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 bis(sulphamidate)

EC Number: **237-396-1**

CAS Number: **13770-89-3**

Index Number: **028-018-00-4**

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Lead Registrant of Nickel sulphamate – JS_Nickel_sulphamate

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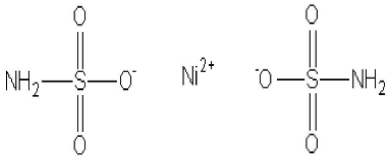
1. IDENTITY OF THE SUBSTANCE

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 bis(sulphamidate), nickel sulphamate, nickel sulfamate, sulfamic acid nickel (2^{++}) salt (2:1), sulfamic acid nickel (2^{+}) salt (2:1), nickel (2^{+}) disulfamate
Other names (usual name, trade name, abbreviation)	nickel sulphamate
ISO common name (if available and appropriate)	not applicable
EC number (if available and appropriate)	237-396-1
EC name (if available and appropriate)	nickel bis(sulphamidate)
CAS number (if available)	13770-89-3
Other identity code (if available)	CLP Annex VI Index number, 028-018-00-4
Molecular formula	H3NO3S.1/2Ni
Structural formula	
SMILES notation (if available)	not applicable
Molecular weight or molecular weight range	250.865
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)	≥ 99.0 — ≤ 100.0 % (w/w)

1.2 Composition of the substance

Name: Nickel sulphamate

Degree of purity: ≥ 99.0 — ≤ 100.0 % (w/w)

Table 2: Nickel sulphamate 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 bis(sulphamidate) EC no.: 237-396-1	≥ 99.0 — ≤ 100.0 % (w/w)	Carc. 1A ; H350i Muta. 2 ; H341 Repr. 1B ; H360D*** STOT RE 1 ; H372** Resp. Sens. 1 ; H334 Skin Sens. 1; H317 Aquatic Acute 1: H400 Aquatic Chronic 1; H410	Acute Tox. 4; H302 Acute Tox. 4; H332

** the classification under 67/548/EEC indicating the route of exposure has been translated into the corresponding class and category according to this Regulation, but with a general hazard statement not specifying the route of exposure as the necessary information is not available; *** indicates a general concern for effects on both fertility and development, the general hazard statement can be replaced by the hazard statement indicating only the property of concern, where either fertility or developmental effects are proven to be not relevant.

Table 3: Nickel sulphamate 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
Unknown impurity	≥ 0 — ≤ 1.0 % (w/w)	Not applicable	Not applicable	No

Table 4: Nickel sulphamate 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 sulphamate tetrahydrate EC no.: 237-396-1	100%	Not applicable	Acute oral toxicity (EPSL, 2008) Toxicokinetics/Read-Across for acute inhalation toxicity: Henderson et al. (2012a, b) and KMHC (2010)	Also referred to as "nickel sulphamate crystals". Supplier reported no presence of impurity which may be dangerous such as in Directives 88/379/EEC and 67/548/EEC.

2. PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6: C&L table

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	028-018-00-4	nickel bis(sulfamidate); nickel sulfamate	237-396-1	13770-89-3	Carc. 1A Muta. 2 Repr. 1B STOT RE 1 Resp. Sens. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H350i H341 H360D*** H372** H334 H317 H400 H410	GHS08 GHS09 Dgr	H350i H341 H360D*** H372** H334 H317 H410		STOT RE 1; H372: C ≥ 1 % STOT RE 2; H373: 0,1 % ≤ C < 1 % Skin Sens. 1; H317: C ≥ 0,01 % M=1	H
Dossier submitters proposal					**Acute Tox.4 (ADD)**	**H302 (ADD) H332 (ADD)**	**GHS07 (ADD)**	**H302 (ADD) H332 (ADD)**			
Resulting Annex VI entry if agreed by RAC and COM	028-018-00-4	nickel bis(sulfamidate); nickel sulfamate	237-396-1	13770-89-3	**Acute Tox. 4 Acute Tox. 4** Carc. 1A Muta. 2 Repr. 1B STOT RE 1 Resp. Sens. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	**H302 H332** H350i H341 H360D*** H372** H334 H317 H400 H410	**GHS07** GHS08 GHS09 Dgr	**H302 H332** H350i H341 H360D*** H372** H334 H317 H410		STOT RE 1; H372: C ≥ 1 % STOT RE 2; H373: 0,1 % ≤ C < 1 % Skin Sens. 1; H317: C ≥ 0,01 % M=1	H

** the classification under 67/548/EEC indicating the route of exposure has been translated into the corresponding class and category according to this Regulation, but with a general hazard statement not specifying the route of exposure as the necessary information is not available; *** indicates a general concern for effects on both fertility and development, the general hazard statement can be replaced by the hazard statement indicating only the property of concern, where either fertility or developmental effects are proven to be not relevant.

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	not applicable (harmonised classification proposed)	Yes
Acute toxicity via dermal route	hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	not applicable (harmonised classification proposed)	Yes
Skin corrosion/ irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	hazard class not assessed in this dossier	No
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	hazard class not assessed in this dossier	No
Germ cell mutagenicity	hazard class not assessed in this dossier	No

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

3. HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The original proposal to include nickel sulphamate (EC No. 237-396-1; CAS No. 13770-89-3) 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 sulphamate for some endpoints (carcinogenicity, reproductive toxicity, mutagenicity, chronic inhalation toxicity, sensitization, and toxicity to the environment) was based on grouping of soluble 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 sulphamate was due to a lack of substance-specific data at the time of entry.

Nickel sulphamate'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.

4. JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Justification that action is needed at Community level is required.

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

Further detail on need of action at Community level

Consumers do not come into contact with nickel sulphamate. 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 sulphamate is not of concern for acute oral and acute inhalation toxicity, where all the other water soluble nickel compounds are classified for these endpoints. Newly available data support classification for acute oral and inhalation toxicity, which would bring nickel sulphamate in line with all other water soluble nickel compounds (with the exception of nickel chloride) which are currently classified as Acute Tox. 4.

5. DATA SOURCES

- Chemical Safety Report (CSR) for Nickel Sulphamate (2015 update), including unpublished laboratory reports referenced within the CSR
- Comprehensive scientific literature search related to toxicokinetics and acute inhalation toxicity of nickel sulphamate
- Searching of the ECHA website and general internet searching related to the history of the previous classification and labelling
- Searching of the ECHA database and the CLP (including ATPs) for registration dossiers of impurities related to classifications and self-classifications

6. PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	solid blue crystalline material	Harlan Labs, 2010	Physical state: solid, crystalline Colour:blue Odour: no data
Melting/freezing point (°C)	Not applicable	Harlan Labs, 2010	Nickel sulphamate decomposes from approximately 415 K. The determination was performed by differential scanning calorimetry, using ASTM E537-86, Method A1 melting/freezing temperature of Commission Regulation (EC) No 440/2008 of 30 May 2008 and method 102 of the OECD guidelines for testing of chemicals, 27 July 1995.
Boiling point (°C)	Not applicable	Not applicable	Data waived - Column 2 of REACH Annex VII provides the following exemption: A study does not need to be conducted for solids which either melt above 300°C or decompose before boiling. Nickel Sulphamate was determined not to melt below 300°C as decomposition was noted at 141.63 C.
Relative density	2.25 at 20°C	Harlan Labs, 2010	The relative density of the test material has been determined to be 2.25 at 20.0 ± 0.5°C, using a gas comparison pycnometer, 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	Column 2 of REACH Annex VII provides the following exemption: A study does not need to be conducted for solids which either melt above 300°C or decompose before boiling. Nickel sulphamate decomposes at 415 K (141.63 °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 sulphamate is not designed or anticipated to have surfactant properties.
Water solubility (mg/l)	49.9 to 60% w/w at 20 °C	Harlan Labs, 2010	The water solubility has been determined to be in the range of 49.9 to 60.0% w/w of solution at 20.0 ± 0.5°C, using a flask method based on 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. Nickel sulphamate is inorganic, 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 sulphamate 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 sulphamate, 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 sulphamate 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 sulphamate is very 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 sulphamate 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 under Sections 5 and 6 of this IUCLID database.
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 for solid substances. The representative substance of Ni sulphamate used for testing of physico-chemical endpoints sulphamate is a solid, and therefore viscosity data were not deemed relevant. However, as Ni sulphamate is typically placed on the market in the EU in solution.

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

Table 9: Summary table of toxicokinetic studies via inhalation

Method	Results	Remarks	Reference
rat (Wistar) male oral: gavage Exposure regime: One dose Doses/conc.: 10 mg of Ni	The absorbed fraction in the rats was estimated to be 0.09% for the Ni-Metal group and 10-34% for the $NiSO_4$, $NiCl_2$, and $Ni(NO_3)_2$ groups. Following administration of a single dose of 10 mg nickel (nickel sulphate in 5% starch saline solution) by gavage to male Wistar rats, the absorbed fraction was 11% 24 hours after oral administration. Ni concentrations in all organs in the rats given $NiSO_4$, $NiCl_2$, and $Ni(NO_3)_2$ were significantly higher than those in the control rats. About 84-87% of the Ni amount was found in the kidneys for the NiCl2 and $NiSO_4$. The percent nickel in the kidneys for the $Ni(NO_3)_2$ and Ni-Metal groups was 73 and 51%, respectively. Soluble Ni compounds (including $NiCl_2$) were excreted within 72 hours after the oral administration. The Ni measured in urine from the $NiCl_2$ group after oral administration was 914 µg/total 24-hour urine volume.	Klimisch score: 2 (reliable with restrictions) key study experimental result read-across from supporting substance (structural analogue or surrogate) **Test material (CAS number): 7786-81-4 Ni Sulphate**	Ishimatsu S, Kawamoto T, Matsuno K, Kodama Y. (1995)
in vitro study In situations where the bioavailability of a metal substance is not known or not feasible to determine experimentally, the amount of metal ion "available for absorption" may be measured using in vitro methods. In this application the dissolution (e.g., elution or extraction) of metal ion from various nickel compounds in surrogate (synthetic) tissue fluids is measured. The resultant value is termed bioaccessibility and is defined as the amount of a substance (e.g., metal ion)	For sample N104 of nickel sulphamate tetrahydrate, the nickel release was: • 83.4% of Ni content after 2 hours in gastric fluid, • 11.8% of Ni content after 24 hours in intestinal fluid, • 49.1% of Ni content and 8.6% of sample weight after 72 hours in interstitial fluid, • 104.4% of Ni content and 18.3% of sample in lysosomal fluid, • 91.7% of Ni content after 24 hours in sweat fluid.	Klimisch score: 2 (reliable with restrictions) supporting study experimental result **Test material (EC name): nickel sulphamate**	KMHC (2010) Henderson, RG, Cappellini D, Seilkop SK, Bates HK and Oller AR (2012a) [gastric and intestinal fluid results only]

available for absorption. This report measured bioaccessibility of nickel substances as a surrogate for bioavailability.	Evaluation of results: Data have been incorporated into read-across assessments for nickel and nickel-containing substances.		
Study type: Toxicokinetics, absorption and retention of nickel from drinking water or food intake **Study 1**: Healthy men without known nickel sensitization. Nickel dose in drinking water was 12 µg Ni/kg body weight. Six different time intervals between nickel-supplemented drinking water and the intake of food (e.g., eggs) were chosen to represent varying degrees of fasting and non-fasting conditions. The volunteers were tested for nickel sensitization after termination of the study using patch tests with nickel sulphate 5% in petrolatum. **Study 2**: Twenty nickel-sensitized and 20 non-nickel-sensitized women with current vesicular hand eczema were dosed with 12 µg Ni/kg body weight in drinking water under fasting conditions. Total nickel and nickel isotope measurements were made for each of the urine and blood samples.	**Study 1:** The range of median half-times of urinary excretion for six schedules was 19.92-26.65 h, with a range of individual means of 21.00-35.78 h. When the eggs were taken 4 h prior to nickel in drinking water, a cumulative median amount of 25.81% (25.00 +/- 11.02) of the administered dose was excreted, while 2.51% (2.95 +/- 1.32) was excreted when the nickel was mixed into the eggs. Range of median Ni serum clearance = 8.15 - 8.40 ml/min Range of median Ni creatinine clearance = 89.34 - 95.23 ml/min Range of median Ni half-times of urinary excretion = 19.92 - 26.65 hours **Study 2:** Serum nickel concentrations showed a peak 3 h after administration of nickel in water (fasting). Peak serum concentrations were 14.93 µg/liter (2.14-31.83) and 15.11 µg/liter (6.52-30.17) for nickel-sensitized and controls, respectively. The cumulated excretion in urine was 10.82% (1.79-29.46) for nickel-sensitized individuals and 11.26% (4.03-25.14) for controls.	Klimisch score: 2 (reliable with restrictions) supporting study experimental result **Test material (Common name): Nickel ion (stable isotope ^{61}Ni)**	Nielsen GD, Søderberg U, Jørgensen PJ, Templeton DM, Rasmussen SN Andersen KE, and Grandjean P. (1999)

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

Toxicokinetic Information

Information characterizing the toxicokinetics of nickel sulphamate was limited to in vitro studies evaluating the dissolution rates of nickel sulphamate in different synthetic fluids. Data on the bioaccessibility of Ni sulphamate in six biological fluids as a surrogate for bioavailability are reported within Section 7.1.1 of this IUCLID file (KMHC, 2010). The read across approach for oral exposure is described in Henderson et al. (2012a). Additional information regarding the toxicokinetic properties of Ni sulphamate are read across from

Ni sulphate as described below.

Oral

Endpoint Summary Information from the 2008/2009 European Union Risk Assessment for Nickel Sulphate:

> "A study on volunteers (Nielsen et al., 1999), in which the nickel compound administered was not specified, showed that 25.8% of the administered dose was excreted in the urine following administration of nickel in drinking water to fasting individuals compared with 2.5% when nickel was mixed into a meal. Based on experimental data from various human studies, Diamond et al. (1998) have used a biokinetic model to estimate nickel absorption; the results showed that estimated nickel absorption ranged from 12-27% of the dose when nickel was ingested after a fast, to 1-6% when nickel was administered either in food, in water, or in a capsule during (or in close proximity to) a meal."

For the purpose of risk characterization performed as part of the Registration, Evaluation, and Authorisation of Chemicals (REACH) Registration for nickel sulphamate, as described in the 2008/2009 European Union Risk Assessment for Nickel Sulphate, a value of 30% was taken forward for the risk characterisation as the absorbed fraction of nickel from the gastrointestinal tract following oral exposure to nickel ion under fasting conditions. This same value was used for Ni sulphamate based on similar nickel ion release as described in the read across approach for oral exposure in Henderson et al. (2012a). For absorption of nickel from food, soil, dust and from water consumed with food, a value of 5% was used. When extrapolating rat exposures from the oral route to the inhalation route, a value of 11% was used for absorption by the oral route (Ishimatsu et al., 1995) and 100% for the inhalation route (respirable fraction, 100% deposition).

Inhalation

Endpoint Summary Information from the 2008/2009 European Union Risk Assessment for Nickel Sulphate:

> "The deposition of particles in the respiratory tract depends on the particle sizes (MMADs) as well as on other characteristics of the particles, and the absorption of nickel from the respiratory tract into the
> blood stream depends on the solubility of the nickel compound inhaled. Soluble nickel compounds, such as nickel nitrate, are expected to be absorbed from the respiratory tract following inhalation exposure.
>
> One study of nickel sulphate in rats (Medinsky et al. 1987) using intratracheal instillation of nickel sulphate (as a solution in saline) showed that 50 to 80% of a dose (dependent on the dose) of nickel sulphate can be absorbed from the respiratory tract. Studies in rats using intratracheal instillation of nickel chloride (Carvalho & Ziemer 1982, English et al. 1981, Clary 1975) showed that up to approximately 97% of a dose of nickel chloride can be absorbed from the respiratory tract. By assuming that the absorption of nickel following inhalation exposure to nickel chloride is similar to absorption following intratracheal instillation, the absorption of nickel from the respiratory tract following inhalation of nickel chloride might be as high as about 97%. Furthermore, an inhalation study on nickel sulphate (Benson et al. 1995) showed that clearance of nickel sulphate from the lungs of rats and mice is extensive (up to 99% in rats and 80 to 90% in mice). By assuming that the clearance of nickel sulphate particles (respirable particles, MMADs ranging from 2.0 to 2.4 μm) from the lungs in the inhalation study is due to absorption rather than to deposition or by mucociliary action, the absorption of nickel from the lungs following inhalation of nickel sulphate might be as high as up to 99% (at concentrations up to 0.11 mg Ni/m^3 in rats and up to 0.22 mg Ni/m^3 in mice).
>
> In conclusion, the available data on nickel sulphate and nickel chloride indicate that the absorption of nickel following inhalation of these nickel compounds might be as high as up to 97-99%; it should be noted that the fraction absorbed apparently depends on the concentration of the nickel compound

> in the inhaled air as well as on the duration of exposure. For the purpose of risk characterisation, a value of 100% will be taken forward to the risk characterisation for the absorbed fraction of nickel from the respiratory tract following exposure by inhalation of nickel nitrate for particulates with an aerodynamic diameter below 5 μm (respirable fraction). For nickel particulates with aerodynamic diameters above 5 μm (non -respirable fraction), the absorption of nickel from the respiratory tract is considered to be negligible as these particles predominantly will be cleared from the respiratory tract by mucociliary action and translocated into the gastrointestinal tract and absorbed. Hence, for the non-respirable fraction, 100% clearance from the respiratory tract by mucociliary action and translocation into the gastrointestinal tract is assumed and the oral absorption figures can be taken."

For the purpose of risk characterization performed as part of the REACH Registration for nickel sulphamate, as described in the 2008/2009 European Union Risk Assessment for Nickel Sulphate, a value of 100% was taken forward as the absorbed fraction of nickel from the respiratory tract following inhalation exposure to nickel sulphamate (respirable size, 100% deposition) in rats.

8. EVALUATION OF HEALTH HAZARDS

Acute toxicity

8.1 Acute toxicity - oral route

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

Method, guideline, deviation(s) if any	Species, strain, sex	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD Guideline 425 (Acute Oral Toxicity: Up-and-Down Procedure) GLP-compliant	rat (Sprague-Dawley derived, albino) female	**Test material: nickel sulphamate tetrahydrate**	Single oral dose via gavage, following up-down procedure for a total of 7 animals Doses included: 175, 550, and 2000 mg/kg	LD_{50}: 1098 mg/kg bw (female) based on: test mat. (95% Profile-likelihood based confidence interval of 550-2000 mg/kg bw)	EPSL (2008) Henderson RG, Durando J, Oller A, Merkel DJ, Marone PA, Bates HK (2012b)

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

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

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

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

8.1.1. Short summary and overall relevance of the provided information on acute oral

Two studies were available to characterize the acute toxicity of nickel sulphamate, both studies reported on lethality following oral exposure. However, only the information provided by EPSL (2008) was of high quality and reliability; the full study report was not available for the other studies and thus the data was difficult to interpret (NRC 1952, NIOSH 2008).

EPSL (2008) conducted an OECD guideline-based acute toxicity up and down procedure in female rats in an effort to estimate the oral LD50. To do so, nickel sulphamate crystals (nickel sulphamate tetrahydrate) were administered via oral gavage as a 60% w/w mixture in distilled water to seven female animals at doses of

175, 550, 2000, 550, 2000, 550, or 2000 mg/kg (administered in this sequence according to the guideline). Animals exposed to 175 or 550 mg/kg of the test substance survived, gained weight, and generally did not demonstrate any signs of gross toxicity, adverse effects, or abnormal behavior throughout the 14-day observation period. All three animals exposed to 2000 mg/kg died within three hours. The oral LD50 was calculated using the maximum likelihood method and was estimated to be 1098 mg/kg body weight in female rats (95% CI of 550 mg/kg to 2000 mg/kg).

8.1.2. Comparison with the CLP criteria

Nickel sulphamate has not been previously classified for acute toxicity, presumably due to a lack of substance-specific data on the compound. However, a newly conducted GLP OECD guideline-compliant study reported an LD_{50} of 1098 mg/kg for nickel sulphamate tetrahydrate in female rats (EPSL, 2008; Henderson et al., 2012b). The new LD_{50} value (1098 mg/kg) now places the toxicity of this compound in the same hazard category as other water-soluble nickel compounds (LD_{50} values ranging from 362 to 2000 mg/kg; Henderson et al., 2012b), which are classified as Acute Tox. 4; H302 and Xn; R22[1]. According to the CLP Regulation, the classification criteria for acute oral toxicity divide substances into four categories based on their LD_{50} values (mg/kg bw): Cat. 1 (<5), Cat. 2 (5–50), Cat. 3 (50–300), and Cat. 4 (300–2000). Substances with toxicity values above 2000 mg/kg do not receive a classification for acute oral toxicity. These results are consistent with the similar solubility (i.e., bioaccessibility of Ni ion) of these compounds observed in gastric and intestinal fluids (Henderson et al., 2012a). In 2012, Henderson et al. published a study using *in vitro* nickel ion bioaccessibility (defined as the amount of a substance available for absorption (Stopford *et al.*, 2003)), as a measure of bioavailability, to read-across toxicity information from data-rich, source substances to data-poor, target substances. The data generated in this study demonstrate that nickel sulphamate has similar Ni ion release compared to other soluble nickel compounds tested (e.g., nickel sulphate and nickel acetate) in synthetic gastric and intestinal fluids. Together, these new data indicate that nickel sulphamate should be classified as Acute Tox. 4; H302 and Xn: R22/2008).

8.1.3. Conclusion on classification and labelling for acute oral toxicity

Nickel sulphamate has not previously been classified for acute oral toxicity in the EU. However, a recently completed OECD-guideline compliant study reported an oral LD_{50} =1098 mg/kg nickel sulphamate in female rats. The newly reported oral LD_{50} value of 1098 mg/kg for nickel sulphamate meets the criteria for classification as Acute Tox. 4; H302 according to the CLP guidelines, which specifies that substances with an LD_{50} value between 300 and ≤2000 mg/kg fall within this category (EC No. 1272/2008). The joint REACH submission for nickel sulphamate reflects self-classification as Acute Tox. 4; H302.

[1] Nickel chloride is currently more stringently classified for acute oral toxicity; however, a separate CLH dossier is being concurrently submitted supporting classification as Acute Tox. 4; H302 and Xn; R22.

8.2 Acute toxicity - inhalation route

Table 13: 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): 7786-81-4 Ni Sulphate hexahydrate (READ-ACROSS)** Powder MMAD: 2.4-3.0 µm	0.063, 0.53, 2.12, 5.08 mg/L 4 hours	LC_{50} (4 h): 2.48 mg $NiSO_4$/L air (male/female)	EPSL (2009b)

Table 14: 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 15 :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 sulphamate are read-across from nickel sulphate. 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 sulphamate should be read-across from nickel sulphate for acute inhalation toxicity. Nickel sulphate hexahydrate has been shown to have an acute inhalation LC_{50} of 2.48 mg nickel sulphate/L. Therefore, application of this read-across paradigm suggests that nickel sulphamate should also be classified as Acute Tox 4; H332.

8.2.2. Comparison with the CLP criteria

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 of this dossier (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 sulphamate should be read-across from nickel sulphate for acute inhalation toxicity, based dissolution in interstitial fluid in the range of 5-15% Ni/sample (Section 9: Other Information). Nickel sulphate hexahydrate has been shown to have an acute inhalation LC_{50} of 2.48 mg nickel sulphate/L . Therefore, application of this read-across paradigm suggests that nickel sulphamate should also be classified 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).

8.2.3. Conclusion on classification and labelling for acute inhalation toxicity

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 sulphamate should be read-across from nickel sulphate for acute inhalation toxicity. Therefore, application of this read-across paradigm suggests that nickel sulphamate should be classified as Acute Tox 4.; H332. The joint REACH submission for nickel sulphamate reflects self-classification as Acute Tox. 4; H322.

9. OTHER INFORMATION

9.1 Justification for read-across (acute inhalation toxicity)

9.1.1. 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)".

This basic premise of science is used in the regulatory setting to reduce the need for animal testing. The most detailed embodiment of the concept of read-across has occurred in the production of Qualitative Structure Activity Relationship (QSAR) models, developed for organic compounds and based on the presence of active groups within the organic molecule that are capable of eliciting certain biological effects. 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.

The primary nickel industry, which has formed the Nickel Consortia, has prepared toxicology data dossiers for nickel compounds under the European Union Registration, Evaluation, and Authorisation of Chemicals (EU REACH) legislation. While harmonized classifications exist in Annex VI of the CLP Regulation for all the nickel compounds registered by the Consortia, the Chemical Safety Reports (CSRs) developed for REACH provide existing data and scientifically appropriate read-across results (including verification testing) following the OECD 2004 Manual for Investigation of HPV Chemicals; Chapter 3: Data Evaluation - Guidance on the Development and Use of Chemical Categories in the HPV Chemicals Programme. Assessments for read-across were made based on bioaccessibility data as a surrogate for bioavailability of nickel and nickel compounds. These data are used within the registration dossier to fulfill data requirements, calculate adjusted DNELs and confirm the validity of previous classifications based on water solubility read-across.

9.1.2. 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. The most simplistic approach to hazard evaluation is to assume that the specific metal-containing compound to be evaluated shows the same hazards as the most water-soluble compounds. This is a conservative approach, since systemic metal ion bioavailability will normally be reduced with decreasing water-solubility and consequently reduced bioavailability (ECHA, 2008). In addition, this approach does not take into account the different chemistries encountered in the three major routes of exposure (i.e., oral, inhalation, and dermal), which will affect different chemical species of a metal in different ways.

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. Water solubility, phagocytosis, bioaccessibility in synthetic biological fluids, and organ deposition and clearance rates are relevant parameters to be considered, depending on the route of exposure (Schoeters and Verougstraete, 2007).

9.1.3. A read-across strategy for metals using bioaccessibility

For metal substances, *in vitro* bioaccessibility data for the metal ion as a surrogate for *in vivo* toxicokinetic data or toxicological data on the source and target substances can be compared and utilized to perform read-across assessments when it can reasonably be assumed that the anion or "counter-ion" does not contribute to observed toxicity. This can be done for classification purposes or for the derivation of Derived No Effect Levels (DNELs). Analyses and read-across can 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. Within an organism the concentration of nickel ion in systemic circulation (for systemic endpoints) or at the cellular target sites (for both local and systemic effects) will be the determining factor in whether or not toxicity will occur.

The source compounds will form a baseline from which to extrapolate the existing data to target substances based upon their bioaccessibility via different routes of exposure. The bioaccessibility-based read-across strategy incorporates the following steps:

Step 1: For the source substances and for the target substances, assess the metal ion release data in the appropriate fluid related to the route of exposure in question for each toxicological endpoint. This enables information about these target substances to be compared to equivalent data from the source substances. At this stage, a preliminary grouping can be done based on these metal ion release data.

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: Assess the most appropriate grouping of substances and 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. Additionally, for endpoints such as carcinogenicity and mutagenicity, bioaccessibility data alone will typically not be sufficient for assessing read-across potential. For these endpoints a more comprehensive assessment that integrates bioaccessibility, physicochemical characteristics, knowledge of the mode of action, *etc*. should be performed.

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. Finally, in the case of water insoluble metal particles, the release amounts or rates adjusted by sample surface area may be of interest. The metrics chosen in each case should be based on the endpoint of concern, the route of exposure, and the units of the *in vivo* parameter that is used for its corresponding verification (e.g., an LD_{50} value expressed as mg metal/kg bw or mg compound/kg bw). 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.

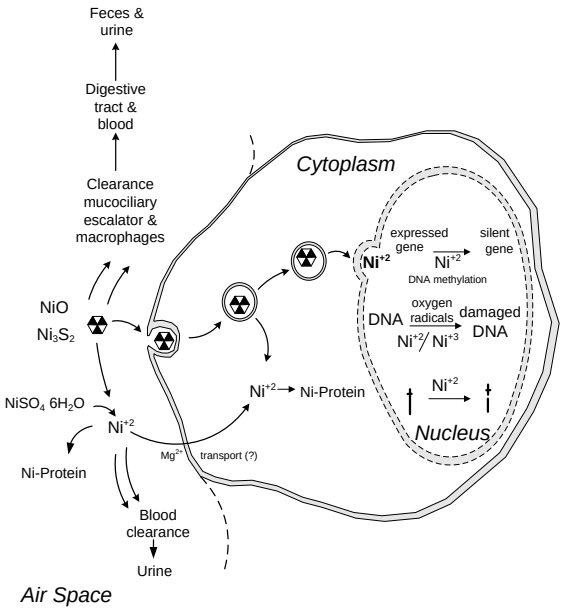
9.1.4. Inhalation toxicity read-across assessment of nickel substances

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. The various factors involved in determining bioavailability of Ni substances in lung epithelial cells have been extensively reviewed and reported in the peer-reviewed literature (Oller et al., 1997; Goodman et al., 2009). 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 Ni substances, 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, sulfidic, or oxidic). Figure 1 illustrates the differing interactions of various forms of nickel with the lung epithelial cell. Highly water-soluble Ni compounds (*e.g.*, Ni sulphate) undergo extensive dissociation in the extracellular fluid. Hence, these compounds are not typically endocytized as particles. The dissociated nickel ions will bind to any available proteins in the extracellular fluid or to proteins in cellular membranes facilitating the inflammatory process seen with inhalation exposure to water soluble nickel compounds. Nickel ions are also transported into the cell via ion channels (*e.g.*, magnesium or calcium channels). Once internalized, free nickel ions rapidly bind to proteins in the cytoplasm. This binding has the potential to disrupt cellular homeostasis which could also exacerbate tissue inflammation. However, in the case of the carcinogenic process, initiation of cancer would require the Ni ions to penetrate into the cell nucleus where the process of tumor formation will be initiated (Costa et al., 1981b). Consideration of the biological interaction of nickel ions released in the extracellular space by dissociation of water soluble nickel salts demonstrates that endocytosis of these salts and subsequent dissolution of the salts in cellular lysosomes does not play an important role in the uptake of nickel ions by lung epithelial cells. Rather, free Ni (II) ions enter lung cells using divalent ion channels. Consequently, evaluation of the bioaccessibility of water soluble nickel salts in lysosomal fluid is irrelevant to the read-across of these water soluble compounds for respiratory non-cancer inflammatory effects.

For water-insoluble Ni compounds *(e.g.,* sulfidic and oxidic), 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 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 considered to be water-insoluble, they have been shown to be partially soluble in some biological fluids (Oller et al., 2009). On the other hand, Ni oxide has been shown not to be solubilized to a significant degree in extracellular fluids. Although Ni oxide particles can be taken up via endocytosis into the cell, this mechanism has been shown to be less efficient than for Ni subsulphide (Sunderman et al., 1987). This is thought to be a contributing factor for the low toxicity observed of Ni oxide compared to other Ni compounds in repeated-dose (and even perhaps in acute) studies. Based on these properties, evaluation of the available bioaccessibility data for various nickel compounds in synthetic intra- and extracellular lung fluids was undertaken in order to perform a read-across hazard assessment for inhalation endpoints.

Figure 1. Interactions of Nickel Compounds with Target (Epithelial) Cells in the Bronchioalveolar Region of the Lungs



9.1.5. Inhalation bioaccessibility data

Bioaccessibility data relevant for the inhalation route of exposure can provide important information regarding the potential bioavailability and subsequent toxicity for inhalation endpoints such as acute and chronic inhalation toxicity (Step 1 of the read-across strategy). To investigate bioavailability via the inhalation route of exposure, source and target nickel substances were subjected to bioaccessibility testing (KMHC, 2010). Samples of nickel compounds tested were provided by the lead registrant company for each substance and they are considered to be representative of the marketed nickel compounds. A brief description of the testing protocol and fluid compositions are provided in **Annex 1**. Ten nickel-containing samples were extracted in three synthetic biological fluids: interstitial, alveolar, or lysosomal, for 2, 5, 24 and 72 hours. Data are reported in Table 16 as the percent of nickel released per gram of sample for extractions performed at 24 or 72 hours in interstitial and lysosomal fluid. 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 16, as results were similar to those obtained from interstitial fluid.

Based on the bioaccessibility results in interstitial lung fluid (with the exception of Ni hydroxycarbonate) a preliminary distinction of 3 main groups could be made: substances releasing > 5% Ni/sample, substances releasing 1-5% Ni/sample, and substances releasing < 1% Ni/sample. Based on the lysosomal results, only one sample (green Ni oxide) has a Ni release different from the others; while green Ni oxide releases < 1% Ni/sample, the rest of the samples release > 10% Ni/sample.

Table 16: 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]	Lysosomal Bioaccessibility (% Ni/sample)[d]	Acute Toxicity inhalation (LC_{50}; mg substance/L)[e]
				24hs - 72 hs	24hs - 72 hs	
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
Other Nickel Compounds						
Ni Hydroxy Carbonate	N128 (N109)	12122-15-5	49 (49)	0.52 - 1.65	47.20 - 47.20[f]	>2.09 (F); 0.24 (M)[g]

a. Source nickel compounds are identified with blue 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 with the exception of Ni hydroxycarbonate (see Footnote 7). The LC_{50} values are inversely related to toxicity. The LC_{50} values in parenthesis are expressed as mg Ni/L. NC, not completed.
f. Value reported after only 2 or 5 hours as samples were determined to be 100% solubilized by these time points.
g. Data from this study suggest that a gender-specific counter-ion effect may be causing increased toxicity in males for Ni hydroxycarbonate. A potential counter-ion effect was also noted in an acute oral toxicity study with this same sample. As noted above, this presence of a counter-ion effect excludes Ni hydroxycarbonate from the read-across paradigm. F, female; M, male.

9.1.6. 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 2-10- and >1000-fold lower (< 0.1%) than that of Ni sulphate, respectively (Benson et al., 1995). This is consistent with a retention $T_{1/2}$ 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 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). While data on inhalation absorption can be relevant for acute toxicity effects, these data may be less relevant for chronic toxicity respiratory effects that are local in nature and independent of systemic absorption. For chronic effects, an assessment of bioavailability at critical sites would be more informative.

9.1.7. In vivo verification: acute toxicity studies

In order to verify that the bioaccessibility data provide reasonable estimations of bioavailability and hence toxicity (as per Step 2 of the read-across strategy), acute inhalation toxicity studies in rats were carried out on three source nickel compounds (nickel sulphate hexahydrate, nickel subsulphide, and green nickel oxide) as well as on black nickel oxide[2] (EPSL 2009a-b;2010b-c). The results of these studies are summarized in Table 18. Each animal study was scored for reliability and reported in Section 7 of the substance-specific IUCLID dossiers and submitted as part of the relevant compound REACH 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. Figure 2 shows the correlation between the LC_{50} (mg Ni/L after a 4 hour exposure) and the bioaccessibility in interstitial or lysosomal lung fluid after 24 hours (% Ni release/g sample) for the four samples mentioned above.

A better regression (ln) fit was found between acute LC_{50} (mg Ni/liter) and Ni ion release in interstitial fluid than between acute LC_{50} and release in lysosomal fluid (R^2 = 0.8756 and 0.541, respectively). Using release values for 5 hours and 72 hours gave similar results. Using LC_{50} values expressed in terms of mg compound/liter demonstrated less of a correlation. It should be noted that for the nickel oxide samples the exact LC_{50} values are not known, but they are predicted to be > 5.08 mg/L (equivalent to > 4 mg Ni/L) based on the limit dose tested in the studies. Using higher LC_{50} values for Ni oxide would improve the regression coefficients.

As discussed earlier, bioaccessibility in interstitial fluid suggests that three groups of nickel substances can be identified. Using data from *in* vivo acute toxicity studies as verification, the interstitial release and acute toxicity data from Figure 2 do not contradict the presence of three main groups of substances but it mainly allows distinction between the nickel oxides that have low interstitial bioaccessibility (< 1%) and low acute toxicity (with LC_{50} values >5 mg substance/L), and the water soluble compounds (Ni sulphate) and water insoluble sulphidic compounds (Ni subsulphide) with interstitial bioaccessibility of 5-15% and 1-5% Ni/g sample, respectively, and LC_{50} values <5 mg substance/L. Based on these groupings, similar classifications (Acute Tox 4; H332) are warranted for Ni sulphate and Ni subsulphide.

For those Ni compounds for which no acute toxicity studies exist (i.e., target compounds), the bioaccessibility data in interstitial fluid (Table 18) can be used to group these substances and select the best source compound to read-across from for acute inhalation toxicity. Ni chloride hexahydrate, Ni acetate tetrahydrate and Ni sulphamate tetrahydrate, all released between 7 and 15% of Ni/g sample. 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% and therefore this compound can be read-across from Ni subsulphide. By contrast, the interstitial release for Ni dihydroxide is <<1% (% Ni/sample) and therefore this compound can be read-across from Ni oxides for acute toxicity. Figure 3 displays the 72 hours release values (% Ni/sample) in interstitial, alveolar and lysosomal fluids, and graphically demonstrates the grouping and read-across application for acute inhalation toxicity discussed here.

[2] Testing with Ni hydroxycarbonate was also conducted at the request of the Ni Consortia to ensure data were relevant for the representative sample (EPSL, 2010a). As mentioned in Table 16 and this sample is not part of the read-across strategy.

Figure 2. Correlations between nickel release in interstitial or lysosomal lung fluid and inhalation LC_{50}

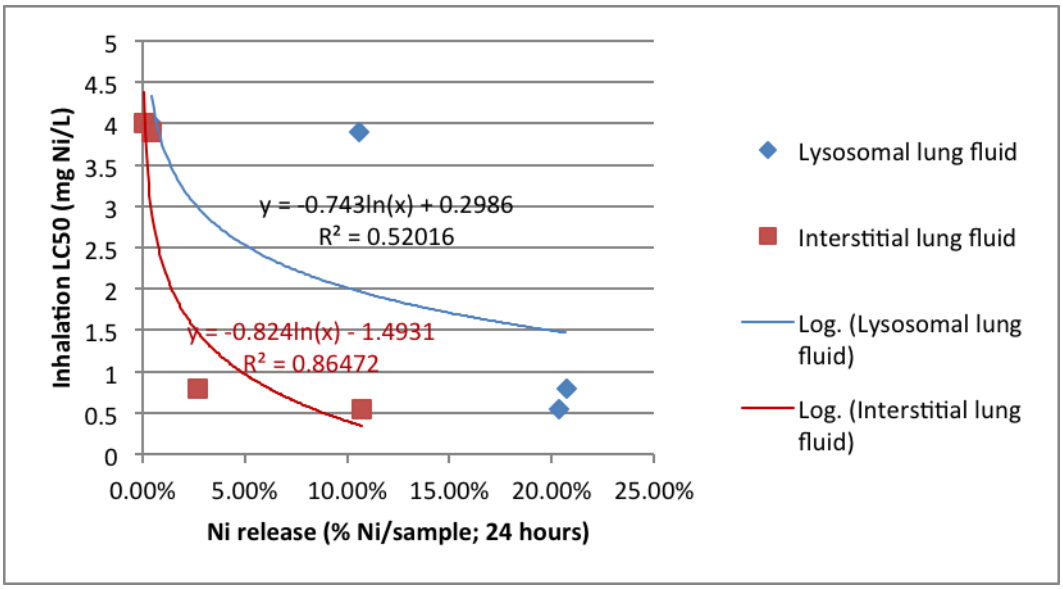
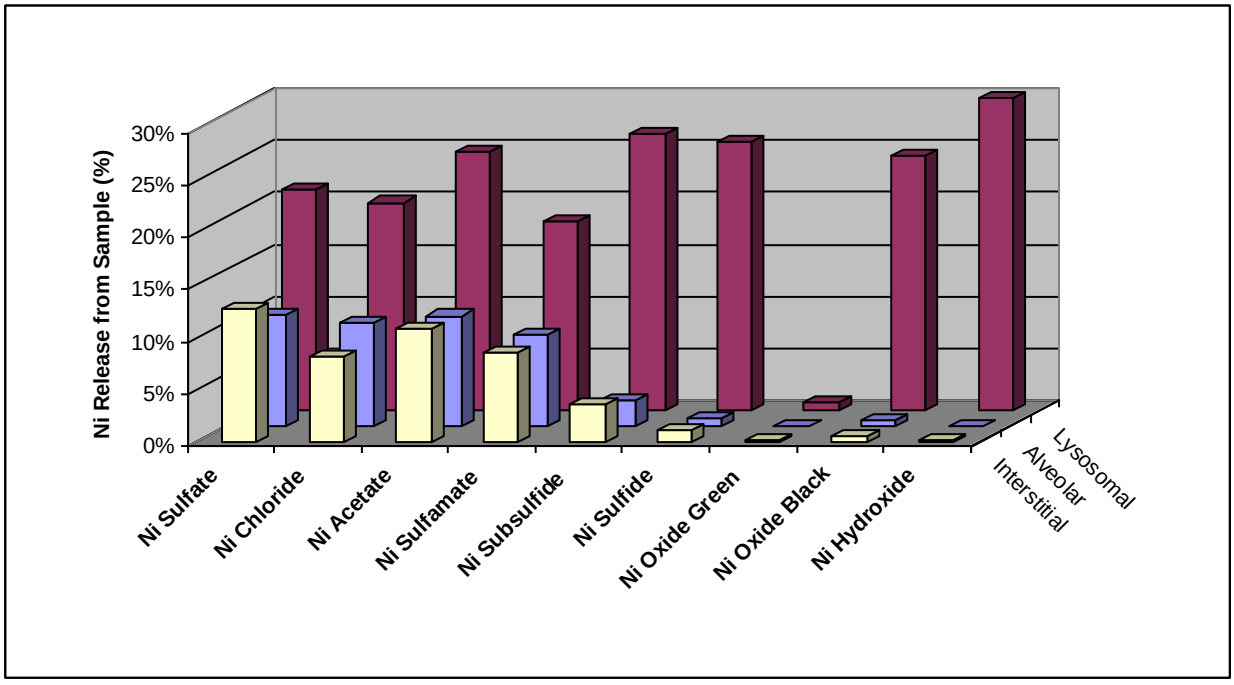


Figure 3. Bioaccessibility data in three synthetic lung fluids support proposed grouping for classification based on read-across from source nickel substance[3]



[3] Interstitial and lysosomal bioaccessibility values are available in Table 18.

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

The bioaccessibility-based paradigm presented in Figure 3 enables grouping of target nickel substances for classification purposes according to bioaccessibility in interstitial and/or lysosomal fluid. The data currently available allow three groups of nickel substances to be identified for read-across with regards to classification for acute 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. It should be noted that in all instances, actual acute inhalation toxicity data are used for classification where available.

Water-Soluble Nickel Compounds

The first group of nickel substances are those that would 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. This group applies to highly water-soluble Ni compounds with dissolution in interstitial fluid in the range of 5-15% Ni/sample. The data in Table 18 demonstrate that all soluble substances tested have similar bioaccessibility (at 24 and 72 hours) in interstitial fluid to the source substance, Ni sulphate. Therefore, this read-across assessment concludes that Ni chloride, Ni acetate, and Ni sulphamate should be read across from Ni sulphate for acute inhalation effects. As Ni sulphamate is not currently classified for this endpoint in Annex VI, a self-classification has been included in the REACH registration dossier.

Sulfidic Nickel Compounds

The second group of Ni substances are those that would be read-across from Ni subsulphide and 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 1-5% Ni/sample and so read-across from Ni subsulphide is warranted. Data on Ni sulphide indicate that it should be read-across from the source substance, Ni subsulphide, for acute effects.

Oxidic Nickel Compounds

The final 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/sample released after 72 hours in interstitial fluid). Oxidic nickel compounds that release less than 1% Ni per gram 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% would be read-across from Ni sulphate or Ni subsulphide, warranting classification as Xn;R20 and Acute Tox 4:H332. 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 (LC_{50} >5mg/L). Ni dihydroxide is currently classified as Xn;R20 and Acute Tox 4:H332 in Annex VI of the CLP Regulation. However, this classification seems inappropriate as the bioaccessibility-based read-across suggests that no classification for acute inhalation toxicity is warranted for Ni dihydroxide. By contrast, Ni hydroxycarbonate, which can be considered as an oxidic Ni compound demonstrated bioaccessibility in interstitial lung fluid that was > 1% and similar to that of Ni sulphide. This would indicate that a classification as Xn;R20 and Acute Tox 4;H332 would be justified for this compound.

9.1.9. 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. Some potential sources of uncertainty 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 based on three compounds.
- Robust data on acute inhalation exposure for four Ni compounds permits further verification of the bioaccessibility data suggesting that grouping based on interstitial (or aveoloar) fluid Ni (II) release is warranted.
- The bioaccessibility-based read-across approach does not address the potential toxicity of any counter-ion that may be present.

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.

9.1.10. Conclusions regarding classification of nickel sulphamate for acute inhalation toxicity

Data on the acute inhalation toxicity of nickel sulphamate are read-across from nickel sulphate. 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 sulphamate should be read-across from nickel sulphate for acute inhalation toxicity. Nickel sulphate hexahydrate has been shown to have an acute inhalation LC_{50} of 2.48 mg nickel sulphate/L. Therefore, application of this read-across paradigm suggests that nickel sulphamate should also be classified as Acute Tox. 4; H332.

10. DETAILED STUDY SUMMARIES

10.1 TOXICOKINETICS

STUDY 1

Study reference:

Ishimatsu S, Kawamoto T, Matsuno K, and Kodama Y. (1995): Distribution of various nickel compounds in rat organs after oral administration. Biological Trace Element Research; 49(1):43–52.

Detailed study summary and results (*from Authors' abstract as included in the registration dossier (IUCLID*)):

In this study, eight kinds of nickel (Ni) compounds were orally administered to Wistar male rats and the distribution of each compound was investigated 24 h after the administration. The Ni compounds used in this experiment were nickel metal [Ni-M], nickel oxide (green) [NiO(G)], nickel oxide (black) [NiO(B)], nickel subsulfide [Ni_3S_2], nickel sulfide [NiS], nickel sulfate [$NiS0_4$], nickel chloride [$NiCl_2$], and nickel nitrate [$Ni(N0_3)_2$]. The solubilities of the nickel compounds in saline solution were in the following order; [$Ni(N0_3)2$ > $NiCl_2$ > $NiS0_4$]>>[NiS > Ni_3S_2] > [NiO(B) > Ni-M >NiO(G)]. The Ni level in the visceral organs was higher in the rats given soluble Ni compounds; $Ni(N0_3)_2$, $NiCl_2$, $NiS0_4$, than that in the rats receiving other compounds. In the rats to which soluble

Ni compounds were administered, 80-90% of the recovered Ni amounts in the examined organs was detected in the kidneys. On the other hand, the Ni concentration in organs administered scarcely soluble Ni compounds; NiO(B), NiO(G), and Ni-M were very low. The estimated absorbed fraction of each Ni compounds was increased with the increase of the solubility. These results suggest that the kinetic behavior of Ni compounds administered orally is closely related with the solubility of Ni compounds, and that the solubility of Ni compounds is one of the important factors for determining the health effect of Ni compounds.

STUDY 2

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 Ni sulphamate is included in Section 9: Other Information.

STUDY 3

Study reference:

Nielsen GD, Søderberg U, Jørgensen PJ, Templeton DM, Rasmussen SN, Andersen KE, and Grandjean P. (1999). Absorption and retention of nickel from drinking water in relation to food intake and nickel sensitivity. Toxicology and Applied Pharmacology; 154:67–75.

Detailed study summary and results (*from Authors' abstract as included from registration dossier (IUCLID*)):

Two studies were performed to examine the influence of fasting and food intake on the absorption and retention of nickel added to drinking water and to determine, if nickel sensitization played any role in this regard. First, eight nonallergic male volunteers fasted overnight before being given nickel in drinking water (12 µg Ni/kg) and, at different time intervals, standardized 1400-kJ portions of scrambled eggs. When nickel was ingested in water 30 min or 1 h prior to the meal, peak nickel concentrations in serum occurred 1 h after the water intake, and the peak was 13-fold higher than the one seen 1 h after simultaneous intake of nickel containing water and scrambled eggs. In the latter case, a smaller, delayed peak occurred 3 h after the meal. Median urinary nickel excretion half-times varied between 19.9 and 26.7 h. Within 3 days, the amount of nickel excreted corresponded to 2.5% of the nickel ingested when it was mixed into the scrambled eggs. Increasing amounts were excreted as the interval between the water and the meal increased, with 25.8% of the administered dose being excreted when the eggs were served 4 h prior to the nickel containing drinking water. In the second experiment, a stable nickel isotope, ^{61}Ni, was given in drinking water to 20 nickel sensitized women and 20 age-matched controls, both groups having vesicular hand eczema of the pompholyx type. Nine of 20 nickel allergic eczema patients experienced aggravation of hand eczema after nickel administration, and three also developed a maculopapular exanthema. No exacerbation was seen in the control group. The course of nickel absorption and excretion in the allergic groups did not differ and was similar to the pattern seen in the first study, although the absorption in the women was less. A sex-related difference in gastric emptying rates may play a role. Thus, food intake and gastric emptying are of substantial significance for the bioavailability of nickel from aqueous solutions.

10.2 HEALTH HAZARDS

10.2.1. Acute oral toxicity - animal data

STUDY 1

Study reference:

Eurofins Product Safety Labs (EPSL; 2008). Acute oral toxicity study in rats, Eurofins PSL Study #25626, Ni sulphamate.

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

Test type

• OECD Guideline 425 (Acute Oral Toxicity: Up-and-Down Procedure)

Test substance

• Test material used in the study is equivalent to the substance identified in the CLH dossier.
• EC number: 237-396-1
• CAS number: 124594-15-6

• Degree of purity: 100%
• Impurities: none
• Batch number: not applicable
• Physicochemical properties that may be important when assessing acute oral toxicity: crystals

Test animals

• Species/strain/sex: Sprague-Dawley, female
• No. of animals per sex per dose: 175 mg/kg: 1 female; 550 mg/kg: 3 females; 2000 mg/kg: 3 females
• Age and weight at the study initiation: 9-12 weeks

Administration/exposure

• Mode of administration: oral gavage
• Duration of test/exposure period: single administration
• Doses/concentration levels, rationale for dose level selection: 175, 550, 2000 mg/kg. A Main Test was conducted using a default starting dose level of 175 mg/kg; remaining doses were determined using the Up and Down procedure (a dose of 550 or 2000 mg/kg was administered to the remaining animals). The decision to proceed with the next animal was based on the survival of the previous animal following dosing.
• Post exposure observation period: 14 days
• Control group and treatment: not applicable
• Vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water): water
• Statistical methods: The Acute Oral Toxicity (Guideline 425) Statistical Program (Weststat, version 1.0, May 2001) was used for all data analyses including: dose progression selections, stopping criteria determinations, and/or LD50 and confidence limit calculations.

Results and reliability (from registration dossier (IUCLID)):

• Mortality:

Dosing Sequence	Animal No.	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
1	3101	175	S	S
2	3102	550	S	S
3	3103	2000	D	D
4	3104	550	S	S
5	3105	2000	D	D
6	3106	550	S	S
7	3107	2000	D	D

S= Survived
D = Death

• LD_{50}: 1098 mg/kg of body weight in female rats with an approximate 95% confidence interval of 550 mg/kg (lower) to 2000 mg/kg (upper)

• Number of deaths at each dose level: 175 mg/kg (1 animal): survived; 500 mg/kg (3 animals): survived; 2000 mg/kg (3 animals): died

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

• Clinical signs:
175 mg/kg (1 animal): appeared active and healthy during the study

500 mg/kg (3 animals): apart from one female exhibiting reduced fecal volume on Day 1, there were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior

2000 mg/kg (3 animals): prior to death, animals were hypoactive and/or exhibited hunched posture and piloerection

•Necropsy finding:
175 mg/kg (1 animal): no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior

500 mg/kg (3 animals): no gross abnormalities were noted for any of the animals when necropsied at the conclusion of the observation period

2000 mg/kg (3 animals): red intestines were observed at necropsy

10.2.2. Acute inhalation toxicity - animal data

STUDY 1

Study reference:

Eurofins Product Safety Labs (EPSL; 2009). Acute inhalation toxicity study in rats, Eurofins PSL Study #27562, Ni sulphate hexahydrate.

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: 232-104-9
• CAS number: 10101-97-0
• Degree of purity: information not available in study report
• Impurities: information not available in study report
• Batch number: not applicable
• Physicochemical properties that may be important when assessing acute oral toxicity: crystalline solid
• Physical form (gas, vapour, dust, mist): aerosol
• Particle size: MMAD = 2.4-3.0 μm
• 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: 8-10 weeks

Administration/exposure
• Type of inhalation exposure and test conditions: nose only inhalation chamber
• Duration of test/exposure period: 4 hours
• Doses/concentration levels: 0.063, 0.53, 2.12, 5.0 mg nickel sulphate/L. Prior to initiation of the full inhalation study, pre-test trials were conducted to establish generation procedures to achieve, to the extent possible, the targeted chamber concentration and desired particle size distribution (mass median aerodynamic diameter between 1 and 4 um).

• Analytical verification of test atmosphere concentrations: Gravimetric samples were withdrawn at six intervals from the breathing zone of the animals during each exposure. Samples were collected using 25 mm glass fiber filters (GFIB 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 dividedby the total volume of air sampled to determine the chamber concentration. Sample airflows were measured using a Mass Flowmeter (Omega, Model #FMA-561 0).
• 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: Biostat 2007 Professional Build 3.6.0.0, AnalystSoft, BioStat - statistical analysis program, Version 2007 was used for data analysis of LC50 and confidence limit calculations.

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

• Incidence of mortality

Exposure Levels (mg/L)	Males	Females	Total
0.063	0/5	0/5	0/10
0.53	0/5	0/5	0/10
2.12	1/5	0/5	1/10
5.08	5/5	5/5	10/10

• The acute inhalation defined LC_{50} of the test substance is 2.48 mg/L in male and female rats with 95% confidence intervals of 1.3 mg/L (lower) to 4.5 mg/L (upper)

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

- Clinical signs:
  0.063 mg/L: Immediately following exposure and throughout the 14-day observation period all animals appeared active and healthy.

  0.53 mg/L: Immediately following exposure and throughout the 14-day observation period all animals appeared active and healthy.

  2.12 mg/L: Immediately following exposure to the test atmosphere, one male appeared hypoactive and exhibited abnormal respiration. This male wasfound dead one day after exposure. All surviving animals appeared active and healthy immediately following exposure and throughout the 14-day observation period.

  5.08 mg/L: Following exposure to the test atmosphere, the surviving rats exhibited clinical signs including abnormal respiration, hypoactivity, abnormal posture and/or ano-genital staining.

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

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

  2.12 mg/L: Gross necropsy of the decedent revealed discoloration of the lungs and liver, and rigor mortis. No gross abnormalities were noted for any of the euthanized animals necropsied at the conclusion of the 14-day observation period.

  5.08 mg/L: Gross necropsy of most decedents revealed discoloration of the lungs, liver and/or intestines, distention of the stomach and/or intestines, and/or rigor mortis. For one male and one female decedent the thymus appeared gray with dark spots.

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12. ANNEX 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 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 µrn 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