The Director General

Maisons-Alfort, 16 January 2014

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

regarding the review of the need to revise the classification for the acute toxicity of nickel dichloride and dihydroxide

ANSES undertakes independent and pluralistic scientific expertise.
ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are made public.

Under the memorandum of understanding signed between ANSES and its supervisory ministries, ANSES received a formal request from the Directorate General for Labour to undertake the following expert appraisal: review of the need to revise the classification for the acute toxicity of nickel dichloride and dihydroxide.

1. BACKGROUND AND PURPOSE OF THE REQUEST

At European level, many nickel compounds have a harmonised classification according to Regulation (EC) no. 1272/2008 on the classification, labelling and packaging of substances and mixtures (known as the CLP Regulation).

In particular, nickel dichloride and dihydroxide are classified for several hazard classes including acute oral toxicity (see table below, full classification in Annex I).

Index No	Chemical name	EC No	CAS No	Harmonised classification for acute oral toxicity
028-011-00-6	Nickel dichloride	231-743-0	7718-54-9	Acute Tox. 3 – H301*: Toxic if swallowed
028-008-00-X	Nickel dihydroxide	235-008-5	12054-48-7	Acute Tox. 4 – H302*: Harmful if swallowed

* minimum classification according to the CLP Regulation, obtained after automatic conversion of the pre-existing classification in Directive 67/548/EEC

For nickel dichloride, it should be noted that the EC number shown in Annex VI of the CLP Regulation corresponds to the general form of nickel dichloride and therefore covers the anhydrous form as well as the various hydrated forms of nickel dichloride (EU RAR 2008). Dihydrated and hexahydrated forms are described in particular in the registration dossiers.

Article 37(6) of the CLP Regulation stipulates that manufacturers, importers and downstream users who have new information which may lead to a change of the harmonised classification shall submit a proposal to revise the classification to the competent authority in one of the Member States.

The Directorate General for Labour thus received a request to revise the acute oral toxicity classification for nickel dichloride and dihydroxide. This request[1] relies on new experimental acute oral toxicity studies and new *in vitro* bioaccessibility studies and proposes:

- to change the acute oral toxicity classification of nickel dichloride from category 3 to category 4.
- to remove the acute oral toxicity classification from nickel dihydroxide.

Based on the information provided by the industry, the Directorate General for Labour asked ANSES:

- to assess the relevance of the new studies presented in the industry's classification revision proposals;
- to determine whether they call into question the current classification of these two nickel compounds for acute oral toxicity.

2. ORGANISATION OF THE EXPERT APPRAISAL

This expert appraisal was carried out in accordance with the French standard NF X 50-110 "Quality in Expertise – General Requirements of Competence for Expert Appraisals (May 2003)".

In response to the request, only the property of acute oral toxicity for which the classification has been called into question by the industry was assessed in detail for each of these two compounds. In relation to this property, relevant information on the oral bioavailability of the two nickel compounds was consulted. Data on their other toxicological properties were not analysed in detail or addressed by specific recommendations.

The first stage of the expert appraisal involved gathering relevant information for the analysis. In particular, the following documents were consulted for each of the two salts:

- The documents and studies that had supported the adoption of the current classification, when they could be located.

[1] Industrial documents forwarded to ANSES by the Directorate General for Labour in the email of 16 April 2013.

- The report on harmonised classification and labelling provided by the industry.
- The registration dossier of the lead registrant in REACH available in July 2013.

All available information was then analysed to draw a conclusion as to the need to revise the classification for the acute toxicity of these two compounds.

3. ANALYSIS AND CONCLUSIONS

3.1 Nickel dichloride ($NiCl_2$)

3.1.1 Data supporting the current classification for acute oral toxicity

No information was found on the data that had been used in a 1st Norwegian classification proposal submitted in 1995 proposing a T; R25[2] acute oral toxicity classification according to Directive 67/548/EEC[3]. This classification is applicable when the LD_{50}[4] is between 25 and 200 mg/kg and equivalent to the Acute Tox. 3 – H301[5] minimum classification[6] according to the CLP Regulation. The classification for this property had been defended in September 1996 by the relevant technical group but this proposal did not lead to a regulatory decision.

Furthermore, nickel dichloride was the subject of a European risk assessment (EU RAR 2008) and a new classification proposal (Danish EPA, 2004a) in tandem with this assessment submitted in 2004.

This classification proposal was based on the oral toxicity studies of **Itskova *et al.* (1969)** and **Study Report A**[7]**,** and concluded that the LD_{50} of nickel dichloride is between 175 and 535 mg $NiCl_2 \cdot 6H_2O$/kg.

Based on these data, the T; R25 classification of nickel dichloride was incorporated into Annex I of Directive 67/548/EEC by its 30th Adaptation to Technical Progress (ATP)[8]. This classification, equivalent to the Acute Tox. 3 H301 minimum classification[4] according to the CLP Regulation, has not been modified since.

[2] Toxic if swallowed

[3] Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances

[4] Lethal dose for 50% of animals

[5] Mentioned as minimum by the CLP Regulation since it was obtained by automatic conversion of the pre-existing classification according to Directive 67/548/EEC

[6] Toxic if swallowed

[7] Reference is confidential

[8] Commission Directive 2008/58/EC of 21 August 2008 amending, for the purpose of its adaptation to technical progress, for the 30th time, Directive 67/548/EEC

3.1.2 Data submitted by the industry and included in the registration dossiers

The classification revision request refers to an additional acute oral toxicity study (**Study report B**, published in Henderson *et al.* 2012b), in addition to the aforementioned Study report A.
The registration dossier for nickel dichloride also mentions the study by **Schafer *et al.* (1985)**.

The classification revision request also takes into account considerations on the bioavailability of nickel dichloride. The amount of nickel ion released *in vitro* after incubation of the substance in various synthetic physiological fluids for a few hours was measured and compared to that released from other nickel compounds (Henderson *et al.* 2012a).

3.1.3 Analysis of acute oral toxicity studies

A detailed analysis of all of the available acute oral toxicity studies is shown in Annex 2.

All of these studies were undertaken with nickel dichloride hexahydrate ($NiCl_2 \cdot 6H_2O$, CAS: 7791-20-0). The studies reported by Study reports A and B are considered to be of good quality while the study by Schafer *et al.* (1985) does not follow the recommendations set out in the guidelines and there is considerable uncertainty regarding the reliability of the study by Itskova *et al.* (1969).
The results of the two good-quality studies are summarised in the table below.

Study	Species	LD_{50} (for $NiCl_2$ $6H_2O$)
Study report A	Rats	Males: 210 mg/kg Females: 175 mg/kg
Study report B (Henderson 2012b)	Rats	Females: 500 mg/kg

The CLP Regulation establishes the acute oral toxicity classification that shall apply to a substance based on its oral LD_{50}:

Category	Acute Tox. 1	Acute Tox. 2	Acute Tox. 3	Acute Tox. 4
LD_{50} (mg/kg)	≤ 5	5-50	50-300	300-2000

Whereas the results of the Study report B correspond to a classification in category 4, those of the Study report A are consistent with the current Acute Tox. 3 classification.

The two studies were undertaken in the same rat strain, with the same vehicle (water), the same mode of administration by gavage and comparable purities. Only females were tested in the Study report B but previous data did not show significant differences in acute toxicity between sexes. Different protocols were used in these two studies. The most recent used the up-and-down method developed to reduce the necessary number of animals. A smaller number of animals was therefore tested in this study (11 females versus 25 in Study report A) which had less statistical power. However, the confidence intervals in these two studies did not overlap and the difference in the sensitivity of the two studies does not appear to be responsible for the observed variation. The difference in results between the two studies has therefore not been explained.

The guide on the application of the CLP criteria[9] specifies that, by default, the classification is based on the lowest available LD_{50} (Section 3.1.2.3.2). Since there is no information indicating that either of these two good-quality studies is more relevant, the most sensitive study (Study report A) should be used for classification.

3.1.4 Analysis of arguments on the bioaccessibility of the substance

Since there are good-quality acute oral toxicity data that can be directly interpreted in terms of classification, data on the *in vitro* bioaccessibility of the substance are not considered essential for a conclusion.

Furthermore, the available data on bioaccessibility (Henderson *et al.* 2012a) and oral toxicokinetics (Ishimatsu *et al.* 1995) indicate that the bioaccessibility and toxicokinetic behaviour of hexahydrated nickel dichloride are similar to those of nickel sulphate. The study by Henderson *et al.* (2012a) mentions an LD_{50} of 362 mg/kg for dihydrated nickel sulphate and an LD_{50} value of 275 mg/kg is reported as the basis for the Xn; R22[10] classification chosen according to Directive 67/548/EEC (Danish EPA, 2004b).

These data confirm that the acute oral toxicity of nickel dichloride and that of nickel sulphate are similar. Nickel sulphate has an Acute Tox. 4 minimum harmonised classification (obtained by converting the Xn; R22 classification according to Directive 67/548/EEC). The data support an Acute Tox. 3 classification which does not conflict with the Acute Tox. 3 classification recommended for dichloride.

3.1.5 Conclusion on classification

On this basis, the current Acute Tox. 3 classification for nickel dichloride is considered appropriate in light of the results of the Study report A.

The data on acute oral toxicity were all obtained with the hexahydrated form. Considering that toxicity is linked to the nickel dichloride entity present and taking into account the difference in the molecular weights of the various anhydrous and hydrated forms, the Acute Tox. 3 classification is relevant for all of the identified forms. It therefore applies to EC number 231-743-0 (general form of nickel dichloride).

[9] Guidance on the application of the CLP criteria. Version 3.0. ECHA. November 2012
[10] Harmful if swallowed

3.2 Nickel dihydroxide ($Ni(OH)_2$)

3.2.1 Data supporting the current classification for acute oral toxicity

No information was found on the data that had been used in the initial German classification proposal that resulted in the Xn; R22 acute oral toxicity classification in the 15th ATP to Directive 67/548/EEC. This classification is applicable when the LD_{50} is between 200 and 2000 mg/kg and equivalent to the Acute Tox. 4 – H302[11] minimum classification[12] according to the CLP Regulation.

A new classification proposal (Danish EPA, 2004c) submitted in 2004 by Denmark referred to an oral toxicity study to maintain this classification: the **Study report C** reported an LD_{50} for nickel dihydroxide of 1500 to 1700 mg $Ni(OH)_2$/kg.

Based on these data, the Xn; R22 classification for nickel dihydroxide was maintained in Annex I of Directive 67/548/EEC by its 31st ATP. This classification has not been modified since.

3.2.2 Data submitted by the industry and included in the registration dossier

The classification revision request refers to an additional acute oral toxicity study (**Study report D**, published in Henderson *et al.* 2012b), in addition to the aforementioned Study report C.
The registration dossier for nickel dihydroxide does not mention any additional studies.

The classification revision request also takes into account considerations on the bioaccessibility of nickel dihydroxide. The amount of nickel ion released *in vitro* after incubation of the substance in various synthetic physiological fluids for a few hours was measured and compared to that released from other nickel compounds (Henderson *et al.* 2012a).

3.2.3 Analysis of acute oral toxicity studies

A detailed analysis of all of the available studies is shown in Annex 3.

The studies reported by Study reports C and D are considered as being of good quality and the results of these two studies are summarised in the table below.

Study	Species	LD_{50} (for $Ni(OH)_2$
Study report C	Rats	Males: 1515 mg/kg Females: 1565 mg/kg
Study report D	Rats	Females: 5000 mg/kg

Whereas the results of the Study report D do not support any classification, those of the Study report C are consistent with the current Acute Tox 4. classification.

The two studies were undertaken in the same rat strain, with the same vehicle (water) and the same mode of administration by gavage. Like for dichloride, the smaller number of animals

[11] Mentioned as minimum by the CLP Regulation since it was obtained by automatic conversion of the pre-existing classification according to Directive 67/548/EEC
[12] Harmful if swallowed

tested (7 females versus 25 in Study report C) and testing in females only in the Study report D do not explain the observed difference in the results.

A difference between the two studies is highlighted in Henderson *et al.* (2012b) regarding the purity of the tested substance. The nickel content in the Study report C is 61% (m/m) and is 54% in the Study report D. Considering the chemical formula of nickel hydroxide, a nickel content of 64% is expected for the 100% pure substance. Based on the nickel content, the corresponding purities are therefore 96% for the Study report C and 85% for the Study report D, which is inconsistent with the >99% purity stated in the latter study. The lower purity of the tested substance in the Study report D may partly explain why a higher LD_{50} was obtained but does not appear sufficient to explain the large difference between the two results.

The most sensitive study undertaken with the substance with the highest nickel content (Study report C) has therefore been used for classification.

3.2.4 Analysis of arguments on the bioaccessibility of the substance

Since there are good-quality acute oral toxicity data that can be directly interpreted in terms of classification, data on the *in vitro* bioaccessibility of the substance are not considered essential for a conclusion.
Moreover, the available bioaccessibility data (Henderson 2012a) indicate that the amount of nickel released from nickel dihydroxide in a synthetic gastric fluid after 2 hours of incubation (143 mg Ni/g) is similar to that released by nickel sulfamate tetrahydrate (148 mg Ni/g) and nickel subsulfide (158 mg Ni/g). The oral LD_{50} values reported for these substances (which have no harmonised classification for acute oral toxicity) are 1098 mg/kg and >11000 mg/kg respectively. These two values are quite different and encompass those obtained with dihydroxide. Therefore, the LD_{50} of 1515 mg/kg for nickel dihydroxide reported in the Study report C cannot be ruled out.

3.2.5 Conclusion on classification

In conclusion, the current Acute Tox. 4 classification for nickel dihydroxide is considered appropriate in light of the results of the Study report C.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety considers that the new data reviewed do not support revising the current harmonised classification of nickel dichloride and dihydroxide for their acute oral toxicity.

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KEYWORDS

Nickel dichloride, CAS 7718-54-9, nickel dihydroxide, CAS 12054-48-7, classification, CLP, acute oral toxicity

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Study report A – confidential reference

Study report B – confidential reference

Study report C – confidential reference

Study report D – confidential reference

ANNEX(ES)

Annex 1 - Harmonised classification of nickel dichloride and dihydroxide

Index No	Chemical name	EC No	CAS No	Classification
028-008-00-X	Nickel dihydroxide	235-008-5	12054-48-7	Acute Tox. 4 – H302* Skin Irrit. 2 – H315 Skin Sens. 1 – H317 Acute Tox. 4 – H332* Resp. Sens. 1 – H334 Muta. 2 – H341 Carc. 1A – H350i Repr. 1B – H360D STOT RE 1 – H372 Aquatic Acute 1 – H400 Aquatic Chronic 1 – H410
028-011-00-6	Nickel dichloride	231-743-0	7718-54-9	Acute Tox. 3 – H301* Skin Irrit. 2 – H315** Skin Sens. 1 – H317** Acute Tox. 3 – H331* Resp. Sens. 1 – H334 Muta. 2 – H341 Carc. 1A – H350i Repr. 1B – H360D STOT RE 1 – H372** Aquatic Acute 1 – H400 Aquatic Chronic 1 – H410

* minimum classification

** concentration limits apply for these hazard classes

Annex 2 - Nickel dichloride - Summary of acute oral toxicity studies

Reference	Species	Test substance	Doses	LD_{50} (for $NiCl_2$ hexahydrate)	Results	Comments and reliability index (Klimisch score)
Itskova 1969	Rats	-	-	Males: 432 mg/kg Females: 535 mg/kg	LD_{50} of 105 mg Ni/kg for males and 130 mg Ni/kg for females.	No additional information available on the study conditions, the form of the test substance or the results. Reliability index: 4 (reliability not assignable)
Study report A	Sprague-Dawley rats (5/sex/ dose)	$NiCl_2$ $6H_2O$ (98.8%)	50, 98, 194, 381, and 750 mg/kg Gavage Vehicle: water	Males: 210 mg/kg Females: 175 mg/kg	Mortality: 50 mg/kg: 0/5 males; 0/5 females 98 mg/kg: 0/5 males; 0/5 females 194 mg/kg: 3/5 males; 4/5 females (dead before day 2) 381 mg/kg: 4/5 males; 5/5 females (dead on day 1) 750 mg/kg: 5/5 males; 5/5 females (dead on day 1) The following clinical signs were observed: reduced activity and salivation from 98 mg/kg, ataxia, swollen limbs from 194 mg/kg. Increased body weight in surviving rats. No lesions observed at autopsy. LD_{50}: Males: 210 mg/kg [95%CI: 159-261] Females: 175 mg/kg [95%CI: 131-219]	GLP study similar to the OECD 401 guideline. Deviations: environmental conditions not reported and administration volumes not specified. These deviations do not call into question the validity of the study results. Reliability index 2 (reliable with restriction).

Reference	Species	Test substance	Doses	LD_{50} (for $NiCl_2$ hexahydrate)	Results	Comments and reliability index (Klimisch score)
Study report B (Henderson 2012b)	Sprague-Dawley rats (1-3 females/ dose)	$NiCl_2$ $6H_2O$ (>97%)	159, 200, 250, 320, 400, 500, 630 and 2000 mg/kg Gavage Vehicle: water	Females: 500 mg/kg	Mortality: 159 mg/kg: 0/1 animal 200 mg/kg: 0/1 animal 250 mg/kg: 0/1 animal 320 mg/kg: 0/1 animal 400 mg/kg: 0/2 animals 500 mg/kg: 2/3 animals (dead within 2 hrs. of administration) 630 mg/kg: 1/1 animal (dead within 24 hrs. of administration) 2000 mg/kg: 1/1 animal (dead within one hour of administration) The following clinical signs were observed: temporary piloerection at 250 mg/kg, hypoactivity and abnormal posture from 500 mg/kg No effect on the body weight of the surviving animals. Discolouration and redness of the intestines were observed at autopsy from 500 mg/kg. LD_{50}: Females: 500 mg/kg [95%CI: 397-642]	GLP study consistent with the OECD 425 guideline (up-and-down method). Reliability index 1 (reliable without restriction).
Schafer 1985	Deer mice (wild type) (5 mice)	$NiCl_2$ $6H_2O$ (purity not specified)	Dose not specified and variable for each animal	888 mg/kg	Twenty-five wheat seeds treated with 2% (w/w) of the substance were offered daily for three days to the animals in addition to conventional laboratory food. Based on the number of seeds consumed and mortality, an average ingested dose that did not kill more than 50% of the animals was calculated and was 888 mg/kg/d.	This study was not undertaken in accordance with a conventional protocol, particularly in terms of the administration mode and period, which were not consistent with the current guidelines. There are uncertainties as to the administered dose, which varied for each animal, and the reliability of the results. Reliability index: 3 (not reliable)

- : no data

Annex 3 - Nickel dihydroxide - Summary of acute oral toxicity studies

Reference	Species	Test substance	Doses	LD_{50} (for $Ni(OH)_2$)	Results	Comments and reliability index (Klimisch score)
Study report C (published in Reagan 1996)	Sprague-Dawley rats (5/sex/dose)	$Ni(OH)_2$ (purity not specified; 61% nickel content cited in Henderson 2012b, correspond-ing to 96% purity)	1000, 1495, 2236, 3344, and 5000 mg/kg Gavage Vehicle: water	Males: 1515 mg/kg Females: 1565 mg/kg	Mortality: 1000 mg/kg: 1/5 males; 0/5 females 1495 mg/kg: 3/5 males; 4/5 females 2236 mg/kg: 3/5 males; 3/5 females 3344 mg/kg: 5/5 males; 5/5 females 5000 mg/kg: 5/5 males; 5/5 females Mortality was observed within 7 days after exposure. The following clinical signs were observed: lethargy, diarrhoea, unsteady gait, bloody faeces and urine. Increased body weight in surviving rats. Red fluid found in the intestines of 15 of the dead animals but no lesions observed at autopsy. LD_{50}: Males: 1515 mg/kg [95%CI: 790-2168] Females: 1565 mg/kg [95%CI: 943-2155]	GLP study similar to the OECD 401 guideline. Deviations: no deviations identified in the abstract in the registration dossier but some information such as the environmental conditions and the administration volumes were not specified. These deviations do not call into question the validity of the study results. Reliability index 2 (reliable with restriction).

Reference	Species	Test substance	Doses	LD_{50} (for $Ni(OH)_2$)	Results	Comments and reliability index (Klimisch score)
Study report D (published in Henderson et al. 2012b)	Sprague-Dawley rats (1-3 females/ dose)	$Ni(OH)_2$ (>99% stated, 54% measured nickel content correspond-ing to 85% purity)	3200, 4000, 5000, 6300 mg/kg Gavage Vehicle: water	Females: 5000 mg/kg	Mortality: 3200 mg/kg: 0/1 animal 4000 mg/kg: 0/1 animal 5000 mg/kg: 2/3 animals (dead within 4 days of administration) 6300 mg/kg: 2/2 animals (dead within 4 days of administration) The following clinical signs were observed: hypoactivity, soft stools, anogenital staining and reduced faecal volume from 5000 mg/kg in the animals that died during the study. No effect on the body weight of the surviving animals. Discolouration of the intestines and/or liver observed at autopsy of the dead animals. LD_{50}: Females: 5000 mg/kg [95%CI: 3390-5800]	GLP study consistent with the OECD 425 guideline (up-and-down method). Deviations: no deviations were reported but the measured nickel content (54%) was not consistent with the stated >99% purity and raises doubts as to the purity of the substance used. Reliability index 2 (reliable with restriction).