungs): distinct transfer (Test No.: #1) Details on metabolites: only measured for Ni Evaluation of results: no bioaccumulation potential based on study results	Klimisch score: 2 (reliable with restrictions) supporting study experimental result **Test material (EC name): trinickel disulphide**	Dunnick JK, Elwell MR, Radovsky AE, Benson JM, Hahn FF, Nikula KJ (1995) NTP (1996)
rat (F344/N) male inhalation: aerosol Exposure regime: 6 hr/day; 5 d/wk for 13 weeks	Transfer (atmosphere to lungs): distinct transfer (Test No.: #1) Details on metabolites: only measured for Ni Evaluation of results: low bioaccumulation	Klimisch score: 2 (reliable with restrictions) supporting study	Dunnick JK, Elwell MR, Benson JM, Hobbs CH, Hahn FF, Haly, PJ, Cheng (1989)

Doses/conc.: 0, 0.15, 0.3, 0.6, 1.2, and 2.5 mg Ni_3S_2/m^3; 0, 0.11, 0.2, 0.4, 0.9, 1.8 mg Ni/m^3 (MMAD = 2.4 μm, GSD = 2.2) Rats were exposed to Ni_3S_2 via inhalation for 13 weeks. Ni levels in lung were quantified.	potential based on study results	experimental result **Test material (EC name): trinickel disulphide**	NTP (1996)
mouse (B6C3F1) male inhalation: aerosol Exposure regime: 6 hr/day; 5 d/wk for 13 weeks Doses/conc.: 0, 0.15, 0.3, 0.6, 1.2, and 2.5 mg Ni_3S_2/m^3; 0, 0.11, 0.2, 0.4, 0.9, 1.8 mg Ni/m^3. (MMAD = 2.4 μm, GSD = 2.2) Mice were exposed to Ni_3S_2 via inhalation for 13 weeks. Ni levels in lung were quantified.	Transfer (atmosphere to lungs): distinct transfer (Test No.: #1) Metabolites identified: no Details on metabolites: only measured for Ni Evaluation of results: low bioaccumulation potential based on study results	Klimisch score: 2 (reliable with restrictions) supporting study experimental result **Test material (EC name): trinickel disulphide**	Dunnick JK, Elwell MR, Benson JM, Hobbs CH, Hahn FF, Haly, PJ, Cheng (1989) NTP (1996)
mouse (BALB/c BYJ) male intratracheal instillation Exposure regime: single exposure Doses/conc.: 11.8 μg (3 μCi) $^{63}Ni_3S_2$ BALB/c BYJ mice were intratracheally instilled with 11.8 μg (roughly equal to 0.5 mg/kg $^{63}Ni_3S_2$; MMD = 1.65 μm; GSD = 1.83) in 20 μL PBS. Tissue samples were collected at 15 minutes, 1, 5 and 20 hours, and 3 and 7 days post exposure; radioactivity was measured and clearance half-life calculated.	Transfer (trachea to lungs): distinct transfer (Test No.: #1) Transfer (trachea to GI tract): distinct transfer (Test No.: #2) Transfer (trachea to kidneys): distinct transfer (Test No.: #3) Transfer (trachea to blood): distinct transfer (Test No.: #4) Toxicokinetic parameters for rapid early and gradual clearance phases: Half-life 1st: 2.0 hr (lung) (Test No.: #1) Half-life 2nd: 119 hr (lung) (Test No.: #1) Half-life 1st: 2.0 (GI tract) (Test No.: #2) Half-life 2nd: 34 hr (GI tract) (Test No.: #2) Half-life 1st: 5.1 hr (kidney) (Test No.: #3) Half-life 2nd: 206 hr (kidney) (Test No.: #3) Half-life 1st: 1.3 hr (blood plasma) (Test No.: #4) Half-life 2nd: 122 hr (blood plasma) (Test No.: #4) Metabolites identified: no Details on metabolites: Only $^{63}Ni_3S_2$ was measured by liquid scintiIlation counting. Evaluation of results: low bioaccumulation potential based on study results	Klimisch score: 2 (reliable with restrictions) supporting study experimental result **Test material (EC name): trinickel disulphide**	Finch GL, Fisher GL, Hayes TL (1987)
rat (Wistar) male inhalation: aerosol Exposure regime: 6 hr day, 5 days/wk, for 6 months Doses/conc.: 0.5 +/- 0.4 mg	Transfer (atmosphere to lungs): distinct transfer (Test No.: #1) Transfer (lungs to liver): distinct transfer (Test No.: #2) Transfer (lungs to kidneys): distinct transfer (Test No.: #3) Transfer (lungs to spleen): no transfer	Klimisch score: 2 (reliable with restrictions) supporting study experimental	Kodama Y, Tanaka I, Matsuno K, Ishimatsu S, Kawamoto T (1993)

Ni_3S_2 /m^3 Wistar rats were exposed to Ni_3S_2 for 6 hr/day, 5 d/wk, for 6 months, followed by a 12-month recovery period. Nickel content in organs was determined by atomic absorption spectrophotometry. MMAD =2.6 µm; GSD = 1.9	detectable (Test No.: #4) Transfer (lungs to blood): no transfer detectable (Test No.: #5) Toxicokinetic parameters: deposition fraction (lung) = 0.5 % (+/- 0.1) (Test No.: #1) clearance rate (lung) = 98% (Test No.: #1) Details on metabolites: Only nickel content was measured. Evaluation of results: bioaccumulation potential cannot be judged based on study results	result **Test material (EC name): trinickel disulphide**	
rat (Fischer 344) male inhalation Exposure regime: single 2 h inhalation Doses/conc.: 13 mg Ni_3S_2 /kg bw (352 mg Ni_3S_2 /m^3) MMAD, GSD of aerosol not provided A single 2 h, nose-only inhalation dose of Ni_3S_2 of 13 mg/kg bw (352 mg/m^3) was administered to F344 rats; Ni content was measured in the nasal mucosa and lungs.	Transfer (transfer to nasal mucosa): distinct transfer (Test No.: #1) Transfer (transfer to lung tissue): distinct transfer (Test No.: #2) Details on metabolites: only measured Ni content Evaluation of results: bioaccumulation potential cannot be judged based on study results	Klimisch score: 2 (reliable with restrictions) supporting study experimental result **Test material (EC name): trinickel disulphide**	Mayer C, Klein RG, Wesch H, Schmezer P (1998)
mouse (A/J) male intratracheal instillation Exposure regime: Single dose Doses/conc.: 11.7 µg $^{63}Ni_3S_2$ (MMAD = 1.66 µm, GSD = 1.83) The pulmonary clearance, tissue distribution, and excretion of particulate $^{63}Ni_3S_2$ was evaluated in strain A/J mice following the intratracheal instillation of 3 µCi (11.7 µg) of $^{63}Ni_3S_2$. Tissue and blood were analyzed at 4 hr and 1, 2, 3, 7, 14, 28, 35 days, while urine and feces were analyzed at 12 and 24 hr after instillation, at 24 hr intervals during the first 7 days, and every 48 hr thereafter up to 35 days after treatment.	Transfer (trachea to lungs): distinct transfer (Test No.: #1) Transfer (lungs to liver): distinct transfer (Test No.: #2) Transfer (lungs to kidney): distinct transfer (Test No.: #3) Transfer (lungs to femur): distinct transfer (Test No.: #4) Transfer (lungs to blood): distinct transfer (Test No.: #5) Toxicokinetic parameters: Half-life 1st: 1.18 +/- 0.7 days (lung) (Test No.: #1) Half-life 2nd: 12.42 +/- 2.1 days (lung) (Test No.: #1) Half-life 1st: 1.58 +/- 0.65 (liver) (Test No.: #2) Half-life 2nd: 32.78 +/- 20.16 (liver) (Test No.: #2) Half-life 1st: 1.3 +/- 0.4 (kidney) (Test No.: #3) Half-life 2nd: 14.61 +/- 7.65 (kidney) (Test No.: #3) Half-life 1st: 2.66 +/- 2.11 (femur) (Test No.: #4) Half-life 2nd: 56.40 +/- 116.0 (femur) (Test	Klimisch score: 2 (reliable with restrictions) supporting study experimental result **Test material (EC name): trinickel disulphide**	Valentine R and Fisher G (1984)

	No.: #4) Half-life 1st: 1.0 +/- 0.11 (blood) (Test No.: #5) Half-life 2nd: 11.31 +/- 1.25 (blood) (Test No.: #5) Half-life 1st: 1.02 +/- 0.11 (urine) (Test No.: #6) Half-life 2nd: 12.08 +/- 1.13 (urine) (Test No.: #6) Half-life 1st: 1.02 +/- 0.54 (feces) (Test No.: #7) Half-life 2nd: 15.34 +/- 3.43 (feces) (Test No.: #7) Evaluation of results: bioaccumulation potential cannot be judged based on study results		
Study type: biological exposure monitoring Type of population: occupational Details on study design: TYPE OF EXPOSURE: -roasting/smelting workers mainly exposed to dry dust containing nickel subsulphide and oxide, corresponding to an average atmospheric concentration of about 0.5 mg Ni per m^3 (range 0.1 to 1.0 mg Ni per m^3) -electrolytic workers are mainly exposed to aerosols of nickel sulphate and chloride, with an average air concentration of about 0.2 mg Ni per m^3 (range 0.1 to 0.5 mg Ni per m^3) -non-process workers are exposed to miscellaneous nickel composites corresponding to an average air concentration of 0.1 mg Ni per m^3 (range 0.01 to 0.5 mg per m^3) EXPOSURE GROUPS / CATEGORIES: -Workers from the Falconbridge Nikkelverk, Kristiansand, Norway -Nickel-exposed group: subjects employed at least 8 years at the plant and working with crushing, roasting, smelting, or electrolysis on 01Oct 76; 20% of remaining non-process workers employed at least 8 years were selected randomly. -Retired nickel workers: 15	Nickel Exposed Group (Active workers: - Mean: in plasma: 6.3 µg Ni/L; in urine: 49.1 µg Ni/L; in nasal mucosa: 273.9 µg Ni per 100g wet weight - Highest: in plasma: 36 µg Ni/L; in urine: 600 µg Ni/L; in nasal mucosa: 3,460 µg Ni per 100g wet weight - statistically significant correlation between plasma and urine nickel concentrations; non-significant correlation between nickel concentrations in plasma and nasal mucosa -283 workers (89%) had nickel values for plasma or urine above normal limit (>4.5 µg/L and >10 µg/L, respectively). -271 workers (85.8%) had nickel levels in the nasal mucosa that were higher than the normal limit (>53 µg/100 g wet wt) Retired Nickel Workers - Mean: in plasma: 2.9 µg Ni/L; in urine: 11.3 µg Ni/L; in nasal mucosa: 114.4 µg Ni per 100g wet weight - Highest: in plasma: 7.0 µg Ni/L; in urine: 42 µg Ni/L; in nasal mucosa: 720 µg Ni per 100g wet weight - statistically significant correlation between plasma and urine nickel concentrations -5 workers (33%) had raised nickel values in plasma or urine -6 workers (40%) had nickel levels in the nasal mucosa that were higher than the normal limit RELATION OF NICKEL CONCENTRATIONS TO EXPOSURE -mean values of nickel in biological samples from the control group are significantly lower than levels in active and retired workers; difference in means between active and retired worker levels in plasma are also significant -differences in mean nickel plasma and urine between the three worker categories; highest levels found in electrolysis followed by roasting/smelting, then non-process workers	Klimisch score: 2 (reliable with restrictions) weight of evidence **Test material (EC name): trinickel disulphide**	Torjussen W, Andersen I (1979)

male pensioners with at least 8 years of previous employment as process workers -Control group: 57 male volunteer patients from the County Hospital SAMPLE COLLECTION - blood, urine and biopsy (nasal mucosa) samples were collected in the morning during the last 3 months of the year 1976 from active and retired nickel workers and during the last 3 months of 1977 from the controls SAMPLE ANALYSIS -measurements of nickel concentrations in the plasma, urine, and nasal mucosa were made with an atomic absorption spectrophotometer STATISTICAL METHODS -student t-test applied to calculate significant differences between means and correlation coefficients -correlation coefficients were calculated between nickel concentrations in nasal mucosa, plasma or urine and the number of years from the first employment at the nickel refinery and between worker categories	-in nasal mucosa, highest mean nickel concentrations were found in subjects from the roasting/smelting department, followed by non-process and electrolytic workers -significant correlations between roasting/smelting work and raised nickel in nasal mucosa and between electrolytic work and raised plasma or urine nickel were observed -significant correlations between length of nickel exposure and nickel concentrations in nasal mucosa, plasma and urine were observed RETENTION AND RELEASE OF NICKEL IN NASAL MUCOSA -the half-life of nickel release from the nasal mucosa was estimated to be about 3.5 years Data are not specific to nickel subsulfide but rather reflects the mixed exposures present in this refining process.		

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

The *in vivo* inhalation toxicokinetic properties of Ni_3S_2 have been evaluated in rats and mice following intratracheal and inhalation administration, for various durations of exposure (1 day to 2 years). The majority of toxicokinetic studies evaluated distribution and clearance of Ni_3S_2 to and from the lung following inhalation; the studies often only evaluated a single dose and compared tissue concentrations of exposed to control animals. Fewer studies comprehensively evaluated tissues other than the lung or evaluated excretion. Lung tissue concentrations were well-characterized by Dunnick and colleagues in a series of inhalation studies designed to evaluate the toxicity of multiple nickel compounds, including Ni_3S_2. These robust studies each included both rats and mice and a range of two or five doses administered over 2 years or 13 weeks, respectively. A dose-dependent increase in the levels of Ni in the lung following Ni_3S_2 exposure was observed for animals exposed up to 13 weeks. However, at later time points (*i.e.*, 7 months and 2 years), similar levels of Ni were measured in the lungs of animals from all dose groups. Collectively these results indicated that little or no bioaccumulation occurred and thus concluded that continuous inhalation of Ni_3S_2 did not result in increased lung burden, suggesting no impaired lung particle clearance. The nickel burden in lung following repeated exposure via inhalation was evaluated in a number of other studies. Benson et al. (1995) examined the nickel lung burden in F344 rats exposed to Ni_3S_2/m^3 for up to 22 days. Over the

course of the exposure period, nickel levels tended to rise rapidly over the first seven days, and then rise more slowly thereafter, resulting in an estimated pulmonary clearance half-time of about 4 days. In a subsequent study, these authors (Benson et al., 1987) examined tissue (lung, kidney, and liver) burden of inhaled Ni_3S_2 in F344/N rats and B6C3F1 mice exposed for 6 hr/d, 5 d/wk, for 12 exposure days (0.6-10 mg/m^3). The authors concluded that the lung and kidney were the only organs containing significant quantities of nickel after 12 days of exposure. Additionally, the study demonstrated no significant differences between lung burdens in males and females.

Similar findings were reported by Kodama et al. (1993) following inhalation exposures to Ni_3S_2 in male Wistar rats for a duration of 6 months, followed by a 12-month recovery period. Nickel content was significantly elevated in the lung, liver and kidneys in animals sacrificed within one day after the last exposure. After 12 months of recovery, the nickel levels in these tissues returned to levels comparable with untreated animals. Nickel concentrations in the lung following an acute exposure have also been evaluated. In a rather robust evaluation, Benson et al. (1994) examined the fate of inhaled radiolabelled $^{63}Ni_3S_2$ in male F344 rats exposed for 120 min and then monitored for up to 32 days after exposure. Nose-only exposures resulted in an estimated 66% of the recovered nickel being deposited in the upper respiratory tract following exposure; nickel was rapidly cleared from the lung with an estimated clearance half-time of 4.6 days. However, a biphasic clearance was reported earlier by Finch et al. (1987), based on the findings of a limited analysis of tissue distribution following instillation of $^{63}Ni_3S_2$. The authors reported an initial half-life of 2 hr followed by a longer half-life of 119 hr. Similar responses were reported in kidneys and blood (206 and 122 hr). In contrast, the longer phase for the GI tract was only 34 hours. The authors concluded that Ni_3S_2 lung burden was quickly cleared by the coughing reflex and subsequently by the GI tract.

Separately, Mayer et al. (1998) administered a single, 2-hour, nose-only inhalation dose of Ni_3S_2 to male F344 rats in an effort to determine the distribution of inhaled Ni_3S_2 particles in the respiratory tract. Relative to control animals, the Ni content (mg/g dry weight) was approximately 40-fold increased in the nasal mucosa and almost 400-fold increased in lung tissues. Similar findings were observed by Valentine and Fisher (1984) in A/J mice exposed to intratracheally-instilled particulate $^{63}Ni_3S_2$. Lung clearance was biexponential; at least 90% of the deposited compound was cleared from the lungs within 35 days. Solubilized Ni_3S_2 was distributed to the liver, kidney, femur, and blood, but was removed from these sites at rates comparable to lung clearance. Collectively, these data indicate that inhaled Ni_3S_2 can distribute to extrapulmonary organs such as the liver and kidney. However, nickel is also cleared or eliminated quickly and does not bioaccumulate in rodents. Though no single study by itself is sufficient to fully characterize the distribution of Ni_3S_2, the data when considered collectively provide a general understanding of the toxicokinetics following inhalation or intratracheal instillation of Ni_3S_2.

In a study of nickel refinery workers (Torjussen and Andersen, 1979), nickel levels in plasma, urine and nasal mucosa of active workers were elevated compared to controls. Statistically significant correlation were observed between plasma and urine nickel concentrations and length of nickel exposure and nickel concentrations in nasal mucosa, plasma and urine. Tissue burden exposures for these workers were mixed, and included a combination of water soluble, oxidic, sulfidic (including Ni_3S_2) and metallic forms of nickel.

8. EVALUATION OF HEALTH HAZARDS

8.1 Acute toxicity - inhalation route

Table 10: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviation(s) if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value LC_{50}**	**Reference**
OECD Guideline 403 (Acute Inhalation Toxicity) GLP-compliant	rat (Sprague-Dawley) male/female 5/sex/group	**Test material (EC name): trinickel disulphide** Powder MMAD: 3.3-3.5 µm	0.2, 1.0, 5.0 mg/L 4 hours	LC50 (4 h): 0.9237 mg/L air (female) LC50 (4 h): 1.352 mg/L air (male) LC50 (4 h): 1.13785 mg/L air (average of males and females)	EPSL (2010)
Intratracheal instillation	mouse (A/J) male	**Test material (EC name): trinickel disulphide** **Fine powder:** MMAD = 1.8 µm; GSD = 1.55 **Course powder:** MMAD = 13.3 µm; GSD = 2.17	4, 20,100 mg Ni_3S_2/kg for single exposure; 0.5-64 mg Ni_3S_2/kg for once per week for 4 weeks	LD_{50}: 4 mg/kg bw (male) (Fine) LD_{50}: 50 mg/kg bw (male) (Coarse)	Fisher GL, Chrisp CE, McNeill KL, McNeill DA, Democko C, Finch GL (1984)
Intratracheal instillation	mouse (BALB/c BYJ) male	**Test material (EC name): trinickel disulphide** MMAD = 1.83 µm; GSD = 2.3	0 or 0.5 mg/kg single intratracheal instillation	lowest observed effect level: increase in polymorphonuclear cells in lavage fluid: 0.5 mg/kg bw (male)	Finch GL, Fisher GL, Hayes TL (1987)
Intratracheal instillations	rat (F344/Crl) male/female	**Test material (EC name): trinickel disulphide**	0.01, 0.10, 1.0 µmol Ni single intratracheal instillation	no observable effect level: 0.01 µmol Ni (male/female) (compared to control levels of lactate dehydrogenase, β-glucuronidase, glutathione peroxidase, glutathione reductase, total protein, sialic acid, total nucleated cells, neutrophils, macrophages, lymphocytes, and eosinophils)	Benson JM, Henderson RF, McClellan RO, Hanson RL, Rebar AH (1986)

single intratracheal instillation	rat (Fischer-344/Lov) male/female 6/sex/group	**Test material (EC name): trinickel disulphide**	0.01, 0.10, 1.0 µmol Ni (0.587, 5.87, 58.7 µg Ni) single intratracheal instillation	no observable effect level: extracellular lactose dehydrogenase (pulmonary cytotoxicity): 0.01 µmol Ni (male/female) no observable effect level: total protein in lavage fluid (pulmonary vascular leakage): 0.01 µmol Ni (male/female) no observable effect level: presence of nucleated cells in lavage fluid (inflammatory response): 0.01 µmol Ni (male/female) no observable effect level: beta glucuronidase (phagocytic activity): 0.1 µmol Ni (male/female) no observable effect level: presence of pulmonary lesions: 0.1 µmol Ni (male/female)	Benson JM, Henderson RF, McClellan RO, Rebar, AH (1984)

Table 11: Summary table of human data on acute inhalation toxicity

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
				None available

Table 12: Summary table of other studies relevant for acute inhalation toxicity

Type of study/data	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
				None available

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

Several studies were identified characterizing the acute or short-term toxicity of Ni_3S_2 in rodents following intratracheal instillation. In addition, one acute inhalation toxicity study was recently completed. The most well-characterized endpoint associated with rodent exposures to Ni_3S_2 was mortality; a GLP guideline-based study was available for inhalation exposure.

Eurofins Product Safety Labs (EPSL, 2010) characterized lethality associated with acute inhalation. This GLP, guideline-based study exposed rats (nose-only) for four hours to Ni_3S_2 (3 dose groups) and monitored survival, body weight, and a number of clinical endpoints. Under the conditions of this study, the acute inhalation defined LC_{50} of $Ni_3S_2$was 1.352 mg/L (95% CI of 0.3371 to 5.422 mg/L) in male rats and 0.9237 mg/L (95% CI of 0.5215 to 1.6359 mg/L) in female rats. The average inhalation LC_{50} is calculated to be 1.14 mg/L (MMAD =3 μm).

LD_{50} estimates were reported in an intratracheal instillation study conducted in mice by Fisher et al. (1984). The findings demonstrated the importance of physical form in the evaluation of pulmonary toxicity and lethality of Ni_3S_2 particles of different size. Fine particles (1.8 μm) were clearly more toxic and lethal than the coarse particles (13.3 μm), with reported LD_{50} values of 4 and 50 mg/kg, respectively; lethality was associated with pulmonary hemorrhage and possible congestion and edema. To compare the mortality results from the inhalation study in rats and the intratracheal instillation study in mice, the delivered dose after 4 hrs of inhalation exposure to 1.14 mg/L (MMAD =3 um) can be calculated, considering a ventilation rate for rats of 0.8 L/min/kg (ECHA, Guidance document: Chapter R.8, 2012) and a deposition fraction in the rats' lungs of 0.116 (MPPD v3.01): 1.14 mg/L x 0.8 L/min kg x 60 x 4 x 0.116= 25.4 mg/kg. This value is consistent with the LD50s found in mice by intratracheal instillation of 4 and 50 mg/kg for fine (1.8 μm) and coarse particles (13.3 μm) respectively.

Several studies with lower doses evaluated cellular, biochemical, and histological endpoints following intratracheal exposures. Benson et al. (1984) exposed F344 rats to 0.01 to 1.0 μmol Ni/rat (0.587 to 58.7 μg Ni/rat) and reported moderate multifocal alveolotis and dose-dependent increases in a number of biochemical parameters. In a 1986 study, this group of authors evaluated similar endpoints and correlated findings with the concentration of nickel in lung tissues following a similar exposure paradigm (0.01 to 1.0 μmol Ni per rat). Levels of Ni in the lung were virtually identical after 1 and 7 days of exposure to Ni_3S_2 (1 μmol Ni), but elevated relative to untreated animals. No significant biochemical, cytological, or histopathological changes were detected in nickel-exposed animals 1 day following administration. However, dose-related responses were observed following 7 days of exposure, indicating toxicity associated with short-term exposure to Ni_3S_2. Acute toxicity following intratracheal exposure in mice (0.5 mg/kg Ni_3S_2 in 20 μL PBS) was also reported by Finch et al. (1987). Observations of lethargy, pulmonary hemmorraging, increased body weight and lung weight, and increased cell yields and polymorphonuclear (PMN) cells in lung lavage fluid led the authors to conclude that Ni_3S_2 can be acutely toxic in mice. Collectively, these data indicate that acute toxicity following intratracheal exposure to Ni_3S_2 in rodents is possible depending on the dose.

8.1.2 Comparison with the CLP criteria

Taken together, the available data indicate that Ni_3S_2 can be acutely toxic in rodents following inhalation and intratracheal instillation under laboratory conditions (inhalation LC_{50} = 1.14 mg/L). The newly reported oral LC_{50} value of 1.14 mg/L for Ni_3S_2meets the criteria for classification as Acute Tox. 4; H332 for inhalation according to the CLP guidelines, which specifies that substances with an LC_{50} value between 1 and ≤5 mg/L fall within this category (EC No. 1272/2008). This is the only route of concern for acute toxicity, as the oral route does not warrant classification (Henderson et al., 2012b).

8.1.3 Conclusion on classification and labelling for acute inhalation toxicity

Ni_3S_2 has not previously been classified for acute inhalation toxicity in the EU. However, a recently completed OECD-guideline compliant study reported an inhalation LC50 =1.14 mg/L Ni_3S_2 after 4 hours of exposure in rats. These newly generated data demonstrate that Ni_3S_2 should be classified as Acute Tox. 4; H332 for inhalation.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The dossier submitter (DS) provided a recent acute inhalation study in rats (according to OECD TG 403 and GLP), two single dose intratracheal studies in rats and two intratracheal studies in mice. In the acute inhalation study, LC_{50} (4 h) values of 0.92 mg/L air (female), 1.35 mg/L air (male) and 1.14 mg/L air (average of males and females) were determined for the dust. LD_{50} values of 4 mg/kg bw (male) (fine) and 50 mg/kg bw (male) (coarse) were derived in one intratracheal study in mice indicating that the particle size affected the acute inhalation toxicity. The other intratracheal studies were performed at lower dose levels and did not induce mortality. The DS used the average LC_{50} value for males and females of 1.14 mg/L air when concluding on the proposed classification, resulting in Acute Tox. 4; H332. No ATE value was proposed.

Comments received during public consultation

Comments were provided by two MSCAs. Both MSCAs suggested deriving an ATE value for acute inhalation toxicity in addition to the classification. Both MSCAs also suggested classification as Acute Tox. 3; H331 based on the LC_{50} value of 0.92 mg/L in female rats as females may be more sensitive. One MSCA proposed an ATE value of 0.5 mg/L based on the converted acute toxicity estimate for the acute inhalation toxicity of dusts according to table 3.1.2 of CLP, Annex I. In addition, one MSCA suggested to take into account the available repeated dose inhalation studies in rats and mice which indicate that mice are more sensitive and could thus also be more sensitive in an acute inhalation study.

The DS responded to the comments, not agreeing to use the most sensitive sex and provided additional information to justify that there is no sex difference in acute inhalation toxicity. The DS did also not agree that the available repeated dose studies showed that mice are more sensitive than rats and provided some additional long-term repeated dose data with rats and mice.

Additional key elements

The following additional summaries of repeated dose inhalation studies in rats and mice were provided during the public consultation by one MSCA and the DS to assess the difference in sensitivity between males and females rats and between rats and mice (see tables below).

***Table**. Short-term inhalation studies with trinickel disulphide in rats*.

Inhalation Studies	Males	Females	Male vs female sensitivity
1-, 2-, 4-, 7-, 12-, and 22-d study, repeated exposure Doses: 0, 0.6, 2.5 mg/m^3 MMAD 0.6 mg/m^3, 2.07 MMAD 2.5 mg/m^3, 1.98 µm 11 males & 11 females/group Benson *et al*. (1995)	Survival at 0.6 & 2.5 mg/m^3 (alive/total): Day 1: 11/11; 11/11 Day 2: 11/11; 11/11 Day 4: 11/11; 11/11 Day 7: 11/11; 10/11 Day 12: 11/11; 11/11 Day 22: 11/11; 11/11	Survival at 0.6 & 2.5 mg/m^3 (alive/total): Day 1: 11/11; 11/11 Day 2: 11/11; 11/11 Day 4: 11/11; 11/11 Day 7: 11/11; 10/11 Day 12: 11/11; 11/11 Day 22: 11/11; 11/11	No difference in sensitivity (survival)
12-d study Doses: 0, 0.6, 1.2, 5, 10 mg/m^3 MMAD 10 mg/m^3 2.8 µm, GSD 2.0 5 males & 5 females/group Dunnick *et al*. (1988); from NTP report (1996)	Survival of controls (alive/total): 5/5 males NO(A)EC for mortality (alive/total): 5 mg/m^3: 5/5 male LO(A)EC for mortality (alive/total): 10 mg/m^3: 4/5 male	Survival of controls (alive/total): 5/5 females NO(A)EC for mortality (alive/total) 10 mg/m^3: 5/5 female LO(A)EC for mortality: >10 mg/m^3	Male more sensitive (i.e. survival was lower); difference not statistically significant
12-d study Doses: 0, 0.6, 1.2, 2.5, 5, 10 mg/m^3 MMAD 10 mg/m^3 2.5-2.6 µm, GSD 1.7-2.0 8 males & 8 females/group; except 5 male & 5 female/group for 1.2 & 5 mg/m^3 doses only Benson *et al*. (1987)	100% survival at 5 mg/m^3 and lower NO(A)EC for mortality (alive/total): 5 mg/m^3: 5/5 male LO(A)EC for mortality (alive/total): 10 mg/m^3: 6/8 male	100% survival at 10 mg/m^3 and lower NO(A)EC for mortality (alive/total): 10 mg/m^3: 8/8 female	Male more sensitive (i.e. survival was lower); difference not statistically significant

Table. Short term repeated dose inhalation studies with trinickel disulphide in rats and mice

Repeated dose inhalation Studies	Rats	Mice	Rat vs Mice Sensitivity
12-d study (6 h/d, 5 d/week) Dose: 0.6, 1.2, 5, 10 mg/m^3 MMAD 10 mg/m^3 2.8 µm, GSD 2.0 Dunnick *et al*. (1988); from the NTP report (1996)	Survival controls (alive/total): 5/5 males and 5/5 females NO(A)EC for mortality (alive/total): 5 mg/m^3; 5/5 male, 5/5 female LO(A)EC for mortality (alive/total): 10 mg/m^3; 4/5 male, 5/5 female	Survival controls (alive/total): 4/5 males, 4/5 females NO(A)EC for mortality (alive/total): 5 mg/m^3; 5/5 male, 5/5 female LO(A)EC for mortality (alive/total): 10 mg/m^3; 0/5 male (day 5-8), 0/5 female (day 5-10)	Mice more sensitive (i.e. survival was lower; also slightly lower in controls)
12-dstudy (6 h/d, 5 d/week) Dose: 0.6, 1.2, 5, 10 mg/m^3 [MMAD 10 mg/m^3 2.5-2.6 µm, GSD 1.7-2.0] Benson *et al*. (1987)	100% survival at 5 mg/m^3 and lower NO(A)EC for mortality (alive/total): 5 mg/m^3; 5/5 male, 5/5 female LO(A)EC for mortality(alive/total): 10 mg/m^3; 6/8 male, 8/8 female	Survival controls (alive/total): 5/8 males, 18/18 females NO(A)EC for mortality (alive/total): 5 mg/m^3; 5/5 male, 5/5 female LO(A)EC for mortality (alive/total): 10 mg/m^3; 0/8 male, 0/17 female	Mice more sensitive (i.e. survival was lower; also lower in male control mice)

Assessment and comparison with the classification criteria

RAC considered the acute inhalation study provided in rats as key for classification because the intratracheal installation studies in rats and mice were not predictive of the acute inhalation effects due to differences in dose rate and site of deposition within the lung.

Groups of 5 rats per dose and sex were exposed nose-only to trinickel disulfide at concentrations of 0.206, 1.02, and 5.15 mg/L for 4 hours in an acute inhalation test according to OECD TG 403 and GLP (EPSL, 2010). The MMAD of the particles were within the recommended range of 1-4 um. However, no information on the geometric standard deviation was provided. LC_{50} values of 1.35 mg/L for male rats, 0.92 mg/L female rats and 1.14 mg/L for male and female rats were determined based on the mortality incidence (see table below). Mortality at 1.02 mg/L occurred between day 4 and 7 and at 5.15 mg/L between day 2 and 5.

***Table**. Mortality incidences after acute inhalation exposure to trinickel disulfide in rats (EPSL, 2010)*

Exposure Levels (mg/L)	Males	Females	Total
0.206	0/5	0/5	0/10
1.02	1/5	3/5	4/10
5.15	5/5	5/5	10/10

As the LC_{50} values were close to the LC_{50} value differentiating between category 3 and 4, application of the LC_{50} value for females would result in a different classification than if the LC_{50} values for males or for the combination of male and female rats were used. The difference could be either due to a difference in sensitivity between males and females or due to a chance finding as the LC_{50} values were in the same range. In line with the suggestions from the DS and the commenting MSCAs, the available short term repeated inhalation studies were assessed for potential difference in sensitivity between male and female rats. The repeated dose rat studies (Benson *et al*., 1987, Benson *et al*., 1995; Dunnick *et al*., 1988) indicated that differences in mortality are small and they did not indicate that female rats are more sensitive than male rats. On the contrary, males seemed to be more sensitive than females (Benson *et al*., 1987; Dunnick *et al*., 1988).

The available single intratracheal instillation studies in rats were not used to assess the sex difference in sensitivity as no information on effects per sex were provided. Long term repeated dose inhalation studies (90-d and chronic) were not assessed as the mortality in such studies may be affected by differences in other parameters, such as accumulation of the substance or of effects, that cannot be extrapolated to mortality after acute exposure.

RAC agreed with the commenting MSCA that where adequate data show that other species are more sensitive the classification should be based on the most sensitive species. Therefore, an assessment of the difference in mortality between rats and mice in short term repeated dose inhalation studies was performed. As for the rat studies, long term repeated dose inhalation studies (90-d and chronic) were not assessed as the mortality in such studies may be affected by differences in other parameters, such as accumulation of the substance or of effects, that cannot be extrapolated to mortality after acute exposure. Also, the available single dose intratracheal instillation studies in rats and male mice were not used because difference in dosing (often low with no mortalities) and because the exposure conditions were either lacking or not comparable (particle size, dose and vehicle volume). Both available 12-d studies (Benson *et al*., 1987; Dunnick *et al*., 1988) showed that mice are more sensitive than rats. The two 12-d studies showed that rats and mice survived after repeated exposure to 0.005 mg/L, whereas mice showed 100% mortality at 0.010 mg/L where rats showed only limited mortality. Based on this, it can be concluded that mice were more sensitive than rats after short term exposure.

According to the CLP guidance (3.1.3.3.5 c. 'Evidence from other toxicity tests'), early effects from repeated dose testing can be used to estimate the acute toxicity when no acute data exists. In the present case, high quality acute toxicity data were available and there was thus no reason to base the LC_{50} on short term repeated dose toxicity studies. The lowest LC_{50} value of 0.92 mg/L was found in females and was below the cut off value of 1 mg/L for category 3. The LC_{50} value for males was just above the cut off value for category 3. In view of these borderline results, RAC looked at other available information. There were no robust acute data that could be used as supportive evidence for differences in sensitivity between sexes and/or species. The short term

repeated dose studies in rats did not indicate a difference in sensitivity between sexes. On the other hand, the short term repeated dose studies in mice did indicate that mice were more sensitive than rats after short term repeated exposure, which could be indicative for a lower LC_{50} value than the LC_{50} value for rats. RAC however noted that repeated dose studies are in general only used when no acute data exist (CLP guidance 3.1.3.3.5) and RAC also noted that the mortalities in these repeated dose studies occurred after 5-10 days of exposure, and that the outcome pointed in different directions as regards sensitivity of species and sexes. Furthermore, the CLP guidance states that classification should be based on the lowest ATE available (3.1.2.3.2). Taking this into account, the classification for acute inhalation toxicity was based on the lowest LC_{50} value of 0.92 mg/L observed in female rats in a robust inhalation toxicity study.

RAC concluded that **classification for acute toxicity via the inhalation as Acute Tox. 3; H331 with an ATE of 0.92 mg/L was warranted.**

9. DETAILED STUDY SUMMARIES

9.1 TOXICOKINETICS

STUDY 1

Study reference:

Kirby Memorial Health Center (KMHC; 2010). Compiled Analysis Reports for 15 Nickel Substances: Solubility in Simulated Fluids. Analyses were conducted during 2008-2010.

Henderson, RG, Cappellini D, Seilkop SK, Bates HK and Oller AR (2012a). Oral bioaccessibility testing and read-across hazard assessment of nickel compounds. Regul Toxicol and Pharmacol. Jun;63(1):20-28.

Detailed study summary and results (*from registration dossier (IUCLID*)):

In situations where the bioavailability of a metal substance is not known or not feasible to determine experimentally, the amount of ion "available for absorption" may be measured using *in vitro* methods. In this application, the dissolution (*e.g.*, elution or extraction) of metal ion from surrogate (synthetic) tissue fluids is measured. The resultant value is termed *bioaccessibility* and is defined as the amount of a substance (*e.g.*, metal ion) available for absorption.

STUDY 2

Study reference:

Benson JM, Carpenter RL, Hahn FF, Haley PJ, Hanson RL, Hobbs CH, Pickrell JA, and Dunnick JK. (1987). Comparative inhalation toxicity of nickel subsulphide to F344/N rats and B6C3F1 mice exposed for 12 days. Fundamental and Applied Toxicology; 9:251-265.

Dunnick JK, Benson JM, Hobbs CH, Hahn FF, Cheng YS, and Eidson AF. (1988). Comparative toxicity of nickel oxide, nickel sulfate hexahydrate, and nickel subsulphide after 12 days of inhalation exposure to F344 rats and B6C3F1 mice. Toxicology; 50:145-156.

National Toxicology Program (NTP; 1996). Toxicology and carcinogenesis studies of nickel subsulphide (CAS no. 12035-72-2) in F344/n rats and B6C3F1 mice (inhalation studies). NIH Publication No. 96-3369. Testing laboratory: National Toxicology Program (NTP). Report no.: NTP TR 453. Owner company: National Institute of Health, Springfield, VA. Washington DC. [Also cited as Dunnick et al., 1989 and Dunnick et al., 1995].

Detailed study summary and results (*from registration dossier (IUCLID*)):

Benson et al. (1987) examined the acute toxicity and tissue burden of inhaled Ni_3S_2 in F344/N rats exposed for 6 hr/d, 5 d/wk, for 12 exposure days (0.6-10 mg/m^3). Chamber concentrations and aerosol size was determined analytically (MMAD: 2.8 μm; GSD 2.0). Animals designated for nickel determination were sacrificed, and the lungs, lung associated lymph nodes (LALN), liver, kidney, nasal turbinates, and testes or ovaries were removed, weighed, dissolved in acid, and nickel content measured by electrothermal atomic absorption spectroscopy. Lung burden was measured in groups exposed to 0.6, 2.5 and 10 mg Ni_3S_2/m^3; nickel was not detected (i.e. < 155 ng) in untreated animals. In males, the concentrations were 7.3, 24.6, and 103 μg Ni/lung at the three doses. Similar values were found in females and there were no significant differences between lung burdens in males and females. Among the other organs examined, nickel levels were only measured at the two highest exposures. At 2.5 mg/m^3, nickel was detected in the LALN, turbinates, and kidneys, but levels below the limit of quantitation (i.e. < 400 ng Ni). At 10 mg/m^3, nickel was detected in all tissues examined but only elevated in the kidney. The authors concluded that the lung and kidney were the only organs containing significant quantities of nickel after 12 days of exposure.

STUDY 3

Study reference:

Benson JM, Barr EB, Bechtold WE, Cheng YS, Dunnick JK, Eastin WE, Hobbs CH, Kennedy CH, and Maples KR. (1994). Fate of inhaled nickel oxide and nickel subsulphide in F344/n rats. Inhalation Toxicology; 6:167-183.

Detailed study summary and results (*from registration dossier (IUCLID*)):

Benson et al. (1994) examined the fate of inhaled radiolabelled $^{63}Ni_3S_2$ in male F344 rats exposed for 120 min. Chamber concentrations and aerosol size were determined analytically (5.7 mg/m3 Ni_3S_2, MMAD: 1.3 μm; GSD 1.5). Four rats were also exposed in plethysmographic chambers in order to measure the potential influence of Ni_3S_2 on minute volume and tidal volume, and were sacrificed within one-hour post exposure in order to determine the deposition and distribution in various tissues. Three rats were exposed nose-only, and kept for excretion analysis in urine and feces for up to 64 days. Additional groups of 3 rats were exposed nose-only and sacrificed at various time points ranging from 1 hr to 64 days post-exposure. Exposure to Ni_3S_2 did not significantly alter minute volume. Based on minute volume and Ni_3S_2 concentration, 175 μg was inhaled and only 24.6 μg (14%) was recovered in all tissues and fluids. Of this recovered Ni, 66% was reported as depositing in the upper respiratory tract (comprised of Ni in skull, nasal turbinates, larynx/trachea, and GI tract) and 34% was deposited in the lower respiratory tract (comprised as blood, soft tissues, lungs, urine, and carcass).

Of the Ni deposited in the respiratory tract, 24% was in the lungs and 74% was in the GI tract, and lower levels of Ni were detected in the blood, kidney, and carcass. Ni was not detected in the lungs of animals sacrificed 32 days after exposure; the calculated clearance half-time was 4.6 days. Data also suggest that some Ni was transferred from the lung to lung associated lymph nodes and subsequently cleared from the lung. On the day of exposure, excretion in the feces and urine was roughly equivalent. In the three days following exposure, excretion in feces and urine was roughly 80-90% and 10%, respectively – potentially indicating physical clearance from the lung and ingestion. Thereafter, excretion in urine rapidly increased from 60-100%. The authors concluded that

Ni_3S_2 is rapidly cleared from the lung and is present in extrarespiratory tissue within a few hours of exposure.

STUDY 4

Study reference:

Benson JM, Cheng YS, Eidson AF, Hahn FF, Henderson RF, and Pickrell JA. (1995). Pulmonary toxicity of nickel subsulphide in F344/N rats exposed for 1-22 days. Toxicology; 103:9-22.

Detailed study summary and results (*from registration dossier (IUCLID*)):

Benson et al. (1995) examined the time course of morphological and biochemical changes in the lungs of F344 rats exposed to 0.6 or 2.5 mg Ni_3S_2/m^3 for up to 22 days, as well as the examined the nickel lung burden in these animals. The morphological and biochemical effects are described in the Repeated Dose Toxicity: Inhalation section. Chamber concentrations and aerosol size were determined analytically for the two exposure concentrations and were 2.07 μm (2.15) and 1.98 μm (2.03), respectively. Animals were sacrificed at 1, 2, 4, 7, 12 and 22 days after the start of exposure. Nickel content in lung was determined by digesting tissues in acids and then measuring Ni via electrothermal atomic absorption spectroscopy. No nickel was detected in control animals (detection limit is 0.66 μg Ni/lung). In treated animals, there were no significant differences between males and females, so the data were pooled. Over the course of the 22-day exposure period, nickel levels tended to rise rapidly over the first seven days, and then rise more slowly thereafter. In the high exposure group, the values for the aforementioned sacrifice days were 6, 11, 15, 28, 24 and 34 μg Ni/g control lung. In the lower exposure group, these values were about a third of the higher group. Combining these early lung burden data points with lung burden data beyond 22 days from a previous publication (Dunnick et al., 1989), the authors calculated a pulmonary clearance half-time of about 4 days. The authors concluded that the data suggest that Ni_3S_2 is “fairly soluble” in lung.

STUDY 5

Study reference:

Dunnick JK, Elwell MR, Radovsky AE, Benson JM, Hahn FF, Nikula KJ, Barr EB, and Hobbs CH. (1995). Comparative carcinogenic effects of nickel subsulphide, nickel oxide, or nickel sulfate hexahydrate chronic exposures in the lung. Cancer Research; 55:5251-5256.

National Toxicology Program (NTP; 1996). Toxicology and carcinogenesis studies of nickel subsulphide (CAS no. 12035-72-2) in F344/n rats and B6C3F1 mice (inhalation studies). NIH Publication No. 96-3369. Testing laboratory: National Toxicology Program (NTP). Report no.: NTP TR 453. Owner company: National Institute of Health, Springfield, VA. Washington DC. [Also cited as Dunnick et al., 1989 and Dunnick et al., 1995].

Detailed study summary and results (*from registration dossier (IUCLID*)):

As part of study evaluating the toxicity and carcinogenicity of inhaled $NiSO_4$, Ni_3S_2 and NiO, Dunnick et al. (1995) described the Ni lung burden at 7 and 15 months following inhalation exposure to Ni_3S_2 in F344/N rats exposed for 6 hr/d, 5 d/wk, for 2 years (0.15, and 1 mg Ni_3S_2/m^3; 0.11 and 0.73 mg Ni/m^3). Chamber concentrations and aerosol size were determined analytically (MMAD: 2.1 μm; GSD 2.0). No significant differences in mortality were observed between control and treated animals. In untreated rats, Ni lung burden was below the limit of detection. In male and female rats exposed to the lower dose, Ni levels in lungs ranged from 4-6 μg/m3; similar levels (3-9 μg/m3) were observed in those exposed to the higher dose. Levels were similar at 7 and 15 months indicating that little or no bioaccumulation occurred. Ni levels following Ni_3S_2 exposure were slightly higher than for $NiSO_4$ and much lower than for NiO. The authors concluded that the nickel

lung burden data showed accumulation of nickel in the lungs of rats after exposure to NiO, whereas nickel was cleared following exposure to Ni_3S_2 and $NiSO_4$. This investigation was part of a comprehensive bioassay conducted

STUDY 6

Study reference:

Dunnick JK, Elwell MR, Benson JM, Hobbs CH, Hahn FF, Haly PJ, Cheng YS, and Eidson AF. (1989). Lung toxicity after 13-week inhalation exposure to nickel oxide, nickel subsulphide, or nickel sulfate hexahydrate in F344/N rats and B6C3F1 mice. Fundamental and Applied Toxicology; 12:584-594.

National Toxicology Program (NTP; 1996). Toxicology and carcinogenesis studies of nickel subsulphide (CAS no. 12035-72-2) in F344/n rats and B6C3F1 mice (inhalation studies). NIH Publication No. 96-3369. Testing laboratory: National Toxicology Program (NTP). Report no.: NTP TR 453. Owner company: National Institute of Health, Springfield, VA. Washington DC. [Also cited as Dunnick et al., 1989 and Dunnick et al., 1995].

Detailed study summary and results (*from registration dossier (IUCLID)*):

As part of a study evaluating the toxicity of $NiSO_4$, Ni_3S_2 and NiO, Dunnick et al. (1989) examined the lung burden of inhaled Ni_3S_2 in F344/N rats exposed for 6 hr/d, 5 d/wk, for 13 weeks (0.15, 0.3, 0.6, 1.2, and 2.5 mg Ni_3S_2 /m^3; 0.11, 0.2, 0.4, 0.9, and 1.8 mg Ni/m^3). This dose range was based on results from an earlier 12-day study where 5 mg Ni_3S_2/m^3 caused lung lesions. Chamber concentrations and aerosol size were determined analytically (MMAD: 2.4 μm; GSD 2.2). Lung Ni levels were measured after 4, 9, and 13 weeks. In rats, there was a clear dose-dependent increase in the levels of Ni in the lung following Ni_3S_2 exposure (e.g., 2.6 and 14.8 μg Ni/g lung at 0.1 and 1.8 mg Ni/m^3, respectively), but little or no time dependence (e.g. 14.8 and 17.6 μg Ni/g lung at 4 and 13 weeks, respectively). The authors concluded that in contrast to NiO, continuous exposure to $NiSO_4$ and Ni_3S_2 did not result in increased lung burden. These results were said to agree with those of other studies, which show that soluble nickel salts are cleared from the rat lung while insoluble nickel salts accumulate in the rat lung. This investigation was part of a comprehensive bioassay conducted by the National Toxicology Program (1996).

STUDY 7

Study reference:

Finch GL, Fisher GL, and Hayes TL. (1987). The pulmonary effects and clearance of intratracheally instilled Ni_3S_2 and TiO_2 in mice. Environmental Research; 42:83-93.

Detailed study summary and results (*from registration dossier (IUCLID)*):

Finch et al. (1987) examined the acute effects of intratracheal exposure to Ni_3S_2, as well as performed a limited analysis of tissue distribution following instillation of $^{63}Ni_3S_2$. In addition to Ni_3S_2, the toxicity of the relatively inert titanium dioxide was also examined. BALB/c BYJ mice were intratracheally instilled with 11.8 μg (roughly equal to 0.5 mg/kg $^{63}Ni_3S_2$; MMD = 1.65 μm; GSD = 1.83) in 20 μL PBS. Tissue samples were collected at 15 minutes, 1, 5 and 20 hours, and 3 and 7 days post exposure. For mice treated with $^{63}Ni_3S_2$, the following tissues were weighed and radioactivity measured: lungs and trachea (distal to larynx), gastrointestinal tract (including esophagus, stomach, and intestines), and kidneys. Blood was also collected via cardiac puncture. Lung weights were slightly elevated after $^{63}Ni_3S_2$ exposure (albeit not significantly); no other measured organs were different from control. In measuring clearance, the authors noted that both

$^{63}Ni_3S_2$ and titanium oxide were initially cleared from the lung through bubbles that led to ingestion. Fifteen minutes after exposure, the lung burden was measured as 75% of the initial dose. Clearance was biphasic, with an initial half-life of 2 hr followed by a longer half-life of 119 hr. Similar responses were reported in kidneys and blood (206 and 122 hr). In contrast, the longer phase for the GI tract was only 34 hours. The authors concluded that Ni_3S_2 lung burden was quickly cleared by the coughing reflex and subsequently by the GI tract.

STUDY 8

Study reference:

Kodama Y, Tanaka I, Matsuno K, Ishimatsu S, and Kawamoto T. (1993). Comparative deposition and clearance of various nickel compounds exposed by inhalation in rats. Biological Trace Element Research; 36:257-269.

Detailed study summary and results (*from registration dossier (IUCLID*)):

Kodama et al. (1993) examined the deposition and clearance of nickel in male Wistar rats exposed to Ni_3S_2 and NiO for 6 hr/day, 5 d/wk, for 6 months, followed by a 12-month recovery period. Chamber concentrations and aerosol size were determined analytically (0.5 mg/m^3; MMAD: 2.6 μm; GSD 1.9). Nickel content in organs was determined by atomic absorption spectrophotometry. There was no significant difference in bodyweight in treated and untreated animals throughout the duration of the exposure and recovery periods. Organ weights were compared for the lung, liver, kidney, and spleen at the end of the 12-month recovery phase. Lung weight was significantly elevated in treated rats relative to control rats (2.9 vs 2.5 g). Nickel content was significantly elevated in the lung, liver and kidneys in animals sacrificed within one day after the last exposure. After 12 months of recovery, the nickel levels in these tissues returned to levels comparable with untreated animals. Nickel levels in the spleen and blood of treated and untreated cells were similar in all animals. Based on the minute volume and nickel concentration in the inhaled air, the inhaled dose was calculated to be 3.8 mg. Based on the recovered nickel in the lung tissues, only about 18 μg, or roughly 0.5% of inhaled dose, was deposited into the lung. By 12 months post exposure, 98% of the nickel was estimated to have cleared from the lung. These data indicated that inhaled Ni_3S_2 can distribute to extrapulmonary organs such as the liver and kidney. The data also showed that nickel levels returned to control levels within 12 months following cessation of exposure. The study design, however, does not allow one to accurately measure a clearance rate.

STUDY 9

Study reference:

Mayer C, Klein KG, Wesch H, and Schmezer P. (1998). Nickel subsulphide is genotoxic *in vitro* but shows no mutagenic potential in respiratory tract tissues of BigBlue™ rats and Muta™Mouse mice *in vivo* after inhalation. Mutation Research; 420:85-98.

Detailed study summary and results (*from registration dossier (IUCLID*)):

Mayer et al. (1998) administered a single 2 h, nose-only inhalation dose of Ni_3S_2 of 13 mg/kg bw (352 mg/m^3) to male F344 rats, which was a dose close to the Maximum Tolerated Dose (MTD). The purpose of this particular study (which was part of a larger study on the in vitro and in vivo mutagenicity and genotoxicity of Ni_3S_2) was to determine the distribution of inhaled Ni_3S_2 particles in the respiratory tract of the experimental animals. The authors measured Ni concentrations in freeze-dried tissue samples of nasal mucosa and lung of treated rats by atom-absorption-spectrometry. Relative to control animals, the Ni content (μg/g dry weight) was approximately 40-fold increased in the nasal mucosa (71 vs 1.9) and almost 400-fold increased in lung tissues (451 vs 1.2).

As the *in vivo* portion of the larger study did not identify DNA damage following inhalative Ni_3S_2 administration in mice and rats, the determination of substantial nickel distribution to the nasal mucosa and lung showed that sufficient particle deposition in the respiratory tract had occurred in rats. However, the authors reported that bioavailability of nickel ions at critical target sites (e.g., heterochromatic regions in the nucleus) seems to correlate with the potential of nickel compounds to directly cause DNA changes. The authors further concluded that these comparative in vitro and in vivo studies confirmed that prediction from in vitro measurements to in vivo situation should be done with caution.

STUDY 10

Study reference:

Valentine R and Fisher G. (1984). Pulmonary clearance of intratracheally administered $^{63}Ni_3S_2$ in strain A/J mice. Environmental Research; 34:328-334.

Detailed study summary and results (*from registration dossier (IUCLID*)):

Valentine and Fisher (1984) studied the toxicokinetics of intratracheally instilled particulate $^{63}Ni_3S_2$ (3 uCi; 1.66 um, MMAD) in A/J mice. Lung and tissue burdens, as well as clearance and elimination kinetics, were determined from 8 groups of 4 mice, using scintillation counting techniques. Animals were sacrificed by intraperitoneal injection of sodium pentobarbital. Blood samples were obtained from the bracheal plexus. Duplicate aliquots of tissue minces, dried feces, or urine samples were digested in 20-ml scintillation vials. Lung clearance over the 35-day observation period was determined to be biexponential in nature, separating into two distinct components with initial and final phase biological half-times corresponding to 1.2 and 12.4 days, respectively. Furthermore, of the deposited Ni_3S_2, at least 90% was cleared from the lungs of strain A/J mice within 35 days. Radioactivity was detected in the blood, liver, kidney, and femur within 4 hr, and was eliminated at rates comparable to that in the lung. Thirty-five days post $^{63}Ni_3S_2$ instillation, excretion involved primarily urinary (60% of dose eliminated), but also fecal (40% of dose eliminated), pathways. The authors point to $^{63}Ni_3S_2$ elimination kinetics in hypothesizing that mucociliary activity, alveolar macrophage transport, and particle dissolution processes were involved in lung clearance. Solubilized Ni_3S_2 was distributed to the liver, kidney, femur, and blood, but was removed from these sites at rates comparable to lung clearance. The authors concluded that the data were consistent with the relatively rapid translocation, solubilization, and elimination of particulate $^{63}Ni_3S_2$ from the body.

STUDY 11

Study reference:

Torjussen W and Andersen I. (1979). Nickel concentrations in nasal mucosa, plasma, and urine in active and retired nickel workers. Annals of Clinical and Laboratory Science; 9(4):289-298.

Detailed study summary and results (*from registration dossier (IUCLID*)):

Torjussen and Andersen (1979) evaluated nickel concentrations in the nasal mucosa, blood and urine of current and retired nickel refinery workers and compared levels to those measured in control subjects. The main study group (n=318) consisted of nickel-exposed workers in the roasting/smelting department, electrolytic workers, and non-process workers who were said to be exposed to dry dust containing nickel subsulfide and oxide, aerosols of nickel sulphate and chloride, or miscellaneous

nickel composites, respectively. Retired workers (n=15) and age-matched control subjects (n=57) were also included in the study. Blood, urine and nasal mucosa biopsy samples were collected between 1976-1977 and analyzed for total nickel using an atomic absorption spectrophotometer. In the nickel-exposed group, mean concentrations of nickel were 6.3 µg/L in plasma, 49.1 µg/L in urine, and 273.9 µg per 100 g wet weight in nasal mucosa. Over 85% of the workers had nickel values that were above the normal limit in all three biological media. Levels were significantly lower in retired workers; mean concentrations of nickel were 2.9 µg/L in plasma, 11.3 µg/L in urine, and 114.4 µg per 100 g wet weight in nasal mucosa.

-mean values of nickel in biological samples from the control group are significantly lower than levels in active and retired workers; difference in means between active and retired worker levels in plasma are also significant

-differences in mean nickel plasma and urine between the three worker categories; highest levels found in electrolysis followed by roasting/smelting, then non-process workers

-in nasal mucosa, highest mean nickel concentrations were found in subjects from the roasting/smelting department, followed by non-process and electrolytic workers

-significant correlations between roasting/smelting work and raised nickel in nasal mucosa and between electrolytic work and raised plasma or urine nickel were observed

-significant correlations between length of nickel exposure and nickel concentrations in mucosa, plasma and urine were observed

RETENTION AND RELEASE OF NICKEL IN NASAL MUCOSA
-the half-life of nickel release from the nasal mucosa was estimated to be about 3.5 year

Data is not specific to nickel subsulfide but rather reflects the mixed exposures present in this refining process.

9.2 HEALTH HAZARDS

9.2.1 Acute inhalation toxicity - animal data

STUDY 1

Study reference:

Eurofins Product Safety Labs (EPSL; 2010). Acute inhalation toxicity study in rats, Eurofins PSL Study #28705, Ni subsulphide

Detailed study summary and results (*from registration dossier (IUCLID*)):

Test type
• OECD Guideline 403 (Acute Inhalation Toxicity); GLP-compliant

Test substance
• Test material used in the study is equivalent to the substance identified in the CLH dossier.
• EC number: 234-829-6
• CAS number: 12035-72-2
• Degree of purity: 100%

• Impurities: none
• Batch number: not applicable
• Physicochemical properties that may be important when assessing acute oral toxicity: powder
• Physical form (gas, vapour, dust, mist): aerosol
• Particle size: MMAD = 3.1, 3.2, and 3.35 μm for doses 0.206, 1.02, and 5.15 mg/L, respectively
• Type or preparation of particles (for studies with aerosols): Wright Dust Generator driven by variable speed motor

Test animals
• Species/strain/sex: Sprague-Dawley, male/female
• No. of animals per sex per dose: 5
• Age and weight at the study initiation: 9-11 weeks; males were 288-385 grams and females were 190-259 grams

Administration/exposure
• Type of inhalation exposure and test conditions: nose only inhalation chamber
• Duration of test/exposure period; 4 hours
• Doses/concentration levels: 0.206, 1.02, 5.15 mg nickel subsulfide/L. After establishing the desired generation procedures during pre-test trials, thirty healthy rats were selected for test and equally distributed into three dose groups.

• Analytical verification of test atmosphere concentrations: Gravimetric samples were withdrawn at five or six intervals from the breathing zone of the animals during each exposure. Samples were collected using 37 mm glass fiber filters (GF/B Whatman) in a filter holder attached by 1/4-inch tygon tubing to a vacuum pump (Reliance Electric, Model #G557X). Filter papers were weighed before and after collection to determine the mass collected. This value was divided by the total volume of air sampled to determine the chamber concentration. Sample airflows were measured using a Mass Flowmeter (Omega, Model #FMA-5610).
• Post exposure observation period: 14 days
• Control group and treatment: none
• Vehicle: identification, concentration and volume used, justification of choice of vehicle: not applicable
• Statistical methods: Probit Analysis; Finney, D.J., Probit Analysis, 3rd ed., Cambridge University Press, Cambridge, Great Britain, 1971, pp.1-333 was used for data analysis of LC_{50} and confidence limit calculations.

Results and discussion (*from registration dossier (IUCLID)*):

• Incidence of mortality

Exposure Levels (mg/L)	Males	Females	Total
0.206	0/5	0/5	0/10
1.02	1/5	3/5	4/10
5.15	5/5	5/5	10/10

• The acute inhalation defined LC_{50} of the test substance is 1.352 mg/L for male rats and 0.9237 mg/L female rats

• Additional information that may be needed to adequately assess data for

reliability:

- Clinical signs:
  0.206 mg/L: Immediately following exposure, all animals appeared active and healthy. Although three males showed signs of facial staining or ocular discharge on Day 1, all rats continued to appear active and healthy over the 14-day observation period. There were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior.

  1.06 mg/L: Immediately following exposure to the test atmosphere, all animals appeared active and healthy. By Day 3, all rats began to show clinical signs including facial staining, irregular respiration, hypoactivity, a thin appearance, reduced fecal volume and/or cold limbs. One male and one female were found dead on Day 4 and two additional females died on Day 5 or 7. All surviving animals recovered from the above symptoms by Day 12.

  5.15 mg/L: Immediately following exposure to the test atmosphere, all animals appeared active and healthy. Two females were found dead on Day 2 and all other rats began to show clinical signs including facial staining, abnormal respiration, hypoactivity, hunched posture, reduced food consumption and/or reduced fecal volume. All remaining animals died within five days of exposure.

- Necropsy findings:
  0.206 mg/L: No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

  1.06 mg/L: Gross necropsy of the decedents revealed discoloration of the lung, liver and/or intestines, and/or rigor mortis, with the lungs displaying the most severe effects. No gross abnormalities were noted for any of the euthanized animals necropsied at the conclusion of the 14-day observation period.

  5.15 mg/L: Gross necropsy of the decedents revealed discoloration of the lungs and/or intestines, with the lungs displaying the most severe effects.

STUDY 2

Study reference:

Benson JM, Henderson RF, McClellan RO, and Rebar AH. (1984). Comparative toxicity of nickel salts to the lung. Progress in Nickel Toxicology; 3rd International Congress on Nickel Metabolism and Toxicology; 85-88.

Detailed study summary and results (*from registration dossier (IUCLID*)):

Test type

•*No guideline* available. Rats were anesthetized, intubated, and exposed to Ni_3S_2 intratracheally (duration not specified). Toxicity in lung was assessed at 1 and 7 days post exposure.

Test substance

• Test material used in the study is equivalent to the substance identified in the CLH dossier.
• EC number: 234-829-6

• CAS number: 12035-72-2
• Degree of purity: the detail was not given in the original study report
• Impurities: the detail was not given in the original study report
• Batch number: not applicable
• Physicochemical properties that may be important when assessing acute oral toxicity: solid
• Physical form (gas, vapour, dust, mist): compound dissolved or suspended in saline
• Particle size: the detail was not given in the original study report
• Type or preparation of particles (for studies with aerosols): not applicable

Test animals
• Species/strain/sex: Fischer-344/Lov rats, male/female
• No. of animals per sex per dose: 6
• Age and weight at the study initiation: the detail was not given in the original study report

Administration/exposure
• Type of inhalation exposure and test conditions: intratracheal instillation
• Duration of test/exposure period: single dose
• Doses/concentration levels: 0.01, 0.10, 1.0 μmol Ni
• Analytical verification of test atmosphere concentrations: not applicable
• Post exposure observation period: 1 or 7 days
• Control group and treatment: the detail was not given in the original study report
• Vehicle: identification, concentration and volume used, justification of choice of vehicle: physiological saline (0.15 M) containing 0.2% gelatin
• Statistical methods: Student's t-test

Results and discussion (*from registration dossier (IUCLID*)):
• Incidence of mortality: not applicable
• The acute inhalation defined LC_{50} of the test substance: not applicable

• Additional information that may be needed to adequately assess data for reliability:

- Ni_3S_2 induced an increase in LDH, BG, TP, and nucleated cells in lavage at 7-day post exposure. Alveolitis lesions were observed 7 days post exposure.
- Clinical signs:
  1 Day Post Exposure: Minimal changes in lactate dehydrogenase (LDH), beta glucuronidase (BG), total protein (TP) in rat lavage fluid.

  7 Days Post Exposure: All Ni compounds increased the TP, but the changes were not dose-dependent. Ni3S2 increased LDH, BG, TP, and nucleated cells in lavage. Alveolitis lesions were observed 7 days post exposure.

STUDY 3

Study reference:

Fisher GL, Chrisp CE, McNeill KL, McNeill DA, Democko C, and Finch GL. (1984). Mechanistic evaluations of the pulmonary toxicology of nickel subsulphide. In: Advances In Modern Environmental Toxicology. MacFarland HN et al. (Ed.). Volume 6. Applied Toxicology Of

Petroleum Hydrocarbons (Symposium). Washington, D. C., USA May 11-13, 1982. Princeton Scientific Publishers: Princeton, N. J., USA

Detailed study summary and results (*from registration dossier (IUCLID*)):

Test type

• *No guideline* available. A/J mice were exposed to Ni_3S_2 particles of different sizes via intratracheal instillation either once or once per week for 4 weeks. Lethality was noted and effects on pulmonary macrophages were characterized.

Test substance

• Test material used in the study is equivalent to the substance identified in the CLH dossier.
• EC number: 234-829-6
• CAS number: 12035-72-2
• Degree of purity: the detail was not given in the original study report
• Impurities: the detail was not given in the original study report
• Batch number: not applicable
• Physicochemical properties that may be important when assessing acute oral toxicity: solid
• Physical form (gas, vapour, dust, mist): compound suspended in phosphate-buffered saline
• Particle size: Fine: MMD =1.8 μm, GSD =1.55; Coarse: MMD =13.3 μm, GSD =2.17
• Type or preparation of particles (for studies with aerosols): not applicable

Test animals

• Species/strain/sex: A/J mice, male
• No. of animals per sex per dose: 10-20
• Age and weight at the study initiation: 8-10 weeks

Administration/exposure

• Type of inhalation exposure and test conditions: intratracheal instillation
• Duration of test/exposure period and doses/concentration levels: 4-100 mg Ni_3S_2/kg for single exposure; 0.5-64 mg Ni_3S_2/kg for once per week for 4 weeks
• Analytical verification of test atmosphere concentrations: not applicable
• Post exposure observation period: 14 days for single dose; 60 days for multiple dose (starting after first exposure)
• Control group and treatment: the detail was not given in the original study report
• Vehicle: identification, concentration and volume used, justification of choice of vehicle: phosphate-buffered saline
• Statistical methods: Macrophage functional data are presented as percent of control values in order to present data from a series of experiments performed at different times. Statistical analyses used data from individual experiments comparing dosage level to control values. Treated and control comparisons were performed using a two-tailed Student t-test with an alpha-value of 0.025.

Results and discussion (*from registration dossier (IUCLID*)):

• The acute inhalation defined LD_{50} of the test substance:

		LD50 (mg/kg)	
Strain	Size: MMD, GSD	Single	Multiple*
A/J	Fine, 1.8 um, 1.55	4	1
A/J	Coarse, 13.3 um, 2.17	50	2

*exposed once per week for 4 weeks

• *Additional information that may be needed to adequately assess data for* reliability:

- Ni_3S_2 induced an increase in LDH, BG, TP, and nucleated cells in lavage at 7-day post exposure. Alveolitis lesions were observed 7 days post exposure.
- Gross pathology: Gross examination of the nickel subsulfide treated mice that died shortly after exposure showed that the lungs were dark red and did not deflate upon opening of the thoracic cavity – indicating that lethality was associated with pulmonary hemorrhage and possible congestion and edema.
- Clinical signs: Toxicity was first clinically manifested by rough hair coats, weight loss, and anorexia.

STUDY 4

Study reference:

Finch GL, Fisher GL, and Hayes TL. (1987). The pulmonary effects and clearance of intratracheally instilled Ni_3S_2 and TiO_2 in mice. Environmental Research; 42:83-93.

Detailed study summary and results (*from registration dossier (IUCLID*)):

Test type

•*No guideline* available. BALB/c BYJ mice were intratracheally instilled with 0.5 mg/kg Ni_3S_2 (MMD = 1.83 μm; GSD = 2.3) in 20 μL PBS. Lung lavage samples were obtained at several time intervals following exposure.

Test substance

• Test material used in the study is equivalent to the substance identified in the CLH dossier.
• EC number: 234-829-6
• CAS number: 12035-72-2
• Degree of purity: the detail was not given in the original study report
• Impurities: the detail was not given in the original study report
• Batch number: not applicable
• Physicochemical properties that may be important when assessing acute oral toxicity: solid
• Physical form (gas, vapour, dust, mist): compound suspended in phosphate-buffered saline
• Particle size: MMD=1.83 μm, GSD =2.3
• Type or preparation of particles (for studies with aerosols): not applicable

Test animals

• Species/strain/sex: BALB/c BYJ mice, male
• No. of animals per sex per dose: 3
• Age and weight at the study initiation: 7-8 weeks

Administration/exposure

• Type of inhalation exposure and test conditions: intratracheal instillation
• Duration of test/exposure period: single dose
• Doses/concentration levels: 0, 0.5 mg/kg
• Analytical verification of test atmosphere concentrations: not applicable
• Post exposure observation period: 7 days

• Control group and treatment: yes, vehicle
• Vehicle: identification, concentration and volume used, justification of choice of vehicle: phosphate-buffered saline
• Statistical methods: To assess possible differences between sample means, the Welch approximation (for unknown, unequal variances) to the Student's t test was used. Radiotracer data were analyzed using a two-compartment model. Mean values and standard deviations from each timepoint were computer fitted using a biexponential fitting program (OFIT program).

Results and discussion (*from registration dossier (IUCLID)*):

• Incidence of mortality: not applicable
• The acute inhalation defined LC_{50} of the test substance: not applicable

• Additional information that may be needed to adequately assess data for reliability:

- Lowest observed effect level, 0.5 mg/kg - increase in polymorphonuclear cells in lavage fluid
- Bodyweights were significantly higher in control and titanium treated animals than Ni_3S_2 treated animals at 3 and 7 days after exposure.
- Clinical signs: Ni_3S_2 -instilled animals appeared lethargic and had rough hair coats approximately 2 or 3 days after dose administration, with an improved appearance over the next few days.

STUDY 5

Study reference:

Benson JM, Henderson RF, McClellan RO, Hanson RL, and Rebar AH. (1986). Comparative acute toxicity of four nickel compounds to F344 rat lung. Fundamental and Applied Toxicology; 7:340-347.

Detailed study summary and results (*from registration dossier (IUCLID)*):

Test type

•*No guideline* available. Rats were exposed to Ni_3S_2 by instillation. Tissues were harvested and analyzed at 1 and 7 days post exposure.

Test substance

• Test material used in the study is equivalent to the substance identified in the CLH dossier.
• EC number: 234-829-6
• CAS number: 12035-72-2
• Degree of purity: 97%
• Impurities: the detail was not given in the original study report
• Batch number: not applicable
• Physicochemical properties that may be important when assessing acute oral toxicity: solid
• Physical form (gas, vapour, dust, mist): compound suspended in saline
• Particle size: the detail was not given in the original study report
• Type or preparation of particles (for studies with aerosols): not applicable

Test animals

• Species/strain/sex: F344/Crl rats, male/female

• No. of animals per sex per dose: 12
• Age and weight at the study initiation: 12-15 weeks

Administration/exposure

• Type of inhalation exposure and test conditions: intratracheal instillation
• Duration of test/exposure period: single dose
• Doses/concentration levels: 0.01, 0.10, 1.0 μmol Ni
• Analytical verification of test atmosphere concentrations: not applicable
• Post exposure observation period: 1 and 7 days
• Control group and treatment: yes, vehicle
• Vehicle: identification, concentration and volume used, justification of choice of vehicle: saline
• Statistical methods: Mean values were calculated for all parameters evaluated for each exposure group, and statistical significance when compared to controls was evaluated using Student's t test adjusted for multiple comparisons (Games, 1977). The criterion for significance was $p < 0.05$.

Results and discussion (*from registration dossier (IUCLID)*):

• Incidence of mortality: not applicable
• The acute inhalation defined LC_{50} of the test substance: not applicable

• Additional information that may be needed to adequately assess data for reliability:

- Alveolitis lesions were observed and significant increases were reported for differential cells in lavage fluid, total nucleated cells, neutrophils, and macrophages in animals exposed to 1 μmol Ni via Ni_3S_2 on day 7.

10. REFERENCES

Benson JM, Barr EB, Bechtold WE, Cheng YS, Dunnick JK, Eastin WE, Hobbs CH, Kennedy CH, and Maples KR. (1994). Fate of inhaled nickel oxide and nickel subsulphide in F344/n rats. Inhalation Toxicology; 6:167-183.

Benson JM, Carpenter RL, Hahn FF, Haley PJ, Hanson RL, Hobbs CH, Pickrell JA, and Dunnick JK. (1987). Comparative inhalation toxicity of nickel subsulphide to F344/N rats and B6C3F1 mice exposed for 12 days. Fundamental and Applied Toxicology; 9:251-265.

Benson JM, Cheng YS, Eidson AF, Hahn FF, Henderson RF, and Pickrell JA. (1995). Pulmonary toxicity of nickel subsulphide in F344/N rats exposed for 1-22 days. Toxicology; 103:9-22.

Benson JM, Henderson RF, McClellan RO, Hanson RL, and Rebar AH. (1986). Comparative acute toxicity of four nickel compounds to F344 rat lung. Fundamental and Applied Toxicology; 7:340-347.

Benson JM, Henderson RF, McClellan RO, and Rebar AH. (1984). Comparative toxicity of nickel salts to the lung. Progress in Nickel Toxicology; 3rd International Congress on Nickel Metabolism and Toxicology; 85-88.

Dunnick JK, Benson JM, Hobbs CH, Hahn FF, Cheng YS, and Eidson AF. (1988). Comparative toxicity of nickel oxide, nickel sulfate hexahydrate, and nickel subsulphide after 12 days of inhalation exposure to F344 rats and B6C3F1 mice. Toxicology; 50:145-156.

Dunnick JK, Elwell MR, Benson JM, Hobbs CH, Hahn FF, Haly PJ, Cheng YS, and Eidson AF. (1989). Lung toxicity after 13-week inhalation exposure to nickel oxide, nickel subsulphide, or nickel sulfate hexahydrate in F344/N rats and B6C3F1 mice. Fundamental and Applied Toxicology; 12:584-594.

Dunnick JK, Elwell MR, Radovsky AE, Benson JM, Hahn FF, Nikula KJ, Barr EB, and Hobbs CH. (1995). Comparative carcinogenic effects of nickel subsulphide, nickel oxide, or nickel sulfate hexahydrate chronic exposures in the lung. Cancer Research; 55:5251-5256.

ECHA (2012). Guidance on information requirements and chemical safety assessment. Chapter R.8: Characterisation of dose [concentration]-response for human health, ECHA-2010-G-19-EN (November, 2012).

Eurofins Product Safety Labs (EPSL; 2010). Acute inhalation toxicity study in rats, Eurofins PSL Study #28705, Ni subsulphide.

European Commission (EC; 2009). Commission Directive 2009/2/EC of 15 January 2009 amending, for the purpose of its adaptation to technical progress, for the 31st time, Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. OJ 16.1.2009.

European Commission (EC; 2009). Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures.

Finch GL, Fisher GL, and Hayes TL. (1987). The pulmonary effects and clearance of intratracheally instilled Ni_3S_2 and TiO_2 in mice. Environmental Research; 42:83-93.

Fisher GL, Chrisp CE, McNeill KL, McNeill DA, Democko C, and Finch GL. (1984). Mechanistic evaluations of the pulmonary toxicology of nickel subsulphide. In: Advances In Modern Environmental Toxicology. MacFarland HN *et al.* (Ed.). Volume 6. Applied Toxicology of Petroleum Hydrocarbons (Symposium). Washington, D. C., USA May 11-13, 1982. Princeton Scientific Publishers: Princeton, N. J., USA.

Harlan Labs (2009). Nickel subsulphide: Determination of hazardous physico-chemical properties. Testing laboratory: Harlan Laboratories Ltd Shardlow Business Park Shardlow Derbyshire DE72 2GD UK. Report no.: 2877-0004. Owner company: Nickel REACH Consortia Members. Report date: 2009-12-13.

Harlan Labs (2010). Nickel subsulphide: Determination of general physico-chemical properties. Testing laboratory: Harlan Laboratories Ltd Shardlow Business Park Shardlow Derbyshire DE72 2GD UK. Report no.: 2877-0003. Owner company: Nickel REACH Consortia Members. Report date: 2010-03-24.

Henderson RG, Cappellini D, Seilkop SK, Bates HK and Oller AR. (2012a). Oral bioaccessibility testing and read-across hazard assessment of nickel compounds. Regul Toxicol and Pharmacol. Jun;63(1):20-8.

Henderson RG, Durando J, Oller AR, Merkel DJ, Marone PA, and Bates HK. (2012b). Acute oral toxicity of nickel compounds. Regul Toxicol and Pharmacol. Apr;62(3):425-432.

Kirby Memorial Health Center (KMHC; 2010). Compiled Analysis Reports for 15 Nickel Substances: Solubility in Simulated Fluids. Analyses were conducted during 2008-2010.

Kodama Y, Tanaka I, Matsuno K, Ishimatsu S, and Kawamoto T. (1993). Comparative deposition and clearance of various nickel compounds exposed by inhalation in rats. Biological Trace Element Research; 36:257-269.

Mayer C, Klein KG, Wesch H, and Schmezer P. (1998). Nickel subsulphide is genotoxic *in vitro* but shows no mutagenic potential in respiratory tract tissues of BigBlue™ rats and Muta™Mouse mice *in vivo* after inhalation. Mutation Research; 420:85-98.

National Toxicology Program (NTP; 1996). Toxicology and carcinogenesis studies of nickel subsulphide (CAS no. 12035-72-2) in F344/n rats and B6C3F1 mice (inhalation studies). NIH Publication No. 96-3369. Testing laboratory: National Toxicology Program (NTP). Report no.: NTP TR 453. Owner company: National Institute of Health, Springfield, VA. Washington DC. [Also cited as Dunnick et al., 1989 and Dunnick et al., 1995]

Torjussen W and Andersen I. (1979). Nickel concentrations in nasal mucosa, plasma, and urine in active and retired nickel workers. Annals of Clinical and Laboratory Science; 9(4):289-298.

Valentine R and Fisher G. (1984). Pulmonary clearance of intratracheally administered $^{63}Ni_3S_2$ in strain A/J mice. Environmental Research; 34:328-334.

Additional references

Benson JM, Cheng YS, Eidson AF, Hahn FF, Henderson RF, and Pickrell JA. (1995). Pulmonary toxicity of nickel subsulfide in F344/N rats exposed for 1-22 days. Toxicology; 103:9-22.

Benson JM, Carpenter RL, Hahn FF, Haley PJ, Hanson RL, Hobbs CH, Pickrell JA, and Dunnick JK. (1987). Comparative inhalation toxicity of nickel subsulphide to F344/N rats and B6C3F1 mice exposed for 12 days. Fundamental and Applied Toxicology; 9:251-265.

Dunnick JK, Benson JM, Hobbs CH, Hahn FF, Cheng YS, and Eidson AF. (1988). Comparative toxicity of nickel oxide, nickel sulfate hexahydrate, and nickel subsulphide after 12 days of inhalation exposure to F344 Rats And B6C3F1 mice. Toxicology; 50:145-156.

NTP (National Toxicology Program). (1996). The toxicology and carcinogenesis studies of nickel subsulfide (CAS no 12035-72-2) in F344/N rats and B6C3F1 mice (inhalation studies). NTP Technical Report No 453. NIH Publication No 96-3369. National Institutes of Health, Springfield (VA). Washington DC.