Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

nickel (II) sulfide; [1]
nickel sulfide; [2]
millerite [3]

EC Number: 240-841-2 [1] 234-349-7 [2] -[3]
CAS Number: 16812-54-7 [1] 11113-75-0 [2]
1314-04-1 [3]

CLH-O-0000001412-86-170/F

Adopted
22 September 2017

DD Month YYY

CLH-O-0000001412-86-170/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **nickel (II) sulfide; [1] nickel sulfide; [2] millerite [3]**

EC Number: **240-841-2 [1] 234-349-7 [2] -[3]**

CAS Number: **16812-54-7 [1] 11113-75-0 [2] 1314-04-1 [3]**

The proposal was submitted by **Terrafame Oy** and received by RAC on **8 August 2016.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Terrafame Oy has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **16 August 2016**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **30 September 2016**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Betty Hakkert**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **22 September 2017** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors, ATE	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	028-006-00-9	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	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]	**Add** Acute Tox. 4	**Add** H332		**Add** H332			
RAC opinion	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]							
Resulting Annex VI entry if agreed by 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]	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			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Article 37(2) of the CLP Regulation states the following:

A manufacturer, importer or downstream user of a substance may submit to the Agency a proposal for harmonised classification and labelling of that substance and, where appropriate, specific concentration limits or M-factors, provided that there is no entry in Part 3 of Annex VI for such a substance in relation to the hazard class or differentiation covered by that proposal.

Consequently, the DS's proposal to revise the hazard class STOT RE 1 in the current Annex VI entry by adding the target organ (lungs) and the route of exposure (inhalation) could not be evaluated by RAC.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

No data on acute inhalation toxicity is available for nickel sulfide. Instead the classification proposal for acute toxicity via inhalation is based on read across from nickel subsulfide. The read-across is based on the bioaccessibility of various nickel compounds tested in synthetic lung fluids combined with *in vivo* verification data for three 'source' nickel compounds. These data suggest, according to the dossier submitter (DS), that read-across from nickel subsulfide to nickel sulfide is justified. A detailed description of the read across assessment is available in Section 9 of the background document.

An OECD TG 403 study (GLP compliant) in Sprague-Dawley rats (5 animals per sex and dose) was performed with 100% pure Ni subsulfide. Doses of 0.206, 1.02 and 5.15 mg/L were used. The results showed an LC_{50} of 1.352 mg/L for male rats and 0.9237 mg/L for female rats. This leads to a mean LC_{50} of 1.14 mg/L.

Nickel subsulfide is not currently classified for acute inhalation toxicity but the available study reports a mean LC_{50} value of 1.14 mg/L which would lead to classification as **Acute Toxicity 4; H332** (CLP criteria for category 4: LC_{50} of between 1 and ≤ 5 mg/L). The DS hence proposed this classification also for nickel sulfide.

Comments received during public consultation

Four Member State Competent Authorities (MSCAs) provided comments in support of the proposed classification. One MSCA however brought forward concerns regarding the proposed read-across approach. The MSCA noted that the this was based on bioelution data and that there is no current agreement on the use of this for regulatory purposes in the context of human health endpoints. They further stated that bioelution/bioaccessibility data is in their opinion not sufficient to address local and inhalation toxicity, as particle-induced effects may also contribute.

Assessment and comparison with the classification criteria

No inhalation data exist for nickel sulfide, only for nickel subsulfide. The DS proposed to read-across from nickel subsulfide based on bioaccessibility data in synthetic lung fluids from various nickel compounds and based on *in vivo* verification data for 3 source nickel compounds. The proposed classification of nickel sulfide as Acute Tox. 4; H332 is based on a new study according to OECD TG 403 where rats were exposed to nickel subsulfide for 4h. In this new study, an average LC_{50} (females and males) of 1.14 mg/L was obtained, hence classification as Acute Tox. 4; H332 is warranted (intervals for Acute Tox. 4; H332 in the CLP Regulation: $1 < LC_{50} \leq 5$ mg/L). At a dose of 0.205 mg/L on the exposure day, three males showed signs of facial staining or ocular discharge while the rest of the rats remained active and healthy over the 14-day observation period. There were no other signs of gross toxicity, adverse pharmacologic effects or abnormal behaviour. At a dose of 1.06 mg/L 3 days after exposure, all rats had clinical signs including facial staining, irregular respiration, hypoactivity, thin appearance, reduced faecal volume and cold limbs. One male and three females died. At a dose of 5.15 mg/L 2 days after exposure, all rats had clinical signs including facial staining, abnormal respiration, hypoactivity, hunched posture, reduced food consumption and reduced faecal volume. All animals died within five days of exposure. Gross necropsy of the rats showed discoloration of the lungs and/or intestines.

Limited information on the acute inhalation of three other nickel compounds is provided besides the LC_{50}, as the DS had proposed a grouping of the nickel compounds classification for acute inhalation based on a comparable bioelution in interstitial fluid as for nickel subsulfide.

The proposed read-across approach is based on the assumption that the toxicity of nickel and nickel compounds is caused by the Ni^{2+} ions released and interacting at the target site. For acute toxicity after inhalation exposure, it is suggested that the respiratory tract is the target site. As no *in vivo* information is available on the presence of Ni^{2+} in the respiratory tract after exposure to the source and the target substances, this is estimated by comparing the bioaccesibility after 24-72 hour bioelution in interstitial fluid. Two groups were defined: group 1 includes highly water soluble Ni salts, with bioaccesability of 7-11%, and Ni sulphide with bioaccesability approximately 1% due to the properties of the counter ion, while group 2 includes nickel salts with a bioaccesability of less than 1%. For the two groups category 4 and no classification is proposed by the DS.

The DS proposed to apply read-across for the classification of one substance to another. 'Read-across' is an accepted method used to interpolate or extrapolate test results from the source(s) to the target substance. For acute inhalation toxicity, an estimated LC_{50}, or LC_{50} range for the target substance should be based on the available information on the source substance(s). This means that direct read-across of a classification from the source substances to the target substance can not be applied. This is particularly important for substances where the LC_{50} is close to the border of a hazard category and where the classification is based on potency.

Furthermore, the assumption that the acute inhalation toxicity of nickel metal and nickel compounds is determined only by the local availability of Ni^{2+} in the respiratory tract is not substantiated by the available information. In the acute inhalation study with nickel subsulfide provided, discoloration of the intestines in addition to the lungs was observed. This shows that a part of the inhaled nickel subsulfide was transported upwards by the respiratory tract, swallowed and entered the intestinal tract. It is known that undissolved inhaled particles can cause local toxicity as shown by the classification of both soluble and insoluble nickel compounds for lung carcinogenicity and the very comparable LOAECs from 2-year studies (table 15 of the background

document). Acute exposure to high dose levels in the lung can induce an inflammatory response. The assumption that Ni^{2+} ions determine the acute inhalation toxicity of nickel and nickel compounds, is therefore considered not to be justified based on theoretical assumptions only and this should be substantiated by verification data.

Although 'bioelution' is applied in the calculation of the external exposure in well defined cases, and is also used on a case by case basis for reading across, there has been little consideration as to whether whether the concept can be used more generally for classification and labelling purposes. There are no internationally agreed guidelines for the conduction of bioelution techniques/studies and no data to show a systematic relationship between bioelution and systemic availability.

The read across proposed by the DS among nickel compounds is based on the results of an *in vitro* study (KMHC, 2010). Several deficiencies with this this bioelution study are noted:

- Lack of information on the particle size of the tested nickel compounds. The first step in the process of absorption via inhalation is deposition and the particle size and shape play a key role in the precise location of deposition and resultant toxicity. The particle size of the aerosol (MMAD and geometric standard deviation, GSD), together with particle density and breathing parameters will eventually thus 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 will 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 may vary also based on their shape and size.
- In addition, the particle size of the nickel compounds used in the bioelution tests should be comparable and preferably the same as in the inhalation test as a difference in particle size results in a difference in surface area and potentially in a difference in dissolution rate. It is noted that, no information is provided on the particle size in the bioelution tests with the nickel compounds.
- Such a bioelution test is at best a simulation of lung conditions and the design is therefore critical. In the CLH report it is indicated that each nickel containing substance was extracted at 2, 5, 24 and 72 hours using 3 different synthetic biological fluids (interstitial, alveolar, and lysosomal). The extractions were performed using one concentration of 0.1 grams nickel sample in 50 mL of fluid. Since the concentration can influence the rate of ion dissolution from the particles, this is a key aspect of the simulation and it is not clear whether the tested concentration is realistic or what it represents.

The above aspects hamper a scientific assessment and justification of the read-across as proposed by the DS. RAC considers that when read-across using the bioelution concept is applied, it should be better supported with relevant study data in order to be valid.

The results of the bioaccessibility (Table 14 of the Background document) of Ni subsulfide and Ni sulfide reveal that for the parameters examined the values of Ni sulfide are lower than those of Ni subsulfide. A lower Ni bioavailability often correlates with a decrease in toxicity and an increase in LC_{50} value. The inhalation acute toxicity LC_{50} value for Ni subsulfide is 1.14 mg/L which is just above 1 mg/L. This would suggest that the LC_{50} value for Ni sulfide is also higher than 1 mg/L. However, the available verification data as provided by the dossier submitter do not provide a strong correlation between bioaccesability and LC_{50} and therefore do not fully support the read across.

Incorporation of the bioaccessibility data into any type of read-across assessment first requires an evaluation of its correlation with *in vivo* data for verification (see table below). For the inhalation route this has been 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 (Ni sulfate hexahydrate, Ni subsulfide, and green Ni oxide, black Ni oxide). 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). Furthermore, the difference in time to mortality for the sulfate and the subsulfide indicates that different parameters may be relevant for these substances. The best results were obtained with the dissolution in interstitial fluid. Based on these (*in vivo* correlation) data, it is not possible to assess whether the bioaccesibility is a good predictor of acute toxicity effects Therefore, the *in vivo* verification does not show that the proposed grouping approach is valid.

Table. Bioelution, water solubility and LC50 values of several nickel compounds.

Sample	**Cas No.**	**Ni Content (%)**	**Water solubility (mg/L)**	**Interstitial Bioaccessibility (% Ni/sample) 24-72 hrs**	**Lysosomal Bioaccessibility (% Ni/sample) 24-72 hrs**	**Acute Toxicity inhalation (LC_{50}; mg substance/L)**
Water-soluble nickel compounds						
Ni sulfate hexahydrate	10101-97-0	22	625000	10.7-12.80	20.35-21.35	2.48 (0.55)
Sulfidic Nickel Compounds						
Ni subsulfide	12035-72-2	61 (70)	16 (144 h)	2.65-3.60	20.7-26.20	1.14 (0.80)
Ni sulfide	**16812-54-7**	**59**	**88**	**0.73 - 1.08**	**14.55 - 25.95**	**not determined**
Oxidic nickel compounds						
Ni oxide green	1313-99-1	77 (81)	0.035	0.08 - 0.10	0.44 - 0.82	>5.08 (>4.1)
Ni oxide black	1313-99-1	75	2.26	0.42 - 0.56	10.60 - 24.50	>5.15 (>3.9)
Other nickel compounds						
Ni hydroxy carbonate	12122-15-5	49 (49)	33	0.52 – 1.65	47.20 – 47.20	>2,09 (F); 0.24 (M)

No information was provided by the dossier submitter on the difference in toxicity between the subsulfide and the sulfide. Nickel sulfide is expected to dissociate into Ni(2+) and S(2-). However, dissociation of Ni_3S_2 into 3 Ni^{2+} and 2 S(2-) also results in 2 remaining electrons, indicating that this dissociation cannot occur directly. Therefore, it is unclear which anions are formed upon

dissociation of nickel subsulfide. Therefore, the absence of an influence of the counter-ion on the toxicity is not shown.

RAC acknowledges that read-across supported by bioavailability information can be useful for assessing the classification of metal salts and this has been applied for nickel compounds under DSD. However, the application of read-across should be properly justified as required under CLP. Currently, insufficient information is available to support the proposed grouping approach. In addition, no information is provided on particle size tested in the bioelution studies and on the anion formed after dissolution. Although the information provided suggests that based on nickel release, the acute inhalation toxicity would either be category 3, 4 or no classification, the available information does not allow to differentiate between these three options. Furthermore, a different classification due to the counter-ion cannot be excluded. This uncertainty is expected to be larger than the uncertainty expected when an acute inhalation study with nickel sulfide is performed. Therefore, the uncertainty is considered too large and a worst-case approach cannot be applied.

In conclusion, the information provided does not justify the proposed classification, therefore **no classification is concluded for the acute inhalation toxicity based on absence of data.**

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).