CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification: nickel sulphide

EC Number: **240-841-2 (also 234-349-7)**

CAS Number: **16812-54-7 (also 11113-75-0 and 1314-04-1)**

Index Number: **028-006-00-9**

Registrant's Identity: Talvivaara Sotkamo Ltd

Lead Registrant of Ni Sulphide – JS_Nickel_sulfide

Date: May 2016

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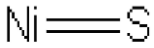
1. IDENTITY OF THE SUBSTANCE

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)	nickel sulphide, nickel sulfide, nickel (II) sulfide, nickel (II) sulphide, millerite
Other names (usual name, trade name, abbreviation)	nickel sulphide
ISO common name (if available and appropriate)	not applicable
EC number (if available and appropriate)	240-841-2 (also covers 234-349-7)*
EC name (if available and appropriate)	nickel sulphide
CAS number (if available)	16812-54-7 (also covers 11113-75-0 and 1314-04-1)*
Other identity code (if available)	CLP Annex VI Index number, 028-006-00-9
Molecular formula	NiS
Structural formula	
SMILES notation (if available)	not applicable
Molecular weight or molecular weight range	90.759
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)	> 88.0 — < 98.0 % (w/w)

* This CLH dossier for nickel sulphide (EC# 240-841-2, CAS# 16812-54-7) is intended to cover all substances identified by CLP index number 028-006-00-9, which includes nickel sulphide (EC# 234-349-7, CAS# 11113-75-0) and millerite (EC# 234-349-7, CAS# 1314-04-1).

1.2 Composition of the substance

Name: Nickel sulphide

Degree of purity: > 88.0 — < 98.0 % (w/w)

Table 2: Nickel sulphide 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)
nickel sulphide* EC no.: 240-841-2	> 88.0 — < 98.0 % (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 STOT RE 1; H372** (target organ/rt of exposure): Lungs/inhalation

* This CLH dossier for nickel sulphide (EC# 240-841-2, CAS# 16812-54-7) is intended to cover all substances identified by CLP index number 028-006-00-9, which includes nickel sulphide (EC# 234-349-7, CAS# 11113-75-0) and millerite (EC# 234-349-7, CAS# 1314-04-1).

** The classification under 67/548/EEC indicating the route of exposure (T48;R23) 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. This indication of the route of exposure should be noted in the CLP classification, rather than relying on a more general hazard statement. It seems that this information was lost or omitted upon transfer from 67/548/EEC DSD to the current CLP classification. Additionally, repeated dose inhalation studies with mice and rats indicate the lungs as the only organ significantly affected with nickel subsulphide (read-across to nickel sulphide). The lungs should be indicated as the target organ. The specification of the route of exposure and target organ for STOT RE1 is applicable to all nickel substances.

Table 3: Nickel sulphide 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
pyrite (FeS2) EC no.: 215-167-7	> 0.1 — < 8.0 % (w/w)	Not currently listed	None identified on ECHA registered substances database	No
zinc sulphide EC no.: 215-251-3	> 0.0 — < 2.0 % (w/w)	Not currently listed	None identified on ECHA registered substances database	No
Other impurities (not known or are in concentrations < 1% that do not affect classification	0.0 — ≤ 11.0 % (w/w)		None identified on ECHA registered substances database	No

Table 4: Nickel sulphide 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
nickel sulphide* EC no.: 240-841-2 (To cover entire entry of CLP index number 028-006-00-9)	100%	Not applicable	Toxicokinetics/Read-Across for acute inhalation toxicity: Henderson et al. (2012), KMHC (2010)	Information as provided by the supplier

* This CLH dossier for nickel sulphide (EC# 240-841-2, CAS# 16812-54-7) is intended to cover all substances identified by CLP index number 028-006-00-9, which includes nickel sulphide (EC# 234-349-7, CAS# 11113-75-0) and millerite (EC# 234-349-7, CAS# 1314-04-1).

2. PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.2 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	028-006-00-9	nickel (II) sulfide; [1] nickel sulfide; [2] millerite [3]	240-841-2 [1] 234-349-7 [2] - [3]	16812-54-7 [1] 11113-75-0 [2] 1314-04-1 [3]	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)** STOT RE 1	**H332 (ADD)** H372**		**H332 (ADD)** H372**			**STOT RE 1** (target organ/rt of exposure): Lungs/inhalation (ADD)**
Resulting Annex VI entry if agreed by RAC and COM	028-006-00-9	nickel (II) sulfide; [1] nickel sulfide; [2] millerite [3]	240-841-2 [1] 234-349-7 [2] - [3]	16812-54-7 [1] 11113-75-0 [2] 1314-04-1 [3]	**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			**STOT RE 1** (target organ/rt of exposure): Lungs/inhalation**

** The classification under 67/548/EEC indicating the route of exposure (T48;R23) 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. This indication of the route of exposure should be noted in the CLP classification, rather than relying on a more general hazard statement. It seems that this information was lost or omitted upon transfer from 67/548/EEC DSD to the current CLP classification. Additionally, repeated dose inhalation studies with mice and rats indicate the lungs as the only organ significantly affected with nickel subsulphide (read-across to nickel sulphide). The lungs should be indicated as the target organ. The specification of the route of exposure and target organ for STOT RE1 is applicable to all nickel substances.

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	not applicable (currently classified, but inclusion of target organ and route of exposure proposed)	Yes*
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

* The STOT RE notation regarding the route of exposure was lost in the transfer from 67/548/EEC DSD (R48/23) to CLP (STOT RE 1) classification. It is important to put back this lost information, which applies to all nickel substances. Additionally, repeated dose inhalation studies with mice and rats indicate the lungs as the only organ significantly affected with nickel subsulphide (read-across to nickel sulphide). The lungs should be indicated as the target organ for STOT RE 1 of nickel sulphide, as well as other nickel compounds.

3. HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Acute toxicity classification

The original proposal to include nickel sulphide (EC No. 240-841-2; CAS No.16812-54-7) as a new entry in Annex I of Council Directive 67/548/EEC was reviewed and agreed for inclusion in the 31st ATP (EC, 2009a). Classification of nickel sulphide 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 (EC, 2008). Therefore, it is assumed that the lack of classification for acute toxicity of nickel sulphide was due to a lack of substance-specific data at the time of entry.

Nickel sulphide'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.

STOT RE classification

According to Annex I of Council Directive 67/548/EEC (DSD), the repeated dose toxicity classification for nickel sulphide and all other identifiers related to Index No. 028-006-00-9 was T;R48/23 indicating Toxic: danger of serious damage to health by prolonged exposure through inhalation. However, the classification that was carried forward into ATP 1 to the CLP Regulation corresponding to ATP 30 and 31 to the 67/548/EEC Directive is STOT RE1 H372, which indicates it causes damage to organs through prolonged or repeated exposure. Unlike the 67/548/EEC DSD classification, the CLP classification is a general hazard statement that does not specify the route of exposure or the target organ, as required in Annex I: 3.9.2.2 of the CLP:

> ***Annex I: 3.9.2.2.*** *The relevant route or routes of exposure by which the classified substance produces damage shall be identified.*

4. JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Justification that action is needed at Community level is required.

Acute toxicity:

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 nickel sulphide. Therefore, the need for action at the community level is focused on occupational exposure in the workplace. The current lack of classification creates the false perception that nickel sulphide is not of concern for acute inhalation toxicity, although available data suggest nickel sulphide warrants classification for this endpoint. Newly available data for nickel subsulphide together with read-across information support classification for acute inhalation toxicity of nickel sulphide as Acute Tox. 4; H332.

STOT RE:

Reason for a need for action at Community level: ***Notification of previous unintentional omission of route of exposure during transition from 67/548/EEC DSD to CLP classification and the indication of lungs as the target organ.***

Further detail on need of action at Community level

Important information regarding repeated exposure toxicity of nickel sulphide was not incorporated in the transition from 67/548/EEC DSD to CLP classification. Inhalation as the specific route of exposure was previously indicated in the DSD classification of R48/23, but there is no such indication in the CLP classification of STOT RE 1. Additionally, repeated inhalation animal studies with respirable size nickel subsulphide (as well as other nickel compounds) provide clear indications that the lungs are the target organs for toxicity. Therefore, the need for action at the community level is focused on an addition to the CLP STOT RE 1 classification for nickel sulphide to include the route of exposure as previously indicated in the 67/548/EEC DSD classification for nickel sulphide, as well as the inclusion of lungs as the target organ. The current CLP classification for this endpoint is incomplete in this regard and should include a notation indicating lungs and inhalation as the target organ and route of exposure, respectively. This notation applies to all of the nickel substances classified as STOT RE 1.

There is no concern regarding other routes of exposure. No target organ toxicity was observed with the most toxic and bioavailable of the nickel compounds (nickel sulphate hexaydrate) after repeated oral exposures for a lifetime (Heim et al., 2007). Dermal exposure is not of relevance either as dermal absorption is estimated to be very low < 1%.

5. DATA SOURCES

- Chemical Safety Report (CSR) for Nickel Sulphide (2015 update), including unpublished laboratory reports referenced within the CSR
- Comprehensive scientific literature search related to toxicokinetics and acute inhalation toxicity of nickel sulphide
- 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	Black solid	Harlan Labs, 2010	Physical state: solid, powder Colour: black Odour: not reported
Melting/freezing point (°C)	> 359.85 (633 K)	Harlan Labs, 2010	Testing by differential scanning caloritry, 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	No determination of boiling temperature was performed in accordance with the exceptions listed in column 2 of REACH Annex VII and section R.7.1.3 'Boiling Point' of the REACH guidance document. This was since the test material was shown not to melt below 573 K (300°C).
Relative density	5.66 x 10^3 at 24°C	Harlan Labs, 2010	Testing using 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	No determination of vapour pressure was performed in accordance with the exceptions listed in column 2 of REACH Annex VII as the the melting temperature of the test material was greater than 300°C.
Surface tension (N/m)	Not applicable	Not applicable	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 nickel sulphide is not designed or anticipated to have surfactant properties.
Water solubility (mg/l)	88 at 20 °C	Harlan Labs, 2010	The water solubility of nickel sulphide has been determined to be 5.69 x 10^{-2}g/L of solution at 20.0 ± 0.5°C monitoring nickel; equivalent to 8.80 x 10^{-2} g/L of test material based on the theoretical nickel content, using the flask method, Method A6 Water Solubility of Commission Regulation (EC) No 440/2008 of 30 May 2008 and Method 105 of the OECD Guidelines for Testing of Chemicals, 27 July 1995.

Partition coefficient n-octanol/water	Not applicable	Not applicable	Column 2 of REACH Annex VII provides the following exemption: A study does not need to be conducted for inorganic substances, hence testing for this endpoint has been waived.
Flash point (°C)	Not applicable	Not applicable	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 nickel sulphide is inorganic.
Stability in organic solvents and identity of relevant degradation products (if relevant)	Not applicable	Not applicable	Stability in organic solvents and identity of relevant degradation products is not an applicable endpoint for inorganic substances such as nickel sulphide, according to Column 2 of Annex IX of REACH regulations.
Dissociation constant	Not applicable	Not applicable	Although the legal text of REACH (Annex IX, Column 2) clearly states that LogKow does not have to be determined for inorganics, a similar exclusion is not made for dissociation constant. Since nickel sulphide is soluble, OECD guideline 112 (the recommended test guideline) is theoretically applicable. However, possible dissociation in metal ion complexes is usually described by a stability constant. The calculations are similar but more involved. The result is usually expressed as LogK rather than pKa. OECD guideline 112 and the methods it contains do not cover these measurements and the stability constant issue is beyond the scope and requirements of OECD 112. Additionally, because nickel sulphide is soluble, the pKa would be negative and negative pKa values are not used in a practical sense. Therefore, data are waived because expressing the dissociation constant of nickel sulphide with a pKa value is impractical. However, OECD guideline 112 is used to establish the dissociation of a chemical in water which is important in assessing its environmental impact. The environmental impact of Ni and Ni-containing compounds has been extensively investigated and adequately reported REACH registration dossier.
Viscosity **dynamic viscosity** (Pas) **kinematic viscosity** (mm^2/s)	Not applicable	Not applicable	According to Column 2 of REACH Annex IX, viscosity data are not required since nickel sulphide is a solid substance.

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

Table 9: Summary table of toxicokinetic studies via inhalation

Method	Results	Remarks	Reference
rat (Wistar) male inhalation: aerosol Exposure regime: 7 hr/day, 5 days/wk, 1 month Doses/conc.: 8.8 +/- 2.8 mg NiS/m3 Rats were exposed to nickel compounds via inhalation for one month. The distribution to organs was determined immediately after exposure and after a 6-month clearance period.	Transfer (atmosphere to lung (after 1 month)): distinct transfer (1.14 +/- 0.19 µg Ni/g (treated) vs. 0.09 +/- 0.05 µg Ni/g (control) - p < 0.01) (Test No.: #1) Transfer (lung to liver (after 1 month)): slight transfer (0.09 +/- 0.03 µg Ni/g (treated) vs. 0.03 +/- 0.01 µg Ni/g (control) - p < 0.01) (Test No.: #2) Transfer (lung to kidney (after 1 month)): distinct transfer (0.96 +/- 0.25 µg Ni/g (treated) vs. 0.1 +/- 0.04 µg Ni/g (control) - p < 0.01) (Test No.: #3) Transfer (lung to spleen (after 1 month)): no transfer detectable (0.25 +/- 0.27 µg Ni/g (treated) vs. 0.25 +/- 0.09 µg Ni/g (control) - p < 0.01) (Test No.: #4)	2 (reliable with restrictions) weight of evidence experimental result **Test material (EC name): nickel sulphide**	Tanaka I, Ishimatsu S, Kodama Y, Haratake J, Horie A, Tsuchiya K, Cho S (1992)
rat (Wistar) male inhalation: aerosol Exposure regime: acute: 4 hr repeated: 7 hr/day, 5 day/wk, 1 month or acutely for 4 hours Doses/conc.: acute: 107 mg/m3 repeated: 8.8 +/- 2.8 mg/m3 Male Wistar rats were exposed via inhalation to 8.8 mg/m3 NiS aerosols (MMAD = 4 µm; GSD =2). Nickel content in several organs were measured; lung clearance half-life was estimated.	Transfer (atmosphere to lung (single dose)): distinct transfer (47.8 +/- 10.0 µg Ni/g (treated) vs. 0.13 +/- 0.06 (control) at 0 hr) (Test No.: #1) Transfer (lung to kidney (single dose)): distinct transfer (3.24 +/- 0.83 µg Ni/g (treated) vs. 0.07 +/- 0.02 (control) at 0 hr) (Test No.: #2) Transfer (lung to liver (single dose)): no transfer detectable (0.20 +/- 0.13 µg Ni/g (treated) vs. 0.03 +/- 0.02 (control) at 0 hr) (Test No.: #3) Transfer (lung to spleen (single dose)): no transfer detectable (0.18 +/- 0.08 µg Ni/g (treated) vs. 0.27 +/- 0.05 (control) at 0 hr) (Test No.: #4) Transfer (atmosphere to lung (repeat dose)): distinct transfer (1.14 +/- 0.19 µg Ni/g (treated) vs. 0.09 +/- 0.05 (control)) (Test No.: #5) Transfer (lung to kidney (repeat dose)): distinct transfer (0.96 +/- 0.25 µg Ni/g (treated) vs. 0.1 +/- 0.04 (control)) (Test No.: #6) Transfer (lung to liver (repeat dose)): not determined (0.09 +/- 0.03 µg Ni/g (treated) vs. 0.03 +/- 0.01 (control)) (Test No.: #7) Transfer (lung to spleen (repeat dose)): no transfer detectable (0.25 +/- 0.27 µg Ni/g (treated) vs. 0.25 +/- 0.09 (control)) (Test No.: #8) Toxicokinetic parameters: Half-life 1st: 20 hr (lung, single dose) (Test No.: #1)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): nickel sulphide**	Tanaka I, Ishimatsu S, Haratake J, Horie A, Kodama Y (1988)
in vitro study In situations where the bioavailability of a metal substance is not known or not feasible to determine experimentally, the	For sample N97 of nickel sulphide, the nickel release was: Part A.9.75% of Ni content after 2 hours in gastric fluid,	Klimisch score: 2 (reliable with restrictions) supporting study	KMHC (2010) Henderson, RG, Cappellini D, Seilkop

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. This report measured bioaccessibility of nickel substances as a surrogate for bioavailability.	Part B.0.18% of Ni content after 24 hours in intestinal fluid, Part C.1.83% of Ni content and 1.08% of sample weight after 72 hours in interstitial fluid, Part D.44% of Ni content and 25.95 % of sample weight after 72 hours in lysosomal fluid, and Part E.8.75% of Ni content after 24 hours in sweat fluid. Evaluation of results: Data have been incorporated into read-across assessments for nickel and nickel-containing substances.	experimental result **Test material (EC name): nickel sulphide**	SK, Bates HK and Oller AR (2012a) [gastric and intestinal fluid results only]

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

Two studies characterizing the in vivo inhalation toxicokinetic properties of NiS in rats were identified. The studies only evaluated a single dose and compared tissue concentrations to control animals. Tanaka et al. (1988; 1992) conducted a series of studies evaluating distribution and retention of NiS in rats following a single or repeated (1-month) inhalation exposure to NiS. Findings indicated that nickel levels in several tissues were elevated immediately after exposure, but decreased to control levels 6 months following exposures. After a single inhalation exposure lung nickel levels were 10-fold higher than any other organ (Tanaka et al., 1988). Kidney showed the second highest values consistent with urine being the main excretory pathway. The authors also concluded that the lung clearance rate was inversely related to solubility. Similar findings were reported by Ishimatsu et al. (1995): only 2.1% of a single oral NiS dose was measurable in organs, blood or urine 24 hr after an oral exposure. The authors suggested that the kinetic behavior of Ni compounds administered orally is closely related with the solubility of Ni compounds. Results reported by Novelli et al. (1995) support these findings; in this study, intramuscular NiS administration resulted in systemic distribution and absorption of nickel in some tissues 72 hours following injection. Collectively, these data indicate that Ni from NiS administered via various routes (e. g., oral, inhalation, and i. m.) can distribute to various organs, such as the lung, kidney and to a lesser extent liver. However, it is cleared or eliminated fairly quickly. Though none of the available studies alone are sufficient to fully characterize the distribution of NiS, the data when considered collectively provide a general understanding of the toxicokinetics following oral, inhalation or i. m. administration of NiS in rats. However, data were not located regarding toxicokinetics in other species.

For the purpose of risk characterisation, the absorption of nickel from inhalation of nickel sulphide in rats is read across from the estimate for nickel subsulphide. For nickel subsulphide, the inhalation absorption in rats is conservatively estimated to be 50%, compared to 100% for nickel sulphate hexahydrate. The retention half time for nickel subsulphide was twice as long as for nickel sulphate hexahydrate (4 days versus 2 days) in the study by Benson et al., (1994). The factor of 2 is also conservative based on the relative Ni ion release in synthetic lung fluids (KMHC, 2010). The relative Ni ion releases in synthetic lung fluids for nickel sulphide and nickel subsulphide are very comparable, further justifying the read across from nickel subsulphide to nickel sulphide (KMHC, 2010).

8. EVALUATION OF HEALTH HAZARDS

8.2 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 (CAS number): 12035-72-2, nickel subsulphide (READ-ACROSS)** Powder MMAD: 3.3-3.5 µm	0.2, 1.0, 5.0 mg/L 4 hours	LC_{50} (4 h): 0.9237 mg/L air (female) LC_{50} (4 h): 1.352 mg/L air (male) LC_{50} (4 h): 1.13785 mg/L air (average of males and females)	EPSL (2010b)

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

8.2.1. Short summary and overall relevance of the provided information on acute inhalation

Data on the acute inhalation toxicity of nickel sulphide are read-across from nickel subsulphide. A comprehensive read-across assessment was recently completed based on bioaccessibility data in synthetic lung fluids of various nickel compounds combined *in vivo* verification data for three source nickel substances. The bioaccessibility-based paradigm presented in Section 9: Other Information (also attached to the accompanying IUCLID file) enables grouping of target nickel substances for classification purposes according to bioaccessibility in interstitial fluid as demonstrated. The outcome of this assessment indicates that nickel sulphide should be grouped with other sulphidic nickel compounds and read-across from nickel subsulphide for acute inhalation toxicity, based dissolution in interstitial fluid in the range of 1-5% Ni/sample (Section 9: Other Information; Table 16). Although nickel subsulphide is not currently classified for acute inhalation toxicity, a recently completed OECD-guideline compliant study reported an LC_{50}=1.14 mg/L and should be classified as Acute Tox. 4; H332. Therefore, application of this read-across paradigm suggests that nickel sulphide should also be classified as Acute Tox 4; H332.

8.2.2. Comparison with the CLP criteria

The newly reported inhalation LC_{50} value of 1.14 mg/L for nickel subsulphide meets the criteria for classification as Acute Tox. 4; H332 according to the CLP guidelines, which specifies that substances with an LC_{50} value between 1 and $\leq$5 mg/L fall within this category (EC No. 1272/2008). The outcome of a bioaccessibility-based read-across assessment (see Section 9: Other Information and Section 13: Appendix 2) suggests that the acute inhalation toxicity of nickel sulphide should be read across from nickel subsulphide and therefore should be classified as Acute Tox 4; H332.

8.2.3. Conclusion on classification and labelling for acute inhalation toxicity

Nickel sulphide has not previously been classified for acute inhalation toxicity in the EU. A comprehensive read-across assessment was recently completed based on bioaccessibility data in synthetic lung fluids of various nickel compounds combined with *in vivo* verification data for three source nickel substances. The bioaccessibility-based paradigm discussed in Section 9: Other Information (also attached to the accompanying IUCLID file) enables grouping of target nickel substances for classification purposes according to bioaccessibility in interstitial fluid as demonstrated. The outcome of this assessment indicates that nickel sulphide should be read-across from nickel subsulphide for acute inhalation toxicity. Therefore, application of this read-across paradigm suggests that nickel sulphide should be classified as Acute Tox 4; H332. The joint REACH submissions for nickel subsulphide and nickel sulphide reflect self-classification as Acute Tox 4; H322.

8.3 STOT RE1 Classification – justification for inclusion of target organ and route of exposure

Table 13. Summary table of animal studies of repeated dose exposure

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
subchronic (inhalation: aerosol) Equivalent or similar to OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day)	rat (F344/N) male/female 8-10 animals/sex/group	**Test material (CAS number): 12035-72-2, nickel subsulphide (READ-ACROSS)** Respirable Powder	0.11, 0.2, 0.4, 0.9, 1.8 mg Ni/m^3 (nominal conc.) Vehicle: no data Exposure: 6 hr/day (5 d/wk, 13wk)	NOEC: 0.1 mg Ni/m^3 (male) (olfactory epithelial atrophy) NOEC: 0.2 mg Ni/m^3 (female) (olfactory epithelial atrophy LOEC: 0.1 mg Ni/m^3 (male/female) (significant increase in lung weight at 13 weeks) LOEC: 0.1 mg Ni/m^3 (male/female) (2-3 animals per sex with chronic active inflammation in lung	Dunnick JK, Elwell MR, Benson JM, Hobbs CH, Hahn FF, Haly PJ, Cheng (1989) Benson JM, Burt DG, Cheng YS, Eidson AF, Gulati DK, Hahn FF, Hobbs (1990) NTP (1996b)
subchronic (inhalation: aerosol) Equivalent or similar to OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day)	mouse (B6C3F1) male/female 8-10 animals/sex/group	**Test material (CAS number): 12035-72-2, nickel subsulphide (READ-ACROSS)** Respirable Powder	0.11, 0.2, 0.4, 0.9, 1.8 mg Ni/m^3 (nominal conc.) Vehicle: no data Exposure: 6 hr/day (5 d/wk, 13wk)	NOEC: 0.4 mg Ni/m^3 (male/female) (no significant increase in lung weight) NOEC: 0.4 mg Ni/m^3 (male/female) (no animals with chronic active inflammation or fibrosis) NOEC: 0.2 mg Ni/m^3 (male/female) (no animals with olfactory epthelial atrophy	Dunnick JK, Elwell MR, Benson JM, Hobbs CH, Hahn FF, Haly PJ, Cheng (1989) Benson JM, Burt DG, Cheng YS, Eidson AF, Gulati DK, Hahn FF, Hobbs (1990) NTP (1996b)

8.3.1. Short summary and overall relevance of the provided information on repeated dose

Toxicity associated with repeated inhalation exposures to nickel subsulphide was well characterized by a series of studies in rats and mice. These studies were generally conducted by the same group of researchers, and were part of, or associated with, a comprehensive bioassay conducted by the National Toxicology Program. Durations of exposure ranged from 12 exposure days up to 2 years. Though general signs of toxicity were evaluated, much of the focus was on toxicity associated with pulmonary endpoints as the respiratory tract was the most sensitive toxicity target. Following 12 days of exposure to nickel subsulphide at doses ranging from 0.6-10 mg/m^3, rats and mice experienced significant toxicity at exposure levels of 5 mg nickel subsulphide /m^3 and higher (Benson et al. 1987). Toxicities included labored respiration, emaciation, dehydration, decreased weight gain, altered organ weights, and mortality in some cases. Histopathological analyses revealed that the respiratory tract was the major target for nickel subsulphide toxicity based on observations of necrotizing pneumonia, emphysema, or fibrosis in exposed rats, and lesions in the nasal epithelium and lung. However, other toxicities, including atrophy of the thymus, spleen, and liver, as well as testicular degeneration were observed in both rats and mice. A more in-depth, time course evaluation of exposure to lower doses (0.6 or 2.5 mg nickel subsulphide /m^3 for up to 22 days resulted in dose- and time-dependent effects (Benson et al. 1995). Exposure-related toxicities included decreases in

body weight, increased lung weight, morphological changes (e.g., nasal lesions, degeneration of olfactory epithelium), and a number of biochemical effects associated primarily with inflammation (e.g., increased alveolar macrophages, hyperplasia of bronchiolar epithelial cells, presence of inflammatory cells in bronchial lumen, LDH activity). Similar findings were noted following 13 weeks of exposure to nickel subsulphide (0.15 to 2.5 mg/m^3; Dunnick et al. 1989) in both rats and mice. No exposure-related mortality was observed, though changes in bodyweight and lung weights were significantly impacted. Additional toxicities included inflammation in the nasal cavity, bronchial lymph nodes and the lung, alveolar macrophage hyperplasia, chronic active inflammation, and olfactory epithelial atrophy. Of interest, rats were more sensitive than mice to the effects of inhaled nickel in this study. In a complimentary study, Benson et al. (1989) reported on additional endpoints in rats and mice exposed to nickel subsulphide for 13 weeks. Biochemical and cytological changes in bronchiolar lavage fluid (BALF) were analyzed in addition to histopathological changes. Significant and dose-dependent effects in a number of biochemical and cytological parameters (e.g., levels of lactate dehydrogenase, β-glucuronidase, percentage of neutrophils and macrophages in lavage fluid) as well as tissue damage (e. g chronic inflammation, macrophage proliferation) were observed. A separate study reported labored breathing, lung foci, enlarged lymph nodes, and nasal and lung lesions (e.g., chronic inflammation associated with this exposure scenario in rodents). Repeated dose toxicities associated with 2 years of exposure to nickel subsulphide included a variety of clinical observations, body and organ weight changes, and altered tissue histopathology (Dunnick et al. 1995). Chronic exposure to concentrations up to 1 mg nickel subsulphide/m^3 were not associated with increased mortality or adverse changes in body weight. However, time- and dose-dependent increases in lung weights were observed, which was thought to be due to inflammation. This conclusion was based on histopathological analyses which revealed alveolar/bronchiolar hyperplasia, inflammation, fibrosis, and lymphoid hyperplasia of the lung-associated lymph nodes. The most critical effects were pulmonary fibrosis, chronic inflammation, and proteinosis. For these effects, a LOAEC of 0.11 mg Ni/m^3 (MMAD = 2.17 µm) was identified in rats and a LOAEC = 0.44 mg Ni/m^3 (MMAD=2.24 µm) was identified in mice.

In summary, repeated inhalation studies with respirable size nickel subsulphide (13 week) in rats and mice have provided clear indications that the lungs are the target organs for toxicity. In fact lung inflammatory effects in rats are the most sensitive marker after inhalation exposure to all nickel compounds. In the 13 week studies with nickel subsulphide, inflammatory effects were detected at exposure levels of 0.14 mg nickel subsulfide/m^3 or 0.1 mg Ni/m^3. This fulfills the criterion for classification as STOT RE1. By oral exposure, no target organ toxicity has been observed with the most toxic and bioavailable of the nickel compounds (nickel sulphate hexaydrate) after repeated exposures for a lifetime (Heim et al., 2007). Dermal exposure is not of relevance either as dermal absorption is estimated to be very low < 1%.

8.3.2. Comparison with the CLP criteria

Annex I: 3.9.2.2 of the CLP requires the identification of the relevant route or routes of exposure by which the classified substance produces damage and also the identification of the target organ. For STOT RE1, the route of exposure is inhalation and the target organ is the lung.

8.3.3. Conclusion on classification and labelling for repeated dose toxicity

A notation should be added to the current STOT RE1 CLP classification entry for Ni sulphide specifying the route of exposure as inhalation and the target organ as the lung.

9. OTHER INFORMATION

9.2 Justification for read-across (acute inhalation toxicity and target organ toxicity)

9.2.1. Background

In its simplest form, read-across is an extrapolation of known data from one substance (data-rich) to another substance (data-poor), based on limited information and some assumptions leading to a conclusion that the two substances will cause similar biological responses. The ability to perform scientifically valid read-across of data from one well-characterized substance (termed "*source*") to another substance with little or no data (termed "*target*") requires that a minimum amount of information pertaining to the unknown substance be compared to the same information from the known substance (ECHA, 2008). For metal substances, comparison of bioavailability data on the source and target substances can be utilized to perform read-across assessments when appropriate. General aspects of read-across are discussed in ECHA's Guidance On Information Requirements And Chemical Safety Assessment, Chapter R.6: QSARs and grouping of chemicals (ECHA, 2008) and in the Application of the CLP Criteria Guidance to Regulation, Section 1.4.3: Read Across (ECHA, 2015; EC, 2009b). The more specific concept of bioavailability of metal compounds is discussed in Section R.6.2.5.6 (ECHA, 2008), which states:

> "The concept of chemical categories has traditionally been widely used for hazard assessment for certain endpoints and risk assessment of inorganic substances. The approaches have generally been based on the occurrence of a common metal ion or anion and the use of read-across to fill data gaps…it is the bioavailability of the metal ion (or a redox form of this ion) at target sites that in most cases determines the occurrence and severity of the effects to be assessed for the read-across of metal substances. Supporting information to assess the bioavailability of the metal ion at the target site can include information on a number of different factors (e.g. physico-chemical properties such as water solubility, degree of dissociation of the metal–containing compound, particle size and structure, in vitro solubility, in vivo data on systemic effects, toxicokinetics)".

For metal substances, it is very important to understand that the simple presence of a metal in a substance does not necessarily impart to that substance the biological properties of the metal ion. It is the bioavailability of the metal ion (or a redox form of this ion) at cellular target sites that needs to be assessed for the read-across of metal substances to be accurate.

9.2.2. Bioavailability and bioaccessibility

Knowledge of bioavailability *(the fraction of the dose that reaches systemic circulation)* is critical for determining toxicity (Klaassen, 2001). Bioavailability can be used as a tool to establish categories of metal substances and facilitate hazard assessment or classification of specific toxicity endpoints. Data on metal substance bioavailability may be derived from *in vivo* sources and estimated from *in vitro* sources. The most complete and useful information on the bioavailability of metal substances is derived from *in vivo* toxicokinetic tests or toxicological tests providing exposure and effect data.

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 in synthetic tissue fluids relevant to the route of exposure in question is measured. The resultant value is termed *bioaccessibility* and is defined as the amount of a substance (*e.g.*, metal ion) available for absorption (Stopford et al., 2003; Henderson et al., 2012a). In cases where insufficient information exists, several kinds of data can be pooled on a weight-of-

evidence basis and applied to all metal substances having similar bioavailability parameters by the read-across method including bioaccessibility in synthetic biological fluids.

9.2.3. A read-across strategy for metals using bioaccessibility

Analyses and read-across need to be conducted for each toxicity endpoint (as opposed to reading-across all endpoints from one single source substance) and should be considered for each route of exposure since the chemical conditions of the gastro-intestinal tract, respiratory tracts and dermal systems are decidedly different and can affect different chemicals in different ways.

For nickel substances, *in vitro* bioaccessibility data for the metal ion can be used as a surrogate for *in vivo* toxicokinetic data or toxicological data on the target substance, Results for target and source nickel compounds can be compared and utilized to perform read-across assessments when the counter ion is the same it can reasonably be assumed that the anion or "counter-ion" does not contribute to observed toxicity. This can be done for classification purposes. The source nickel compound(s) will form a baseline from which to extrapolate the existing data to target substances based upon their bioaccessibility via different routes of exposure. The lung fluid bioaccessibility-based read-across strategy for inhalation toxicity incorporates the following steps:

Step 1: For the source substance (e.g., nickel subsulphide) and for the target substance (e.g., **nickel sulphide**), assess the metal ion release data in the appropriate lung fluid. This enables information about these target substances to be compared to equivalent data from the source substances.

Step 2: Incorporate *in vivo* toxicity or toxicokinetic data to verify that the bioaccessibility data correlate with toxicity. The ability to read-across the relevant effects data can then be evaluated.

Step 3: Identify the source substance for each category or target substance based on relative bioaccessibility data.

Step 4: Use the new paradigm to read-across toxicological data from source substances to target substances based on relative bioaccessibility. Incorporate bioaccessibility data into an overall weight-of-evidence approach that considers all available information, in particular in cases where extrapolation for repeated dose effects (e.g., chronic inhalation toxicity) is desired.

Bioaccessibility data can be expressed in many different ways. For example, bioaccessibility can be reported as mass of metal ion released per mass of sample (e.g., µg metal ion/g sample), or mass of metal ion released per mass of available metal in the sample (e.g., µg metal ion/g of available metal in the sample), after a given time of elution. The rate of release over time can also be calculated, e.g., µg metal ion released per hour, day or week. Different metrics can result in different degrees of correlation between bioaccessibility and *in vivo* data when evaluating verification; therefore, the strength of the regressions can guide the best metrics to use for grouping of substances and determining the scientifically appropriate read-across.

9.2.4. Inhalation toxicity read-across assessment of nickel substances

The potential for most nickel-containing substances to cause adverse effects in the respiratory tract has been shown to be dependent upon the bioavailability of the Ni (II) ion at the target sites. Available data indicate that the primary factor for lung toxicity may be solubility in the respiratory tract including dissociation in extracellular (e.g., interstitial and alveolar) and/or intracellular (e.g., lysosomal) fluids for those particles that are easily taken up by the cells. In the case of toxicity or mortality (e.g., LC_{50}) after acute exposure, the target site may be the whole respiratory tract. Extracellular dissolution may be more important for acute toxicity effects than the intracellular one since *in vivo* acute toxicity studies usually involve exposure for a few hours followed by observation for a few days, and as such the potential for intracellular dissolution is more limited. Release of Ni (II) ion in relevant lung fluids can provide information on the mechanism of action and ultimately on the potential to cause toxicity. Therefore, bioaccessibility data in synthetic lung fluids (as a surrogate for bioavailability) have been utilized in this read-across assessment for acute

inhalation toxicity of **nickel sulphide**, recognizing that additional factors may play a role on respiratory toxicity (i.e. surface reactions).

Solubility in biological fluids varies depending upon the chemical form of nickel (*e.g.*, water-soluble, sulphidic, or oxidic). For water-insoluble Ni compounds *(e.g.*, sulphidic), extracellular dissolution is limited. Therefore, the primary mechanism for uptake into the cell is via endocytosis of the Ni-containing particles (Benson *et al.*, 1992; Goodman et al., 2009). In this case, not only extracellular dissolution but also uptake into the cell and subsequent intracellular dissolution are critical in determining potential for toxicity (primarily after long-term exposures) and are dependent upon several factors. These factors include chemical form, particle size, structure, and surface charge (Costa et al., 1981a; Abbrachio et al, 1981; Miura et al., 1989). There have been many studies investigating the differences in cellular uptake between Ni substances in vitro (Oller et al., 1997; Goodman et al., 2009; 2011). Crystalline **Ni sulphide** and subsulphide have been shown to be readily endocytized *in vitro* (Costa et al., 1981a; Abbrachio et al, 1981). Although these compounds are water-insoluble, they have been shown to be partially soluble in some biological fluids (Oller et al., 2009). Based on these properties, evaluation of the available bioaccessibility data for nickel subsulphide and **nickel sulphide** in synthetic intra- and extracellular lung fluids was undertaken in order to perform a read-across hazard assessment for inhalation endpoints.

9.2.5. Inhalation bioaccessibility data

Bioaccessibility data relevant for the inhalation route of exposure can provide important information regarding the potential systemic bioavailability of the Ni ion and subsequent probability of systemic effects. Regarding local effects such as acute and chronic respiratory tract toxicity, bioaccessibility data provide one of several datasets that need to be considered together in a weight-of evidence approach. While bioaccessibility can inform the bioavailability at local target sites, other factors such as phagocytosis, immune responses, surface reactions can modulate the ultimate bioavailability (e.g., by affecting particle uptake) and/or contribute to the toxicity of the Ni particles. To investigate bioaccessibility via the inhalation route of exposure (Step 1 of the read-across strategy), source and target nickel substances were subjected to bioelution testing (KMHC, 2010). Samples of Ni compounds tested were provided by the lead registrant company for each substance and they are considered to be representative of the marketed Ni compounds. A brief description of the testing protocol and fluid compositions are provided in Appendix 1 in Section 12. In most cases, nickel-containing samples were extracted in three synthetic biological fluids: interstitial, alveolar, and lysosomal, for 2, 5, 24 and 72 hours. Data for 24 and 72 hours in interstitial and lysosomal fluids are reported in Table 14 as the percent of nickel released per gram of sample. The relative bioaccessibility data presented here are comparable to previously published data for selected Ni compounds in simulated lung fluids (Oller et al., 2009). Data obtained in alveolar fluid are not reported in Table 14, as results were similar to those obtained from interstitial fluid.

Based on the bioaccessibility results in interstitial lung fluid at 24 hours a preliminary distinction of two main groups of compounds could be made: substances releasing > approximately 1% Ni/g sample, and substances releasing < 1% Ni/g sample. Based on the lysosomal results, only one sample (green Ni oxide) releases several-fold less Ni than the other samples. It should be noted that the composition of lysosomal fluid includes citric acid which is added to the synthetic fluid to mimic the presence of proteins. However, citric acid binds Ni (and other metal ions) tightly and can exacerbate the dissolution of certain nickel compounds beyond what may happen in vivo. Therefore, there could be some uncertainty associated with predictions that are based solely on releases in synthetic lysosomal fluid, not only because it can overestimate the releases in lysosomal fluid in vivo but also because only the fraction of particles that are taken up by macrophages or epithelial cells will be subject to dissolution in this fluid.

Table 14: Summary interstitial and lysosomal bioaccessibility and corresponding in vivo verification data of nickel substances

Sample[a]	ID Code(s)[b]	CAS No.	Ni Content (%)[c]	Interstitial Bioaccessibility (% Ni/sample)[d] 24hrs – 72hrs	Lysosomal Bioaccessibility (% Ni/sample)[d] 24hrs – 72hrs	Acute Toxicity inhalation(LC_{50}; mg substance/L)[e]
Water-Soluble Nickel Compounds						
Ni Sulphate Hexahydrate	**N58-72**	**10101-97-0**	**22**	**10.7 - 12.80**	**20.35 - 21.35**	**2.48 (0.55)**
Ni Chloride Hexahydrate	N98	7791-20-0	25	7.5 - 8.10	25.05 - 25.05	NC
Ni Acetate Tetrahydrate	N103	6018-89-9	24	8.35 - 10.90	24.85 - 24.85	NC
Ni Sulphamate Tetrahydrate	N104	13770-89-3	18	8.25 - 8.60	18.30 - 18.30	NC
Sulfidic Nickel Compounds						
Ni Subsulphide	**N129 (N18)**	**12035-72-2**	**61 (70)**	**2.65 - 3.60**	**20.7 - 26.20**	**1.14 (0.80)**
Ni Sulphide	N97	16812-54-7	59	0.73 - 1.08	14.55 - 25.95	NC
Oxidic Nickel Compounds						
Ni Oxide Green	**N9/N46 (N126)**	**1313-99-1**	**77 (81)**	**0.08 - 0.10**	**0.44 - 0.82**	**>5.08 (>4.1)**
Ni Oxide Black	N105	1313-99-1	75	0.42 - 0.56	10.60 - 24.50	>5.15 (>3.9)
Ni Hydroxide	N106	12054-48-7	54	0.01 - 0.02	33.90 - 55.80	NC

a. Reference nickel compounds are identified in bold text.
b. Internal sample identification code(s). If more than one sample of a substance was used, samples were confirmed to have similar particle size distributions. The ID code of the sample tested *in vivo* is listed in parentheses.
c. Ni content determined by PIXE or ICP-MS. Content of sample tested *in vivo* is listed in parentheses.
d. Bioaccessibility reported as % Ni released in synthetic fluid per sample (g Ni/g sample x 100), after 24 or 72 hours. Reported values are mean values from duplicate experiments.
e. Acute inhalation LC_{50} values for source compounds determined by OECD Guidelines for the Testing of Chemicals, Procedure 403. Values are reported as average of male and female data. The LC_{50} values are inversely related to toxicity. The LC_{50} values in parenthesis are expressed as mg Ni/L. NC, not completed.

9.2.6. In vivo verification: inhalation toxicokinetic studies

Information on inhalation absorption of various nickel compounds is sparse. Available data indicate that, while the majority (80-90%) of inhaled nickel sulphate particles with mass median aerodynamic diameter (MMAD) of 2-3 µm are absorbed in rodents, the absorption of Ni subsulphide and green Ni oxide samples of the same particle size range is 2-to-10-fold and >1000-fold lower (< 0.1%) than that of Ni sulphate, respectively (Benson et al., 1995). This is consistent with a retention T1/2 in the lungs of 2 days for Ni sulphate, 4-5 days for Ni subsulphide and 120 days for green Ni oxide (Benson et al., 1994; Benson et al., 1995). It is important to note that the first step in the process of inhalation absorption is deposition. The particle size of the aerosol (MMAD and geometric standard deviation, GSD), together with particle density and breathing parameters will determine the deposited dose in different regions of the respiratory tract. Undissolved particles deposited in the upper airways and tracheobronchial region of the lung will be removed by the mucociliary escalator and be absorbed via the gastrointestinal tract. Undissolved particles in the alveolar region will be removed by macrophages to the lymph nodes and the airway lumen. As a result, the absorption mechanisms of different nickel substances vary and in addition, different particle size aerosols of even the same substance are expected to have different deposition and removal rates in various regions of the respiratory tract. This can result in differences in absorption for samples of the same compounds that

differ in particle size. In the case of inhalation, if verification of read-across for acute effects is conducted using toxicokinetic studies, the sameness of the samples used in both studies is critical.

The bioaccessibility data in interstitial lung fluids predicts that the inhalation absorption would be highest for Ni sulphate, about 4-fold lower for Ni subsulphide and >100-fold lower for nickel oxide. Although the number of samples is limited, the results are consistent with the relative inhalation absorption rates observed in vivo with these 3 compounds (as per Step 2 of read-across strategy). Data on inhalation absorption may be less relevant for chronic toxicity respiratory effects (e.g., respiratory tumors) that are local in nature and independent of systemic absorption. For chronic effects, an assessment of bioavailability at critical sites would be more informative.

9.2.7. In vivo verification: acute toxicity studies

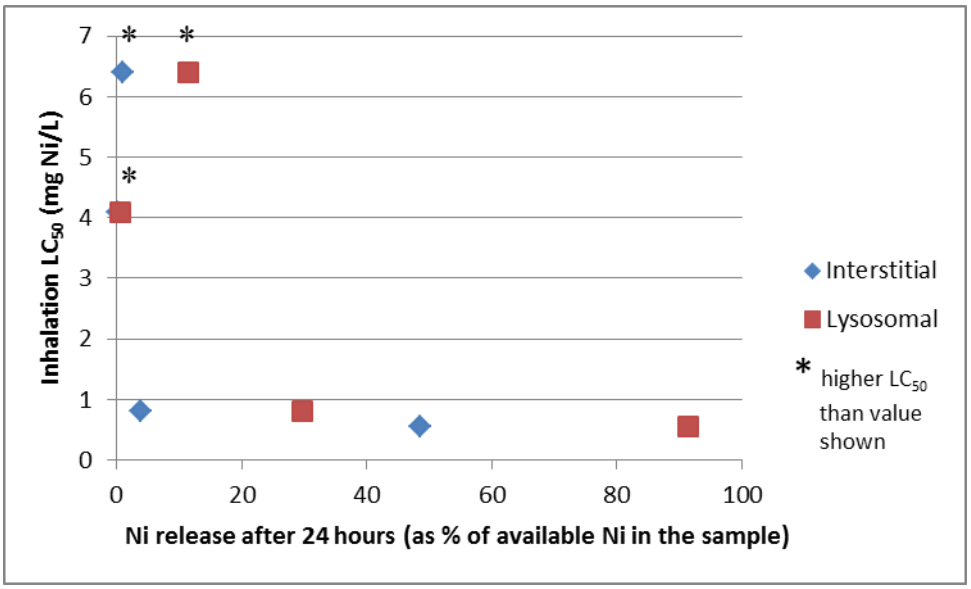
In order to verify that the bioaccessibility data provide reasonable estimations of respiratory tract bioavailability and hence toxicity (as per Step 2 of the read-across strategy), acute inhalation toxicity studies in rats were carried out on three reference nickel compounds (Ni sulphate hexahydrate, Ni subsulphide, and green Ni oxide) as well as on two samples of black Ni oxide (EPSL 2009a-b;2010b-c). Both green and black Ni oxide samples had 100% animal survival at the highest exposure tested in these studies (~5-8 mg compound/L). The results indicated that an exposure level of up to 8.3 mg Ni oxide/L for 4 hours had no effect on animal survival (EPSL, 2013). The results of these studies are summarized in Table 14. Each animal study was scored for reliability and reported in Section 7 of the IUCLID dossiers and submitted as part of the relevant compound registration .

Incorporation of the bioaccessibility data into any type of read-across assessment first requires an evaluation of its correlation with in vivo verification data. For the inhalation route this could be done by looking at the correlation between the LC_{50} (mg compounds/L or mg Ni/L after a 4 hour exposure) and the bioaccessibility in interstitial or lysosomal lung fluid after 5, 24, or 72 hours (% Ni release/g sample or % Ni release of available Ni) for the four compounds mentioned above[1]. These analyses, however, did not yield very meaningful relationships due to the few datapoints available and the fact that for black and green Ni oxide samples the true LC_{50} values are not known (LC_{50} > 5-8 mg Ni oxide/L; > 4-6 mg Ni/L). As an example, Figure 1 below shows the relationship between LC_{50} (mg Ni/L) and 24- hour release in lysosomal and interstitial fluids (% Ni release/of available Ni)[2].

[1] The average bioaccessibility results for the two samples of Ni oxide black were used for black Ni oxide. For the purposes of verification, it should be noted that the LC_{50} value for black Ni oxide is > 8.3 mg compound/L (or > 6.2 mg Ni/L) and the LC_{50} value for green Ni oxide is > 5.1 mg compound/L (or >4.1 mg Ni/L) and may well be > 8.3 mg compound/L (or > 6.2 mg Ni/L).

[2] Twenty-four hour bioaccessibility data was selected as a worst case for acute effects after a 4-h inhalation.

Figure 1. Correlations between 24 hour Ni release in interstitial or lysosomal lung fluid and inhalation LC_{50}.



Based on these data, it is not possible to assess whether lysosomal or interstitial fluid is a better predictor of acute toxicity effects. As discussed earlier, bioaccessibility in interstitial fluid suggests that there are at least two groups of nickel substances. This is confirmed by data from *in vivo* acute toxicity studies. The interstitial release and acute toxicity data (Table 14) mainly allow distinction between the nickel oxides that have low interstitial bioaccessibility (< approximately 1% Ni/g sample or 1% of available Ni at 24 hours) and low acute toxicity (with LC_{50} values >5-8 mg substance/L or > 4-6 mg Ni/L), and the group of water soluble compounds (Ni sulphate) and water insoluble sulphidic compounds (Ni subsulphide) with interstitial bioaccessibility higher than 1% Ni/g sample or >1 % of available Ni for 24 hours and LC_{50} values <3 mg substance/L (< 1.0 mg Ni/L). Based on this grouping similar classifications (Acute Tox 4; H332) are warranted for Ni sulphate and Ni subsulphide.

The bioaccessibility data in interstitial fluid from cource and target Ni compounds (Table 14) can be used to group these substances and select the best source compound to read-across to for acute inhalation toxicity. Ni chloride hexahydrate, Ni acetate tetrahydrate and Ni sulphamate tetrahydrate, all released between 7 and 11% of Ni/g sample (24 hours). These compounds are expected to have similar acute toxicity to Ni sulphate hexahydrate and can be assigned the same acute toxicity classification. The interstitial release for **Ni sulphide** is around 1% Ni/g sample and therefore this compound can be read-across from Ni subsulphide. By contrast, the interstitial release for Ni dihydroxide is <<1% Ni/g sample (24 hours) and therefore this compound can be read-across from Ni oxides for acute inhalation toxicity. The bioaccessibility data in lysosomal fluid consistent with the grouping based on interstitial fluid data although the fraction of particles that can be internalized and come into contact with lysosomal fluid is likely to be relatively small.

9.2.8. In vivo verification: Repeated Exposure Toxicity Studies

In the case of repeated exposure inhalation toxicity and local respiratory effects it is important to gather additional information on repeated exposure toxicokinetics or perform verification with chronic toxicity data to assess whether read-across based on acute effects can be directly extrapolated to repeated exposure effects.

Chronic toxicity studies (13 weeks and two-years in duration) have been conducted in mice and rats with three Ni compounds (Ni sulphate hexahydrate, Ni subsulphide and green Ni oxide) (NTP, 1996a,b,c). For the

two-year studies, the most sensitive lowest observed adverse effect concentration (LOAEC) for chronic inflammation effects in the alveolar region of the lung are shown below together with the Maximum Tolerated Exposure levels (MTD) used in these studies (Table 15). The longer term (72 hours) bioaccessibility results are shown for completeness.

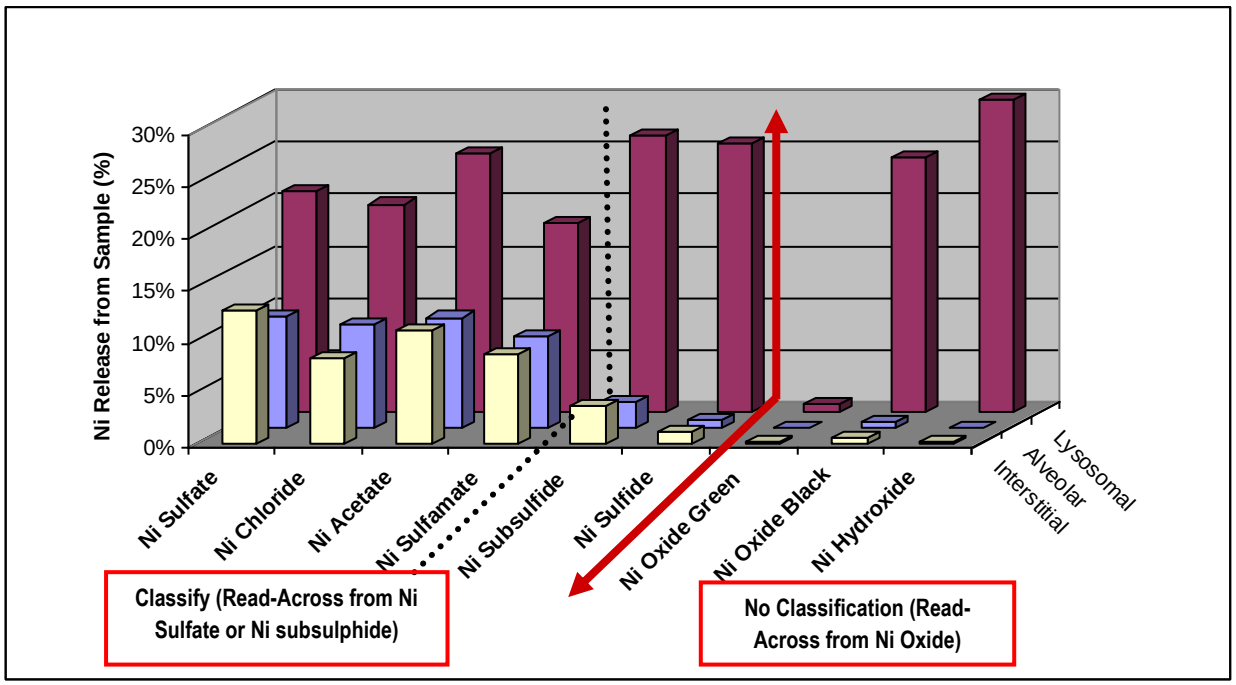
Table 15. Bioaccessibility data (72 hours, % Ni/ sample) and LOAEC values (mg Ni/m3) for chronic inflammation in lifetime inhalation studies in rats.

Sample	MTD 2-year study (mg/m^3) Rats	LOAEC 2-year study (mg/m^3) Rats	Interstitial Bioaccessibility (% Ni/g sample) 72 hours	Lysosomal Bioaccessibility (% Ni/g sample) 72 hours	Interstitial + Lysosomal Bioaccessibility (% Ni/g sample) 72 hours
Water-Soluble Nickel Compound					
Ni Sulphate Hexahydrate	0.5	0.25	12.80	21.35	34.2
Sulphidic Nickel Compound					
Ni Subsulphide	1.0	0.15	3.60	26.20	30.2
Oxidic Nickel Compound					
Ni Oxide Green	2.5	0.62	0.10	0.82	0.92

Again, the analyses are limited by the few datapoints (three) available and no real distinction can be made between the predictive power of Ni releases in one fluid over another. Similar conclusions can be reached using LOAEC data from the 13 week or 2-year inhalation studies. Therefore, grouping and read-across for repeated exposure effects may require consideration of bioaccessibility in both interstitial and lysosomal fluid. The bioaccessibility data in lysosomal fluid is considered more relevant for repeated exposure effects than for acute effects since the duration of exposure is longer (months) and the fraction of particles that potentially could be internalized and come into contact with lysosomal fluid is larger. Still, as previously noted, the potential for lysosomal fluid (based on presence of citric acid) to overpredict the in vivo intracellular bioavailability remains.

Figure 2 displays the 72 hours release values (% Ni/g sample) in interstitial, alveolar and lysosomal fluids, and graphically demonstrates the grouping and read-across application for acute inhalation toxicity discussed here.

Figure 2. Bioaccessibility data in three synthetic lung fluids and proposed grouping for classification based on read-across from source nickel substance.



9.2.9. Grouping for inhalation toxicity classification based on read-across approach

Bioaccessibility in interstitial and/or lysosomal fluid for target nickel substances allow two main groups of nickel substances to be identified for read-across with regards to classification for acute and repeated dose inhalation toxicity. The following read-across assessment for substances registered under the Nickel Consortia for REACH is based on these groupings for hazard classification using the CLP system and data for the three source Ni substances. It should be noted that in all instances, actual acute inhalation toxicity data are used for classification where available.

Group 1

Water-Soluble Nickel Compounds

These substances can be read across from Ni sulphate and receive an acute inhalation toxicity classification of Xn;R20 and Acute Tox 4;H332, for DSD and CLP, respectively. These are highly water-soluble Ni compounds with dissolution in interstitial fluid in the range of 7-11% Ni/g sample (24 hours). The data in Table 14 demonstrate that all soluble substances tested have similar bioaccessibility (at 24 and 72 hours) in interstitial fluid to the source substance, Ni sulphate. The same read-across assessment appears to be justified for repeated exposure toxicity, when considering the results of 72 hour bioaccessibility in lysosomal fluid (18-25% Ni/g sample). For these compounds the counter ion is not suspected of imparting additional toxicity. As such, repeated exposure classification as T;R48/23 and STOT RE 1; H372 (inhalation route and lung target organ) would be appropriate. Therefore, this read-across assessment concludes that Ni chloride, Ni acetate, and Ni sulphamate should be read across from Ni sulphate for both acute and repeated dose inhalation effects. *As Ni sulphamate is not currently classified for this endpoint in Annex VI, a self-classification is being proposed.*

Sulfidic Nickel Compounds

The first group of Ni substances also include those that would be read-across from Ni subsulphide and also receive an acute inhalation toxicity classification of Xn;R20 and Acute Tox 4:H332, for DSD and CLP, respectively. For **Ni sulphide**, its bioaccessibility data (at 24 and 72 hours) in interstitial fluid is in the range of approximately 1% Ni/g sample and so read-across from Ni subsulphide is warranted. Furthermore, both compounds share the same counter ion (sulfur) and are known to have similar surface properties; in vitro studies indicate similar toxicities and cellular uptakes. For repeated exposure toxicity effects, bioaccessibility results in lysosomal fluid should also be considered (15-30% Ni/g sample) supporting read-across from Ni subsulphide and classification of **Ni sulphide** as T;R48/23 and STOT RE 1; H372 (inhalation route and lung target organ). Data on **Ni sulphide** indicate that it should be read-across from the source substance, Ni subsulphide for acute and chronic effects as well as target organ toxicity after repeated inhalation exposure (see Appendix 2 for details on read-across justification for acute inhalation).

Group 2

Oxidic Nickel Compounds

The second group for read-across consists of Ni substances that can be read-across from green Ni oxide and thus should carry no classification for acute inhalation toxicity. This group is relevant for other oxidic Ni compounds where extracellular dissolution is negligible (<1% Ni/g sample released after 24 hours in interstitial fluid). Oxidic nickel compounds that release less than 1% Ni/g sample in interstitial fluid could be read-across from green Ni oxide and hence carry no classification for acute inhalation toxicity. Oxidic substances releasing >1% Ni/g sample in interstitial fluid would be read-across from Ni sulphate or Ni subsulphide, warranting classification as Xn;R20 and Acute Tox 4:H332. All oxidic nickel substances would classify for repeated dose toxicity as T;R48/23 and STOT RE 1; H372.

The outcome of this assessment indicates that both black Ni oxide and Ni dihydroxide fit within this third group (<1 % Ni/sample released in interstitial fluid) and should be read-across for acute toxicity from green Ni oxide. In the case of black Ni oxide, its own in vivo acute toxicity data confirm that this is the correct read-across (LC50 >5mg/L). Ni dihydroxide is currently classified as Xn;R20 and Acute Tox 4:H332 in Annex VI of the CLP Regulation. This classification had been read-across from the oral route of exposure. However, this classification seems inappropriate as the lung bioaccessibility-based read-across suggests that no classification for acute inhalation toxicity may be warranted for Ni dihydroxide.

For repeated exposure toxicity effects, bioaccessibility results in lysosomal fluids could also be considered. While green Ni oxide releases very little Ni in lysosomal fluid (<1% after 72hs), black Ni oxide releases up to 20%. Similarly, Ni dihydroxide releases in lysosomal fluid are similar to those of sulphidic Ni compounds. Therefore, conservatively, a read-across from Ni subsulphide to these two compounds for chronic toxicity effects could be warranted. In practice this is not an issue since Ni subsulphide and Ni oxides have the same classification for repeated dose toxicity (i.e., STOT RE 1; H372).

Classification in the EU Based on In vivo Data

Results of in vivo acute inhalation toxicity studies conducted in this program were evaluated with respect to the existing classifications as listed in Annex VI of the CLP for each source substance. The reported LC_{50} values of >5.08 mg/L (green Ni oxide) and >8.3 mg/L (black Ni oxide) confirm that Ni oxides should not be classified for acute inhalation toxicity. As data were not available at the time of classification, the current CLP classification for acute inhalation toxicity of Ni sulphate is based on read-across from oral data. The LC_{50} of 2.48 mg/L reported here now confirms that Ni sulphate was correctly classified as Xn;R20 and Acute Tox. 4;H332. Finally, Ni subsulphide has not been classified for acute inhalation toxicity. However, the newly generated data demonstrate that Ni subsulphide should carry the same classification as Ni sulphate, Xn;R20 and Acute Tox. 4; H332. Therefore, the REACH registration files for Ni subsulphide and **Ni sulphide** (based on read-across) include self-classifications for this endpoint and a separate CHL dossier has been submitted for nickel subsulphide.

All substances included in this program are currently classified for chronic respiratory toxicity according to Annex VI of the CLP (T;R48/23 and STOT RE 1;H372 route of exposure inhalation, target organ lung). The current bioaccessibility-based paradigm does not contradict this classification.

9.2.10. Uncertainties in read-across for acute inhalation toxicity

The inhalation read-across described here for nickel compounds overcomes uncertainties in previous read-across efforts based solely on water solubility by incorporating results in biological relevant fluids to the inhalation route of exposure and *in vivo* verification. However, remaining uncertainties and how they were addressed are listed below.

- Limited toxicokinetic data on Ni absorption after single inhalation exposure does not permit full verification of bioaccessibility data but allowed for a preliminary assessment between absorption and interstitial fluid Ni (II) release data for three source compounds.

- Robust data on acute inhalation exposure are only available for four Ni compounds. Correlations between LC50 and bioaccessibility in lung fluids do not reliably allow us to select one fluid over another one, but suggests that preliminary grouping based on interstitial fluid Ni (II) release is reasonable, since there is considerable uncertainty associated with bioaccessibility data in this fluid overpredicting absolute intracellular Ni releases after acute exposures.

- Similarly, for chronic toxicity effects, only data from 3 compounds are available for verification of bioaccessibility data, indicating that release in lysosomal fluid should also be considered.

- The bioaccessibility-based read-across approach does not address the potential toxicity of any counter-ion that may be present, although none of the nickel compounds included here are suspected of having counter ions with toxic effects (e.g., acetate, sulphamate, chloride).

- In the case of inhalation exposure and local effects there are factors other than Ni bioaccessibility that could contribute to the observed toxicities (e.g., particle uptake, particle surface effects, immune effects). A weight-of-evidence approach to read-across must be applied.

Most of the limitations listed above are overcome by considering the weight of evidence of all available, relevant data and by always adopting the most conservative read-across approach.

9.2.11. Conclusions regarding classification of nickel sulphide for acute inhalation toxicity

Data on the acute inhalation toxicity of **nickel sulphide** are read-across from nickel subsulphide (see Appendix 2 for details on read-across justification for acute inhalation). The comprehensive read-across assessment presented above included *in vivo* verification data for three source nickel substances. The bioaccessibility-based paradigm presented in Section 9: Other Information (also attached to the accompanying IUCLID file) enables grouping of target nickel substances for acute toxicity classification purposes according to bioaccessibility in interstitial fluid as demonstrated. The outcome of this assessment indicates that **nickel sulphide** should be read-across from nickel subsulphide for acute inhalation toxicity. Other evidence that can be considered in a weight-of-evidence approach include similar surface charateristics of crystalline samples (e.g., negative surface charge), similar in vitro cellular uptake and toxicity profiles, and lack of toxicity of counterion (sulphide) since Ni subsulphide has no classification for oral toxicity (Henderson et al., 2012b). Although nickel subsulphide is not currently classified for acute inhalation toxicity, a recently completed OECD-guideline compliant study reported an LC_{50}=1.14 mg/L nickel subsulphide (LC_{50}=0.83 mg Ni/L) after 4 hours of exposure. This newly generated data demonstrate that nickel subsulphide and **nickel sulphide** (calculated LC_{50}=1.3 mg nickel sulphide/L) should be classified as Acute Tox. 4; H332. The REACH registration for **nickel sulphide** reflects self-classification as Xn; R20 and Acute Tox. 4; H332.

9.2.12. Conclusions regarding STOT RE1 target organ and route of exposure

The STOT RE1 classification for Ni subsulphide is based on the lung inflammatory effects observed in rats and mice after repeated inhalation exposure. This classification has been read-across to **Ni sulphide** (CLP). Notation regarding the relevant route of exposure (inhalation) and the target organ for toxicity (lung) can also be read across to **Ni sulphide**.

10. DETAILED STUDY SUMMARIES

10.2 TOXICOKINETICS

STUDY 1

Study reference:

Tanaka I, Ishimatsu S, Haratake J, Horie A, and Kodama Y. (1988). Biological half-time in rats exposed to nickel monosulfide (amorphous) aerosol by inhalation. Biological Trace Element Research; 17:237-246.

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

The retention of NiS in rats following repeated inhalation was evaluated (note: the study also included toxicity evaluations; see Repeated Dose Toxicity section). Male Wistar rats were exposed via inhalation to 8.8 mg/m^3 NiS aerosols for 7 hours/day, 5 days/week up to 1 month. The MMAD and GSD were 4.0 μm and 2.2, respectively. Some rats were sacrificed 24 hr after the termination of exposure while some rats were kept for an additional 6-month clearance period. In addition, some rats were exposed acutely to 107 mg/m^3 NiS for 4 hours and sacrificed 0, 24, 48, 72, and 76 hours after exposure. Nickel content was measured in the lungs, liver, spleen, kidneys, blood, and urine via flameless absorption spectrophotometry. .

In the acutely exposed rats, exposure to NiS resulted in no significant differences in body or organ weights. Nickel content in organs, blood, and urine after a single 4 hr exposure were reported, but statistical significance not provided. In the lung, control values were 0.13 μg/g tissue, whereas values immediately after exposure, at 0, 24, and 72 hrs were 47.8, 25.4 and 3.2 μg/g, respectively. In the liver, nickel levels were 0.03 μg/g and 0.20 μg/g in control animals and in those immediately after exposure. Liver nickel levels returned to normal by 48 hours post-exposure. Spleen nickel levels were unaffected, whereas kidney, urine, and blood levels increased after exposure and remained detectable after 72 hours. Nickel levels were slightly elevated in rats exposed to NiS for 1 month (statistical significance not provided), but were the same as control animals in those rats that were kept for a 6-month clearance period. Based on data from the 4 hr and 1-month exposures, the authors estimated the biological half-time for lung clearance of NiS to be 20 hr. The authors speculated that NiS is deposited into the lung, "dissolved easily into the blood," transferred rapidly to the kidney, and subsequently excreted into the urine.

STUDY 2

Study reference:

Tanaka I, Ishimatsu S, Kodama Y, Haratake J, Horie A, Tsuchiya K, and Cho S. (1992). Durability of various kinds of nickel compounds in rats administered by inhalation. Nickel and Human Health, Current Perspectives. Nieboer E and Nriagu JO (Eds); Advances in Environmental Science and Technology; 25:481-490.

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

Tanaka et al. (1992) reported on the durability (defined as degradation in vivo) of various kinds of nickel compounds in rats administered by inhalation as part of a book on Nickel and Human Health. Specifically, the book chapter described the differences of nickel distribution in rat organs (lungs, liver, kidneys, and spleen) following aerosol inhalation of NiS (amorphous nickel monosulfide, NiS[A]), green nickel oxide (NiO), nickel metal, or Ni(III) oxide (Ni_2O_3). Rats were exposed to

nickel compounds via inhalation for one month (8.8 +/- 2.8 mg NiS/m^3; MMAD and GSD were 3.9 µm and 2.6, respectively). The distribution to organs was determined immediately after the 1-month exposure or 6 months after exposure (referred to as the clearance period). Nickel contents in the organs and whole blood were measured by flameless atomic absorption spectrometry. Exposure to NiS had no affect on bodyweight; however, lung and spleen weights were significantly increased and decreased, respectively. In animals exposed for one month, the nickel concentrations were elevated in the lung, liver, kidney, and whole blood relative to controls. In animals exposed for a month and then monitored for 6 months, the lung weights remained significantly elevated, while kidney weights became elevated and spleen weight returned to control level. In rats kept for 6 months, there were no significant differences in nickel concentration in the lung, liver, kidney, spleen, or blood between treated and control animals. The authors concluded that NiS (as well as Ni_2O_3 and nickel metal) was deposited and cleared simultaneously. Furthermore, the authors concluded that the lung clearance rate was different for the various nickel compounds and suggested that the biological half-time in the lungs (estimated to be: NiO >> Ni_2O_3 > nickel metal >> NiS) was generally inversely related to solubility (NiS >> Ni_2O_3 > NiO > nickel metal).

STUDY 3

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.

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

A complete summary of the study design and data from KMCH (2010) and its incorporation into the read-across assessment for acute inhalation toxicity of NiS is included in Section 9: Other Information.

10.3 HEALTH HAZARDS

10.3.1. Acute inhalation toxicity - animal data

STUDY 1

Study reference:

Eurofins Product Safety Labs (EPSL; 2010b). 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 not 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 0.205, 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

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 ofthe 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 LC50 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 ofthe decedents revealed discoloration of the lung, liver and/or intestines, and/or rigor mortis. 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.

10.3.2. Repeated dose toxicity - animal data

STUDY 1

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 subsulfide, or nickel sulfate hexahydrate in F344/N rats and B6C3F1 mice. Fundamental and Applied Toxicology; 12:584-594.

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

Test type

• OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day)

Test substance

• Test material used in the study is not equivalent to the substance identified in the CLH dossier.

• EC number: 234-829-6

• CAS number: 12035-72-2

• Degree of purity: >97%

• 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 = 2.4 μm, with GSD = 2.2 for doses 0.11, 0.2, 0.4, 0.9, and 1.8 mg Ni/m^3

• Type or preparation of particles (for studies with aerosols): Nebulizer

Test animals

• Species/strain/sex: rat, F344/N, male/female

• No. of animals per sex per dose: 10

• Age and weight at the study initiation: 7-8 weeks

Administration/exposure

• Duration of test/exposure period; 6 hours/day; 5 days/wk; 13 weeks

• Doses/concentration levels: 0.11, 0.2, 0.4, 0.9, and 1.8 mg nickel/m^3.

• Analytical verification of test atmosphere concentrations: The aerosol concentration in the exposure chambers was monitored by taking three 2-hr filter samples during the 6-hr exposure day that were found to be within ± 10% of target levels. The mean daily concentration in the exposure chambers was calculated from the filter samples.

• Control group and treatment: yes; filtered air

• Vehicle: identification, concentration and volume used, justification of choice of vehicle: not applicable

• Statistical methods: The significance of differences between dosed and control group means was assessed using multiple comparison procedures with two-tailed tests.

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

•Effect levels

Endpoint	Exposure Level (mg Ni/m^3)	Sex	Basis for effect level
LOEC	0.2	male	Significant decrease in body weight at this and higher conc (except 0.9 mg Ni/m^3)
LOEC	0.1	male/female	Significant increase in lung weight at 13 weeks
LOEC	0.1	male/female	10/10 animals with alveolar macrophage hyperplasia
LOEC	0.1	male/female	2-3 animals per sex with chronic active inflammation in lung (out of 8-10 animals per sex)
NOEC	0.1	male	0 animals with olfactory epithelial atrophy (8-10 animals)
NOEC	0.2	female	0 animals with olfactory epithelial atrophy (8-10 animals)

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

reliability:

- Clinical signs: no effects
- Body weight and weight gain: bodyweight was significantly reduced (4-7%) in three of the four highest dose groups (but not in second highest group) in males only
- Organ weights: lung weight increased in all animals at all doses
- Histopathology (non-neoplastic): Inflammatory changes were seen in the lung, nasal cavity, and bronchial lymph node after exposure to Ni compounds.

  The lung lesions included alveolar macrophage hyperplasia, inflammation, and fibrosis. Alveolar macrophage hyperplasia consisted of an increase in the number of macrophages within alveolar spaces.

  The change in the bronchial lymph nodes consisted of minimal to mild lymphoid hyperplasia.

STUDY 2

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 subsulfide, or nickel sulfate hexahydrate in F344/N rats and B6C3F1 mice. Fundamental and Applied Toxicology; 12:584-594.

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

Test type

• OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day)

Test substance

• Test material used in the study is not equivalent to the substance identified in the CLH dossier.

• EC number: 234-829-6

• CAS number: 12035-72-2

• Degree of purity: >97%

• 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 = 2.4 μm, with GSD = 2.2 for doses 0.11, 0.2, 0.4, 0.9, and 1.8 mg Ni/m^3

• Type or preparation of particles (for studies with aerosols): Nebulizer

Test animals

• Species/strain/sex: mouse, B6C3F1, male/female

• No. of animals per sex per dose: 10

• Age and weight at the study initiation: 7-8 weeks

Administration/exposure

• Duration of test/exposure period; 6 hours/day; 5 days/wk; 13 weeks

• Doses/concentration levels: 0.11, 0.2, 0.4, 0.9, and 1.8 mg nickel/m^3.

• Analytical verification of test atmosphere concentrations: The aerosol concentration in the exposure chambers was monitored by taking three 2-hr filter samples during the 6-hr exposure day that were found to be within ± 10% of target levels. The mean daily concentration in the exposure chambers was calculated from the filter samples.

• Control group and treatment: yes; filtered air

• Vehicle: identification, concentration and volume used, justification of choice of vehicle: not applicable

• Statistical methods: The significance of differences between dosed and control group means was assessed using multiple comparison procedures with two-tailed tests.

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

•Effect levels

Endpoint	Exposure Level (mg Ni/m³)	Sex	Basis for effect level
NOEC	1.8	male/female	No significant change in body weight
NOEC	0.4	male/female	No significant increase in lung weight
NOEC	0.1	male/female	No animals with alveolar macrophage hyperplasia
NOEC	0.4	male/female	No animals with chronic active inflammation
NOEC	0.4	male/female	No animals with fibrosis (8-10 animals)
NOEC	0.3	female	No animals with olfactory epithelial atrophy (8-10 animals)

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

- Clinical signs: no effects
- Body weight and weight gain: no effect
- Organ weights: lung weight increased in mice exposed to ≥ 0.9 mg Ni/m^3
- Histopathology (non-neoplastic): Inflammatory changes were seen in the lung, nasal cavity, and bronchial lymph node after exposure to NiO.

The lung lesions included alveolar macrophage hyperplasia, inflammation, and fibrosis. Alveolar macrophage hyperplasia consisted of an increase in the number of macrophages within alveolar spaces.

The change in the bronchial lymph nodes consisted of minimal to mild lymphoid hyperplasia.

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12. APPENDIX 1: BIOACCESSIBILITY PROTOCOLS

General protocol:

The samples were extracted in leaching fluids (interstitial, alveolar, and lysosomal). The samples were extracted for up to four time periods (2, 5, 24, or 72 hrs). The extractions were performed using 0.1 gram of sample in 50 ml of fluid. A shaker water bath at a temperature of 37°C was used. All extractions were performed in duplicate. Blank fluids were extracted in duplicate at one time period. The extracts were analyzed for soluble nickel using ICP/MS. Results were reported as µg Ni/g sample and as % of total available Ni released.

Specific information on each synthetic fluid:

Simulated Interstitial Fluid

A. Extraction:
Each extraction was performed using 0.1 gram of sample in 50 ml of Gamble's Solution (pH = 7.4 ± 0.2). Samples were weighed into acid washed 250 ml amber Erlenmeyer flasks. Gamble's Solution was added to the flasks and they were then swirled to mix compound and fluid. The pH was checked for each solution and adjusted if necessary with 2N HCl or 1N NaOH. To keep the extraction pH at 7.4, 5% CO_2 in Nitrogen was bubbled into the solution at a rate of 50 cc/min. The bubbling solutions were placed in a preheated 37° C reciprocal shaker bath. The samples were bubbled and allowed to shake for the required extraction times. Once complete, the solutions were removed from the bath. The pH was checked and the solutions were filtered through a 0.45 µm filter. The filtrates were collected in 8 oz. disposable plastic bottles and kept in a 35° C incubator until analyzed.

B. Analysis:
EPA Method #200.8 (ICP/MS)

C. Gamble's Solution:

	g/L of DI Water
Magnesium chloride hexahydrate	0.2033
Sodium chloride	6.0193
Potassium chloride	0.2982
Dibasic sodium phosphate (anhydrous)	0.1420
Sodium sulphate (anhydrous)	0.0710
Calcium chloride dihydrate	0.3676
Sodium acetate trihydrate	0.9526
Sodium bicarbonate	2.6043
Sodium citrate dihydrate	0.0970

Simulated Alveolar Fluid

A. Extraction:
Each extraction was performed using 0.1 gram of sample in 50 ml of Synthetic Lung Fluid (pH = 7.4 ± 0.2). Samples were weighed into acid washed 250 ml amber Erlenmeyer flasks. Synthetic Lung Fluid was added to the flasks and they were then swirled to mix compound and fluid. The pH was checked for each solution and adjusted if necessary with 2N HCl or 1N NaOH. To keep the extraction pH at 7.4, 5% CO_2 in Nitrogen was bubbled into the solution at a rate of 50 cc/min. The bubbling solutions were placed in a preheated 37° C reciprocal shaker bath. The samples were bubbled and allowed to shake for the required extraction times.

Once complete, the solutions were removed from the bath. The pH was checked and the solutions were filtered through a 0.45 µm filter. The filtrates were collected in 8 oz. disposable plastic bottles and kept in a 35° C incubator until analyzed.

B. Analysis:
EPA Method #200.8 (ICP/MS)

C. Synthetic Alveolar Lung Fluid:

	g/L of DI Water
Magnesium chloride hexahydrate	0.2033
Sodium chloride	6.0193
Potassium chloride	0.2982
Dibasic sodium phosphate (anhydrous)	0.1420
Sodium sulphate (anhydrous)	0.0710
Calcium chloride dihydrate	0.3676
Sodium acetate trihydrate	0.9526
Sodium bicarbonate	2.6043
Sodium citrate dihydrate	0.0970
Phosphatidyl choline	0.1000

Simulated Lysosomal Fluid

A. Extraction:
Each extraction was performed using 0.1 gram of sample in 50 ml of Simulated Lysosomal Fluid (pH= 4.5- 5.0). Samples were weighed into acid washed 250 ml amber Erlenmeyer flasks. Lysosomal Fluid was added to the flasks and they were then swirled to mix compound and fluid. The pH was checked for each solution and adjusted if necessary with 2N HCl or 1 N NaOH . The opening of the flasks were covered with parafilm and aluminum foil. The flasks were then placed in a preheated 37° C reciprocal shaker bath. The samples were allowed to shake for the required extraction times. Once complete, the solutions were removed from the bath. The solutions were filtered through a 0.45 µm filter and the pH was verified. The filtrates were collected in 8 oz. disposable plastic bottles and kept in a 35° C incubator until analyzed.

B. Analysis:
EPA Method #200.8 (ICP/MS)

C. Synthetic Lysosomal Lung Fluid:

	g/L of DI Water
Sodium chloride	3.21
Sodium hydroxide	6.00
Citric acid	20.8
Calcium chloride	0.097
Sodium phosphate heptahydrate	0.179
Sodium sulphate	0.039
Magnesium chloride hexahydrate	0.106
Glycine	0.059
Sodium citrate dehydrate	0.077
Sodium tartrate dehydrate	0.090
Sodium lactate	0.085
Sodium pyruvate	0.086
Formaldehyde	1.0 mL

13. APPENDIX 2: READ ACROSS FOR ACUTE INHALATION TOXICITY: NI SUBSULPHIDE TO NI SULPHIDE

Table 16 below follows the 2015 ECHA Read Across Assessment Framework (RAAF) suggested format.

A categorical approach was selected for read-across, where quantitative variations in effects are predicted based on quantitative variations in the lung interstitial fluid bioaccessibility of Ni ion among category members. All Ni compounds are considered to transform (to different extents) upon inhalation into the common soluble Ni ion (scenario 3). See Sections 9.1.1 to 9.1.3.

Table 16. Read across for acute inhalation toxicity for nickel sulphide

Assessment Elements	Assessment Element Type	Justification	Assessment Option Score
C1	Substance characterisation	Source: Ni subsulphide. CAS 12035-72-2, EC 234-829-6 Target: Ni sulphide. CAS 16812-54-7, EC 240-841-2. Substance has >88% and < 98% purity (w/w). Impurities include pyrite (<8%) and Zn sulphide (<2%), with other impurities < 1%.	5
C2	Structural similarity and category hypothesis	All Ni-containing compounds have been shown to release soluble Ni (II) ions to different extents, depending on the compound and the medium (route of exposure) tested. The absorbed soluble Ni ion is considered to be the toxic moiety responsible for the acute toxicity of nickel compounds. All Ni compounds are considered to transform (to different extents) upon inhalation into a common soluble Ni (II) ion (see Sections 9.1.4 and 9.1.5).	5
C3	Link of structural similarity and differences with proposed regular pattern	The potential for most nickel-containing substances to cause adverse effects in the respiratory tract has been shown to be dependent upon the bioavailability of the Ni (II) ion at the target sites. This enables grouping of target Ni substances for classification purposes according to bioaccessibility in interstitial and/or lysosomal fluid. The data currently available allow two main groups of nickel substances to be identified for read-across with regards to classification for acute and repeated dose inhalation toxicity. Within one group, the data indicate that Ni subsulphide and Ni sulphide, bioaccessibility data in interstitial fluid is similar (e.g. approximately 1% Ni/g sample), so read-across from Ni subsulphide to Ni sulphide is warranted (see Sections 9.1.5 to 9.1.9).	4
C4	Consistency of effects on data matrix	The bioaccessibility-based approach presented above enables grouping of target Ni substances for classification purposes according to bioaccessibility in interstitial and/or lysosomal fluid. While there was not a meaningful correlation between the LC_{50} (mg compounds/L or mg Ni/L after a 4-hour	5

		exposure) and the bioaccessibility in interstitial or lysosomal lung fluid after 5, 24, or 72 hours (% Ni release/g sample or % Ni release of available Ni) due to the few data points available and lack of a true LC_{50} for nickel oxide, data on bioaccessibility in interstitial fluid suggests that there are at least two groups of nickel substances. This is confirmed by data from *in vivo* acute toxicity studies. A distinction can be made between the nickel oxides that have low interstitial bioaccessibility (< ~ 1% Ni/g sample or 1% of available Ni at 24 hours) and low acute toxicity (with LC_{50} values >5-8 mg substance/L or > 4-6 mg Ni/L), and the group of water soluble compounds (Ni sulphate) and water insoluble sulphidic compounds (Ni subsulphide) with interstitial bioaccessibility higher than 1% Ni/g sample or >1 % of available Ni for 24 hours and LC_{50} values <3 mg substance/L (< 1.0 mg Ni/L) (see Section 9.1.6 and 9.1.7).	
C5	Reliability and adequacy of source studies	The source study to derive an LC_{50} suitable to assess appropriate classification for acute inhalation toxicity is an acute toxicity inhalation study with Ni subsulphide (CAS 12035-72-2), (EPSL 2010). Study follows GLP and OECD 403.	5
3.1	Formation of common (identical) compound	Both source (Ni subsulphide) and target (Ni sulphide) samples released soluble Ni (II) ions in synthetic alveolar and interstitial fluid (see Section 9.1.5).	5
3.2	The biological targets for common compound	Available data indicate that the primary factor for lung toxicity of nickel substances may be solubility in the respiratory tract. In the case of mortality (e.g., LC_{50} studies) after acute exposure, the target site for nickel substances may be the whole respiratory tract. Extracellular (e.g., interstitial and alveolar) dissolution may be more important for acute toxicity effects than the intracellular (e.g. lysosomal) one (particularly for water soluble and partially soluble compounds) since *in vivo* acute toxicity studies usually involve exposure for a few hours followed by observation for a few days, and as such the potential for intracellular dissolution is more limited. After exposure and dissolution, the dissociated nickel ions will bind to any available proteins in cellular membranes or, once transported into the cell via ion channels (e.g., magnesium or calcium channels), rapidly bind to proteins in the cytoplasm. This binding has the potential to disrupt cellular homeostasis and cause acute toxicity.	4
3.3	Exposure of biological target to common compounds	During inhalation exposure the bioavailability of Ni ions to lung cells will influence the level of exposure to the common compound (e.g. Ni ion). The bioaccessibility data in interstitial lung fluids predicts that the inhalation absorption would be highest for Ni sulphate, about 4-fold lower for Ni subsulphide and >100-fold lower for nickel oxide. Although the number of samples is limited, the results are consistent	4

		with the relative inhalation absorption rates observed in vivo with these 3 compounds.	
3.4	The impact of parent compounds	Both source (Ni subsulphide) and target (Ni sulphide) are very similar in chemical structure and surface properties (e.g. surface charge). In addition, results from in vitro tests indicate similar uptake of sulphide and subsulphide particles into mammalian cells.	5
3.5	Formation and impact of non-common compounds	Both source (Ni subsulphide) and target (Ni sulphide) are expected to have similar non-common compounds (sulphide moiety) and this moiety does is not expected to influence the overall acute inhalation toxicity to any significant extent. This is supported by the fact that no acute oral toxicity was observed with Ni subsulphide (Henderson et al., 2012a).	4
C6	Bias that influences the prediction	Summary: The inhalation read-across for nickel compounds described here relies on relative bioaccessibility data with support from physico-chemical and in vitro uptake data (in a weight-of-evidence approach) to determine the most appropriate grouping of substances for read-across, overcoming uncertainties in read-across efforts based solely on water solubility. Consistency of effects in the data matrix have been shown with data on bioavailability as well as acute and repeated dose inhalation toxicity. The substances included in this category have structural similarity as well as similar Ni2+ ion release in the appropriate synthetic biological fluids.	5

Assessment option score: 5, acceptable with high confidence; 4, acceptable with medium confidence; 3, acceptable with just sufficient confidence