Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

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

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

CLH-O-0000001412-86-272/F

Adopted

15 March 2019

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON TRINICKEL DISULPHIDE; NICKEL SUBSULFIDE; [1] HEAZLEWOODITE [2]

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: trinickel disulphide; nickel subsulfide; [1] heazlewoodite [2]
EC number: 234-829-6 [1] - [2]
CAS number: 12035-72-2 [1] 12035-71-1 [2]
Dossier submitter: Johnson Matthey Chemicals GmbH

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
04.09.2018	Germany		MemberState	1
Comment received				
The structural formula (Ni=Ni=Ni=S=S) in chapter 1.1 of the CLH report is wrong and should be removed.				
Dossier Submitter's Response				
The Dossier Submitter thanks the German MSCA for pointing out an error in the structural formula for trinickel disulphide. The Dossier Submitter agrees that the current structural formula is not accurate for trinickel disulphide, as it is difficult to represent in a linear formula. The current structural formula will be replaced with a crystalline structure in the technical and registration dossier. In conclusion, we thank Germany for their comment which prompted a review of the structural formula for trinickel disulphide.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
06.09.2018	France		MemberState	2
Comment received				
FR is of the opinion that the use of the LC50 of female (0.9237 mg/L) should have been considered for the classification of trinickel disulphide, since without justification, in general, classification is based on the lowest LC50 available. The classification would then be Acute Tox 3. If the LC50 of 1.14 mg/L is considered more relevant, it should have been justified in the CLH report. Moreover, an ATE should have been specified in the report, for classification of mixtures containing trinickel disulphide.				

Dossier Submitter's Response

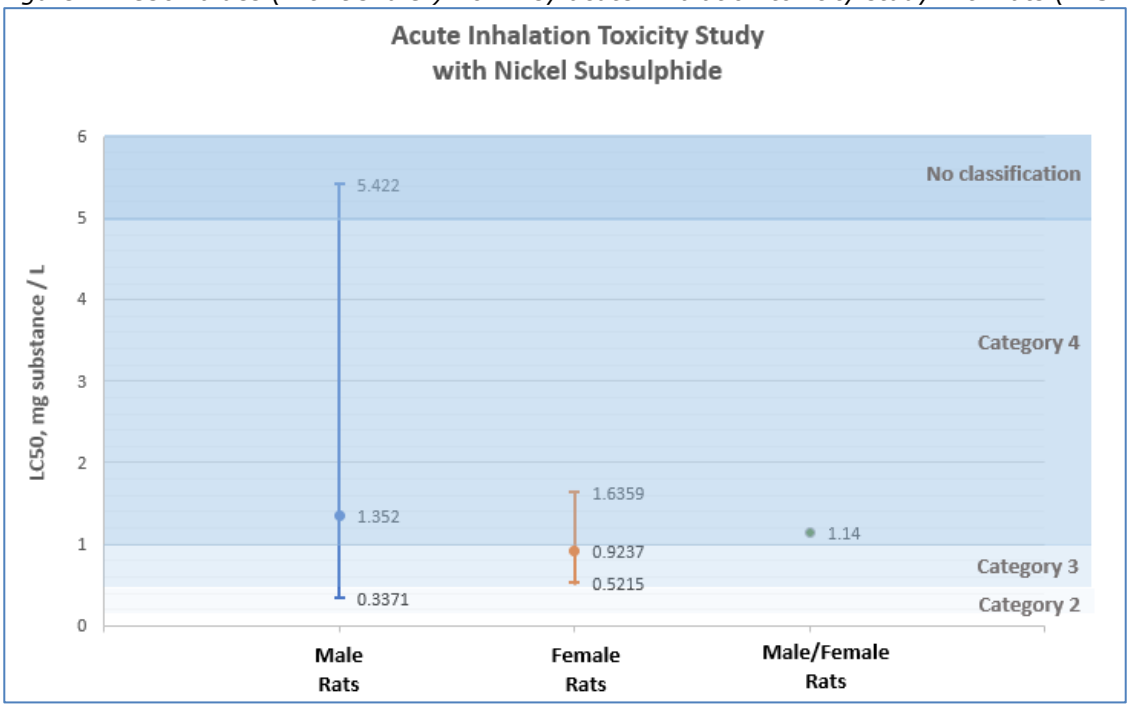
The Dossier Submitter thanks the French MSCA for their review of the data noting their consideration of female rats with a lower LC50 value as the most sensitive sex in the absence of a justification in favour of using the mean (male, female) value, and the absence of the acute toxicity estimate (ATE) value.

Male vs female data

In general, acute toxicity classification is based on the lowest species LC50 available rather than the lowest LC50 in terms of sex (male or female). The French MSCA suggests that the lowest LC50 available should be used, unless otherwise justified. The Dossier Submitter therefore offers the following considerations in support of using the mean (male and female) value. There is no statistical difference between the LC50 values for male and female rats. The results of the OECD TG 403 study with trinickel disulphide (EPSL, 2010) indicate that both the male and overall LC50 are within the range for Cat. 4 classification, while the female LC50 is only slightly below the threshold for Cat. 4 classification. The Dossier Submitter considers it appropriate to take the overall study LC50 value of 1.14 mg substance /L air (≥1 mg substance/L cutoff for Category 4) for purposes of classification and proposes an Acute tox 4 classification.

Figure 1 below shows the LC50 values (and 95% Confidence Intervals) from the key study (EPSL 2010) as well as the cutoff values for classification for acute toxicity, with an LC50 between 1 and ≤5 mg/L representing an Acute Toxicity Category 4 classification and an LC50 between 0.5 and ≤1 mg/L representing an Acute Toxicity Category 3 classification.

Figure 1. LC50 values (with 95% CI) from key acute inhalation toxicity study with rats (EPSL 2010).



The above assessment is further supported by the raw data for the acute study. As seen in Table 1 below, the survival results for males and females are identical except for a difference of just 2 deaths in the middle exposure group (1 dead male versus 3 dead females). The average survival for the concentration levels of 1.02 mg /L is 60% consistent with the combined LC50 of 1.14 mg/L. While the LC50 is at the lower end of the Acute 4 range, it is definitely within this range as shown in Figure 1.

Table 1. Mortality (raw data) from key acute inhalation toxicity study with rats (EPSL 2010).

Exposure Level (mg/L)	Number Dead/Number Tested		
	Males	*Females*	*Total*
0.206	0/5	0/5	0/10
1.02	1/5	3/5	4/10
5.15	5/5	5/5	10/10

Furthermore, female rats are not consistently more sensitive to the toxicity effects of trinickel disulphide as evidenced by the acute and the short-term repeated exposure data presented in Table 2. The data in Table 2 indicate no statistical or consistent difference in male or female sensitivity (survival) to the short term (1- to 22-day exposures) effects of trinickel disulphide inhalation.

Table 2. Short-term inhalation studies with trinickel disulphide

Inhalation Studies	Males	Females	Male vs Female Sensitivity
Acute toxicity			
4-hour study EPSL (2010) Doses: 0.2, 1, 5 mg/L 5 males & 5 females/group	LC50: 1.352 mg substance /L air (95%CI 0.3371-5.422) Survival (alive/total): at 0.2 mg/L: 5/5 at 1.0 mg/L: 4/5 at 5.0 mg/L: 0/5 Average LC50, for male&female: 1.14 mg substance /L air	LC50: 0.9237 mg substance/L air (95%CI 0.5215-1.6359) Survival (alive/total): at 0.2 mg/L: 5/5 at 1.0 mg/L: 2/5 at 5.0 mg/L: 0/5 Average LC50, for male&female: 1.14 mg substance /L air	LC50 for females is lower, with no statistically significant difference
Short-term repeated dose			
1,2,4,7,12, and 22-day study Benson et al (1995) repeated exposure Doses: 0, 0.6, 2.5 mg/m^3 MMAD 0.6 mg/m^3, 2.07 MMAD 2.5 mg/m^3, 1.98 µm 11 males & 11 females/group	Survival at 0.6 & 2.5 mg/m^3 (alive/total): Day 1: 11/11; 11/11 Day 2: 11/11; 11/11 Day 4: 11/11; 11/11 Day 7: 11/11; 10/11 Day 12: 11/11; 11/11 Day 22: 11/11; 11/11	Survival at 0.6 & 2.5 mg/m^3 (alive/total): Day 1: 11/11; 11/11 Day 2: 11/11; 11/11 Day 4: 11/11; 11/11 Day 7: 11/11; 10/11 Day 12: 11/11; 11/11 Day 22: 11/11; 11/11	No difference in sensitivity (survival)
12-day study Dunnick et al (1988); from NTP report (1996) Doses: 0, 0.6, 1.2, 5, 10 mg/m^3 MMAD 10 mg/m^3 2.8 µm, GSD 2.0 5 males & 5 females/group	Survival of controls (alive/total): 5/5 males NO(A)EC –mortality (alive/total) 5 mg/m^3: 5/5 male LO(A)EC –mortality (alive/total) 10 mg/m^3: 4/5 male	Survival of controls (alive/total): 5/5 females NO(A)EC –mortality (alive/total) 10 mg/m^3: 5/5 female LO(A)EC –mortality: >10 mg/m^3	Males survival is lower (more sensitive) with no statistically significant difference

12-day study Benson et al (1987) Doses: 0, 0.6, 1.2, 2.5, 5, 10 mg/m^3 MMAD 10 mg/m^3 2.5-2.6 µm, GSD 1.7-2.0 8 males & 8 females/group; except 5 male & 5 female/group for 1.2 & 5 mg/m^3 doses only	100% survival at 5 mg/m^3 and lower NO(A)EC –mortality (alive/total) 5 mg/m^3: 5/5 male LO(A)EC –mortality (alive/total) 10 mg/m^3: 6/8 male	100% survival at 10 mg/m^3 and lower NO(A)EC –mortality (alive/total) 10 mg/m^3: 8/8 female	Males survival is lower (more sensitive) with no statistically significant difference

The Dossier Submitter does not consider it appropriate to use the LC50 for the female rat as the determinate for acute toxicity classification. The overall study LC50 value of 1.14 mg substance /L air, combining both the male and female data, is a more appropriate representation of the data and should be the basis for classification of acute toxicity for the following main reasons:

- No statistically significant difference between the male and female rat LC50 values of the key acute toxicity study
- No statistically significant difference in sensitivity/survival between the male and female rats in several short-term repeated dose studies, although for these studies male rats, not female rats, are shown to be slightly more sensitive
- The CLP Guidance does not mention that for a given animal species the ATE value should be based on the most sensitive sex

The acute and short-term repeated dose toxicity data do not support the suggestion that female rats are more sensitive to the effects of trinickel disulphide. Therefore, the overall study LC50 value of 1.14 mg substance /L air, with a proposed Acute Toxicity of Category 4 is proposed.

Appropriate ATE value

The Dossier Submitter did not provide an Acute Toxicity Estimate (ATE) value for the classification of trinickel disulphide in the CLH dossier submission. Although ATE values are described in the CLP Guidance, the Dossier Submitter understood that they are not required for CLH dossier submission nor acceptance of registration dossiers. However, the Dossier Submitter does agree with the value of this information for the use of trinickel disulphide in mixtures. As indicated in the CLP Guidance [see CLP options available to derive ATE for mixture below; Note (b) to Table 3.1.1], an available LD50 or LC50 is used as the ATE value, otherwise an appropriate conversion value can be used.

Notes to Table 3.1.1:

(a) The acute toxicity estimate (ATE) for the classification of a substance is derived using the LD_{50}/LC_{50} where available.

(b) The acute toxicity estimate (ATE) for the classification of a substance in a mixture is derived using:

- *the LD_{50}/LC_{50} where available,*
- *the appropriate conversion value from Table 3.1.2 that relates to the results of a range test, or*
- *the appropriate conversion value from Table 3.1.2 that relates to a classification category.*

An LC50 from the EPSL study (2010) is available and should be used as the ATE value. So, the Dossier Submitter proposes an ATE value of 1.1 mg/L (rounded to one decimal point) based on the EPSL (2010) acute toxicity study overall LC50 of 1.14 mg/L, combining both

male and female rat data. An ATE of 1.1 mg/L for trinickel disulphide in mixtures will be more protective than the conversion value of 1.5 mg/L based on a Category 4 classification (if an LC50 or LD50 was not available).

In conclusion, we thank France for their comments which prompted a review of additional inhalation studies and the inclusion of an ATE value. We believe the information reviewed here further supports the classification of trinickel disulphide as Acute Tox 4.

References

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

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

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

ECHA. (2017). Guidance on the Application of the CLP Criteria. Version 5.0 (Section 3.1. Acute Toxicity).

EPSL. (2010). Acute inhalation toxicity study in rats - Defined LC_{50}. Testing laboratory: Eurofins Product Safety Laboratories. Report no.: 28705. Nickel subsulphide. Owner company: Nickel REACH Consortia Members. Report date: 2010-04-02.

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

RAC's response

RAC agrees that an ATE value should be derived.
As the LC50 values in male and female rats were in the same range but just above or below the LC50 value of 1 mg/L that differentiates between category 3 and 4, RAC considered that robust additional data should be available to justify not using the lowest LC50 value. As the available short term repeated dose inhalation studies do not provide adequate data to deviate from using the lowest LC50, RAC used the lowest LC50.

Date	Country	Organisation	Type of Organisation	Comment number
04.09.2018	Germany		MemberState	3

Comment received

The dossier submitter (DS) is of the opinion that trinickel disulphide is to be classified as Acute Tox. 4 (H332) for inhalation (page 20). The DE-CA does not agree with this proposed classification. Moreover, the DE-CA notes that the DS did not report/calculate any acute toxicity estimate (ATE) values, as described in the CLP Guidance (section3.1.2.2).

The key study (Anonymous, 2010) – performed according to OECD TG 403 (GLP compliant)

in rats – resulted in different LC50 values for males and females. The male LC50 (4 h) was 1.35 mg/L, whereas the LC50 for female rats (4 h) was 0.92 mg/L. The combined LC50 for males and females was calculated to be 1.14 mg/L (MMAD3.3 – 3.5 µm). According to these results, female rats are more sensitive to trinickel disulphide compared to male rats.
The female LC50 is, moreover, within the ranges of the acute toxicity estimates (ATE) for acute inhalation toxicity, Cat. 3 (0.5 < ATE ≤ 1.0 mg/L; H331), and the male and combined LC50 is only slightly above this threshold (1.35 mg/L and 1.14 mg/L, respectively).

Thus the DE-MSCA is of the opinion that trinickel disulphide should be classified accordingly as Acute Tox. 3, H331 for inhalation, as the CLP-Guidance states: "In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested" (section: 3.1.2.3.2).

Supporting evidence for this argumentation can be found in the repeated dose toxicity studies mentioned in the CLH report as well as in the technical dossier of trinickel disulphide.

In a repeated dose test with Fischer rats, Benson et al. (1995) reported a significant drop in body weights of male and female rats after 7 days of exposure to trinickel disulphide at 0.0025 mg/L (test concentration approx. 370-fold lower than the female LC50 in the acute toxicity test: 0.92 mg/L). Moreover, one male and one female rat died during day 7 of exposure at 0.0025 mg/L (MMAD 1.98 µm; GSD: 2.03).

Benson et al. (1987) exposed mice and rats repeatedly to trinickel disulphide (6h/d, 5d/wk, 2 wks; whole body; n = 18/sex/ concentration) at concentrations much lower than used for acute toxicity testing. The test concentration at which mortality occurred (0.011 mg/L; MMAD 2.5 – 2.64 µm, GSD: 1.7 – 2.01) was approximately 83-fold lower than the LC50 for females determined in the acute toxicity test by Anonymous (2010). While 2/18 male rats died during trinickel disulphide exposure at 0.011 mg/L (no specifics on time of death reported), no female rats died, although signs of labored breathing, emaciation, dehydration and decreased body weight gain, as well as effects on lung and thymus were observed in all animals exposed to the substance at ≥ 0.0051 mg/L. In addition, in the same study 8/18 male mice died before the end of the exposure to trinickel disulphide at 0,011 mg/L, whereas 18/18 female mice died at that test concentration (no specifics on time of death, except that 9 of them had to be sacrificed moribund 9-10 days after the exposure and 1 died accidentally). These results indicate that mice are more sensitive than rats with respect to inhalation toxicity of trinickel disulphide.

Similar results were described by Dunnick et al. (1988), who reported mortality in rats (1/5 male rats) but especially in mice (5/5 male and 5/5 female mice) after repeated inhalation of trinickel disulphide at 0.0073 mg/L (MMAD: 2.8µm; GSD: 0.2). This exposure concentration was approx. 126-fold lower than the female LC50 in the acute toxicity test (0.92 mg/L). Unfortunately no details on time of death were reported in this study. However, study results again indicate that mice are more susceptible than rats when inhaling trinickel disulphide.

In NTP (1996) repeated dose inhalation studies (6h/d, 5d/w for 12 exposure days), similar results were obtained. Again, rats and mice lost weight when exposed to trinickel disulphide at 0.01 mg/L (MMAD: 2.79 µm, GSD: 1.95) and did not gain weight at 0.005 mg/L trinickel disulphide (MMAD: 2.65, GSD: 2.12). While 1/5 male rats died at 0.01 mg/L trinickel disulphide (day 14 of the exposure period), 5/5 male and 5/5 female mice died at that exact exposure concentration (approx. 92-fold lower than the female LC50 in the acute toxicity test: 0.95 mg/L). The male mice died at days 5, 6, 6, 7 and 8 of the exposure, and the

female mice died at days 5, 9, 10, 10 of the exposure. One female died accidentally at day 6. No mortality was observed at 0.005 mg/L.

It is noted that no mortality was reported in the NTP studies for the exposure days 0-3 and no information on time of death was reported in the other above mentioned repeated dose studies. The CLP Guidance states: "Mortalities during the first 72 h after first treatment (in a repeated dose study) may also be considered for the assessment of acute toxicity" (section 3.1.1). Nevertheless, in summary these repeated dose studies are indicative of two facts:

1) Female mice and rats seem to be more sensitive after inhalation of trinickel disulphide than males of the same species
2) Mice seem to me much more sensitive than rats with respect to inhalation toxicity of trinickel disulphide.

The DS cites several supportive studies, in which mice or rats were instilled once intratracheally (IT instillation) with the test substance, but did not cite the supportive repeated dose inhalation studies.

In one of the cited IT instillation study (Fisher, 1984), a LD50 of 4 mg/kg bw (one bolus dose of fine trinickel disulphide particles; MMAD = 1.8 µm) was determined, whereas the other studies used much lower doses, as they were designed to evaluate different endpoints than mortality such as cellular, biochemical and histological endpoints (Benson et al., 1984; Benson et al., 1986; Finch et al., 1987). To be able to compare the mortality results of the IT instillation study by Fisher (1984) to the inhalation study by Anonymous (2010), the DS calculated the delivered dose after the 4h of inhalation exposure.
The DE-CA considers this approach as not appropriate for determining the acute inhalation toxicity of trinickel disulphide, as the inhalation route is the physiologically relevant route, whereas IT instillation represents a non-physiological route. The CLP Guidance states that findings from studies using non-physiological routes may provide useful information (especially when evaluating carcinogenicity), however, results from such studies need to be considered with caution, as usually dosing via these routes provides a high bolus dose which gives different toxicokinetics to normal routes (section 3.6.2.3.2).
In this regard, various scientists compared the deposition and distribution of dust in the lung (of rats) using two methods for delivery: aerosol inhalation and IT instillation (Brain et al., 1976; Driscoll et al., 2000; Osier et al., 1997; Pritchard et al., 1985). All authors reported that IT instillations resulted in non-uniform distribution patterns with preferential deposition in the dependent portions of the lung (focally high doses of material without distribution to the periphery), whereas animals exposed to aerosol showed a more homogeneous distribution with preferential deposition in the apical lobes. These differences in dose distribution were shown to influence clearance pathways, doses to certain cells and to tissues, and the degree and site of systemic absorption. It was concluded that these differences in distribution do not allow for establishing an absolute dose-response relationship when using the IT instillation method and that the method of administration can affect the systemic bioavailability, with inhaled materials being more bioavailable than those delivered by instillation. Differences in distribution and deposition were further suggested to be due to the dose and type of vehicle, the volume of material administered, as well as the dose and type of the anesthetic agent when performing IT instillations. Moreover, a key difference between the two exposure methods was shown to be the dose rate, i.e. administration of a dose within a few seconds with IT instillation, as opposed to minutes, hours, days or weeks when the material is inhaled. The authors also indicated that another important difference between the two exposure techniques is that inhalation can result in deposition within the upper respiratory tract, the extent of which depends upon the characteristics of the airborne material. Because instillation bypasses this portion of the

respiratory tract, it is not a viable technique if responses in this region are endpoints of concern.

Taken together, the DE-CA is of the opinion that the repeated dose inhalation studies are more suitable to be used as supporting studies compared to IT instillation studies, especially when evaluating acute inhalation toxicity.

In summary, the key acute inhalation toxicity study (Anonymous, 2010) resulted in an LC50 for female rats within the ranges of the ATE for inhalation toxicity, Cat. 3 (0.92 mg/L). Additionally – based on repeated dose inhalation toxicity data – rats are expected to be less sensitive compared to mice. The CLP-Guidance states: "In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested" (section: 3.1.2.3.2).
Having this in mind, the DE-MSCA considers a classification of trinickel disulphide as Acute Tox. 3 for inhalation (H 331) as appropriate using the LC50 for female rats ($0.5 < ATE \leq 1.0$ mg/L).

In addition, for the classification for acute inhalation toxicity of mixtures containing trinickel disulphide an ATE value of 0.5 mg/L is proposed for the calculation with the additivity formula according to Annex I, part 3, and section 3.1.3.6.1. and 3.1.3.6.2.3 of the CLP regulation. The ATE value of 0.92 mg/L (inhalation) for the substance trinickel disulphide is converted from the acute toxicity point estimate of acute hazard category 3 (see Table 3.1.2 of the CLP regulation).

References

Anonymous (2010): Acute inhalation toxicity study in rats with nickel subsulfide

Benson J.M., Carpenter R.L., Hahn F.F., Haley P.J., Hanson R.L., Hobbs C.H., Pickrell J.A., and Dunnick J.K. (1987): Comparative Inhalation Toxicity of Nickel Subsulfide to F344/N Rats and B6C3F1 Mice Exposed for 12 Days. Fundamental and Applied Toxicology 9 (2), 251-265. DOI: Doi 10.1016/0272-0590(87)90047-9

Benson J.M., Cheng Y.S., Eidson A.F., Hahn F.F., Henderson R.F., and Pickrell J.A. (1995): Pulmonary toxicity of nickel subsulfide in F344/N rats exposed for 1-22 days. Toxicology 103 (1), 9-22. DOI: Doi 10.1016/0300-483x(95)03098-Z

Benson J.M., Henderson R.F., and Mcclellan R.O. (1984): Comparative Toxicity of Nickel Salts to the Lung. Annals of Clinical and Laboratory Science 14 (5), 405-405. <Go to ISI>://WOS:A1984TF43500024

Benson J.M., Henderson R.F., Mcclellan R.O., Hanson R.L., and Rebar A.H. (1986): Comparative Acute Toxicity of 4 Nickel Compounds to F344 Rat Lung. Fundamental and Applied Toxicology 7 (2), 340-347. DOI: Doi 10.1016/0272-0590(86)90164-8

Brain J.D., Knudson D.E., Sorokin S.P., and Davis M.A. (1976): Pulmonary distribution of particles given by intratracheal instillation or by aerosol inhalation. Environmental Research 11 (1), 13-33. DOI: https://doi.org/10.1016/0013-9351(76)90107-9

Driscoll K.E., Costa D.L., Hatch G., Henderson R., Oberdorster G., Salem H., and Schlesinger R.B. (2000): Intratracheal instillation as an exposure technique for the evaluation of respiratory tract toxicity: uses and limitations. Toxicological Sciences 55 (1),

24-35

Dunnick J.K., Benson J.M., Hobbs C.H., Hahn F.F., Cheng Y.S., and Eidson A.F. (1988): Comparative Toxicity of Nickel-Oxide, Nickel Sulfate Hexahydrate, and Nickel Subsulfide after 12 Days of Inhalation Exposure to F344/N Rats and B6c3f1 Mice. Toxicology 50 (2), 145-156. DOI: Doi 10.1016/0300-483x(88)90087-X

Finch G.L., Fisher G.L., and Hayes T.L. (1987): The pulmonary effects and clearance of intratracheally instilled Ni3S2 and TiO2 in mice. Environ Res 42 (1), 83-93. https://www.ncbi.nlm.nih.gov/pubmed/3803346

Fisher G., Chrisp, CE, McNeill, KL, McNeill, DA, Democko, C, Finch GL, (1984): Mechanistic evaluations of the pulmonary toxicology of nickel subsulphide. In: Advances In Modern Environmental Toxicology ((Ed.) M.H.e.a., ed.). Washington, D. C., USA May 11-13, 1982. Princeton Scientific Publishers: Princeton, N. J., USA

NTP (1996): Toxicology and carcinogenesis studies of nickel subsulphide (CAS no. 12035-72-2) in F344/n rats and B6C3F1 mice (inhalation studies). NTP TR 453 / NIH Publication No. 96-3369. Testing laboratory: National Toxicology Program (NTP)

Osier M., Baggs R., and Oberdörster G. (1997): Intratracheal instillation versus intratracheal inhalation: influence of cytokines on inflammatory response. Environmental Health Perspectives 105 (Suppl 5), 1265

Pritchard J.N., Holmes A., Evans J.C., Evans N., Evans R.J., and Morgan A. (1985): The distribution of dust in the rat lung following administration by inhalation and by single intratracheal instillation. Environmental Research 36 (2), 268-297. DOI: https://doi.org/10.1016/0013-9351(85)90025-8.

Dossier Submitter's Response

The dossier submitter thanks the German MSCA for their comments indicating that trinickel disulphide should be classified as Acute Tox 3 based on what they view as differential results for females and males, with supporting information from repeated dose toxicity studies via inhalation that were interpreted to indicate that mice are more sensitive to toxicity of trinickel disulphide than rats.

The CLH dossier included a proper citation for the acute inhalation toxicity study with trinickel disulphide (key study) that was conducted at Eurofins Product Safety Labs (www.productssafetylabs.com) under the direction of Jennifer Durando and completed in April 2, 2010: *Eurofins Product Safety Labs (EPSL; 2010). Acute inhalation toxicity study in rats, Eurofins PSL Study #28705, Ni subsulphide*. We hope the information provided is sufficient to warrant a citation of this study as "EPSL, 2010" and not "Anonymous, 2010" as suggested by Germany; the latter connotes a high degree of unreliability which is not warranted for a GLP compliant study.

Appropriate ATE value

The Dossier Submitter did not provide an Acute Toxicity Estimate (ATE) value for the classification of trinickel disulphide in the CLH dossier submission. Although the values are described in the CLP Guidance, the Dossier Submitter understood that they are not required for CLH dossier submission nor acceptance of registration dossiers. However, the Dossier Submitter does agree with the value of this information for the use of trinickel disulphide in mixtures. As indicated in the CLP Guidance [see CLP options available to derive ATE for mixture below; Note (b) to Table 3.1.1], an available LD50 or LC50 is used as the ATE

value, otherwise an appropriate conversion value can be used. An LC50 from the EPSL study (2010) is available and should be used as the ATE value.

> *Notes to Table 3.1.1:*
>
> *(a) The acute toxicity estimate (ATE) for the classification of a substance is derived using the LD_{50}/LC_{50} where available.*
>
> *(b) The acute toxicity estimate (ATE) for the classification of a substance in a mixture is derived using:*
>
> - *the LD_{50}/LC_{50} where available,*
> - *the appropriate conversion value from Table 3.1.2 that relates to the results of a range test, or*
> - *the appropriate conversion value from Table 3.1.2 that relates to a classification category.*

Additionally, the CLP Guidance states that ATE values should be based on the lowest ATE value of the most sensitive appropriate species, but the guidance does not mention consideration of the most sensitive sex. Although the LC50 for female rats is slightly lower than for male rats in the key acute toxicity study, there is no statistically significant difference between the male and female rat LC50 values (EPSL 2010). Several short-term repeated dose studies also show no statistically significant difference between male and female mortality data (Benson et al, 1987, 1995; Dunnick et al 1988) (see Table 2 above in the response to the French MSCA). So, the Dossier Submitter proposes an ATE value of 1.1 mg/L (rounded to one decimal point) based on the EPSL (2010) acute toxicity study overall LC50 of 1.14 mg/L,combining male and female rat data. An ATE of 1.1 mg/L for trinickel disulphide in mixtures will be more protective than the conversion value of 1.5 mg/L based on a Category 4 classification (if an LC50 or LD50 was not available).

Inhalation studies vs intratracheal instillation studies

The DE-MSCA does not favor the inclusion of intratracheal instillation studies in evaluation of the CLH dossier, as these studies are not appropriate to determine acute inhalation toxicity of trinickel disulphide. The Data Submitter agrees that the existing intratracheal instillation studies with trinickel disulphide do not provide relevant information for acute toxicity classification. These studies were included in the CLH dossier for completeness. The OECD-guideline compliant inhalation study (EPSL 2010) is the primary study on which the proposal for a harmonized classification for acute toxicity is based.

Acute toxicity vs repeated dose toxicity

DE-MSCA commented on the significantly lower test concentrations, in which adverse effects were observed, for the repeated dose studies compared to the observed acute toxicity LC50 values. Due to differences in exposure frequency, it is not unusual that lower doses result in adverse effects for repeated dose toxicity studies compared to higher treatment doses in acute toxicity studies.

However, the study noted by the German MSCA (Benson et al 1995) may not be a good example of a sex differential (male vs female) for survival in acute versus repeated toxicity studies. While there were 2 unusual deaths after 7 days of repeated exposure (one male and one female), all animals in separate exposure groups survived exposure for 12 and 22 days. Even in this instance, there was no apparent susceptibility of the females to the toxicity (see Table 2 above in the response to the French MSCA).

Rats vs mice

The German MSCA notes that the repeated dose studies indicate that mice are more

sensitive than rats with respect to inhalation toxicity of trinickel disulphide. Additional data from repeated dose studies with rats and mice is presented in Table 3. While it is indeed accurate that in the 12-day repeated dose studies (Benson et al., 1987; Dunnick et al., 1988) mice appear to be more sensitive to the toxicity of trinickel disulphide than rats, the survival of the control mice was also lower than for rats indicating a non-treatment (trinickel disulphide) related difference. In the 13-week and 2-year studies with trinickel disulphide, not only is a higher mice sensitivity not observed but to the contrary, higher exposure levels were selected for mice than for rats.

Table 3. Repeated dose inhalation studies with trinickel disulphide

Repeated Dose Inhalation Studies	Rats	Mice	Rat vs Mice Sensitivity
12-day study Dunnick et al (1988); from the NTP report (1996) Dose: 0.6, 1.2, 5, 10 mg/m^3 MMAD 10 mg/m^3 2.8 µm, GSD 2.0	Survival controls (alive/total): 5/5 males and 5/5 females NO(A)EC –mortality (alive/total): 5 mg/m^3; 5/5 male, 5/5 female LO(A)EC –mortality (alive/total): 10 mg/m^3; 4/5 male, 5/5 female	Survival Controls (alive/total): 4/5 males, 4/5 females NO(A)EC –mortality (alive/total): 5 mg/m^3; 5/5 male, 5/5 female LO(A)EC –mortality (alive/total): 10 mg/m^3; 0/5 male, 0/5 female	Mice more sensitive (controls & exposed)
12-day study Benson et al (1987) Dose: 0.6, 1.2, 5, 10 mg/m^3 [MMAD 10 mg/m^3 2.5-2.6 µm, GSD 1.7-2.0]	100% survival at 5 mg/m^3 and lower NO(A)EC –mortality (alive/total): 5 mg/m^3; 5/5 male, 5/5 female LO(A)EC –mortality(alive/total): 10 mg/m^3; 6/8 male, 8/8 female	Survival Controls (alive/total): 5/8 males, 18/18 females NO(A)EC –mortality (alive/total): 5 mg/m^3; 5/5 male, 5/5 female LO(A)EC –mortality (alive/total): 10 mg/m^3; 0/8 male, 0/17 female	Mice more sensitive (controls & exposed)
13-week study Dunnick et al (1989) & Benson et al (1990); from the NTP report (1996) Dose: 0.15, 0.3, 0.6, 1.2, 2.5 mg/m^3 MMAD 2.5 mg/m^3 2.6 µm, GSD 2.2	All animals (10 per sex per group) survived 13 weeks at 0, 0.3, 0.6, 1.2 and 2.5 mg/m^3	Survival Controls (alive/total): 8/10 males, 10/10 females At highest concentration (2.5 mg/m^3): 10/10 males, 8/10 females Survival not different from control	Equal sensitivity
2-year study Dunnick et al (1995); from the NTP report (1996) Dose: 0.15, 1.0 mg/m^3 rats Dose: 0.6, 1.2 mg/m^3 mice MMAD 1.0 mg/m^3 2.0 µm, GSD 2.0 MMAD 1.2 mg/m^3 2.2 µm, GSD 1.9	NO(A)EC – mortality; survival was similar to controls Controls (alive/total): 13/63 males and 25/63 females 1.0 mg/m^3 (alive/total): 18/63 male, 28/63 female	NO(A)EC – mortality; survival was exactly the same for control and 1.2 mg/m^3 (alive/total): 26/80 male/ 36/80 female	Equal sensitivity

Thus, in the absence of an acute inhalation toxicity study in mice, the relevance of these findings to support the classification of trinickel disulphide as Acute Tox 3 is unclear. The DE-MSCA quotes from the CLP-Guidance that "In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested" (section: 3.1.2.3.2). However, the guidance goes on to state that "The preferred

test species for evaluation of acute toxicity by the oral and inhalation routes is the rat…" (section: 3.1.2.3.2, Annex I: 3.1.2.2.1).

The key study for the acute toxicity inhalation endpoint used rat as the test species, and there is no acute inhalation study conducted in mice Consideration of species sensitivity in repeated dose toxicity inhalation studies is inconsistent and should not supersede the evidence provided by the acute toxicity key study or the preference for rat as the test species as indicated in the CLP Guidance.

The Dossier Submitter does not consider it appropriate to base the acute toxicity classification of trinickel disulphide on just the LC50 for the female rat in anticipation that an acute study in mice might have yielded a lower LC50 value. The overall acute toxicity inhalation study LC50 value of 1.14 mg substance /L air, combining both the male and female rat data, is an appropriate representation of the data and should be the basis for classification of acute toxicity of trinickel disulphide for the following main reasons:

- No statistically significant difference between the male and female rat LC50 values of the key acute toxicity study
- No statistically significant difference in sensitivity/survival between the male and female rats in several short-term repeated dose studies, although for these studies male rats, not female rats, are shown to be slightly more sensitive
- Inconsistent sensitivity for mice and rats to the inhalation effects of trinickel disulphide in repeated dose toxicity studies
- CLP Guidance indicates rats as the preferred species for acute toxicity inhalation studies

In conclusion, we thank the German MSCA for their comments which had prompted a review of additional inhalation studies. We believe the information reviewed here further supports the classification of trinickel disulphide as Acute Tox 4, with an LC50 value of 1.14 mg/L for the substance trinickel disulphide, rounded to an ATE of 1.1 mg/L for trinickel disulphide in mixtures.

References

Benson JM, Burt DG, Cheng YS, Eidson AF, Gulati DK, Hahn FF, Hobbs CH, and Pickrell JA. (1990). Subchronic inhalation toxicity of nickel subsulphide to rats and mice. Inhalation Toxicology; 2:1-19.

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

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

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

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

ECHA. (2017). Guidance on the Application of the CLP Criteria. Version 5.0 (Section 3.1. Acute Toxicity).

EPSL. (2010). Acute inhalation toxicity study in rats - Defined LC_{50}. Testing laboratory: Eurofins Product Safety Laboratories. Report no.: 28705. Nickel subsulphide. Owner company: Nickel REACH Consortia Members. Report date: 2010-04-02.

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

RAC's response

The points raised by the MSCA were considered. However, RAC had doubts about the predictiveness of repeated dose studies for acute inhalation mortality. Therefore, in line with the CLP guidance, RAC concluded on the classification based on the lowest LC50 value of 0.92 mg/L in the acute toxicity study in rat and the ATE was derived accordingly.