

**Committee for Risk Assessment**  
**RAC**

Annex 1  
**Background document**  
to the Opinion proposing harmonised classification  
and labelling at EU level of  
**Cadmium hydroxide**

**EC number: 244-168-5**  
**CAS number: 21041-95-2**

CLH-O-0000001412-86-80/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

**Adopted**  
**4 December 2015**



## **CLH report**

### **Proposal for Harmonised Classification and Labelling**

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

**Substance Name: Cadmium hydroxide**

**EC Number:** 244-168-5

**CAS Number:** 21041-95-2

**Index Number:** included in group entry 048-001-00-5

**Contact details for dossier submitter:**

Swedish Chemicals Agency

Esplanaden 3a, P.O Box 2

SE-172 13 Sundbyberg, Sweden

[kemi@kemi.se](mailto:kemi@kemi.se)

+46 8 519 41 100

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# Part A.

## 1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

### 1.1 Substance

**Table 1: Substance identity**

<b>Substance name:</b>	<i>Cadmium hydroxide</i>
<b>EC number:</b>	<i>244-168-5</i>
<b>CAS number:</b>	<i>21041-95-2</i>
<b>Annex VI Index number:</b>	<i>Included in group entry 048-001-00-5</i>
<b>Degree of purity:</b>	<i>&gt; 80 - &lt; 100 (typical value 98.5) % (w/w)</i>
<b>Impurities:</b>	<i>Nickel (catalyst) is present as NiSO<sub>4</sub> (aq) at the concentration range <math>\geq 0.0</math> - <math>\leq 1.05</math> (typical value <math>&lt; 0.1</math>) % (w/w).</i>

### 1.2 Harmonised classification and labelling proposal

**Table 2: The current Annex VI entry and the proposed harmonised classification**

	<b>CLP Regulation</b>
<b>Current entry in Annex VI, CLP Regulation</b>	Acute Tox. 4*; H302 Acute Tox. 4*; H312 Acute Tox. 4*; H332 Aquatic Acute 1; H400 Aquatic Chronic 1; H410
<b>Current proposal for consideration by RAC</b>	Carc. 1B; H350 Muta. 1B; H340 STOT RE 1; H372 (kidney, bone)
<b>Resulting harmonised classification (future entry in Annex VI, CLP Regulation)</b>	Carc. 1B; H350 Muta. 1B; H340 STOT RE 1; H372 (bone, kidney)

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	Acute Tox. 4*; H302 Acute Tox. 4*; H312 Acute Tox. 4*; H332 Aquatic Acute 1; H400 Aquatic Chronic 1; H410
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**1.3 Proposed harmonised classification and labelling based on CLP Regulation****Table 3: Proposed classification according to the CLP Regulation**

<b>CLP Annex I ref</b>	<b>Hazard class</b>	<b>Proposed classification</b>	<b>Proposed SCLs and/or M-factors</b>	<b>Current classification <sup>1)</sup></b>	<b>Reason for no classification <sup>2)</sup></b>
<b>2.1.</b>	Explosives	None		None	Hazard class not assessed in this dossier
<b>2.2.</b>	Flammable gases	None		None	Hazard class not assessed in this dossier
<b>2.3.</b>	Flammable aerosols	None		None	Hazard class not assessed in this dossier
<b>2.4.</b>	Oxidising gases	None		None	Hazard class not assessed in this dossier
<b>2.5.</b>	Gases under pressure	None		None	Hazard class not assessed in this dossier
<b>2.6.</b>	Flammable liquids	None		None	Hazard class not assessed in this dossier
<b>2.7.</b>	Flammable solids	None		None	Hazard class not assessed in this dossier
<b>2.8.</b>	Self-reactive substances and mixtures	None		None	Hazard class not assessed in this dossier
<b>2.9.</b>	Pyrophoric liquids	None		None	Hazard class not assessed in this dossier
<b>2.10.</b>	Pyrophoric solids	None		None	Hazard class not assessed in this dossier
<b>2.11.</b>	Self-heating substances and mixtures	None		None	Hazard class not assessed in this dossier
<b>2.12.</b>	Substances and mixtures which in contact with water emit flammable gases	None		None	Hazard class not assessed in this dossier
<b>2.13.</b>	Oxidising liquids	None		None	Hazard class not assessed in this dossier
<b>2.14.</b>	Oxidising solids	None		None	Hazard class not assessed in this dossier
<b>2.15.</b>	Organic peroxides	None		None	Hazard class not assessed in this dossier



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<b>2.16.</b>	Substance and mixtures corrosive to metals	None		None	Hazard class not assessed in this dossier
<b>3.1.</b>	Acute toxicity - oral	None		Acute Tox. 4*; H302	Hazard class not assessed in this dossier
	Acute toxicity - dermal	None		Acute Tox. 4*; H312	Hazard class not assessed in this dossier
	Acute toxicity - inhalation	None		Acute Tox. 4*; H332	Hazard class not assessed in this dossier
<b>3.2.</b>	Skin corrosion / irritation	None		None	Hazard class not assessed in this dossier
<b>3.3.</b>	Serious eye damage / eye irritation	None		None	Hazard class not assessed in this dossier
<b>3.4.</b>	Respiratory sensitisation	None		None	Hazard class not assessed in this dossier
<b>3.4.</b>	Skin sensitisation	None		None	Hazard class not assessed in this dossier
<b>3.5.</b>	Germ cell mutagenicity	Muta. 1B; H340		None	Harmonised classification proposed
<b>3.6.</b>	Carcinogenicity	Carc. 1B; H350		None	Harmonised classification proposed
<b>3.7.</b>	Reproductive toxicity	None		None	Hazard class not assessed in this dossier
<b>3.8.</b>	Specific target organ toxicity –single exposure	None		None	Hazard class not assessed in this dossier
<b>3.9.</b>	Specific target organ toxicity – repeated exposure	STOT RE 1; H372 (kidney, bone)		None	Harmonised classification proposed
<b>3.10.</b>	Aspiration hazard	None		None	Hazard class not assessed in this dossier
<b>4.1.</b>	Hazardous to the aquatic environment	None		Aquatic Acute 1; H400 Aquatic Chronic 1; H410	Hazard class not assessed in this dossier
<b>5.1.</b>	Hazardous to the ozone layer	None		None	Hazard class not assessed in this dossier

<sup>1)</sup> Including specific concentration limits (SCLs) and M-factors

<sup>2)</sup> Data lacking, data inconclusive, data conclusive but not sufficient for classification, hazard class not assessed in this dossier, or harmonized classification proposed.

**Labelling:**

Signal word:

Danger

Hazard statements:

H340; H350; H372 (bone, kidney)

Precautionary statements:

No precautionary statements are proposed since precautionary statements are not included in Annex VI of Regulation EC no. 1272/2008.

Pictogram:

GHS08



**Proposed notes assigned to an entry:**

None.

## **2 BACKGROUND TO THE CLH PROPOSAL**

### **2.1 History of the previous classification and labelling**

There is no harmonised classification for cadmium hydroxide other than the harmonised classification justified by the Annex VI group entry with index number 048-001-00-5, i.e. Acute Tox. 4\* (H302, H312, H332), Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410). However, specific harmonised classification exists for other cadmium compounds (see the Classification & Labelling Inventory (ECHA, 2015a)).

The registrant has classified cadmium hydroxide in:

- Carc. 1B; H350
- Muta. 2; H341
- Repr. 2; H361
- STOT RE 1; H372 (kidney, lung, bone) (Inhalation)
- Acute Tox. 2; H330
- Aquatic Acute 1; H400

- Aquatic Chronic 1; H410

## **2.2 Short summary of the scientific justification for the CLH proposal**

In mammalian toxicity, the toxicity of inorganic cadmium compounds is regarded to result from the intrinsic properties of the  $\text{Cd}^{2+}$  ion. Conversely, it could be argued that the toxicity of any diffusible, and thus bioavailable, inorganic cadmium compound could be used to determine the intrinsic properties of the  $\text{Cd}^{2+}$  ion, i.e. information on toxicity could be read across from one cadmium compound to another (please refer to Section 4). Taken into consideration available data from the registration and reviews of cadmium toxicity by other regulatory bodies (JRC, 2007; EFSA, 2009; ATSDR, 2012), the dossier submitter concludes that cadmium hydroxide should be classified in:

- Carc. 1B; H350, since treatment related tumours were observed in two species (rat and mouse), in three different studies in one species (rat), in both sexes of one species (rat), and that tumours occurred at multiple sites and/or were of different types.
- Muta 1B; H340, since structural chromosome aberrations were induced in somatic cells of mice, micronuclei were induced in somatic cells of mice and rats, and that numerical and structural chromosome aberrations were induced in the germ cells of mice.
- STOT RE 1 (kidney, bone); H372, since significant toxicity in humans was demonstrated in kidney and bone

## **2.3 Current harmonised classification and labelling**

### **2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation**

Acute Tox. 4\*; H302, H312, H332

Aquatic Acute 1; H400

Aquatic Chronic 1; H410

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2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Table 5: Self-classification according to the Classification and Labelling inventory January 23, 2015 (ECHA, 2015a).

Classification		Labelling			Specific Concentration limits, M-Factors	Notes	Number of Notifiers	Joint Entries
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code(s)				
Acute Tox. 4	H302	H302		GHS07 GHS09 Wng			23	
Acute Tox. 4	H312	H312						
Acute Tox. 4	H332	H332						
Aquatic Acute 1	H400							
Aquatic Chronic 1	H410	H410						
Acute Tox. 4	H302	H302		GHS07 GHS09 Wng		<u>Note A</u> <u>Note 1</u>	15	
Acute Tox. 4	H312	H312						
Acute Tox. 4	H332	H332						
Aquatic Acute 1	H400	H400						
Aquatic Chronic 1	H410	H410						
Acute Tox. 2	H330	H330		GHS06 GHS09 GHS08 Dgr	M(Chronic)=10 M=10		4	✔
Muta. 2	H341	H341						
Carc. 1B	H350	H350						
Repr. 2	H361	H361						

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		(H361fd is exact statement (translation of R60-61))						
STOT RE 1	H372 (kidney, lung, bone) (Inhalation)	H372						
Aquatic Acute 1	H400							
Aquatic Chronic 1	H410	H410						
		H410						
		H312						
		H400		GHS07 GHS09 Wng			2	
		H332						
		H302						

**RAC general comment****Background information in support of the proposal**

Cadmium hydroxide is currently among a number of cadmium salts classified in CLP Annex VI under the group entry with Index No. 048-001-00-5. This entry indicates a classification of Acute Tox 4\*, H302, Acute Tox 4\*; H312, Acute Tox 4\*; H332, Aquatic Acute 1; H400 and Aquatic Chronic 1; H410.

The Dossier Submitter (DS) has proposed a new entry specifically for cadmium hydroxide itself. This would carry across without any change the current classification provided in the group entry and then proposes to add classification for repeated dose toxicity (STOT RE 1; H372 – kidney, bone), germ cell mutagenicity (Muta. 1B; H340) and carcinogenicity (Carc. 1B; H350).

The DS has described how the systemic toxicity of inorganic cadmium compounds is commonly regarded to result from the intrinsic properties of the Cd<sup>2+</sup> ion. Those compounds from which Cd<sup>2+</sup> is readily bioavailable will therefore share common hazards. As there is no definitive data available on the systemic toxicity of cadmium hydroxide, data on the bioavailability of Cd<sup>2+</sup> from this compound were used by the DS to predict its toxicity.

For those compounds with high water solubility, a high degree of bioavailability can be assumed. At lower levels of water solubility, bioavailability may also depend on other factors.

As shown in the following table, received from the International Cadmium Association during the Public Consultation, the water solubility of cadmium hydroxide is rather low.

Ranking of solubility	Cadmium compound	Water solubility (mg/L) <sup>2</sup>	Harmonized classification <sup>1</sup>		
			Carcinogenicity	Mutagenicity	STOT RE
Very soluble	Cadmium sulphate	540 x 10 <sup>3</sup>	1B; H350	1B; H340	1; H372
	Cadmium nitrate	507 x 10 <sup>3</sup>			
	Cadmium chloride	457 x 10 <sup>3</sup>	1B; H350	1B; H340	1; H372
	Cadmium fluoride	35 x 10 <sup>3</sup>	1B; H350	1B; H340	1; H372
Slightly soluble	Cadmium hydroxide	69.5			
	Cadmium carbonate	3.2			
	Cadmium metal	2.3	1B; H350	2; H341	1; H372
	Cadmium oxide	2.1	1B; H350	2; H341	1; H372
Insoluble	Cadmium sulphide	6.10 <sup>-7</sup>	1B; H350	2; H341	1; H372

<sup>1</sup> Only harmonised classifications for carcinogenicity, mutagenicity and STOT RE are presented, since that is within the scope of the present CLH report. However, cadmium sulphate, chloride and fluoride also have harmonised classifications for acute toxicity, reproductive toxicity, acute and chronic aquatic toxicity (ECHA, 2015).

<sup>2</sup> Solubility data as presented in the CSR part of the REACH registration (2015), except for cadmium fluoride where solubility data was from ECB (1997).

However, the DS also summarised a study showing 87% solubility of cadmium carbonate (94% for cadmium oxide and 5% for cadmium sulphide) in artificial gastric juice (pH = 1.47) during a 2-hour incubation period at 37°C. RAC notes though that the solubility in artificial body fluids depends on many factors, including the loading ratio (how much substance is added to how much solvent), the temperature, solubilisation time, presence of soluble impurities and particle size. However, it can be predicted that Cd<sup>2+</sup> ions would be evolved in the stomach following oral ingestion of cadmium carbonate and that, in a similar fashion to the systemically toxic, highly water soluble salts, there will be uptake into the general circulation. For example, the DS commented that Cd<sup>2+</sup> can be absorbed from the proximal duodenum either by simple

diffusion or can be mediated by the metal-ion transporter DMT1. Although there is no data to show that cadmium hydroxide is soluble in gastric juice, RAC considers that the evidence demonstrating that cadmium carbonate is highly soluble in gastric juice can be extrapolated to cadmium hydroxide because both have similarly low water solubilities. This study showed that low solubility in water does not necessarily equate to low solubility *in vivo* i.e. a cadmium compound that is poorly soluble in water may be considerably more soluble in gastric juice and thus the toxic Cd<sup>2+</sup> ion will be evolved *in vivo* and become systemically available following oral exposure.

The ECHA guidance on the application of the CLP criteria (CLP Guidance) states that, "*Bioavailability of a substance or a mixture is normally assumed if there are in vitro studies available which show the solubility of a substance or mixture in body fluids or artificial simulated body fluids.*"

Cadmium oxide, a substance with similar water solubility to cadmium hydroxide, showed bioavailability following inhalational exposure of rats, evidence by systemic effects on fertility and reproductive organs, as well as general and developmental toxicity. This indicates that the systemic effects of Cd<sup>2+</sup> ions are likely to occur following inhalational exposure to less water soluble cadmium compounds, including cadmium hydroxide.

RAC therefore agrees with the DS that it is reasonable to consider the systemic hazards of cadmium hydroxide using data from other inorganic cadmium compounds, for which Cd<sup>2+</sup> bioavailability is apparent, although there is some uncertainty on the extent of solubility in (artificial) body fluids of these slightly water soluble cadmium compounds, and hence on the degree of bioavailability of Cd<sup>2+</sup> (especially following inhalation exposure).

The DS has proposed a new entry specifically for cadmium hydroxide itself. This would carry across without any change the classification provided in the group entry and add classification for repeated dose toxicity (STOT RE 1; H372 – kidney, bone), germ cell mutagenicity (Muta. 1B; H340) and carcinogenicity (Carc. 1B; H350). However, only repeated dose toxicity, mutagenicity and carcinogenicity were assessed in the CLH report.

The DS has provided an assessment only of repeated dose toxicity, mutagenicity and carcinogenicity in their CLH report.

RAC has not addressed the scientific validity of the proposal from the Dossier Submitter to retain by default the existing classifications for acute toxicity and aquatic toxicity. No data were provided in the CLH report for such an assessment to be made and this was outside the scope of the public consultation. Similarly, although some inorganic cadmium compounds are classified for reproductive toxicity in Annex VI of the CLP Regulation, this endpoint was also not assessed by RAC.

### 3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Cadmium hydroxide has CMR properties. Harmonised classification and labelling for CMR and respiratory sensitisation is a community-wide action under article 36 of the CLP regulation.

The reason for a need for action at Community level regarding STOT RE is differences in self-classification. The dossier submitter has focussed on toxicity in kidney and bone, since these organs are the most sensitive from a STOT RE perspective. Even though the lung is also a known target organ, the most sensitive effect caused by lung exposure is lung cancer, for which harmonised classification is proposed and, therefore, toxicity in the lung has not been evaluated with respect to specific target organ toxicity after repeated exposure in this CLH report.

# Part B.

## SCIENTIFIC EVALUATION OF THE DATA

### 1 IDENTITY OF THE SUBSTANCE

#### 1.1 Name and other identifiers of the substance

**Table 6: Substance identity**

<b>EC number:</b>	244-168-5
<b>EC name:</b>	Cadmium hydroxide
<b>CAS number (EC inventory):</b>	21041-95-2
<b>CAS number:</b>	21041-95-2
<b>CAS name:</b>	Cadmium hydroxide
<b>IUPAC name:</b>	Cadmium dihydroxide
<b>CLP Annex VI Index number:</b>	Included in group entry 048-001-00-5
<b>Molecular formula:</b>	Cd(OH) <sub>2</sub>
<b>Molecular weight range:</b>	146.4257

**Structural formula:**





## 1.2 Composition of the substance

**Table 7: Constituents (non-confidential information)**

Constituent	Typical concentration	Concentration range	Remarks
Cadmium hydroxide	98.5 % (w/w)	> 80 - < 100.0 % (w/w)	

Current Annex VI entry: included in the group entry 048-001-00-5

**Table 8: Impurities (non-confidential information)**

Impurity	Typical concentration	Concentration range	Remarks
Nickel	< 0.1 % (w/w)	$\geq 0.0 - \leq 1.05$ % (w/w)	Ni is present as NiSO <sub>4</sub> (aq)

Current Annex VI entry: 028-009-00-5

**Table 9: Additives (non-confidential information)**

Additive	Function	Typical concentration	Concentration range	Remarks
-				

Current Annex VI entry: None

### 1.2.1 Composition of test material

## 1.3 Physico-chemical properties

**Table 10: Summary of physico - chemical properties**

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	The physical state of the substance is solid homogeneous powder, its colour is greenish white, it is odourless.	REACH registration (ECHA 2015b)	No guideline followed. Appearance and physical state were determined by visual inspection, odour by smell (organoleptic).
Melting/freezing point	The melting and decomposition of the material starts in nitrogen at 186°C and at 188°C in air.	REACH registration (ECHA 2015b)	EU Method A.1 (Melting / Freezing Temperature)
Boiling point		Data waived in REACH registration (ECHA 2015b)	
Relative density	The density of the substance is 4.73 g/cm <sup>3</sup> .	REACH registration (ECHA 2015b)	EU Method A.3 (Relative Density)
Vapour pressure		Data waived in REACH registration (ECHA 2015b)	
Surface tension		Data waived in REACH registration (ECHA 2015b)	
Water solubility	The experimentally determined average solubility at 20°C of Cd in the substance is 69.5 mg/l (slightly soluble). The corresponding value for the pure compound was also calculated with HSC 7.0 software. The obtained value was 2.5 mg/l. The calculated solubility product for Cd(OH) <sub>2</sub> was found to be in good agreement with the value found in the literature .	REACH registration (ECHA 2015b)	OECD Guideline 105 (Water Solubility)
Partition coefficient n-octanol/water		Data waived in REACH registration (ECHA 2015b)	
Flash point		Data waived in REACH registration (ECHA 2015b)	
Flammability		Data waived in REACH registration (ECHA 2015b)	
Explosive properties		Data waived in REACH registration (ECHA 2015b)	

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Self-ignition temperature		Data waived in REACH registration (ECHA 2015b)	
Oxidising properties	No oxidation occurred, the sample is very stable	REACH registration (ECHA 2015b)	No guideline followed. The Pourbaix diagram of the substance was calculated using the EpH module of the HSC 7.0 program.
Granulometry	The D50 of the substance is 146 µm, the D80 is 384 µm.	REACH registration (ECHA 2015b)	ASTM E323-09 standard specification for perforated plate sieves for testing purposes.
Stability in organic solvents and identity of relevant degradation products		Data waived in REACH registration (ECHA 2015b)	
Dissociation constant		Data waived in REACH registration (ECHA 2015b)	
Viscosity		Data waived in REACH registration (ECHA 2015b)	

## 2 MANUFACTURE AND USES

### 2.1 Manufacture

Not relevant for this CLH report.

### 2.2 Identified uses

- Tonnage band: 1000-10 000 (ECHA, 2015b)
- Uses (Danish Environmental Protection Agency, 2013):
  - Component for production of inorganic cadmium compounds
  - Component for production of organic cadmium compounds
  - Component for production of inorganic pigments
  - Cadmium production by pyrometallurgy
  - Batteries / Fuel cells
  - Electroplating and electroplating
  - Laboratory reagent

### 3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this report.

### 4 HUMAN HEALTH HAZARD ASSESSMENT

#### Equivalence between cadmium salts in mammalian toxicity

In mammalian toxicity, the toxicity of any inorganic cadmium compound, i.e. cadmium salt, is regarded to result from the intrinsic properties of the  $\text{Cd}^{2+}$  ion; see for example the European Union Risk Assessment Report (EU RAR) – Volume 74 cadmium metal, Part II Human Health (JRC, 2007). The solubility of a cadmium salt in body fluids at sites where exposure occurs is a factor affecting the bioavailability of the  $\text{Cd}^{2+}$  ion and, accordingly, the level of systemic exposure of the  $\text{Cd}^{2+}$  ion may be different for different cadmium salts. However, the solubility in water of a cadmium salt may not be indicative of its solubility in a certain body fluid. Plain examples of this are cadmium oxide (very low solubility in water), cadmium carbonate (very low solubility in water) and cadmium sulphide (practically insoluble in water), for which the solubility in artificial gastric juice (pH 1.47) has been demonstrated to be 94 %, 87 % and 5 %, respectively, and in artificial intestinal juice (pH 8.20) 0.151 %, 0.015 % and 0.112 %, respectively, after incubation for 2 hours at 37 °C (Wada et al., 1972). Information on solubility in artificial simulated body fluids is considered to be of importance for evaluating the bioavailability of a substance, as indicated by the following statement in section 1.3.2.1 of the ECHA guidance on the application of the CLP criteria (ECHA, 2013): “*Bioavailability of a substance or a mixture is normally assumed if there are in vitro studies available which show the solubility of a substance or mixture in body fluids or artificial simulated body fluids.*”

The high solubility of the above cadmium salts in artificial gastric juice compared to their solubility in water is related to the low pH of gastric juice caused by its content of hydrochloric acid. Thus, one characteristic of the gastric chyme is the occurrence of an excess of chloride ions derived from the gastric juice. This implies that, following dissociation of any cadmium salt in the gastric chyme, the  $\text{Cd}^{2+}$  ions would be encircled by chloride ions at a much higher concentration than that of its counter ion. Accordingly, the chemical conditions in the stomach and the proximal duodenum arising from oral administration of a cadmium salt would be comparable for all cadmium salts soluble in gastric juice. As the gastric chyme is expelled by the stomach into the duodenum, a low pH prevails in the proximal part of the duodenum, from which ionic cadmium can be absorbed either by simple diffusion or mediated by the metal-ion transporter DMT1 (Tallkvist et al. 2001, Park et al., 2002). Hence, it can be considered that the  $\text{Cd}^{2+}$  ion of cadmium oxide, cadmium carbonate and cadmium sulphide is bioavailable following oral exposure.

In more distal parts of the intestine the pH is higher due to the pH of the intestinal juice, in which the solubility of a cadmium salt would be lower, as reflected by the solubility in artificial intestinal juice of the three cadmium salts in the study referred to above (Wada et al., 1972). However, since a cadmium salt administered orally would dissociate already in the stomach when reaching the gastric chyme, it would enter into the distal part of the intestine in the ionic state. Consequently, this would mean that conditions in other parts of the intestine that might have an influence on the toxicity of a cadmium salt, for example the increase in pH occurring already in the distal duodenum, would be similar regardless of the cadmium salt used in a study.

Obviously, the conditions described above also apply to cadmium chloride, which is the cadmium salt that has been used in most experimental studies conducted to evaluate the toxicity of the  $\text{Cd}^{2+}$

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ion in animals. Hence, results from oral studies with cadmium chloride would be relevant for evaluating the toxic hazards of any cadmium salt considered to be soluble in gastric juice.

Bioavailability of the  $\text{Cd}^{2+}$  ion following inhalation was observed in a carcinogenicity study in male Wistar (TNO/W75) rats exposed for 23 hours per day, 7 days a week for 18 months to cadmium chloride aerosols with cadmium concentrations of 0, 12.5, 25 and 50  $\mu\text{g}/\text{m}^3$  (Takenaka *et al.*, 1983). After the end of the exposure period, the rats were observed for an additional 13 months before the study was ended. It is stated in the publication that the remaining cadmium concentrations in the lungs were relatively high at the end of the study and that they were almost at the same level as those observed in the livers.

Studies on absorption of cadmium chloride and cadmium sulphide in rats after inhalation exposure 6 hours per day during 10 days showed that, for each salt, 40 % of the deposited material was cleared during the exposure period and that, at 3 months after the last exposure (end of the study), 56 % and 81 % of the lung burden at the end of the exposure period was cleared for cadmium chloride and cadmium sulphide, respectively (Klimish, 1993). Accumulation of cadmium in the kidney at the end of the study was 35 % and 1 % for cadmium chloride and cadmium sulphide, respectively, indicating bioavailability for both, although the latter is practically insoluble in water.

Of the few existing studies addressing systemic toxicity following inhalation of cadmium oxide in animals, the study by Dunnick (1995) has the highest reliability. In this study systemic toxicity was demonstrated in rats by effects on fertility and reproductive organs (males and females) as well as by general and developmental toxicity, and in mice by general and developmental toxicity. According to the ECHA guidance on the application of the CLP criteria (ECHA, 2013), information on systemic toxicity is of importance for evaluating the bioavailability of a substance, as expressed in the following statement in section 1.3.2. of the guidance: *“In general, bioavailability is not explicitly evaluated in hazard classification – the observation of systemic toxicity implicitly demonstrates a degree of bioavailability.”* Hence, from the results of the study by Dunnick (1995), it can be considered that the  $\text{Cd}^{2+}$  ion of cadmium oxide, which has very low solubility in water, is bioavailable following inhalation exposure.

In conclusion, there is experimental support for bioavailability of the  $\text{Cd}^{2+}$  ion of cadmium salts with very low solubility in water, i.e. cadmium oxide, cadmium carbonate and cadmium sulphide after oral exposure, and cadmium oxide and cadmium sulphide after inhalation exposure. Hence, it is reasonable to consider that the  $\text{Cd}^{2+}$  ion of other water-soluble cadmium salts would also be bioavailable after oral and inhalation exposure, since the solubility of these cadmium salts in the body fluids at the sites of exposure would not be expected to be significantly lower than for the cadmium salts referred to above. Turned into a general concept, this would mean that any cadmium salt could in principle be used in experimental studies to evaluate the health hazards of the  $\text{Cd}^{2+}$  ion, although systemic toxicity of the  $\text{Cd}^{2+}$  ion may be more efficiently detected by using cadmium salts with high solubility in the body fluids at the sites of exposure, since this may result in higher systemic doses of the  $\text{Cd}^{2+}$  ion than those that would be obtained by using less soluble cadmium salts. Accordingly, it would be adequate to evaluate the health hazards of cadmium hydroxide by using data from other cadmium salts, of which cadmium chloride, which is highly soluble in water, has been used in most experimental studies.

On theoretical grounds it would be possible to argue that the counter ion of cadmium hydroxide could potentially contribute to the toxic effects to be evaluated. However, such potential contributions would not affect the classification for carcinogenicity and mutagenicity, since these classifications are based on animal studies in which animals were exposed to cadmium chloride (and in some studies also a few additional cadmium compounds), which is classified in the highest possible category based on animal data, i.e. Carc. 1B and Muta. 1B. Neither would the classification

for specific target organ toxicity after repeated exposure be affected, since the proposed classification is based on studies in humans exposed to cadmium resulting in classification in the highest possible category, i.e. STOT RE 1.

The basis for the general concept presented above has also been expressed in a human health assessment for cadmium oxide produced by the National Industrial Chemicals Notification and Assessment Scheme under the Department of Health in Australia, in which it is stated that the high solubility of cadmium oxide in gastric juice means that water soluble cadmium salts such as cadmium chloride are appropriate analogues for orally administered cadmium oxide (NICNAS, 2013). A similar approach was presented in the EU RAR for cadmium metal (JRC, 2007) by stating that it is reasonable to assume that gastrointestinal absorption of cadmium oxide is not significantly different from that of other cadmium salts, mainly because of the high solubility of cadmium oxide in gastric juice and, therefore, data from studies conducted with other cadmium salts are judged relevant for assessing the gastrointestinal absorption of cadmium oxide. Further, in the Toxicological Profile for Cadmium produced by the Agency for Toxic Substances and Disease Registry (ATSDR, 2012) it is noted that the mechanism of action for an observed toxic effect involves the effect of the cadmium cation and that the cation is the same regardless of the anion species, and that cadmium oxide and cadmium carbonate (which are relatively insoluble in water but may dissolve at gastric pH) appear to be similar in absorption and toxicity to soluble cadmium.

#### **4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)**

Please refer to Section 4 for relevant information.

#### **4.2 Acute toxicity**

Not evaluated in this report.

#### **4.3 Specific target organ toxicity – single exposure (STOT SE)**

Not evaluated in this report.

#### **4.4 Irritation**

Not evaluated in this report.

##### **4.4.1 Eye irritation**

Not evaluated in this report.

##### **4.4.2 Respiratory tract irritation**

Not evaluated in this report.

#### **4.5 Corrosivity**

Not evaluated in this report.

## **4.6 Sensitisation**

### **4.6.1 Skin sensitisation**

Not evaluated in this report.

### **4.6.2 Respiratory sensitisation**

Not evaluated in this report.

## **4.7 Specific target organ toxicity – repeated exposure**

Scientific reviews of repeated dose toxicity studies on cadmium compounds have been produced by several parties, for example, JRC (2007, EU RAR), EFSA (2009, Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on cadmium in food) and ATSDR (2012, Toxicological Profile for Cadmium). Cadmium has been shown to affect many organs after repeated exposure. This CLH report will, however, only focus on the toxic effects on kidney and bone, since these organs are highlighted in scientific reviews as being critical target organs for cadmium-induced toxicity.

As is evident from the reviews referred to above, evidence for cadmium toxicity in kidney and bone is available from reliable studies in humans. Such evidence meets the criteria for a STOT RE classification in Category 1. Given that, results from studies in animals would not add any information necessary for the classification. Thus, summaries of studies in animals are not included in this CLH report. To summarise studies in humans demonstrating cadmium toxicity in kidney and bone, the study summaries from the scientific review produced by ATSDR (2012) have been incorporated into the CLH report (see Section 4.7.2).

### **4.7.1 Non-human information**

Not evaluated in this CLH report for the reason stated in Section 4.7.

### **4.7.2 Human information**

#### **4.7.2.1 Effects on kidney**

Table 10: Summary table of repeated dose toxicity studies relevant for classification as STOT RE according to the CLP regulation (adapted from ATSDR (2012))

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Method	Results	Remarks	Reference
<b>Oral exposure</b>			
<ul style="list-style-type: none"> <li>Type of population: general population (Belgium); 1699 males, females, 20-80 years old</li> <li>Effect biomarker: <math>\beta</math>2M, protein, NAG, amino acids, calcium</li> </ul>	<ul style="list-style-type: none"> <li>Significant correlation between urinary cadmium and effect biomarkers</li> <li>Dose-response relationship between urinary cadmium and prevalence of abnormal effect biomarker levels.</li> </ul>	-	Buchet et al. (1990)
<ul style="list-style-type: none"> <li>Type of population: residents in cadmium-polluted area (includes occupationally exposed subjects (Sweden)</li> <li>Effect biomarker: pHC</li> </ul>	<ul style="list-style-type: none"> <li>Mean urinary cadmium level: 0.81 <math>\mu</math>g/g creatinine (M), 0.66 <math>\mu</math>g/g creatinine (F)</li> <li>Linear relationship between urinary cadmium and pHC (relationship remained significant after removal of occupationally exposed subjects.</li> </ul>	-	Järup et al. (2000)
<ul style="list-style-type: none"> <li>Type of population: general population (United States); 88 males, 71 females, 6-17 years old; 71 males, 80 females <math>\geq</math>18 years old</li> <li>Effect biomarker: <math>\beta</math>2M, NAG, AAP, albumin</li> </ul>	<ul style="list-style-type: none"> <li>0.07 <math>\mu</math>g/g creatinine (M, child), 0.08 <math>\mu</math>g/g creatinine (F, child), 0.24 <math>\mu</math>g/g creatinine (M, adult), 0.23 <math>\mu</math>g/g creatinine (F, adult)</li> <li>No significant associations (after correction for age, sex) between urinary cadmium and effects biomarkers in children.</li> <li>Significant association after age and gender adjustment) between urinary cadmium and NAG and AAP in adults.</li> <li>Dose-response relationship between urinary cadmium and NAG and AAP.</li> </ul>	-	Noonan et al. (2002)
<ul style="list-style-type: none"> <li>Type of population: residents in cadmium-polluted area (Japan); 878 males, 972 females</li> <li>Effect biomarker: <math>\beta</math>2M</li> </ul>	<ul style="list-style-type: none"> <li>Dose-response relationship between cadmium in rice and effect biomarker.</li> </ul>	-	Nogawa et al. (1989)
<ul style="list-style-type: none"> <li>Type of population: general population (Japan); 558 males, 743 females, <math>\geq</math>50 years old</li> <li>Effect biomarker: <math>\beta</math>2M, total protein, NAG</li> </ul>	<ul style="list-style-type: none"> <li>Mean urinary cadmium level: 1.3 <math>\mu</math>g/g creatinine (M), 1.3 <math>\mu</math>g/g creatinine (F)</li> <li>Significant correlation between urinary cadmium and effect biomarkers (NAG was only significant in females).</li> <li>Dose-response relationship between urinary cadmium and prevalence of abnormal effect biomarker levels</li> </ul>	-	Yamanaka et al. (1998)
<ul style="list-style-type: none"> <li>Type of population: general population (Japan); 568 males, 742 females, <math>\geq</math>50 years old</li> </ul>	<ul style="list-style-type: none"> <li>Mean urinary cadmium level: 2.2-3.4 <math>\mu</math>g/g creatinine (M), 2.8-3.9 <math>\mu</math>g/g creatinine (F)</li> </ul>	-	Oo et al. (2000)



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<ul style="list-style-type: none"> <li>• Effect biomarker: total protein, NAG, <math>\beta</math>2M</li> </ul>	<ul style="list-style-type: none"> <li>• Significant correlation (with age adjustment) between urinary cadmium and effect biomarkers.</li> </ul>		
<ul style="list-style-type: none"> <li>• Type of population: general population (Japan); 1105 males, 1648 females, <math>\geq</math>50 years old</li> <li>• Effect biomarker: <math>\beta</math>2M, total protein, NAG</li> </ul>	<ul style="list-style-type: none"> <li>• Mean urinary cadmium level: 1.8 <math>\mu</math>g/g creatinine (M), 2.4 <math>\mu</math>g/g creatinine (F)</li> <li>• Significant correlation between urinary cadmium and protein and <math>\beta</math>2M.</li> <li>• Dose-response relationship between urinary cadmium and prevalence of abnormal effect biomarker levels.</li> </ul>	-	Suwazono et al. (2000)
<ul style="list-style-type: none"> <li>• Type of population: residents in cadmium-polluted area (China); 118 males, 170 females, high exposure group; 80 males, 158 females, moderate exposure group</li> <li>• Effect biomarker: <math>\beta</math>2M, RBP, albumin</li> </ul>	<ul style="list-style-type: none"> <li>• Mean urinary cadmium level: 11.18 <math>\mu</math>g/g creatinine (high exposure group), 3.55 <math>\mu</math>g/g creatinine (moderate exposure group)</li> <li>• Significant correlation between urinary cadmium and effect biomarkers.</li> <li>• Dose-response relationship between urinary cadmium and prevalence of abnormal effect biomarker levels.</li> </ul>	-	Jin et al. (2002)
<ul style="list-style-type: none"> <li>• Type of population: residents in cadmium-polluted area (China); 118 males, 170 females, high exposure group; 80 males, 158 females, moderate exposure group</li> <li>• Effect biomarker: <math>\beta</math>2M, NAG, NAG-B, RBP, albumin</li> </ul>	<ul style="list-style-type: none"> <li>• Dose-response relationship between urinary cadmium and prevalence of abnormal effect biomarker levels.</li> </ul>	-	Jin et al. (2004a)
<b>Inhalation exposure</b>			
<ul style="list-style-type: none"> <li>• Type of population: zinc-cadmium smelter workers (n=87)</li> </ul>	<ul style="list-style-type: none"> <li>• Effect: age-related decline in maximal GFR was exacerbated in workers with cadmium-induced microproteinuria.</li> <li>• Adverse effect level (U-Cd): 11.1 <math>\mu</math>g/g creatinine</li> </ul>	-	Roels et al. (1991)
<ul style="list-style-type: none"> <li>• Type of population: workers using cadmium pigments in plastic production or using cadmium in welding (n=27)</li> </ul>	<ul style="list-style-type: none"> <li>• Effect: significant increase in urinary <math>\beta</math>2M and NAG levels.</li> <li>• Adverse effect level (U-Cd): 5 <math>\mu</math>g/g creatinine</li> </ul>	-	Verschoor et al. (1987)
<ul style="list-style-type: none"> <li>• Type of population: cadmium alloy workers (n=164)</li> </ul>	<ul style="list-style-type: none"> <li>• Effect: higher incidence of increased urinary <math>\beta</math>2M levels (<math>&gt;</math>250 <math>\mu</math>g/L cut-off) when urinary cadmium levels exceeded 10 <math>\mu</math>g/g creatinine on one or more occasions, as compared to workers who never exceeded the 10 <math>\mu</math>g/g</li> </ul>	-	Toffoletto et al. (1992)

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	<p>creatinine level.</p> <ul style="list-style-type: none"> <li>• Adverse effect level (U-Cd): 10 µg/g creatinine</li> </ul>		
<ul style="list-style-type: none"> <li>• Type of population: cadmium smelter workers (n=53)</li> </ul>	<ul style="list-style-type: none"> <li>• Effect: significant increase in urinary protein and β2M levels.</li> <li>• Adverse effect level (U-Cd): 13.3 µg/g creatinine</li> </ul>	-	Shaikh et al. (1987)
<ul style="list-style-type: none"> <li>• Type of population: non-ferrous smelter workers (n=58)</li> </ul>	<ul style="list-style-type: none"> <li>• Effect: significant increase in urinary β2M, RBP protein, pHc, albumin, and transferrin levels.</li> <li>• Adverse effect level (U-Cd): &gt;10 µg/g creatinine</li> </ul>	-	Bernard et al. (1990)
<ul style="list-style-type: none"> <li>• Type of population: workers exposed to cadmium pigment dust (n=58)</li> </ul>	<ul style="list-style-type: none"> <li>• Significant correlation between urinary cadmium and NAG levels.</li> <li>• Significant correlation with β2M at one of the two time points.</li> <li>• Adverse effect level (U-Cd): 1.1-1.4 µg/g creatinine</li> </ul>	-	Kawada et al. (1989)
<ul style="list-style-type: none"> <li>• Type of population: zinc-cadmium smelter workers (n=50)</li> </ul>	<ul style="list-style-type: none"> <li>• Significant association between urinary cadmium levels and urinary levels of NAG, albumin, and transferrin</li> <li>• At higher urinary cadmium levels (10 µg/g creatinine), there were significant associations with RBP and β2M.</li> <li>• Adverse effect level (U-Cd): 4 µg/g creatinine</li> </ul>	-	Roels et al. (1993)
<ul style="list-style-type: none"> <li>• Type of population: battery workers (n=561)</li> </ul>	<ul style="list-style-type: none"> <li>• 10 % prevalence of abnormal β2M levels (220 µg/g creatinine cut-off).</li> <li>• Adverse effect level (U-Cd): 1.5 µg/g creatinine for ≥60 years of age; 5 µg/g creatinine for &lt;60 years of age</li> </ul>	-	Järup and Elinder (1994)
<ul style="list-style-type: none"> <li>• Type of population: alkaline battery factory workers (n=102)</li> </ul>	<ul style="list-style-type: none"> <li>• 10 % prevalence of renal dysfunction (β2M &gt;380 µg/g creatinine; RBP &gt;130 µg/g creatinine).</li> <li>• Adverse effect level (U-Cd): 10-15 µg/g creatinine.</li> </ul>	-	Jakubowski et al. (1987)
<ul style="list-style-type: none"> <li>• Type of population: workers at a factory using cadmium-containing solders (n=60)</li> </ul>	<ul style="list-style-type: none"> <li>• 25 % prevalence of abnormal β2M levels (300 µg/g creatinine cut-off).</li> <li>• Adverse effect level (U-Cd): 2-5 µg/g creatinine.</li> </ul>	-	Elinder et al. (1985a)

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<ul style="list-style-type: none"> <li>• Type of population: workers at nickel-cadmium battery factory (n=92)</li> </ul>	<ul style="list-style-type: none"> <li>• Significant increase in pHC and NAG levels (after adjustment for age, gender, and race).</li> <li>• Adverse effect level (U-Cd): 5-10 µg/g creatinine.</li> </ul>	-	Chia et al. (1992)
<ul style="list-style-type: none"> <li>• Type of population: cadmium smelter workers (n=85)</li> </ul>	<ul style="list-style-type: none"> <li>• Significant increases in β2M and NAG levels and increased prevalence of abnormal levels of these biomarkers.</li> <li>• Adverse effect level (U-Cd): 5-10 µg/g creatinine.</li> </ul>	-	Chen et al. (2006a, 2006b)
<ul style="list-style-type: none"> <li>• Type of population: alkaline battery factory workers (n=141)</li> </ul>	<ul style="list-style-type: none"> <li>• 10 % prevalence of renal dysfunction (β2M &gt;300 µg/g creatinine; RBP &gt;300 µg/g creatinine).</li> <li>• Adverse effect level (B-Cd): 300 µg-years/L (30 years of 10 µg/L)</li> </ul>	-	Jakubowski et al. (1992)
<ul style="list-style-type: none"> <li>• Type of population: battery workers (n=440)</li> </ul>	<ul style="list-style-type: none"> <li>• Approximately 10 % prevalence of abnormal β2M levels (35 µg/mmol creatinine cut-off).</li> <li>• Adverse effect level (B-Cd): 5.6 µg/L; cumulative exposure 691 µg-years/m<sup>3</sup></li> </ul>	-	Järup et al. (1988)
<ul style="list-style-type: none"> <li>• Type of population: cadmium recovery plant workers (n=45)</li> </ul>	<ul style="list-style-type: none"> <li>• Significant association between cumulative exposure and urinary β2M, RBP, phosphate, and calcium and serum creatinine levels.</li> <li>• Adverse effect level: cumulative exposure 300 mg/m<sup>3</sup></li> </ul>	-	Thun et al. (1989)
<ul style="list-style-type: none"> <li>• Type of population: workers exposed to cadmium fumes (n=33)</li> </ul>	<ul style="list-style-type: none"> <li>• Increased urinary β2M and protein levels (mean 6375 µg/g creatinine and 246 mg/g creatinine, respectively) in 7 workers (mean in remaining 23 workers 53 µg/g creatinine and 34 mg/g creatinine)</li> <li>• Adverse effect level: cumulative exposure 1137 µg/m<sup>3</sup>/years</li> </ul>	-	Falck et al. (1983)

AAP = alanine aminopeptidase; β2M = β2-microglobulin; F = female; M = male; NAG = N-acetyl-β-glucosaminidase; pHC = human complex-forming glycoprotein, also referred to as α1M; RBP = retinol binding protein; U-Cd = urinary cadmium; B-Cd = blood cadmium; GFR = glomerular filtration rate

#### 4.7.2.1.1 Oral exposure

Strong evidence is available that the kidney is one of the main target organs of cadmium toxicity following extended oral exposure. The effects are similar to those seen following inhalation exposure (see Section 4.7.2.1.2). Study summaries from ATSDR (2012) are reproduced below.

*[Start of text from ATSDR (2012)]*

Buchet et al. (1990) examined 1,699 non-occupationally exposed males and females (aged 20–80 years) living in Belgium. Urinary cadmium levels significantly correlated with urinary  $\beta$ 2-microglobulin, retinol binding protein, NAG, amino acid, and calcium levels; the partial  $r^2$  values were 0.0036, 0.0210, 0.0684, 0.0160, and 0.0168, respectively. The probability that individuals would have abnormal values for the renal biomarkers (defined as >95th percentile for subjects without diabetes or urinary tract diseases and who did not regularly take analgesics) was estimated using logistic regression models with adjustments for age, gender, smoking, disease, and use of analgesics. It was estimated that >10% of  $\beta$ 2-microglobulin, retinol binding protein, amino acid, and calcium values would be abnormal when 24-hour urinary cadmium levels were >3.05, 2.87, 2.74, 4.29, or 1.92  $\mu\text{g}/24$  hour, respectively.

Järup et al. (2000) examined 1,021 individuals living near a nickel-cadmium battery plant in Sweden for at least 5 years ( $n=799$ ) or employed as battery workers ( $n=222$ ). The mean urinary cadmium levels were 0.81 and 0.65  $\mu\text{g}/\text{g}$  creatinine in males and females, respectively. Urinary cadmium levels were significantly associated with urinary human complex-forming glycoprotein (pHC; also referred to as  $\alpha$ 1-microglobulin) levels, after adjustment for age. The relationship remained statistically significant after removal of the cadmium workers from the analysis. The prevalence of abnormal pHC values (defined as exceeding the 95th percentile in a Swedish reference population; >7.1 and 5.3  $\text{mg}/\text{g}$  creatinine for males and females, respectively) was estimated to increase by 10% at urinary cadmium levels of 1  $\mu\text{g}/\text{g}$  creatinine. The European Chemicals Bureau (2007) recalculated the probability of HC proteinuria (using the raw data from Järup and associates) to account for the differences in age of the reference population (mean of 40 years) and study population (mean of 53 years). Based on these recalculations, the urinary cadmium level associated with a 10% increased probability of abnormal pHC values (20% total probability) was 2.62  $\mu\text{g}/\text{g}$  creatinine for the total population. In the environmental exposed subgroup, a urinary cadmium level of 0.5  $\mu\text{g}/\text{g}$  creatinine was associated with a 13% probability (doubling of the probability in reference population) of abnormal pHC values.

Noonan et al. (2002) examined residents in Pennsylvania living near a defunct zinc smelting facility (geometric mean urinary cadmium level of 0.14  $\mu\text{g}/\text{g}$  creatinine) and a reference community located 10 miles from the facility (geometric mean urinary cadmium levels of 0.12  $\mu\text{g}/\text{g}$  creatinine). The data from the two communities were pooled because there were no differences in urinary cadmium levels between them.  $\beta$ 2-microglobulin, NAG, alanine aminopeptidase (AAP), and albumin levels were measured as biomarkers of renal dysfunction. The geometric mean urinary cadmium levels were 0.07 and 0.08  $\mu\text{g}/\text{g}$  creatinine in 88 males and 71 females aged 6–17 years and 0.24 and 0.23  $\mu\text{g}/\text{g}$  creatinine in 71 males and 80 females aged  $\geq 18$  years. No significant correlations between urinary cadmium levels and renal biomarkers were observed in the children, after adjustment for creatinine, age, and gender. In adults, significant correlations (after adjustment for creatinine, age, gender, smoking, and self-reported diabetes or thyroid disease) between urinary cadmium and NAG (partial correlation coefficient of 0.20, 95% CI of 0.05–0.36) and AAP (partial correlation coefficient of 0.21 and 95% CI of 0.05–0.36) were observed. Significant dose-effect relationships were also found for these two biomarkers. Urinary cadmium levels were not significantly associated with elevated levels of  $\beta$ 2-microglobulin or albumin.

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Nogawa et al. (1980; *presumably a typing error; the reference of 1989 is assumed to be correct; dossier submitter's note*) examined 878 males and 972 females aged  $\geq 50$  years living in the Kakehashi River basin in Japan; the Kakehashi River, cadmium polluted from an upstream mine, was used to irrigate rice fields.  $\beta 2$ -Microglobulin measured in morning urine samples was used as a biomarker of renal dysfunction and cadmium intake was estimated from rice samples collected in 1974. Cadmium levels in rice were considered to be representative of cadmium intake because over 70% of the total cadmium intake has been shown to come from rice. Cadmium in the rice ranged from 0.10 to 0.69  $\mu\text{g/g}$ .  $\beta 2$ -Microglobulin levels were significantly higher in the study population compared to a reference population of 113 males and 161 females living in a nearby area. A significant dose-related association between total cadmium intake and prevalence of abnormal  $\beta 2$ -microglobulin values (defined as  $\beta 2$ -microglobulin levels of  $\geq 1,000 \mu\text{g/g}$  creatinine) was found. The total cadmium intake, which resulted in a prevalence of abnormal  $\beta 2$ -microglobulin levels equal to the control group, was 1,678 mg in males (prevalence in controls was 6.0%) and 1,763 mg in females (prevalence in controls was 5.0%). A further analysis of the exposed subjects (Hochi et al. 1995) found that the prevalence of abnormal  $\beta 2$ -microglobulin levels (using a cut-off level of 1,000  $\mu\text{g/g}$  creatinine) exceeded the prevalence in the reference population when cadmium intake was  $\geq 2$  g and the subjects were divided into subgroups by age. The prevalence of abnormal  $\beta 2$ -microglobulin levels at a given cadmium intake increased with age.

Yamanaka et al. (1998) examined 558 males and 743 females aged  $\geq 50$  years living in a cadmium nonpolluted area in Japan. Urinary cadmium level was used as a biomarker of exposure and urinary  $\beta 2$ -microglobulin, total protein, and NAG as biomarkers of renal dysfunction. The geometric mean urinary cadmium levels were 1.3 and 1.3  $\mu\text{g/g}$  creatinine in males and females, respectively. Significant correlations (after adjustment for age) between urinary cadmium levels and total protein,  $\beta 2$ -microglobulin, and NAG were found. Abnormal levels of renal biomarkers were defined as exceeding the 84% upper limit value calculated from a referent group of 2,778 non-exposed individuals; the cut-off values were 124.8 and 120.8 mg/g creatinine for total protein in males and females, 492 and 403  $\mu\text{g/g}$  creatinine for  $\beta 2$ -microglobulin, and 8.0 and 8.5 U/g creatinine for NAG. Dose-response relationships between urinary cadmium levels and prevalence of abnormal levels of  $\beta 2$ -microglobulin, total protein, and NAG were found. The odds ratios (95% CI) were 6.589 (3.383–12.833), 3.065 (1.700–5.526), and 1.887 (1.090–3.268) for protein,  $\beta 2$ -microglobulin, and NAG in males and 17.486 (7.520–40.660), 5.625 (3.032–10.435), and 2.313 (1.399–3.824) for protein,  $\beta 2$ -microglobulin, and NAG in females.

Another study of residents living in a cadmium nonpolluted area of Japan examined 346 males and 529 females from one area (area A) and 222 males and 413 females in another area (area B); all subjects were  $\geq 50$  years of age and were not occupationally exposed to heavy metals (Oo et al. 2000). The geometric mean urinary cadmium levels were 2.2 and 2.8  $\mu\text{g/L}$  in males and females in area A and 3.4 and 3.9  $\mu\text{g/L}$  in area B. Significant correlations (with adjustment for age) were found between urinary cadmium and urinary levels of protein,  $\beta 2$ -microglobulin (not significant in males in area B) and NAG levels. A significant association between urinary cadmium levels and the prevalence (cut-off levels from same referent population as Yamanaka et al. 1998) of abnormal levels of urinary protein (cut-off level of 113.8 and 96.8  $\mu\text{g/L}$  in males and females),  $\beta 2$ -microglobulin (378 and 275  $\mu\text{g/L}$ ) (only significant in females in area A), and NAG (8.0 and 7.2  $\mu\text{g/L}$ ). The odds ratios (95% CI) for an increase in prevalence of abnormal renal biomarkers were 8.810 (3.401–22.819) and 11.282 (3.301–38.362) for protein in males in areas A and B, respectively, 8.234 (3.696–18.343) and 23.901 (8.897–64.210) for protein in females in areas A and B; 2.558 (1.246–5.248) for  $\beta 2$ -microglobulin in females in area A; 47.944 (14.193–161.954) and 9.940 (3.153–31.340) for NAG in males in areas A and B; and 72.945 (21.873–243.263) and 25.374 (9.452–68.117) for NAG in females in areas A and B.

In a re-examination of the populations studied by Yamanaka et al. (1998) and Oo et al. (2000), Suwazono et al. (2000) measured cadmium levels in blood and urine and urinary levels of total protein,  $\beta$ 2-microglobulin, and NAG in 1,105 males and 1,648 females over the age of 50 years. The geometric mean concentrations of cadmium in urine were 1.8 and 2.4  $\mu\text{g/g}$  creatinine in males and females, respectively, and blood cadmium levels were 2.0 and 1.8  $\text{ng/g}$  in males and females. After adjustment for age, significant associations between urinary cadmium levels and urinary protein and  $\beta$ 2-microglobulin in males and females were found. Additionally, blood cadmium levels were significantly associated with urinary protein and NAG levels in males and urinary protein,  $\beta$ 2-microglobulin, and NAG levels in females. Cut-off levels (defined as the 84% upper limit values from 424 male and 1,611 female nonsmoking subjects) of 157.4 and 158.5  $\text{mg/g}$  creatinine for protein in males and females, respectively, 507 and 400  $\mu\text{g/g}$  creatinine for  $\beta$ 2-microglobulin in males and females, respectively, and 8.2 and 8.5  $\mu\text{g/g}$  creatinine for NAG in males and females, respectively, were used to evaluate the prevalence of abnormal levels of renal biomarkers. Logistic regression analysis demonstrated significant associations between urinary cadmium levels and increased prevalence of abnormal levels of total protein (odds ratio of 3.923, 95% CI of 2.2028–7.590) and  $\beta$ 2-microglobulin (odds ratio of 2.259, 95% CI of 1.372–3.717) in males; in females, significant associations were found for total protein (odds ratio of 7.763; 95% CI of 4.231–14.243),  $\beta$ 2-microglobulin (odds ratio of 2.259, 95% CI of 1.879–4.281), and NAG (odds ratio of 1.882, 95% CI of 1.311–2.702). For blood cadmium levels, the only significant association found was for an increased prevalence of abnormal total protein levels in females (odds ratio of 3.490, 95% CI of 1.661–7.331).

Jin et al. (2002) examined three populations living various distances from a nonferrous metal smelter. The geometric mean levels of urinary cadmium were 11.18 and 12.86  $\mu\text{g/g}$  creatinine in males ( $n=294$ ) and females ( $n=171$ ) in the highly polluted area, 3.55 and 4.45  $\mu\text{g/g}$  creatinine in males ( $n=243$ ) and females ( $n=162$ ) in the moderately polluted area, and 1.83 and 1.79  $\mu\text{g/g}$  creatinine in males ( $n=253$ ) and females ( $n=155$ ) in the control area. Significant correlations were found between urinary (and blood) cadmium levels and renal biomarkers ( $\beta$ 2-microglobulin, retinol binding protein, and albumin). Cut-off values for  $\beta$ 2-microglobulin, retinol binding protein, and albumin of 300  $\mu\text{g/g}$  creatinine, 300  $\mu\text{g/g}$  creatinine, and 15  $\text{mg/g}$  creatinine, respectively, were used to assess possible dose-response relationships (no additional information was provided); although 300  $\mu\text{g/g}$  creatinine was reported as the cut-off values for  $\beta$ 2-microglobulin, subsequent analysis of this data set (Jin et al. 2004a) reported a cut-off value of 800  $\mu\text{g/g}$  creatinine. Significant dose-response relationships between urinary (and blood) cadmium and the prevalence of abnormal levels of renal markers of kidney dysfunction were found.

*[End of text from ATSDR (2012)]*

#### **4.7.2.1.2 Inhalation exposure**

Strong evidence is available that the kidney is one of the main target organs of cadmium toxicity following extended inhalation exposure. The effects are similar to those seen following oral exposure (see Section 4.7.2.1.1). Study summaries from ATSDR (2012) are reproduced below.

*[Start of text from ATSDR (2012)]*

One of the first signs of kidney effects is tubular dysfunction characterized by an increased urinary excretion of low-molecular-weight proteins such as  $\beta$ 2-microglobulin, human complex-forming glycoprotein (pHC) (also referred to as  $\alpha$ 1-microglobulin), and retinol binding protein or increased urinary levels of intracellular enzymes such as N-acetyl- $\beta$ -glucosaminidase (NAG) (European Chemicals Bureau 2007 (*in this CLH report cited as JRC, 2007; dossier submitter's note*); Järup et

al. 1998). Numerous occupational exposure studies have reported increases in urinary levels of these biomarkers (Bernard et al. 1990; Chen et al. 2006a, 2006b; Chia et al. 1992; Elinder et al. 1985b; Falck et al. 1983; Jakubowski et al. 1987, 1992; Järup and Elinder 1994; Järup et al. 1988; Kawada et al. 1989; Roels et al. 1993; Shaikh et al. 1987; Thun et al. 1989; Toffoletto et al. 1992; Verschoor et al. 1987). At higher exposure levels, increased urinary levels of high-molecular-weight proteins such as albumin have been reported (Bernard et al. 1979, 1990; Chen et al. 2006a, 2006b; Elinder et al. 1985b; Mason et al. 1988; Roels et al. 1989, 1993; Thun et al. 1989), but there is some debate as to whether this represents glomerular damage (Bernard et al. 1979; Roels et al. 1989) or severe tubular damage (Elinder et al. 1985a; Mason et al. 1988; Piscator 1984).

Chronic exposure to very high cadmium levels can result in glomerular damage resulting in decreases in glomerular filtration rate (GFR) (Friberg 1950; Järup et al. 1995; Roels et al. 1991). Järup et al. (1995) found a dose-response relationship between blood cadmium levels and GFR in cadmium workers. At blood cadmium levels of 5.6 to <8.4 µg/L, 33.3% of the workers had decreased GFR (defined as <80% of referents); whereas all subjects with blood cadmium levels of ≥8.4 µg/L exhibited a decreased GFR.

Another study did not find alterations in GFR in workers with urinary cadmium levels of approximately 11 µg/g creatinine; however, an exacerbation of the age-related decline in maximal GFR was observed (Roels et al. 1991). Other studies reported increases in serum creatinine levels, which are suggestive of impaired GFR (Roels et al. 1989; Thun et al. 1989).

*[End of text from ATSDR (2012)]*

#### **4.7.2.2 Effects on bone**

Table 11: Summary table of repeated dose toxicity studies relevant for classification as STOT RE according to the CLP regulation (adapted from ATSDR (2012))

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON CADMIUM HYDROXIDE

Method	Results	Remarks	Reference
<b>Oral exposure</b>			
<ul style="list-style-type: none"> <li>Type of population: women environmentally exposed to cadmium (Sweden)</li> </ul>	<ul style="list-style-type: none"> <li>Mean urinary cadmium level: 0.52 µg/L</li> <li>Negative relationship between urinary cadmium levels and bone mineral density.</li> </ul>	-	Åkesson et al. (2005)
<ul style="list-style-type: none"> <li>Type of population: residents in cadmium-polluted area (Sweden)</li> </ul>	<ul style="list-style-type: none"> <li>Significant decreases in bone mineral density for &gt;60 years of age with blood cadmium levels of ≥0.56 µg/L.</li> </ul>	-	Alfvén et al. (2002)
<ul style="list-style-type: none"> <li>Type of population: Subjects, of which approximately 10 % were environmentally or occupationally exposed to cadmium (Sweden)</li> </ul>	<ul style="list-style-type: none"> <li>Increased risk of bone fractures for &gt;50 years of age with urinary cadmium levels of &gt;2 µg/g creatinine.</li> </ul>	-	Alfvén et al. (2004)
<ul style="list-style-type: none"> <li>Type of population: Subjects, of which approximately 10 % were environmentally or occupationally exposed to cadmium (Sweden)</li> </ul>	<ul style="list-style-type: none"> <li>Increased risk of osteoporosis among men &gt;60 years of age with urinary cadmium levels of &gt;5 µg/g creatinine.</li> </ul>	-	Alfvén et al. (2000)
<ul style="list-style-type: none"> <li>Type of population: residents living near zinc smelters (Belgium)</li> </ul>	<ul style="list-style-type: none"> <li>Decrease in proximal and distal forearm bone density of approximately 0.1 g/cm<sup>2</sup> was associated with a two-fold increase in urinary cadmium level in postmenopausal women.</li> </ul>	-	Staessen et al. (1999)
<ul style="list-style-type: none"> <li>Type of population: women living near zinc smelters</li> </ul>	<ul style="list-style-type: none"> <li>Suggestive evidence that cadmium has a direct osteotoxic effect.</li> </ul>	-	Schutte et al. (2008)
<ul style="list-style-type: none"> <li>Type of population: residents in cadmium-polluted area (Poland)</li> </ul>	<ul style="list-style-type: none"> <li>Significant decrease in bone mineral density in males with urinary cadmium levels of &gt;2 µg/g creatinine.</li> </ul>	-	Trzcinka-Ochocka et al. (2010)
<ul style="list-style-type: none"> <li>Type of population: residents in cadmium-polluted area (China)</li> </ul>	<ul style="list-style-type: none"> <li>Significant increases in prevalence of low forearm bone mineral density in postmenopausal women with urinary cadmium levels of &gt;20 µg/g creatinine.</li> <li>Significant increases in prevalence of low forearm bone mineral density in men, premenopausal women, and postmenopausal women with blood cadmium levels of &gt;20 µg/g creatinine.</li> </ul>	-	Nordberg et al. (2002)
<ul style="list-style-type: none"> <li>Type of population: residents in cadmium-polluted area (China)</li> </ul>	<ul style="list-style-type: none"> <li>Increase in bone fractures in males (mean urinary cadmium level 9.20 µg/g creatinine) and females (mean urinary cadmium level 12.86</li> </ul>	-	Wang et al. (2003)



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	µg/g creatinine)		
<ul style="list-style-type: none"> <li>• Type of population: residents in cadmium-polluted area (China)</li> </ul>	<ul style="list-style-type: none"> <li>• Significant dose-response relationship between urinary cadmium levels and the prevalence of osteoporosis.</li> </ul>	-	Jin et al. (2004b), Wang et al. (2003), Zhu et al. (2004)
<ul style="list-style-type: none"> <li>• Type of population: residents in areas with moderate or heavy cadmium pollution ten years after the source of rice was switched to commercially available rice from cadmium-nonpolluted areas (China)</li> </ul>	<ul style="list-style-type: none"> <li>• Significant decreases in forearm bone mineral density in women from the moderately polluted area and in men from the heavily polluted area.</li> <li>• Decreases in bone mineral density in women 60-69 or ≥70 years old from both polluted areas, and in men ≥70 years old from the heavily polluted area.</li> <li>• Significantly higher prevalence of osteoporosis in women from the polluted areas which increased with urinary cadmium levels.</li> </ul>	-	Chen et al. (2009)
<ul style="list-style-type: none"> <li>• Type of population: residents in cadmium-polluted area (China)</li> </ul>	<ul style="list-style-type: none"> <li>• Higher prevalence of osteoporosis in women with renal dysfunction or tubular damage</li> <li>• Significantly lower bone mineral density levels in women with tubular damage</li> <li>• No significant associations between the prevalence of osteoporosis or bone mineral density and alterations in renal biomarkers in men.</li> </ul>	-	Chen et al. (2011)
<ul style="list-style-type: none"> <li>• Type of population: residents living near an industrial complex (Korea)</li> </ul>	<ul style="list-style-type: none"> <li>• Significant associations between high urinary cadmium levels (≥1.0 µg/g creatinine) and osteopenia</li> <li>• Bone mineral density negatively associated with urinary cadmium levels.</li> </ul>	-	Shin et al. (2011)
<ul style="list-style-type: none"> <li>• Type of population: health-survey population (Sweden)</li> </ul>	<ul style="list-style-type: none"> <li>• Significantly lower urinary cadmium levels bone mineral density in postmenopausal women with elevated urinary cadmium levels (median 1.1 µg/g creatinine) compared to women with low urinary cadmium levels (median 0.36 µg/g creatinine)</li> <li>• Significant changes of biomarkers indicative of increased bone resorption in the high urinary cadmium group.</li> </ul>	-	Engström et al. (2009)

## ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON CADMIUM HYDROXIDE

• Type of population: general population (USA)	• Significant association between urinary cadmium levels and osteopenia and osteoporosis in adults with urinary cadmium levels of >1 µg/g creatinine.	-	Wu et al. (2010)
• Type of population: general population (USA)	• 43 % increased risk of osteoporosis in women ≥50 years of age with urinary cadmium levels of 0.50-1.00 µg/g creatinine, as compared to women with urinary cadmium levels of <0.50 µg/g creatinine.	-	Gallagher et al. (2008)
<b>Inhalation exposure</b>			
• Case study: alkaline battery workers	• Osteomalacia observed	-	Adams et al. (1969)
• Case study: battery plate maker	• Osteomalacia observed	-	Blainey et al. (1980)
• Case study: cadmium workers	• Hypercalciuria and osteomalacia observed	-	Kazantzis 1979
• Case study: cadmium-exposed workers	• Hypercalciuria and calcium deficit observed	-	Scott et al. (1980)

### 4.7.2.2.1 Oral exposure

Strong evidence is available that bone is one of the main target organs of cadmium toxicity following extended oral exposure. The effects are similar to those seen following inhalation exposure (see Section 4.7.2.2.2). Study summaries from ATSDR (2012) are reproduced below.

In several studies of populations exposed to elevated levels of cadmium in the diet, the occurrence of osteomalacia, osteoporosis, bone fractures and decreased bone mineral density have been established. The first observation of an association between these types of bone effects and cadmium exposure was reported in residents living in the cadmium-contaminated area of the Jinzu River basin in Japan. The condition was termed Itai-Itai disease (Tsuchiya, 1969a, b). Studies of populations living in other areas of Japan and in other countries, and of populations exposed to low levels of cadmium, have confirmed the association between cadmium exposure and bone effects.

*[Start of text from ATSDR (2012)]*

In a study of Swedish women environmentally exposed to cadmium, a significant negative relationship between urinary cadmium levels and bone mineral density was observed (Åkesson et al. 2005); the mean urinary cadmium level of the population was 0.52 µg/L. In Swedish residents living in an area with known cadmium pollution from battery manufacturing facilities, significant associations were noted between blood cadmium levels and bone mineral density and between urinary cadmium levels and risk of fractures and osteoporosis. There were significant decreases in bone mineral density in environmentally exposed subjects older than 60 years of age with blood cadmium levels of ≥0.56 µg/L (Alfvén et al. 2002). Increases in the risk of bone fractures were observed in subjects (approximately 10% of all subjects examined had environmental and occupational exposure to cadmium) older than 50 years of age with urinary cadmium levels >2 µg/g creatinine; no significant associations were found in subjects under 50 years of age (Alfvén et al.

2004). Another study of this population found significant increases in the risk of osteoporosis among men >60 years of age with urinary cadmium levels  $\geq 5$   $\mu\text{g/g}$  creatinine; however, an increased risk of osteoporosis was not observed in women (Alfvén et al. 2000). A Belgian study in which residents living near zinc smelters found a 2-fold increase in cadmium exposure (as assessed via urinary cadmium levels) was associated with a decrease in proximal and distal forearm bone density of approximately  $0.1 \text{ g/cm}^2$  among postmenopausal women (Staessen et al. 1999). For women with urinary cadmium levels  $>1$   $\mu\text{g/day}$ , the incidence of bone fracture was 13.5 per 1,000 person-years. Another study of a subset of the women living near a zinc smelters (Schutte et al. 2008) provides suggestive evidence that cadmium has a direct osteotoxic effect. Significant associations between urinary cadmium levels and the levels of two pyridinium crosslinks of collagen (urinary levels of hydroxylysylpyridinoline and lysylpyridinoline), proximal forearm bone mineral density, and serum parathyroid hormone levels were found. In almost all of the examined women, urinary levels of retinol binding protein were below the cut-off level of 338  $\mu\text{g/day}$ , suggesting no cadmium-induced effect on renal tubular function. Several biomarkers of bone damage were examined in a subsequent follow-up study of some of the women (Schutte et al. 2008) only 1 of the 294 women examined had evidence of renal dysfunction (increased retinol binding protein). Significant associations between urinary cadmium excretion and two biomarkers of bone resorption (urinary hydroxylysylpyridinoline and lysylpyridinoline) were found. Although significant associations between urinary cadmium levels and biomarkers of renal dysfunction were observed in Polish adults living in a cadmium-polluted area, the only association between urinary cadmium and bone biomarkers was a significant decrease in bone mineral density among males with urinary cadmium levels of  $\geq 2$   $\mu\text{g/g}$  creatinine (Trzcinka-Ochocka et al. 2010). Similar results have been observed in several studies of residents living in areas of China with moderate or high cadmium pollution levels (Jin et al. 2004b; Nordberg et al. 2002; Wang et al. 2003; Zhu et al. 2004). There were significant increases in the prevalence of low forearm bone mineral density in postmenopausal women with urinary cadmium levels  $>20$   $\mu\text{g/g}$  creatinine and in men, premenopausal women, and postmenopausal women with blood cadmium levels  $>20$   $\mu\text{g/L}$  (Nordberg et al. 2002). An increase in bone fractures was observed in males and females over the age of 40 years living in the area of high cadmium exposure (mean urinary cadmium levels in the area were 9.20 and 12.86  $\mu\text{g/g}$  creatinine in the males and females, respectively) (Wang et al. 2003). A significant dose-response relationship between urinary cadmium levels and the prevalence of osteoporosis was observed (Jin et al. 2004b; Wang et al. 2003; Zhu et al. 2004); the Jin et al. (2004b) study found that 23 of the 31 subjects with osteoporosis also exhibited signs of renal dysfunction. A subsequent study by this group examined 316 male and female residents living in areas with moderate or heavy cadmium pollution 10 years after the source of rice was switched to commercially available rice from nonpolluted areas (Chen et al. 2009). As in the earlier studies, significant decreases in forearm bone mineral density were observed in the women living in the moderately polluted area and in the men and women living in the heavily polluted areas. When the subjects were divided by age, decreases in bone mineral density were observed in women 60–69 or  $\geq 70$  years old in both cadmium polluted areas and in men  $\geq 70$  years living in the heavily polluted area. A significantly higher prevalence of osteoporosis was also observed in women living in the polluted areas and the prevalence increased with increasing urinary cadmium levels. In another study of this population, Chen et al. (2011) found a higher prevalence of osteoporosis (assessed in 2006) among women with renal dysfunction (urinary albumin  $>15$   $\text{mg/g}$  creatinine and urinary NAG  $\geq 12$   $\text{IU/g}$  creatinine; renal biomarkers assessed in 1998) or tubular damage (urinary NAG  $\geq 12$   $\text{IU/g}$  creatinine); no significant association was found for glomerular damage (urinary albumin  $\geq 15$   $\text{mg/g}$  creatinine). Significantly lower bone mineral density levels were also found in women with tubular damage, as compared to those without tubular damage. In men, no significant associations between the prevalence of osteoporosis or bone mineral density and alterations in renal biomarkers were found. Chen et al. (2011) also compared the change in bone mineral damage from 1998 to

2006 in subjects with and without evidence of kidney damage and found a significantly greater decrease in bone mineral density among women with tubular damage. In a study of adults living near an industrial complex in Korea, significant associations between high urinary cadmium levels ( $\geq 1.0$   $\mu\text{g/g}$  creatinine) and osteopenia were observed in males and females (Shin et al. 2011). Bone mineral density was also negatively associated with urinary cadmium levels.

In a substudy of a population-based health survey in Sweden (Engström et al. 2009), a significantly lower bone mineral density was observed in postmenopausal women with elevated urinary cadmium levels (median of 1.1  $\mu\text{g/g}$  creatinine, 5–95<sup>th</sup> percentile of 0.69–1.7  $\mu\text{g/g}$  creatinine) as compared to women with low urinary cadmium levels (median of 0.36  $\mu\text{g/g}$  creatinine; 5–95<sup>th</sup> percentile of 0.18–0.73  $\mu\text{g/g}$  creatinine). Significant decreases in serum parathyroid hormone levels and increases in urinary deoxypyridinoline levels (indicative of increased bone resorption) were also found in the high urinary cadmium group; however, there were no significant alterations in serum 1,25-dihydroxyvitamin D levels. Significant elevations in biomarkers of renal dysfunction (urinary NAG and pHC and estimated glomerular filtration rate) were also in the high urinary cadmium group. In the U.S. general population (using data collected during NHANES III, 1988–1994), a significant association was found between urinary cadmium levels and osteopenia and osteoporosis among adults with urinary cadmium levels of  $>1$   $\mu\text{g/g}$  creatinine (Wu et al. 2010). Using the same NHANES data set, Gallagher et al. (2008) found a 43% increased risk of osteoporosis (hip bone mineral density defined) (odds ratio of 1.43; 95% CI of 1.02–2.00) among women  $\geq 50$  years of age with urinary cadmium levels of 0.50–1.00  $\mu\text{g/g}$  creatinine, as compared to women with urinary cadmium levels of  $<0.50$   $\mu\text{g/g}$  creatinine.

*[End of text from ATSDR (2012)]*

#### **4.7.2.2.2 Inhalation exposure**

Strong evidence is available that bone is one of the main target organs of cadmium toxicity following extended inhalation exposure. The effects are similar to those seen following oral exposure (see Section 4.7.2.2.1). Study summaries from ATSDR (2012) are reproduced below.

*[Start of text from ATSDR (2012)]*

Case studies indicate that calcium deficiency, osteoporosis, or osteomalacia can develop in some workers after long-term occupational exposure to high levels of cadmium (Adams et al. 1969; Blainey et al. 1980; Bonnell 1955; Kazantzis 1979; Scott et al. 1980).

*[End of text from ATSDR (2012)]*

#### **4.7.3 Other relevant information**

#### **4.7.4 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure**

In humans, significant associations between cadmium levels in urine and biomarkers for kidney damage, i.e. excretion of low molecular weight proteins (such as  $\beta 2$ -microglobulin, human complex forming glycoprotein (pHC) (also referred to as  $\alpha 1$ -microglobulin), and retinol binding protein), increased levels of intracellular enzymes (for example, N-acetyl- $\beta$ -glucosaminidase (NAG)) and increased excretion of calcium), or significant increases in the prevalence of abnormal levels of these biomarkers, have been observed in numerous reliable studies of workers occupationally exposed to cadmium (Roels et al., 1991; Verschoor et al., 1987; Toffoletto et al., 1992; Shaikh et

al., 1987; Bernard et al., 1990; Kawada et al., 1989; Roels et al., 1993; Järup and Elinder, 1994; Jakubowski et al., 1987; Elinder et al., 1985a; Chia et al., 1992; Chen et al., 2006a, b; Jakubowski et al., 1992; Järup et al., 1988; Thun et al., 1989; Falck et al., 1983) and in numerous reliable studies of people living in areas contaminated with cadmium (Buchet et al., 1990; Järup et al., 2000; Noonan et al., 2002; Nogawa et al., 1989; Yamanaka et al., 1998; Oo et al., 2000; Suwazono et al., 2000; Jin et al., 2002; Jin et al., 2004a). Hence, relevant information on kidney toxicity of cadmium after oral and inhalation repeated exposure is available in humans and, accordingly, the dossier submitter considers that there is sufficient evidence to conclude that cadmium is toxic to the kidney in humans.

In humans, the occurrence of osteomalacia, osteoporosis, bone fractures and decreased bone mineral density have been established in numerous reliable studies of populations exposed to elevated levels of cadmium in the diet (Åkesson et al., 2005; Alfvén et al., 2000, 2002, 2004; Staessen et al., 1999; Schutte et al., 2008; Trzcinka-Ochocka et al., 2010; Nordberg et al., 2002; Wang et al., 2003; Jin et al., 2004b; Zhu et al., 2004; Chen et al., 2009, 2011; Shin et al., 2011; Engström et al., 2009; Wu et al., 2010; Gallagher et al., 2008), and similar effects have been observed in reliable case studies of workers occupationally exposed to cadmium (Adams et al. 1969; Blainey et al. 1980; Bonnell 1955; Kazantzis 1979; Scott et al. 1980). Hence, relevant information on bone toxicity of cadmium after oral and inhalation repeated exposure is available in humans and, accordingly, the dossier submitter considers that there is sufficient evidence to conclude that cadmium is toxic to bone in humans.

In this connection, the dossier submitter would like to point out that it is discussed in the literature whether or not effects on bone are linked to effects on kidney, since the occurrence of effects on bone usually are recorded at the same dose level as effects on kidney (see ATSDR, 2012). In a recent publication by Åkesson et al. (2014) it is concluded that available data point toward a direct effect of cadmium on bone, since low-level environmental exposure to cadmium seems to mobilise minerals from bone, even in the absence of cadmium-induced renal tubular dysfunction.

In mammalian toxicity, the toxicity of cadmium is regarded to result from the intrinsic properties of the  $\text{Cd}^{2+}$  ion. As outlined in Section 4, it is reasonable to assume that the  $\text{Cd}^{2+}$  ion is bioavailable after oral and inhalation exposure of cadmium hydroxide. Hence, the toxic effects of cadmium observed in the studies summarised above are relevant also for cadmium hydroxide. Accordingly, it is concluded that cadmium hydroxide is toxic to kidney and bone in humans after oral and inhalation repeated exposure.

#### **4.7.5 Comparison with the CLP criteria**

According to the criteria in the CLP regulation, Annex I: 3.9.2.2, classification in Category 1 is reserved for substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Further, in Annex I: 3.9.2.10.2 of the CLP regulation it is stated that, when well-substantiated human data are available showing a specific target organ toxic effect that can be reliably attributed to repeated or prolonged exposure to a substance, the substance shall normally be classified. Positive human data, regardless of probable dose, predominates over animal data. On consideration of the human data referred to in this CLH report, they are considered to demonstrate significant toxicity in humans in kidney and bone.

In view of these considerations, the evidence referred to is deemed to match the criteria for classification of cadmium hydroxide in Category 1 (kidney and bone) for specific target organ toxicity-repeated exposure.

Where no evidence is available from human studies, or from animal studies that can support a presumption that significant toxicity in humans will be produced following repeated exposure, classification in Category 2 is based on animal studies providing evidence that a substance can be presumed to have the potential to be harmful to human health following repeated exposure. Since evidence for significant toxicity is available from human studies, classification in Category 2 is not justified.

The available evidence shows that cadmium toxicity is produced following oral or inhalation exposure. Data supporting that other routes of exposure would not produce toxicity is not available, and thus no route of exposure should be specified.

#### 4.7.6 Conclusions on classification and labelling for STOT RE

Cadmium hydroxide should be classified in STOT RE 1 (kidney, bone); H372.

### **RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)**

#### **Summary of the Dossier submitter’s proposal**

The DS proposed the classification of cadmium hydroxide as STOT RE 1 (H372) with bone and kidney as target organs and used the updated Toxicological Profile for Cadmium issued by the Agency for Toxic Substances and Disease Registry (ATSDR) as a data source. In the CLH dossier, the DS assumed that the toxic species of cadmium salts is the cadmium ion and did not differentiate among different cadmium salts. The dossier provided evidence to support classification for STOT RE only from epidemiological studies and did not contain data from studies in animals on the understanding that these studies would not add any information necessary for the classification.

#### *Effects on kidney*

Some 25 different epidemiological studies were used to support the classification of STOT RE 1 for kidney. In these studies it was demonstrated that the prevalence of abnormal values of biomarkers of kidney injury was correlated with cadmium exposure. The biomarkers of renal damage were mainly proteinuria (detection of low molecular weight proteins in urine such as  $\beta$ 2-microglobulin, human complex-forming glycoprotein, N-acetyl- $\beta$ -glucosaminidase and retinol binding protein). Sometimes reductions in glomerular filtration rate were also found to be associated with cadmium exposure. The studied populations included general population residents in more or less cadmium contaminated areas and occupational populations (smelters, workers using cadmium pigments in plastic production or in welding and battery workers). The routes of exposure were oral (for the general population) and inhalation (for occupational populations).

#### *Effects on bone*

Twenty different epidemiological studies were used to support the classification of STOT RE 1 for bone. In these studies, two types of populations were studied, general population (environmentally exposed by oral route) and occupational population (cadmium workers exposed by inhalation). Positive correlations were found between urinary cadmium level and reduced bone mineral density (osteoporosis, osteopenia, osteomalacia) and increased risk of bone fractures.

#### **Comments received during public consultation**

During Public Consultation the International Cadmium Association (a non-profit organisation

acting on behalf the International Zinc Association) supported the proposed classification.

Two MS supported the classification of cadmium hydroxide as STOT RE 1 (H372).

One of the two MS requested to set an SCL but the DS responded that it is impossible to assess the potency of cadmium hydroxide itself since the hazardous property is extrapolated from other substances.

The other MS suggested including an overview about the significance of the biomarkers for kidney damage and in response the DS referred to the information included in the EU RAR for cadmium on this issue. This same MS drew attention to a study by Navas-Acien *et al.* (2009) on the impact of low-level cadmium exposure on clinical renal outcomes. RAC has addressed both comments in the section "Additional key elements".

### **Additional key elements**

#### *Other evidence of association between cadmium exposure and renal damage*

Navas-Acien *et al.* (2009) correlated environmental exposure to cadmium in a population of 14778 adults who participated in the National Health and Nutrition Examination Survey (1999–2006) with clinical renal outcomes. The geometric mean level of cadmium in the blood was 0.41 µg/L (3.65 nmol/L) and after adjustment for survey year, sociodemographic factors, chronic kidney disease risk factors, and blood lead, the odds ratios for albuminuria (>30 mg/g creatinine), reduced estimated glomerular filtration rate (<60 mL/minute/1.73 m<sup>2</sup>), and both albuminuria and reduced estimated glomerular filtration rate were 1.92, 1.32 and 2.91, respectively. According to the authors these findings support consideration of cadmium as a chronic kidney disease risk factor in the general population.

#### *Biomarkers of effect for renal damage (taken from the EU RAR for cadmium)*

Glomerular function is usually assessed by measuring the glomerular filtration rate (GFR). The GFR can be assessed in intact animals and humans by measuring the urinary excretion and plasma level of a substance "x" that is freely filtered through the glomeruli and neither secreted nor reabsorbed by the tubules. The GFR in an average sized normal man is approximately 125 mL/min. Its magnitude correlates fairly well with body surface area, and values in women are 10% lower than those in men even after correction for body surface area.

Various disease processes in the kidneys may alter the glomerular filtration leading to excessive protein leakage into the urine. Normally only plasma substances with a molecular weight less than 50 000 are filtered through the glomerulus to form primary urine. Heavy proteinuria, with the appearance of large plasma molecules such as albumin and immunoglobulins in the urine reveals a glomerular lesion with increased permeability.

Analysis of low-molecular weight proteins (LMW) in urine, such as β<sub>2</sub>-microglobulin (β<sub>2</sub>M), Retinol-Binding Protein (RBP), α<sub>1</sub>-microglobulin (α<sub>1</sub>M, also termed protein HC) or intracellular enzymes (e.g. N-acetyl-β-D-glucosaminidase; NAG) is used to detect tubular effects. Under physiological conditions, proteins in glomerular filtrate are actively reabsorbed by proximal tubular cells and catabolised in the lysosomal subcellular compartment.

Although the determination of β<sub>2</sub>M has been widely used for the screening of proximal tubular effects, this test presents a major pitfall arising from the instability of this protein in acid urine (pH < 5.5-6.0). This degradation is very rapid at 37°C and can therefore occur in the bladder, necessitating neutralising urine by the ingestion of sodium bicarbonate several hours before collection of the urinary specimen. Since this complicated procedure was not applied in most epidemiological studies that used β<sub>2</sub>M, it should be considered that some of them may have underestimated the urinary β<sub>2</sub>M levels in subjects with acid urine. Proteins such as RBP and

$\alpha$ 1-M are more resistant to degradation and are now preferred to  $\beta$ 2M.

Increased NAG activity is interpreted as reflecting damage to the lysosomal compartment of the proximal tubular cells. The association between increased urinary excretion of NAG and renal damage has not been fully determined and may reflect normal increased lysosomal activity.

### Assessment and comparison with the classification criteria

The DS has used an array of epidemiological studies published in the open scientific literature and previously employed by the Agency for Toxic Substances and Disease Registry (ATSDR) to build its updated toxicological profile for cadmium. It is noted that in all cases the toxicity is attributed to the cadmium cation regardless of the original chemical species (cadmium salt, cadmium oxide or cadmium hydroxide).

#### Effects on kidney

The relevant findings from available epidemiological studies are summarised in the table below.

Method (inc. type of population)	Results	Remarks	Reference
General population (Belgium); 1699 males, females, 20-80 years old  Effect biomarker: $\beta$ 2M, RBP, NAG, amino acids, calcium	Significant correlation between U-Cd and effect biomarkers.  Dose-response relationship between U-Cd and prevalence of abnormal effect biomarker levels.	When 24-hour U-Cd levels were >3.05, 2.87, 2.74, 4.29, or 1.92 $\mu$ g the probability of displaying abnormal values of $\beta$ 2-M, RBP, amino acid, and calcium values would be higher than 10%, respectively.	Buchet <i>et al.</i> (1990)
799 residents in cadmium-polluted area + 222 occupationally exposed subjects (Sweden)  Effect biomarker: pHc	Mean U-Cd level: 0.81 $\mu$ g/g creatinine (M), 0.66 $\mu$ g/g creatinine (F)  Linear relationship between U-Cd and pHc.	Relationship remained significant after removal of occupationally exposed subjects.  U-Cd level associated with a 10% increased probability of abnormal pHc values was 2.62 $\mu$ g/g creatinine for the total population.	Järup <i>et al.</i> (2000)
General population (United States); 88 males, 71 females, 6-17 years old; 71 males, 80 females $\geq$ 18 years old  Effect biomarker: $\beta$ 2M, NAG, AAP, albumin	U-Cd levels: 0.07 $\mu$ g/g creatinine (M, child), 0.08 $\mu$ g/g creatinine (F, child), 0.24 $\mu$ g/g creatinine (M, adult), 0.23 $\mu$ g/g creatinine (F, adult).  Significant association and dose-response relationship (after age and gender adjustment) between U-Cd and NAG and AAP in adults.	No significant associations (after correction for age, sex) between U-Cd and effects biomarkers in children.  U-Cd levels in adults were not significantly associated with elevated levels of $\beta$ 2M or albumin.	Noonan <i>et al.</i> (2002)



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<p>Residents in cadmium-polluted area (Japan); 878 males, 972 females. ≥50 years old</p> <p>Effect biomarker: β2M</p>	<p>Dose-response relationship between cadmium in rice and effect biomarker.</p>	<p>Cadmium levels in rice were considered to be representative of cadmium intake because over 70% of the total cadmium intake has been shown to come from rice.</p>	<p>Nogawa <i>et al.</i> (1989)</p>
<p>General population (Japan); 558 males, 743 females, ≥50 years old</p> <p>Effect biomarker: β2M, total protein, NAG</p>	<p>Mean U-Cd level: 1.3 µg/g creatinine (both M and F).</p> <p>Significant correlation between U-Cd and effect biomarkers (NAG was only significant in females).</p> <p>Dose-response relationship between U-Cd and prevalence of abnormal effect biomarker levels</p>	<p>The odds ratios were 6.589, 3.065, and 1.887 for protein, β2M and NAG in males and 17.486, 5.625, and 2.313 for protein, β2M, and NAG in females.</p>	<p>Yamanaka <i>et al.</i> (1998)</p>
<p>General population (Japan); 568 males, 742 females, ≥50 years old</p> <p>Effect biomarker: total protein, NAG, β2M</p>	<p>Mean U-Cd level: 2.2-3.4 µg/g creatinine (M), 2.8-3.9 µg/g creatinine (F)</p> <p>Significant correlation (with age adjustment) between U-Cd and effect biomarkers.</p>	<p>-</p>	<p>Oo <i>et al.</i> (2000)</p>
<p>General population (Japan); 1105 males, 1648 females, ≥50 years old</p> <p>Effect biomarker: β2M, total protein, NAG</p>	<p>Mean U-Cd level: 1.8 µg/g creatinine (M), 2.4 µg/g creatinine (F).</p> <p>Significant correlation between U-Cd and protein and β2M.</p> <p>Dose-response relationship between urinary cadmium and prevalence of abnormal effect biomarker levels.</p>	<p>Blood cadmium levels were significantly associated with urinary protein and NAG levels in males and urinary protein, β2M and NAG levels in females.</p>	<p>Suwazono <i>et al.</i> (2000)</p>
<p>Residents in cadmium-polluted area (China); 118 males, 170 females, high exposure group; 80 males, 158 females, moderate exposure group</p> <p>Effect biomarker: β2M, RBP, albumin</p>	<p>Mean U-Cd level: 11.18 (M) and 12.86 (F) µg/g creatinine (high exposure group), 3.55 (M) and 4.45 (F) µg/g creatinine (moderate exposure group)</p> <p>Significant correlation between U-Cd and effect biomarkers.</p>	<p>Dose-response relationship between U-Cd and prevalence of abnormal effect biomarker levels.</p>	<p>Jin <i>et al.</i> (2002)</p>

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Residents in cadmium-polluted area (China); 118 males, 170 females, high exposure group; 80 males, 158 females, moderate exposure group  Effect biomarker: $\beta$ 2M, NAG, NAG-B, RBP, albumin	Dose-response relationship between U-Cd and prevalence of abnormal effect biomarker levels.	-	Jin <i>et al.</i> (2004a)
Zinc- cadmium smelter workers (n=87)	Effect: age-related decline in maximal GFR was exacerbated in workers with cadmium-induced microproteinuria.  Adverse effect level (U-Cd): 11.1 $\mu$ g/g creatinine.	-	Roels <i>et al.</i> (1991)
Workers using cadmium pigments in plastic production or using cadmium in welding (n=27)	Effect: significant increase in urinary $\beta$ 2M and NAG levels.	Adverse effect level (U-Cd): 5 $\mu$ g/g creatinine.	Verschoor <i>et al.</i> (1987)
Cadmium alloy workers (n=164)	Effect: higher incidence of increased urinary $\beta$ 2M levels (>250 $\mu$ g/L cut-off) when U-Cd levels exceeded 10 $\mu$ g/g creatinine on one or more occasions, as compared to workers who never exceeded the 10 $\mu$ g/g creatinine level.	Adverse effect level (U-Cd): 10 $\mu$ g/g creatinine.	Toffoletto <i>et al.</i> (1992)
Cadmium smelter workers (n=53)	Effect: significant increase in urinary protein and $\beta$ 2M levels.	Adverse effect level (U-Cd): 13.3 $\mu$ g/g creatinine.	Shaikh <i>et al.</i> (1987)
Non-ferrous smelter workers (n=58)	Effect: significant increase in urinary $\beta$ 2M, RBP protein, pHc, albumin, and transferrin levels.	Adverse effect level (U-Cd): >10 $\mu$ g/g creatinine.	Bernard <i>et al.</i> (1990)
Workers exposed to cadmium pigment dust (n=58)	Significant correlation between U-Cd and NAG levels.  Significant correlation with $\beta$ 2M at one of the two time points.	Adverse effect level (U-Cd): 1.1-1.4 $\mu$ g/g creatinine.	Kawada <i>et al.</i> (1989)
Zinc- cadmium smelter workers (n=50)	Significant association between U-Cd levels and urinary levels of NAG, albumin, and transferrin.  At higher urinary	Adverse effect level (U-Cd): 4 $\mu$ g/g creatinine.	Roels <i>et al.</i> (1993)

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	cadmium levels (10 µg/g creatinine), there were significant associations with RBP and β2M.		
Battery workers (n=561)	10% prevalence of abnormal β2M levels (220 µg/g creatinine cut-off).	Adverse effect level (U-Cd): 1.5 µg/g creatinine for ≥60 years of age; 5 µg/g creatinine for <60 years of age.	Järup and Elinder (1994)
Alkaline battery factory workers (n=102)	10% prevalence of renal dysfunction (β2M >380 µg/g creatinine; RBP >130 µg/g creatinine).	Adverse effect level (U-Cd): 10-15 µg/g creatinine.	Jakubowski <i>et al.</i> (1987)
Workers at a factory using cadmium-containing solders (n=60)	25% prevalence of abnormal β2M levels (300 µg/g creatinine cut-off).	Adverse effect level (U-Cd): 2-5 µg/g creatinine.	Elinder <i>et al.</i> (1985a)
Workers at nickel-cadmium battery factory (n=92)	Significant increase in pHC and NAG levels (after adjustment for age, gender, and race).	Adverse effect level (U-Cd): 5-10 µg/g creatinine.	Chia <i>et al.</i> (1992)
Cadmium smelter workers (n=85)	Significant increases in β2M and NAG levels and increased prevalence of abnormal levels of these biomarkers.	Adverse effect level (U-Cd): 5-10 µg/g creatinine.	Chen <i>et al.</i> (2006a, 2006b)
Alkaline battery factory workers (n=141)	10% prevalence of renal dysfunction (β2M >300 µg/g creatinine; RBP >300 µg/g creatinine).	Adverse effect level (B-Cd): 300 µg-years/L (30 years of 10 µg/L).	Jakubowski <i>et al.</i> (1992)
Battery workers (n=440)	Approximately 10% prevalence of abnormal β2M levels (35 µg/mmol creatinine cut-off).	Adverse effect level (B-Cd): 5.6 µg/L; cumulative exposure 691 µg-years/m <sup>3</sup> .	Järup <i>et al.</i> (1988)
Cadmium recovery plant workers (n=45)	Significant association between cumulative exposure and urinary β2M, RBP, phosphate, and calcium and serum creatinine levels.	Adverse effect level: cumulative exposure 300 mg/m <sup>3</sup> .	Thun <i>et al.</i> (1989)
Workers exposed to cadmium fumes (n=33)	Increased urinary β2M and protein levels (mean 6375 µg/g creatinine and 246 mg/g creatinine, respectively) in 7 workers (mean in remaining 23 workers 53 µg/g creatinine and 34 mg/g creatinine).	Adverse effect level: cumulative exposure 1137 µg/m <sup>3</sup> /years.	Falck <i>et al.</i> (1983)

Abbreviations: AAP = alanine aminopeptidase; β2M = β2-microglobulin; F = female; M = male; NAG = N-acetyl-β-glucosaminidase; pHC = human complex-forming glycoprotein, also referred to as α1M; RBP = retinol binding protein; U-Cd = urinary cadmium; B-Cd = blood cadmium; GFR = glomerular filtration rate.

*Effects on bone*

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The relevant findings from available epidemiological studies are summarised in the table below.

Method (inc. type of population)	Results	Reference
Women environmentally exposed to cadmium (Sweden)	Mean urinary cadmium level: 0.52 µg/L Negative relationship between blood cadmium levels and bone mineral density.	Åkesson <i>et al.</i> (2005)
Residents in cadmium-polluted area (Sweden)	Significant decreases in bone mineral density for >60 years of age with blood cadmium levels of ≥0.56 µg/L.	Alfvén <i>et al.</i> (2002)
Subjects, of which approximately 10% were environmentally or occupationally exposed to cadmium (Sweden)	Increased risk of bone fractures for >50 years of age with urinary cadmium levels of >2 µg/g creatinine.	Alfvén <i>et al.</i> (2004)
Subjects, of which approximately 10% were environmentally or occupationally exposed to cadmium (Sweden)	Increased risk of osteoporosis among men >60 years of age with urinary cadmium levels of >5 µg/g creatinine. Effect not observed in women.	Alfvén <i>et al.</i> (2000)
Residents living near zinc smelters (Belgium)	Decrease in proximal and distal forearm bone density of approximately 0.1 g/cm <sup>2</sup> was associated with a two-fold increase in urinary cadmium level in postmenopausal women.	Staessen <i>et al.</i> (1999)
Women living near zinc smelters	Suggestive evidence that cadmium has a direct osteotoxic effect.	Schutte <i>et al.</i> (2008)
Residents in cadmium-polluted area (Poland)	Significant decrease in bone mineral density in males with urinary cadmium levels of >2 µg/g creatinine.	Trzcinka-Ochocka <i>et al.</i> (2010)
Residents in cadmium-polluted area (China)	Significant increases in prevalence of low forearm bone mineral density in postmenopausal women with urinary cadmium levels of >20 µg/g creatinine.  Significant increases in prevalence of low forearm bone mineral density in men, premenopausal women, and postmenopausal women with blood cadmium levels of >20 µg/g creatinine.	Nordberg <i>et al.</i> (2002)
Residents in cadmium-polluted area (China)	Increase in bone fractures in males (mean urinary cadmium level 9.20 µg/g creatinine) and females (mean urinary cadmium level 12.86 µg/g creatinine).	Wang <i>et al.</i> (2003)
Residents in cadmium-polluted area (China)	Significant dose-response relationship between urinary cadmium levels and the prevalence of osteoporosis.	Jin <i>et al.</i> (2004b), Wang <i>et al.</i> (2003), Zhu <i>et al.</i> (2004)
Residents in areas with moderate or	Significant decreases in forearm bone mineral density in women from the moderately polluted	Chen <i>et al.</i> (2009)

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heavy cadmium pollution ten years after the source of rice was switched to commercially available rice from cadmium- non-polluted areas (China)	<p>area and in men from the heavily polluted area.</p> <p>Decreases in bone mineral density in women 60-69 or <math>\geq 70</math> years old from both polluted areas, and in men <math>\geq 70</math> years old from the heavily polluted area.</p> <p>Significantly higher prevalence of osteoporosis in women from the polluted areas which increased with urinary cadmium levels.</p>	
Residents in cadmium-polluted area (China)	<p>Higher prevalence of osteoporosis in women with renal dysfunction or tubular damage.</p> <p>Significantly lower bone mineral density levels in women with tubular damage.</p> <p>No significant associations between the prevalence of osteoporosis or bone mineral density and alterations in renal biomarkers in men.</p>	Chen <i>et al.</i> (2011)
Residents living near an industrial complex (Korea)	<p>Significant associations between high urinary cadmium levels (<math>\geq 1.0</math> <math>\mu\text{g/g}</math> creatinine) and osteopenia.</p> <p>Bone mineral density negatively associated with urinary cadmium levels.</p>	Shin <i>et al.</i> (2011)
Health- survey population (Sweden)	<p>Significantly lower urinary cadmium levels bone mineral density in postmenopausal women with elevated urinary cadmium levels (median 1.1 <math>\mu\text{g/g}</math> creatinine) compared to women with low urinary cadmium levels (median 0.36 <math>\mu\text{g/g}</math> creatinine).</p> <p>Significant changes of biomarkers indicative of increased bone resorption in the high urinary cadmium group.</p>	Engström <i>et al.</i> (2009)
General population (USA)	Significant association between urinary cadmium levels and osteopenia and osteoporosis in adults with urinary cadmium levels of $>1$ $\mu\text{g/g}$ creatinine.	Wu <i>et al.</i> (2010)
General population (USA)	43% increased risk of osteoporosis in women $\geq 50$ years of age with urinary cadmium levels of 0.50-1.00 $\mu\text{g/g}$ creatinine, as compared to women with urinary cadmium levels of $<0.50$ $\mu\text{g/g}$ creatinine.	Gallagher <i>et al.</i> (2008)
Case study: alkaline battery workers	Osteomalacia observed.	Adams <i>et al.</i> (1969)
Case study: battery plate maker	Osteomalacia observed.	Blainey <i>et al.</i> (1980)
Case study: cadmium workers	Hypercalciuria and osteomalacia observed.	Kazantzis 1979
Case study: cadmium-exposed workers	Hypercalciuria and calcium deficit observed	Scott <i>et al.</i> (1980)

A potential issue of concern is a possible link between effects on bone and kidney. However, it

is remarkable that in the studies by Schuttle *et al.* (2008) only 1 of 294 women examined displayed evidence of renal dysfunction (increased retinol binding protein). In a recent publication by Åkesson *et al.* (2014) it was concluded that the available data point towards a direct effect of cadmium on bone and therefore there are no links between adverse effects on bone and kidney since tubular proteinuria is associated with Cd exposure at  $> 4 \mu\text{g/g}$  creatinine and/or blood concentration  $> 4 \mu\text{g/L}$ ; while the available information shows that associations with bone effects occur in population strata with Cd urinary levels as low as 0.5–2  $\mu\text{g/g}$  creatinine. RAC agrees that both effects should be independently assessed and classified.

RAC notes that no information was available regarding the chemical species of cadmium to which the assessed populations were exposed. Therefore, there was no experimental evidence that cadmium hydroxide was specifically able to induce any of the previously reported effects in humans. Bioavailability of cadmium arising from cadmium hydroxide might be another issue of concern, especially taking into consideration that its solubility in water is three orders of magnitude lower than the solubility of cadmium nitrate. These two considerations (absence of empirical information with the hydroxide and doubts about bioavailability) might lead to a different classification than the highly soluble salts.

However, the dossier contains results of an *in vitro* study where it was demonstrated that cadmium oxide (slightly soluble in water), cadmium carbonate (slightly soluble in water) and cadmium sulphide (practically insoluble in water) were dissolved after 2 hours at 37 °C in artificial gastric juice (pH 1.47) to the extent of 94%, 87% and 5%, respectively. The rationale to explain these results is that the acid pH of the stomach is able to solubilize cadmium regardless of the original chemical species. Furthermore, despite again no specific information for cadmium hydroxide having been found, it seems plausible that this mechanism also applies for cadmium hydroxide and according to section 1.3.2.1 of the CLP Guidance, this is enough grounds upon which to assume bioavailability for CLH purposes.

Other experimental studies have also probed that cadmium sulphide (the cadmium salt with the lowest solubility in water) is bioavailable after inhalation (although to a lower extent than other very soluble salts). Studies on absorption of cadmium chloride and cadmium sulphide in rats after inhalation exposure 6 hours per day during 10 days showed that accumulation of cadmium in the kidney at the end of the study was 35% and 1% for cadmium chloride and cadmium sulphide, respectively, indicating bioavailability for both.

Therefore, RAC concludes that cadmium hydroxide has enough solubility in body fluids to release cadmium ions into the blood and cause typical adverse effects attributable to cadmium ions.

In conclusion, the DS supplied a large body of evidence linking the cadmium exposure (mainly through urinary cadmium excretion) in humans to the following alterations:

1. Excretion of low molecular weight proteins typically considered as biomarkers of kidney injury, such as  $\beta_2$ -microglobulin, human complex forming glycoprotein and retinol binding protein.
2. Excretion of calcium and N-acetyl- $\beta$ -glucosaminidase (also suggesting kidney alterations).
3. Increases in the prevalence of abnormal levels of the former biomarkers in occupational population and non-occupational population living in areas contaminated with cadmium.
4. Increases in the occurrence of osteomalacia, osteoporosis and bone fractures in environmentally or occupationally exposed population versus control population.

According to the Guidance on the Application of the CLP Criteria (Version 4.0, November 2013) specific target organ toxicity (repeated exposure) means significant health effects that can

impair function as consequence of a repeated exposure to a substance. The above described effects on kidney and bone can be considered as qualifying for STOT RE classification because they are toxicologically relevant and have affected the function of the kidney (caused proteinuria and increased calcium excretion) and bone morphology (caused osteoporosis and osteomalacia).

According to the Criteria in the CLP Regulation "*substances that have produced significant toxicity in humans*" are classified in Category 1 on the basis of "*reliable and good quality evidence from human cases or epidemiological studies*".

Thus, taking into consideration all the above stated information, **RAC agrees with the DS that cadmium hydroxide warrants classification as STOT RE 1; H372 (kidney, bone).**

#### 4.8 Germ cell mutagenicity (Mutagenicity)

In mammalian toxicity, the toxicity of cadmium salts is regarded to result from the intrinsic properties of the Cd<sup>2+</sup> ion. Therefore, the term cadmium used in this section refers to the Cd<sup>2+</sup> ion.

##### 4.8.1.1 Non-human information

###### 4.8.1.1.1 In vitro data

Studies on the mutagenic potential of cadmium *in vitro* have been reviewed by the International Agency for Research on Cancer (IARC, 1993, 2012) and the Agency for Toxic Substances and Disease Registry (ATSDR, 2012). The available data show that cadmium induces chromosome aberrations and gene mutations in cultured mammalian cells, while most studies on the induction of gene mutations in bacteria produced negative results. This overall picture of the results is reflected in the conclusions presented in the reviews referred to above, which involve that cadmium causes chromosome aberrations and gene mutations in mammalian cells *in vitro*. The dossier submitter agrees with this conclusion.

## 4.8.1.1.2 In vivo data

Table 12: Summary table of relevant in vivo mutagenicity/genotoxicity studies

Method	Results	Remarks	Reference
<ul style="list-style-type: none"> <li>• Test material: cadmium chloride</li> <li>• Study type: bone-marrow chromosome aberration test, bone-marrow micronucleus test and sister chromatid exchange test in bone-marrow</li> <li>• Test animals: male Swiss albino mice</li> <li>• Administration: intraperitoneal injection</li> <li>• Doses: 0, 0.42, 0.84, 1.68, 3.37 or 6.75 mg/kg bw</li> </ul>	<ul style="list-style-type: none"> <li>• Bone-marrow chromosome aberration test: positive</li> <li>• Bone-marrow micronucleus test: positive</li> <li>• Sister chromatid exchange test in bone-marrow: positive</li> </ul>	<ul style="list-style-type: none"> <li>• Key study</li> <li>• Reliability code: 2</li> <li>• Experimental result</li> </ul>	Mukherjee et al. (1988)
<ul style="list-style-type: none"> <li>• Test material: cadmium chloride</li> <li>• Study type: bone-marrow chromosome aberration test, bone-marrow micronucleus test, sister chromatid exchange test in bone-marrow and spermatogonial chromosome aberration test</li> <li>• Test animals: white Swiss mice</li> <li>• Administration: intraperitoneal injection</li> <li>• Doses: 0, 1.9, 5.7 or 9.5 mg/kg bw (bone-marrow chromosome aberration test); 0, 1.9, 5.7 or 7.6 mg/kg bw (micronucleus test and sister chromatid exchange test); 0, 0.9, 1.9, 5.7 or 9.5 mg/kg bw (spermatogonial chromosome aberration test)</li> </ul>	<ul style="list-style-type: none"> <li>• Bone-marrow chromosome aberration test: positive</li> <li>• Bone-marrow micronucleus test: positive</li> <li>• Sister chromatid exchange test in bone-marrow: positive</li> <li>• Spermatogonial chromosome aberration test: positive</li> </ul>	<ul style="list-style-type: none"> <li>• Key study</li> <li>• Reliability code: 2</li> <li>• Experimental result</li> </ul>	Fahmy and Aly (2000)
<ul style="list-style-type: none"> <li>• Test material: cadmium chloride</li> <li>• Study type: bone-marrow micronucleus test</li> <li>• Test animals: male Swiss albino mice</li> <li>• Administration: intraperitoneal injection</li> <li>• Doses: 0, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0 or 2.0 mg/kg bw</li> </ul>	<ul style="list-style-type: none"> <li>• Positive</li> </ul>	<ul style="list-style-type: none"> <li>• Key study</li> <li>• Reliability code: 2</li> <li>• Experimental result</li> </ul>	Jagetia and Adiga (1994)
<ul style="list-style-type: none"> <li>• Test material: cadmium chloride</li> <li>• Study type: erythrocyte micronucleus assay and alkaline comet assay</li> <li>• Test animals: Wistar rats</li> <li>• Administration: oral or subcutaneous injection</li> <li>• Doses: 0.5 mg/kg bw daily for 9</li> </ul>	<ul style="list-style-type: none"> <li>• Erythrocyte micronucleus test: positive</li> <li>• Alkaline comet assay: equivocal</li> </ul>	<ul style="list-style-type: none"> <li>• Key study</li> <li>• Reliability code: 2</li> <li>• Experimental result</li> </ul>	Kašuba et al. (2002)



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days (oral) or 0.5 mg/kg bw (single subcutaneous injection)			
<ul style="list-style-type: none"> <li>• Test material: cadmium chloride</li> <li>• Study type: alkaline comet assay in nasal epithelial cells, lung, whole blood, liver, kidney, bone marrow, brain and testicle</li> <li>• Test animals: male CD-1 mice</li> <li>• Administration: inhalation</li> <li>• Doses: 0.08 µg/cm<sup>3</sup>, 60 minutes inhalation twice a week (for exposure groups 2-4 below); four exposure groups, i.e. 1 (single inhalation), 2 (two weeks of exposure, three inhalations), 3 (three weeks of exposure, five inhalations), 4 (four weeks of exposure, seven inhalations)</li> </ul>	Positive in all tissues	<ul style="list-style-type: none"> <li>• Key study</li> <li>• Reliability code: 2</li> <li>• Experimental result</li> </ul>	Valverde et al. (2000)
<ul style="list-style-type: none"> <li>• Test material: cadmium chloride</li> <li>• Study type: alkaline comet assay</li> <li>• Test animals: male Swiss Albino mice</li> <li>• Administration: oral</li> <li>• Doses: 0, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0 or 128.0 mg/kg bw</li> </ul>	Positive	<ul style="list-style-type: none"> <li>• Key study</li> <li>• Reliability code: 2</li> <li>• Experimental result</li> </ul>	Devi et al. (2001)
<ul style="list-style-type: none"> <li>• Test material: cadmium chloride</li> <li>• Study type: oocyte aneuploidy test</li> <li>• Test animals: female golden hamsters</li> <li>• Administration: subcutaneous injection</li> <li>• Doses: 0, 1, 2 or 4 mg/kg bw</li> </ul>	Positive	<ul style="list-style-type: none"> <li>• Key study</li> <li>• Reliability code: 2</li> <li>• Experimental result</li> </ul>	Watanabe et al. (1979)
<ul style="list-style-type: none"> <li>• Test material: cadmium chloride</li> <li>• Study type: blastocyst aneuploidy test</li> <li>• Test animals: female ddY/F mice</li> <li>• Administration: subcutaneous injection</li> <li>• Doses: 0, 1.5 or 3.0 mg/kg bw</li> </ul>	Positive	<ul style="list-style-type: none"> <li>• Key study</li> <li>• Reliability code: 2</li> <li>• Experimental result</li> </ul>	Watanabe and Endo (1982)
<ul style="list-style-type: none"> <li>• Test material: cadmium chloride</li> <li>• Study type: oocyte aneuploidy test</li> <li>• Test animals: female CD-1 mice</li> <li>• Administration: subcutaneous injection</li> <li>• Doses: 0, 2, 4 or 6 mg/kg bw</li> </ul>	Negative	<ul style="list-style-type: none"> <li>• Key study</li> <li>• Reliability code: 2</li> <li>• Experimental result</li> </ul>	Mailhes et al. (1988)
<ul style="list-style-type: none"> <li>• Test material: cadmium chloride</li> <li>• Study type: spermatocyte</li> </ul>	Positive	<ul style="list-style-type: none"> <li>• Key study</li> <li>• Reliability code: 2</li> </ul>	Miller and Adler (1992)

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<ul style="list-style-type: none"> <li>aneuploidy test</li> <li>• Test animals: male (102/E1 x C3H/E1)F<sub>1</sub> mice</li> <li>• Administration: intraperitoneal injection</li> <li>• Doses: 0, 1, 3 or 6 mg/kg bw</li> </ul>		<ul style="list-style-type: none"> <li>• Experimental result</li> </ul>	
<ul style="list-style-type: none"> <li>• Test material: cadmium chloride</li> <li>• Study type: dominant lethal test</li> <li>• Test animals: male ICR/Ha Swiss mice</li> <li>• Administration: intraperitoneal injection</li> <li>• Doses: 5.4 or 7.0 mg/kg bw (one experiment) and 1.35 or 2.70 mg/kg bw (another experiment)</li> </ul>	Negative	<ul style="list-style-type: none"> <li>• Key study</li> <li>• Reliability code: 2</li> <li>• Experimental result</li> </ul>	Epstein et al. (1972)
<ul style="list-style-type: none"> <li>• Test material: cadmium chloride</li> <li>• Study type: dominant lethal test and heritable translocation test</li> <li>• Test animals: male BALB/c mice</li> <li>• Administration: intraperitoneal injection</li> <li>• Doses: 0 or 1.75 mg/kg bw</li> </ul>	<ul style="list-style-type: none"> <li>• Dominant lethal test: negative</li> <li>• Heritable translocation test: negative</li> </ul>	<ul style="list-style-type: none"> <li>• Key study</li> <li>• Reliability code: 2</li> <li>• Experimental result</li> </ul>	Gillivod and Léonard (1975)
<ul style="list-style-type: none"> <li>• Test material: cadmium chloride</li> <li>• Study type: dominant lethal test</li> <li>• Test animals: female (101 x C<sub>3</sub>H)F<sub>1</sub> mice</li> <li>• Administration: intraperitoneal injection</li> <li>• Doses: 0 or 2 mg/kg bw</li> </ul>	<ul style="list-style-type: none"> <li>• Dominant lethal test: negative</li> </ul>	<ul style="list-style-type: none"> <li>• Key study</li> <li>• Reliability code: 2</li> <li>• Experimental result</li> </ul>	Suter (1975)
<ul style="list-style-type: none"> <li>• Test material: cadmium chloride</li> <li>• Study type: dominant lethal test</li> <li>• Test animals: male Sprague-Dawley rats</li> <li>• Administration: oral</li> <li>• Doses: 0, 0.1, 1 or 10 mg/kg bw/day for 9 weeks</li> </ul>	<ul style="list-style-type: none"> <li>• Dominant lethal test: negative</li> </ul>	<ul style="list-style-type: none"> <li>• Key study</li> <li>• Reliability code: 2</li> <li>• Experimental result</li> </ul>	Sutou et al. (1980a, 1980b)

### Studies in somatic cells

In a study by Mukherjee et al. (1988), the mutagenicity/genotoxicity of cadmium in male Swiss albino mice exposed to cadmium chloride by intraperitoneal injection was evaluated using the bone-marrow chromosome aberration test, the bone-marrow micronucleus test and the sister chromatid exchange test in bone-marrow. Mice (5 per dose group in the chromosome aberration test and sister chromatid exchange test; 4 per dose/sampling group in the micronucleus test), 8-10 weeks old, were administered a dose of cadmium chloride of 0, 0.42, 0.84, 1.68, 3.37 or 6.75 mg/kg bw. Mitomycin C was used as a positive control.

In the chromosome aberration test, bone marrow was sampled 24 hours after the intraperitoneal injection of cadmium chloride. Scoring of chromosome aberrations was done in 60 metaphase cells

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(only with 40 chromosomes) per animal. The frequency of metaphases with chromosome aberrations (gaps excluded) at the doses 0, 0.42, 0.84, 1.68, 3.37 and 6.75 mg/kg bw was 0.996, 4.32, 5.12, 5.60 and 6.6 %, respectively, and the number of chromosome aberrations per cell at the corresponding concentrations was 0.009, 0.043, 0.0512, 0.056, 0.066 and 0.093, respectively. The increases with dose in the frequency of cells with chromosome aberrations and in the number of chromosome aberrations per cell were statistically significant ( $P < 0.05$ ), as were pairwise comparisons between the negative control and single dose groups as regards the number of chromosome aberrations per cell.

In the micronucleus test, bone marrow was sampled 24 and 48 hours after the intraperitoneal injection of cadmium chloride. From each animal 500 polychromatic erythrocytes were scored for the presence of micronuclei, and 1000 erythrocytes per animal were scored to determine the ratio of PCE/NCE (polychromatic/normochromatic erythrocytes) as a measure of cytotoxicity. At the dose of 0, 0.42, 0.84, 1.68, 3.37 and 6.75 mg/kg bw the frequency of polychromatic erythrocytes with micronuclei at the sampling time 24 hours was 0.2, 0.25, 0.30, 0.35, 0.45 and 0.60 %, respectively, and at the sampling time 48 hours the frequency at the corresponding doses was 0.15, 0.20, 0.25, 0.30, 0.35 and 0.45 %, respectively. At both sampling times the increase with dose in the frequency of polychromatic erythrocytes with micronuclei were statistically significant ( $P < 0.05$ ), as was the pairwise comparison between the negative control and the 6.75 mg/kg bw dose group. No statistically significant effects on the ratio of PCE/NCE were observed.

In the sister chromatid exchange test, bone-marrow cells were sampled 24 hour after the intraperitoneal injection of cadmium chloride. Examination of sister chromatid exchanges was done in 20 metaphase cells (only with 40 chromosomes) per animal. To evaluate effects on cell proliferation, frequencies of first-, second- and third-generation metaphases was determined in 100 consecutive metaphases per animal to calculate a proliferation rate index. At the dose of 0, 0.42, 0.84, 1.68, 3.37 and 6.75 mg/kg bw the number of sister chromatid exchanges per cell was 2.30, 2.79, 3.07, 3.50, 4.82 and 5.53, respectively. The increase with dose in the number of sister chromatid exchanges per cell was statistically significant ( $P < 0.0001$ ). Pairwise comparison between the negative control and single dose groups showed that the increases in the number of sister chromatid exchanges per cell were statistically significant ( $P < 0.05$ ) for all doses except the lowest (0.42 mg/kg bw). No statistically significant effects on bone marrow cell replication were established in the analyses of the proliferation rate index.

In a study by Fahmy and Aly (2000), the mutagenicity/genotoxicity of cadmium in white Swiss mice exposed to cadmium chloride by intraperitoneal injection was evaluated using the bone-marrow chromosome aberration test (8-12 weeks old mice), the bone-marrow micronucleus test (6-8 weeks old mice) and the sister chromatid exchange test in bone-marrow (3-4 months old mice). In the chromosome aberration test, mice (5 per dose group) were administered a single dose of cadmium chloride of 0, 1.9, 5.7 or 9.5 mg/kg bw, and in the micronucleus test and the sister chromatid exchange tests, mice (5 per dose group) were administered a single dose of cadmium chloride of 0, 1.9, 5.7 or 7.6 mg/kg bw, and

In the bone-marrow chromosome aberration test, bone marrow was sampled 24 hours after the intraperitoneal injection of cadmium chloride. For the treatment with the dose 9.5 mg/kg bw, additional sampling was done at 12 and 48 hours. Mitomycin *c* was used as a positive control. Scoring of chromosome aberrations was done in 75-100 metaphase plates per animal. At the 24 hours sampling time the frequency of metaphases with chromosome aberrations (gaps excluded) at the doses 0, 1.9, 5.7 and 9.5 mg/kg bw was 1.75, 3.28, 5.75 and 11.00 %, respectively. For the two highest doses the increase in the frequency of cells with chromosome aberrations was statistically significant ( $P < 0.01$ ) as compared to the negative control, and the values show that the increase was dose related. At the highest dose 9.5 mg/kg bw, the frequency of cells with chromosome aberrations

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in the additional samples taken at 12 and 48 hours after treatment was 8.00 and 5.25 %, respectively. The increases were statistically significant ( $P < 0.01$ ) as compared to the negative control. However, both values were lower than 11.00 % observed for cells sampled 24 hours after the treatment. Polyploidy and endomitosis was observed in treated groups only.

In the bone-marrow micronucleus test, bone marrow was sampled 24 hours after the intraperitoneal injection of cadmium chloride. Mitomycin *c* was used as a positive control. A statistically significant increase in the frequency of polychromatic erythrocytes with micronuclei as compared to the negative control value of 0.57 % was observed at all doses of cadmium chloride, i.e. at 1.9, 5.7 and 7.6 mg/kg bw a frequency of 1.3 ( $P < 0.05$ ), 1.6 ( $P < 0.05$ ) and 2.1 ( $P < 0.01$ ) %, respectively, was observed. The values demonstrate that the increase was dose related. Bone marrow toxicity was induced at the two highest doses, as indicated by statistically significant increases in the frequency of polychromatic erythrocytes as compared to the negative control, confirming that the test substance reached the target cells.

In the sister chromatid exchange test, bone-marrow cells were sampled for examination for sister chromatid exchanges in 40 well-spread metaphases per animal. Mitomycin *c* was used as a positive control. The mean number of sister chromatid exchanges per cell at the doses 0, 1.9, 5.7 and 7.6 mg/kg bw was 4.6, 5.44, 6.67 and 7.35. The increase in the mean number of sister chromatid exchanges per cell was statistically significant at the mid ( $P < 0.05$ ) and high ( $P < 0.01$ ) dose as compared to the negative control, and the values show that the increase was dose related.

In a study by Jagetia and Adiga (1994), the mutagenicity of cadmium in male Swiss albino mice exposed to cadmium chloride by intraperitoneal injection was evaluated by the bone-marrow micronucleus test. Mice (4 per exposure group), 6-8 weeks old, were administered a dose of cadmium chloride of 0, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0 or 2.0 mg/kg bw. Bone marrow was sampled from the animals at 24 hours after the administration of cadmium chloride. A minimum of 2000 polychromatic erythrocytes in each animal were scored for the presence of micronuclei, and at least 4000 erythrocytes per animal were scored to determine the ratio of PCE/NCE (polychromatic/normochromatic erythrocytes) as a measure of cytotoxicity. At the dose of 0, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0 and 2.0 mg/kg bw the frequency of polychromatic erythrocytes with micronuclei was 2.28, 3.41, 4.68, 5.34, 6.46, 7.00, 7.90 and 8.67 per 1000 cells, respectively. The frequency increased in a dose-related manner. In comparisons with the negative control, statistically significant increases were established at 0.05 mg/kg bw ( $P < 0.05$ ) and at 0.1, 0.25, 0.5, 1.0 and 2.0 mg/kg bw ( $P < 0.01$ ). A dose-related decline in the PCE/NCE ratio was observed, and the decrease was statistically significant at 0.25 mg/kg bw (1.08) and higher doses (0.87, 0.85, 0.80), as compared to the negative control (1.40).

In a study by Kašuba et al. (2002), the mutagenicity/genotoxicity of cadmium in 6 days old suckling Wistar rats exposed orally to cadmium chloride and in 5 days old suckling Wistar rats exposed subcutaneously to cadmium chloride was evaluated with the erythrocyte micronucleus assay and the alkaline comet assay. Rats (10 per dose group) were orally administered daily doses of cadmium of 0.5 mg/kg bw as cadmium chloride for 9 days, or subcutaneously administered a single injection cadmium of 0.5 mg/kg bw as cadmium chloride. Rats were sacrificed at the age of 16 days, whereupon blood was sampled for analyses with the erythrocyte micronucleus assay and the comet assay.

In the micronucleus assay, 10 animals were analysed in each exposed group and scoring of micronuclei was done in 2000 reticulocytes per animal. In animals exposed to cadmium chloride, a statistically significant ( $P < 0.05$ ) increase in the mean number of micronuclei was observed as compared to the negative control (5 animals analysed). The mean number of micronuclei was 16.6 and 14.3 in orally and subcutaneously exposed groups, respectively, and 3.2 in the negative control.

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In the comet assay, 4 orally exposed and 3 subcutaneously exposed animals were analysed and scoring of comet tail intensity and comet tail length was done in 100 cells per animal. No increase in comet tail intensity was observed in groups exposed to cadmium chloride as compared to the negative control (5 animals analysed). However, a statistically significant ( $P < 0.05$ ) increase in comet tail length was observed in exposed groups as compared to the negative control. The comet tail length was 59.68 and 58.42 in orally and subcutaneously exposed groups, and 21.17 in the negative control. Due to the lack of an increase in tail intensity, the result of the study is considered to be equivocal.

In a study by Valverde et al. (2000), the genotoxicity of cadmium in male CD-1 mice exposed to cadmium chloride by inhalation was evaluated by the comet assay in nasal epithelial cells, lung, whole blood, liver, kidney, bone marrow and brain. Mice (4 per exposure group), 45 days old, were exposed to cadmium chloride at the concentration of  $0.08 \mu\text{g}/\text{cm}^3$ . The duration of each inhalation treatment was 60 minutes, and groups receiving multiple treatments were treated twice a week. The study involved four exposure groups, i.e. 1, single inhalation; 2, two weeks of exposure, three inhalations; 3, three weeks of exposure, five inhalations; 4, four weeks of exposure, seven inhalations. For each group, two control animals exposed to deionised water for the same time were used. Animals were sacrificed 24 hours after the last treatment, and immediately thereafter blood was sampled and the nasal septum, lung, liver, kidney, femur and brain were removed. Scoring of comet tail length as a measure of DNA damage was done in 100 cells/tissue/animal.

After the first exposure, a statistically significant increase in comet tail length was observed in all tissues except the kidney, in which a statistically significant increase was first observed after two weeks of exposure. In the nasal epithelial cells, lung, bone marrow and brain the level of DNA damage remained rather constant during the exposure period of four weeks, while in the liver, kidney and leucocytes a remarkable increase in DNA damage was observed in the last week of exposure. The viability of cells was  $>80\%$  in all tissues as determined by the trypan blue exclusion technique.

In a study by Devi et al. (2001), the genotoxicity of cadmium in male Swiss Albino mice exposed to cadmium chloride by oral intubation was evaluated with the alkaline comet assay. Mice (5 per dose group), four weeks of age, were administered a single dose of cadmium chloride of 0, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0 or 128.0 mg/kg bw. Cyclophosphamide was used as a positive control. Blood was sampled from the animals at 24, 48, 72 and 96 hours after the administration of cadmium chloride. Following completion of electrophoresis 50 leucocytes from each animal were scored for comet tail length as an estimate of DNA damage. Cell viability ranged from 92 to 96 %, as determined by the trypan blue exclusion technique. At the 24 hours sampling time a statistically significant ( $P < 0.05$ ) increase in mean comet tail-length was observed at all nine dose levels as compared to the negative control. Moreover, a clear dose-response relationship was observed, with an approximate 14-fold increase in mean comet tail-length at the highest dose as compared to the negative control. At the later sampling times, i.e. 48, 72 and 96 hours, a gradual decrease in mean comet tail-length with time was observed at all dose levels.

### Studies in germ cells

In a study by Watanabe et al. (1979), the mutagenicity of cadmium in female golden hamsters (*Mesocricetus auratus*) exposed to cadmium chloride by subcutaneous injection was evaluated by scoring chromosome aberrations in meiotic metaphase II oocytes. Hamsters (20 per dose group), 8-12 weeks old, were administered a dose of cadmium chloride of 0, 1, 2 or 4 mg/kg bw 5 hours before the time of estimated ovulation. The animals were anaesthetised 12 hours after treatment, whereupon oocytes were extracted for cytogenetic analysis. Oocytes with adequately spread meiotic

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metaphase II plates were examined for structural and numerical chromosome aberrations. In total, 536 oocytes from cadmium-treated groups and the control group were analysed. Only numerical chromosome aberrations were observed, i.e. hypohaploidy (n-1), hyperhaploidy (n+1) and diploidy (2n). In addition, meiotic anaphase I oocytes with two separate haploid chromosome sets and no degenerated polar body occurred in cadmium treated hamsters. The overall frequency of oocytes with numerical chromosome aberrations at the dose of 0, 1, 2 and 4 mg/kg bw was 2.8, 3.7, 27.6 and 26.3 %, respectively. The increase with dose in the overall frequency of oocytes with numerical chromosome aberrations was statistically significant ( $P < 0.01$ ). Pairwise comparison between the negative control and single dose groups showed that the increases in the overall frequency of oocytes with numerical chromosome aberrations were statistically significant ( $P < 0.01$ ) at the two highest doses. Statistically significant increases were also observed for specific chromosome aberrations, except hypohaploidy.

In a study by Watanabe and Endo (1982), the mutagenicity of cadmium in female ddY/F mice exposed to cadmium chloride by subcutaneous injection was evaluated by scoring blastocysts (preimplantation embryos) for numerical chromosome aberrations. Mice were induced to superovulate by treatment with pregnant mare's serum and human chorionic gonadotropin. Three hours after the superovulation treatment and mating, the females were administered a dose of cadmium chloride of 0, 1.5 or 3.0 mg/kg bw. Animals were sacrificed about 82 hours after mating, whereupon blastocysts were flushed from the uterus. Metaphase cells of the blastocysts were analysed for numerical chromosome aberrations. The frequency of blastocysts with numerical chromosome aberrations (hypodiploidy, hyperdiploidy and triploidy) at the dose of 0, 1.5 and 3.0 mg/kg bw was 3.3, 12.3 and 15.9 %, respectively. The increase in numerical chromosome aberrations was statistically significant ( $P < 0.05$ ) at the high dose.

In a study by Mailhes et al. (1988), the mutagenicity of cadmium in female CD-1 mice exposed to cadmium chloride by subcutaneous injection was evaluated by scoring hyperploidy in meiotic metaphase II oocytes. Mice (50 per dose group), 8-12 weeks old, were induced to superovulate by treatment with pregnant mare's serum and human chorionic gonadotropin, and immediately thereafter mice were administered a dose of cadmium chloride of 0, 2, 4 or 6 mg/kg bw. The animals were sacrificed 17 hours after treatment, whereupon oocytes were extracted for cytogenetic analysis. No increase in hyperploidy was observed.

In a study by Miller and Adler (1992), the mutagenicity of cadmium in male (102/E1 x C3H/E1) $F_1$  mice exposed to cadmium chloride by intraperitoneal injection was evaluated by scoring spermatocytes for aneuploidy. Mice (6 per dose group), 10-14 weeks old, were administered a dose of cadmium chloride of 0, 1, 3 or 6 mg/kg bw. Colcemid and econazole were used as positive controls. Germ cells were sampled from the animals 6, 14 and 22 hours after the intraperitoneal injection of cadmium chloride. Scoring of hypoploidy and hyperploidy was done in 100 meiotic metaphase II cells per animal. With the cadmium chloride doses of 1, 3 and 6 mg/kg bw, 0.7-0.8 % of the meiotic metaphase II cells had more than 20 chromosomes, i.e. were hyperploidy, compared to 0.3 % in the negative control. For none of the individual dose groups a statistically significant difference from the frequency in the positive control group could be demonstrated, but a statistically significant ( $P < 0.05$ ) induction of hyperploidy was established when all dose groups were compared to the negative control group by the Cochran-Armitage trend test. A statistically significant ( $P < 0.01$ ) induction was also established for hypoploidy when all dose groups were compared to the negative control group by the trend test.

In a study by Fahmy and Aly (2000), the mutagenicity of cadmium in white Swiss mice exposed to cadmium chloride by intraperitoneal injection was evaluated by the spermatogonial chromosome aberration test. Mice (5 per dose group), 8-12 weeks old, were administered a single dose of cadmium chloride of 0, 0.9, 1.9, 5.7 or 9.5 mg/kg bw. Cyclophosphamide was used as a positive

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control. Germ cells were sampled from the animals 12 days after the intraperitoneal injection of cadmium chloride. Scoring of chromosome aberrations was done in 75-100 metaphase plates per animal. The results showed that male mice spermatogenesis is sensitive to cadmium chloride, as demonstrated by reduction in the number of spermatocytes at the lower doses and failure of spermination at the highest dose of 9.5 mg/kg bw. The frequency of spermatocytes with chromosome aberrations (X-Y univalents, autosomal univalent, fragments and translocations) at the doses 0, 0.9, 1.9 and 5.7 mg/kg bw was 4.0, 4.25, 6.0 and 8.5 %, respectively. The increase in spermatocytes with chromosome aberrations was statistically significant ( $P < 0.05$ ) at the dose of 5.7 mg/kg bw. There was an increase in both numerical and structural aberrations; however the majority of the aberrations were numerical.

In a study by Epstein et al. (1972), the mutagenicity of cadmium in male ICR/Ha Swiss mice exposed to cadmium chloride by intraperitoneal injection was evaluated by the dominant lethal test. Male mice (7 and 9 in the low and high dose group, respectively), 8-10 weeks old, were in one experiment administered a dose of cadmium chloride of 5.4 or 7.0 mg/kg bw and, in another experiment, 1.35 or 2.70 mg/kg bw. The negative control group consisted of 10 males. Each male was mated with 3 untreated females, 8-10 weeks old, which were replaced weekly for 8 consecutive weeks. Females were dissected and analysed. No increase in dominant lethals was observed in any of the experiments.

In a study by Gilliavod and Léonard (1975), the mutagenicity of cadmium in male BALB/*c* mice exposed to cadmium chloride by intraperitoneal injection was evaluated with the dominant lethal test and the heritable translocation assay. Male mice (number not specified), 11-13 weeks old, were administered a dose of cadmium chloride of 0 or 1.75 mg/kg bw. In the dominant lethal test each male was mated with 3 untreated females (age not specified), which were replaced weekly for 3 consecutive weeks. Females were dissected and analysed. No increase in dominant lethals was observed. In the heritable translocation assay, germ cells were sampled from 120 F<sub>1</sub> males from matings between cadmium chloride treated males and untreated females. From each F<sub>1</sub> male 25 spermatocytes were analysed for the occurrence of translocations. No translocations were observed.

In a study by Suter (1975), the mutagenicity of cadmium in female (101 x C<sub>3</sub>H)F<sub>1</sub> mice exposed to cadmium chloride by intraperitoneal injection was evaluated by the dominant lethal test. Mice (85 females), age not indicated, were administered a dose of cadmium chloride of 0 or 2 mg/kg bw and were mated 0.5-4.5 days later. A negative control group of 79 females was included. Females were sacrificed 12-15 days after copulation and were then dissected and analysed. No increase in dominant lethals was observed.

In a study by Sutou et al. (1980a, 1980b), the mutagenicity of cadmium in male Sprague-Dawley rats orally exposed to cadmium chloride was evaluated using the dominant lethal test. Rats (14 per dose group), 4 weeks old, were administered a dose of cadmium chloride of 0, 0.1, 1 or 10 mg/kg bw/day for 9 weeks and were then mated with 2 females per week for 6 weeks. Pregnant females were sacrificed on the 13<sup>th</sup> day of gestation and were thereafter dissected and analysed. No increase in dominant lethals was observed.

In a study by Valverde et al. (2000), the genotoxicity of cadmium in male CD-1 mice exposed to cadmium chloride by inhalation was evaluated by the comet assay in the testicle. Mice (4 per exposure group), 45 days old, were exposed to cadmium chloride at the concentration of 0.08 µg/cm<sup>3</sup>. The duration of each inhalation treatment was 60 minutes, and groups receiving multiple treatments were treated twice a week. The study involved four exposure groups, i.e. 1, single inhalation; 2, two weeks of exposure, three inhalations; 3, three weeks of exposure, five inhalations; 4, four weeks of exposure, seven inhalations. For each group, two control animals exposed to deionised water for the same time were used. Animals were sacrificed 24 hours after the last

treatment, and immediately thereafter the testicles were removed. Scoring of comet tail length as a measure of DNA damage was done in 100 cells/animal.

After the first exposure, a statistically significant increase in comet tail length was observed. The level of DNA damage remained rather constant during the exposure period of four weeks. The viability of cells was >80 % as determined by the trypan blue exclusion technique.

### 4.8.1.2 Human information

To investigate the mutagenic potential of cadmium in somatic cells (peripheral blood lymphocytes) in humans, studies have been performed in workers from different occupational fields (i.e. cadmium plant; alkaline battery factory; manufacture of cadmium pigments; production of cadmium, zinc, silver and copper alloys; smelter; zinc industry; zinc smelting plant), and in people living in cadmium-polluted areas. In some of the studies an increase in the frequency of chromosome aberrations was observed. However, from the evaluation of the studies in the EU RAR (2007) it is apparent that the studies were afflicted with shortcomings regarding study designs, exposure assessments or consideration of confounding factors limiting their value as evidence for a causal relationship between exposure to cadmium and mutagenicity. The dossier submitter agrees with the view presented in the EU RAR (2007) regarding the imperfect quality of the studies. Hence, the data would not be sufficient for the purpose of classification according to CLP and, therefore, summaries of the studies have not been included in the present CLH report.

No studies on the mutagenic potential of cadmium in germ cells of humans are available.

### 4.8.2 Other relevant information

### 4.8.3 Summary and discussion of mutagenicity

The mutagenic potential of cadmium has been investigated *in vitro* in bacterial cells and mammalian cells, and *in vivo* in somatic cells of mice and rats and in germ cells of mice, rats and golden hamsters. Studies in somatic cells of humans were generally afflicted with shortcomings limiting their value as evidence for a causal relationship between exposure to cadmium and mutagenicity. Hence, the data would not be sufficient for the purpose of classification according to CLP and, therefore, the studies have not been evaluated in the present CLH report. No studies on the mutagenic potential of cadmium in germ cells of humans are available.

Results from studies *in vitro* showed that cadmium induces chromosome aberrations and gene mutations in cultured mammalian cells, while most studies on the induction of gene mutations in bacteria produced negative results (IARC, 1993, 2012; ATSDR, 2012).

Cadmium chloride induced chromosome aberrations in somatic cells *in vivo* after intraperitoneal injection, as demonstrated by positive results from cytogenetic studies in the bone marrow of mice (Mukherjee et al., 1988; Fahmy and Aly, 2000) and micronucleus studies in the bone marrow of mice (Mukherjee et al., 1988; Jagetia and Adiga, 1994; Fahmy and Aly, 2000). In rats, a micronucleus study in blood was positive after oral administration of cadmium chloride (Kasuba et al., 2002).

Cadmium chloride induced DNA damage in somatic cells *in vivo* detected by the alkaline comet assay, as demonstrated by positive results from a study in blood of mice after oral administration (Devi et al., 2001), and a study in nasal epithelial cells, lung, whole blood, liver, kidney, bone marrow and brain of mice exposed by inhalation (Valverde et al., 2000). One study in blood of orally exposed mice produced equivocal results (Kasuba et al., 2002).



Cadmium chloride induced sister chromatid exchanges in somatic cells *in vivo* after intraperitoneal injection, as demonstrated by positive results from studies in mice (Mukherjee et al., 1988; Fahmy and Aly, 2000).

Cadmium chloride induced numerical and structural chromosome aberrations in germ cells *in vivo*, as demonstrated by positive results from studies in mice exposed by intraperitoneal injection (Miller and Adler, 1992; Fahmy and Aly, 2000) and subcutaneous injection (Watanabe and Endo, 1982). One study on numerical chromosome aberrations in germ cells of mice exposed to cadmium chloride by subcutaneous injection was negative (Mailhes et al., 1988).

Cadmium chloride administered by intraperitoneal injection did not induce dominant lethals in germ cells of mice (Epstein et al., 1972; Gillivod and Léonard, 1975; Suter, 1975) and rats (Sutou et al., 1980), or heritable translocations in mice (Gillivod and Léonard, 1975).

The dossier submitter considers that there is sufficient evidence to conclude that cadmium induces structural chromosome aberrations and micronuclei in somatic cells *in vivo*, and numerical and structural chromosome aberrations in germ cells *in vivo*. The potential to induce numerical chromosome aberrations in germ cells, and observations of polyploidy and endomitosis in somatic cells, suggests that also the aneugenic potential of cadmium contributed to the induction of micronuclei observed in somatic cells. The potential of cadmium to induce chromosome aberrations was not detected in germ cells using the dominant lethal test. However, the dominant lethal test is generally considered to be rather insensitive.

The positive results from *in vivo* genotoxicity studies measuring the induction of DNA damage (comet assay) and sister chromatid exchanges in somatic cells are in agreement with the positive results from the *in vivo* mutagenicity studies referred to above.

In mammalian toxicity, the toxicity of cadmium salts is regarded to result from the intrinsic properties of the  $\text{Cd}^{2+}$  ion. As outlined in Section 4, it is reasonable to assume that the  $\text{Cd}^{2+}$  ion is bioavailable after oral and inhalation exposure of cadmium hydroxide. Hence, the mutagenic effects of other cadmium salts, such as cadmium chloride used in the studies summarised above, are relevant also for cadmium hydroxide. Accordingly, it is concluded that cadmium hydroxide is mutagenic in germ cells of experimental animals.

#### 4.8.4 Comparison with criteria

According to the criteria in the CLP Regulation, Annex I: 3.5.2.2, classification in Category 1A is reserved for substances known to induce heritable mutations in the germ cells of humans. No studies on the mutagenic potential of cadmium in germ cells of humans are available. Therefore, classification in Category 1A is not justified.

Where no evidence from human studies is available, classification in Category 1B or 2 is based on the type of evidence that is available from animal studies and *in vitro* studies. Classification in Category 1B is for substances for which there are positive results from *in vivo* heritable germ cell mutagenicity tests in mammals; or positive results from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations in germ cells; or positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny (i.e. substances to be regarded as if they induce heritable mutations in the germ cells of humans). Classification in Category 2 is for substances for which there is positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from somatic cell mutagenicity tests *in vivo* in mammals; or other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro*

mutagenicity assays (i.e. substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans). On consideration of the *in vivo* data referred to in this CLH report, they are considered to demonstrate positive results from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations in germ cells. This is based on the facts that structural chromosome aberrations were induced in somatic cells of mice, micronuclei were induced in somatic cells of mice and rats, and that numerical and structural chromosome aberrations were induced in the germ cells of mice.

In view of these considerations, the evidence referred to is deemed to match the criteria for classification of cadmium hydroxide as a Category 1B mutagen.

#### 4.8.5 Conclusions on classification and labelling

Cadmium hydroxide should be classified in Muta. 1B (H340).

#### **RAC evaluation of germ cell mutagenicity**

##### **Summary of the Dossier submitter's proposal**

The DS proposed a Muta. 1B; H340 classification for cadmium hydroxide.

The DS noted that studies in human somatic cells were generally affected by shortcomings limiting their value as evidence for a causal relationship between exposure to cadmium and mutagenicity. Hence, the data would not be sufficient for the purpose of classification according to CLP and, therefore, the studies were not evaluated in the CLH report.

The mutagenic potential of cadmium has been investigated *in vitro* in bacterial cells and mammalian cells, and *in vivo* in somatic cells of mice and rats and in germ cells of mice, rats and golden hamsters.

*In vitro* studies showed that cadmium induces chromosome aberrations and gene mutations in cultured mammalian cells, while most studies on the induction of gene mutations in bacteria produced negative results.

Cadmium chloride induced chromosome aberrations in somatic cells *in vivo* after intraperitoneal injection, as demonstrated by positive results from cytogenetic studies in the bone marrow of mice and micronucleus studies in the bone marrow of mice. In rats, a micronucleus study in blood was positive after oral administration of cadmium chloride.

Cadmium chloride induced DNA damage in somatic cells *in vivo* detected by the alkaline comet assay, as demonstrated by positive results from a study in blood of mice after oral administration, and a study in nasal epithelial cells, lung, whole blood, liver, kidney, bone marrow and brain of mice exposed by inhalation. One study in blood of orally exposed mice produced equivocal results.

Cadmium chloride induced numerical and structural chromosome aberrations in germ cells *in vivo*, as demonstrated by positive results from studies in mice exposed by intraperitoneal or subcutaneous injection. One study on numerical chromosome aberrations in germ cells of mice exposed to cadmium chloride by subcutaneous injection was negative.

Cadmium chloride administered by intraperitoneal injection did not induce dominant lethals in germ cells of mice and rats, or heritable translocations in mice.

The DS concluded that there was sufficient evidence that cadmium induces structural chromosome aberrations and micronuclei in somatic cells *in vivo*, and numerical and structural

chromosome aberrations in germ cells *in vivo*. The potential of cadmium to induce chromosome aberrations was not detected in germ cells using the dominant lethal test. However, according to the DS, the dominant lethal test is generally considered to be rather insensitive.

The DS considered the toxicity of cadmium salts to result from the intrinsic properties of the Cd<sup>2+</sup> ion. As bioavailability of the Cd<sup>2+</sup> ion could be assumed after oral and inhalation exposure to cadmium hydroxide, the mutagenic effects of other cadmium salts, such as cadmium chloride used in the studies summarised above, were considered relevant for cadmium hydroxide. Accordingly, the DS concluded that cadmium hydroxide is mutagenic in germ cells of experimental animals.

Classification in Category 1A was not considered justified because there are no available studies on the mutagenic potential of cadmium in human germ cells.

Based on the observations that structural chromosome aberrations were induced in somatic cells of mice, that micronuclei were induced in somatic cells of mice and rats, and that numerical and structural chromosome aberrations were induced in the germ cells of mice, the DS considered that there was sufficient evidence to demonstrate that cadmium hydroxide warrants classification as a Category 1B mutagen i.e. there were positive results from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations in germ cells.

### **Comments received during public consultation**

One MS agreed explicitly with the classification proposal for Muta. 1B; H340.

The International Cadmium Association (ICdA) did not consider that there was adequate justification for the proposed Muta. 1B; H340 classification. Based on the read across principles, the ICdA commented that the cadmium hydroxide should be classified according to the previous harmonised classification for cadmium compounds belonging to the same water-solubility range group i.e. cadmium oxide and cadmium metal and, therefore, that cadmium hydroxide should be classified as Muta. 2; H341. The ICdA referred to the fact that cadmium oxide received a Muta. Cat. 3: R68 classification (corresponding to CLP classification Muta 2; H340) rather than Muta. Cat. 2: R46 (corresponding to CLP classification Muta. 1B; H340) because there was no positive evidence for cadmium oxide itself. The positive results of *in vivo* somatic cell mutagenicity studies that led to the Muta. Cat. 3: R68 classification for cadmium oxide under the Dangerous Substances Directive **67/548/EEC** (DSD) were from other cadmium compounds.

In response to the comment from the ICdA, the DS stated that they did not agree with the approach taken by industry to classify cadmium hydroxide. The DS stated that available data support that cadmium salts release the toxic species Cd<sup>2+</sup> which is bioavailable even with cadmium salts of low water solubility. Consequently, cadmium chloride (very soluble) is an appropriate analogue for cadmium hydroxide (slightly soluble). Since data from studies with cadmium chloride provided evidence that the hazardous properties of the Cd<sup>2+</sup> ion include mutagenicity in germ cells, cadmium hydroxide should be classified in Muta. 1B; H340.

### **Additional key elements**

#### Regulatory background

The DS proposal to classify cadmium hydroxide in Cat. 1B for mutagenicity is based on the existing classification of several other cadmium compounds: cadmium chloride, cadmium sulphate and cadmium fluoride. The Muta. 1B classification of these substances is not open to review, but meeting records of the Commission Working Group on the Classification and Labelling of Dangerous Substances from 1998 indicate that this was agreed only after considerable discussion. The Netherlands, Germany and France initially favoured Muta. Cat. 3;

R40, whereas the UK and Sweden favoured Muta. Cat. 2; R46 (classification according to the criteria of the Dangerous Substances Directive 67/548/EEC). The eventual agreement to classify these salts with Muta. Cat. 2; R46 (equivalent to Muta. 1B under CLP) was based on a combination of the clear genotoxic effects seen in somatic cells *and* evidence of germ cell exposure in several rodent fertility studies involving oral or inhalational treatment with cadmium chloride. The germ cell mutagenicity studies presented by the DS in their proposal to classify cadmium hydroxide are essentially the same as those discussed for cadmium chloride, cadmium sulphate and cadmium fluoride in 1998, but which were not in themselves considered sufficient to justify the higher level of classification.

In contrast to the chloride, sulphate and fluoride salts, cadmium metal, cadmium oxide and cadmium sulphide have a harmonised classification for mutagenicity as Muta. 2; H341. The equivalent classification under DSD (Muta. Cat. 3; R40) was agreed by the Commission Working Group on the Classification and Labelling of Dangerous Substances in 2002. At that time, the debate was whether mutagenicity classification for these three substances was appropriate or not, i.e. the options were either to classify with Muta. Cat. 3; R68 or not to classify at all. The final decisions in favour of Muta. Cat. 3; R68 (equivalent to Muta. 2; H341 under CLP) for the metal, oxide and sulphide were based on concerns about the general potential for cadmium bioavailability in combination with the general positive evidence suggesting that cadmium can induce genotoxic effects in somatic tissues. However, no Member State argued that these substances should be classified with Muta. Cat. 2; R46 (equivalent to Muta. 1B under CLP). The lower water solubility and possibly lower bioavailability of Cd<sup>2+</sup> ions from the metal, oxide and especially the sulphide compared to the chloride were thus also taken into account.

#### **Assessment and comparison with the classification criteria**

RAC agrees with the DS that the available data show clearly that highly water soluble cadmium salts, exemplified by cadmium chloride, have mutagenic potential. Cadmium chloride induces chromosome aberrations and gene mutations in cultured mammalian cells and this genotoxicity has been confirmed in somatic cells *in vivo* in several mouse bone marrow or peripheral erythrocyte micronucleus and chromosome aberration tests.

In germ cells, negative results have been reported in three mouse dominant lethal assays, a rat dominant lethal assay and a mouse heritable translocation assay. However, none of these tests were conducted according to the most stringent recommended conditions (e.g. in comparison to the OECD test guidelines) and the negative results cannot be regarded as robust evidence that systemically available Cd<sup>2+</sup> will lack genotoxic activity in the germ cells.

In contrast, a positive response (increased tail length) was found recently in a comet assay of testicular DNA from mice exposed once and multiple times for 60 min to 0.08 µg/cm<sup>3</sup> cadmium chloride by inhalation (details provided in the CLH report). In this test, positive results were also found in DNA from several somatic tissues, including liver, kidney, bone marrow and brain. This study appears to show that bioavailable Cd<sup>2+</sup> also has potential to damage germ cell DNA. Further support for this is provided in an unconventional, but well performed test for aneuploidy in the spermatocytes of mice treated once with 1, 3 or 6 mg/kg bw cadmium chloride by intra-peritoneal injection. Statistically significant increases in hyperploidy and hypoploidy were seen in comparisons made with a negative control group.

Additionally, a positive result was reported in an *in vivo* mouse spermatogonial chromosome aberration test, in which mice were administered cadmium chloride intra-peritoneally (0.9, 1.9, 5.7 and 9.5 mg/kg bw). Studies employing non-standard methodology to investigate genetic damage in mice and hamster oocytes *in vivo* gave inconsistent results; overall they provide limited weight in the analysis of the mutagenic potential of Cd<sup>2+</sup>.

As concluded by the DS, RAC's opinion is that these data indicate that bioavailable Cd<sup>2+</sup> has the potential to damage the genetic material in somatic and germ cells. Although some of the studies described above used non-physiological routes of administration, there was clear

evidence from studies involving oral and inhalational exposure which are of the greatest relevance for assessing this hazard in humans.

On the basis of these studies, it can be concluded that systemically available  $\text{Cd}^{2+}$  poses a mutagenic hazard to germ cells in animals. As oral and inhalational exposure to cadmium hydroxide can be expected to yield systemically available  $\text{Cd}^{2+}$  ions, it can be assumed that cadmium hydroxide will thus possess this hazard. Therefore classification in Category 1B for mutagenicity is justified. In the absence of any data informing on germ cell mutagenicity in humans, Category 1A is inappropriate.

An alternative option would be to take note of the current Category 2 mutagenicity classification of cadmium metal and cadmium oxide in Annex VI of the CLP Regulation. These substances have a very similar water solubility to cadmium hydroxide and might therefore be considered to be better models for the potential bioavailability of  $\text{Cd}^{2+}$  from this compound than the highly water soluble salts. However, there are very limited data available on the mutagenicity of these less water soluble substances. In addition to negative results in the bacterial mutagenicity tests, a negative result was reported in a peripheral blood erythrocyte micronucleus test in mice exposed for 13-weeks to cadmium oxide by inhalation (Dunnick *et al*, 1995; cited by the International Cadmium Association in their submission during the public consultation). Full details of this micronucleus test were not provided to RAC during public consultation, and it was not included in the CLH report from the DS. However, this test was not performed according to a standard regulatory protocol and the study only addressed inhalational exposure. It cannot be taken as evidence for a lack of  $\text{Cd}^{2+}$  bioavailability and absence of a mutagenic hazard, especially as in other tests conducted by the same laboratory, inhalation of this substance did cause systemic effects in rats (reproductive toxicity). It seems likely that the sensitivity of the micronucleus test was not optimised.

On the basis that mutagenicity is a hazard that can be caused even by very low concentrations of a genotoxic species (i.e. mutagenicity is regarded routinely for regulatory purposes as a "non-threshold" hazard), RAC's opinion is that even relatively limited bioavailability of  $\text{Cd}^{2+}$  will present an inherent mutagenic hazard to germ cells. On this basis, RAC concluded that classification in **category 1B for mutagenicity** was warranted.

#### 4.9 Carcinogenicity

In mammalian toxicity, the toxicity of cadmium salts is regarded to result from the intrinsic properties of the  $\text{Cd}^{2+}$  ion. Therefore, the term cadmium used in this section refers to the  $\text{Cd}^{2+}$  ion.

#### 4.9.1 Non-human information

**Table 13: Summary table of relevant carcinogenicity studies**

Method	Results	Remarks	Reference
<ul style="list-style-type: none"> <li>• Test material: cadmium chloride</li> <li>• Test animals: male Wistar (WF/NCr) rats</li> <li>• Administration: oral (diet)</li> <li>• Doses: 0, 25, 50, 100 and 200 ppm in the diet for 77 weeks</li> </ul>	Positive: increases in the incidences of proliferative lesions in the prostate, leukemia (large granular lymphocytes) and testicular tumours (interstitial cell tumours).	<ul style="list-style-type: none"> <li>• Key study</li> <li>• Reliability code: 2</li> <li>• Experimental result</li> </ul>	Waalkes and Rehm (1992)
<ul style="list-style-type: none"> <li>• Test material: cadmium chloride</li> <li>• Test animals: male Wistar (TNO/W75) rats</li> <li>• Administration: inhalation</li> <li>• Doses: 0, 12.5, 25 and 50 <math>\mu\text{g}/\text{m}^3</math> for 23 hours per day, 7 days a week for 18 months</li> </ul>	Positive: increase in the incidence of primary lung carcinomas (mostly adenocarcinomas but also epidermoid carcinomas and mucoepidermoid carcinomas).	<ul style="list-style-type: none"> <li>• Key study</li> <li>• Reliability code: 2</li> <li>• Experimental result</li> </ul>	Takenaka et al. (1983)
<ul style="list-style-type: none"> <li>• Test materials: cadmium chloride, cadmium sulphate, cadmium sulphide, cadmium oxide dust and cadmium oxide fume</li> <li>• Test animals: Wistar (BOR-WISW) rats</li> <li>• Administration: inhalation</li> <li>• Doses: 0, 30 and 90 <math>\mu\text{g}/\text{m}^3</math> (cadmium chloride, cadmium oxide dust); 0 and 90 <math>\mu\text{g}/\text{m}^3</math> (cadmium sulphate); 0, 90, 270, 810 and 2430 <math>\mu\text{g}/\text{m}^3</math> (cadmium sulphide); 0, 10 and 30 <math>\mu\text{g}/\text{m}^3</math> (cadmium oxide fume) for 22 hours per day, 7 days a week for up to 18 months</li> </ul>	Positive: all cadmium compounds increased the incidence of primary lung tumours (mostly adenomas and adenocarcinomas but bronchioalveolar adenomas and squamous-cell carcinomas were also observed in a few rats)	<ul style="list-style-type: none"> <li>• Key study</li> <li>• Reliability code: 2</li> <li>• Experimental result</li> </ul>	Glaser et al. (1990)
<ul style="list-style-type: none"> <li>• Test material: cadmium chloride, cadmium sulphate, cadmium sulphide, cadmium oxide dust and cadmium oxide fume</li> <li>• Test animals: female Han:NMRI mice, and male and female Hoe:SYHK Syrian golden hamsters</li> <li>• Administration: inhalation</li> <li>• Doses: 0, 30 and 90 <math>\mu\text{g}/\text{m}^3</math> (cadmium chloride, cadmium sulphate); 0 and 90 <math>\mu\text{g}/\text{m}^3</math> (cadmium sulphate); 0, 90, 270 and 1000 <math>\mu\text{g}/\text{m}^3</math> (cadmium sulphide); 10, 30, 90 and 270 <math>\mu\text{g}/\text{m}^3</math> (cadmium oxide dust); 10, 30 and 90 <math>\mu\text{g}/\text{m}^3</math> (cadmium oxide fume); mice were exposed for 9 or 18 hours per day, 5 days a</li> </ul>	<ul style="list-style-type: none"> <li>• Mice: positive; cadmium oxide dust and cadmium oxide fume increased the incidence of lung tumours (histopathological types not reported)</li> <li>• Syrian golden hamster: negative</li> </ul>	<ul style="list-style-type: none"> <li>• Key study</li> <li>• Reliability code: 2</li> <li>• Experimental result</li> </ul>	Heinrich et al. (1989)

week for 6 to 69 weeks, and Syrian golden hamsters for 19 or 8 hours per day, 5 days a week for 13 to 65 weeks			
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#### 4.9.1.1 Carcinogenicity: oral

The effect of chronic dietary zinc deficiency on the carcinogenicity of cadmium given to male Wistar (WF/NCr) rats in the diet was evaluated in a study by Waalkes and Rehm (1992). Rats, two weeks of age, were exposed for 77 weeks to cadmium at 0, 25, 50, 100 and 200 ppm as cadmium chloride mixed with a zinc-adequate diet or a marginally zinc-deficient diet (28 rats/exposed group and 56 rats from pooled controls).

The incidence of proliferative lesions (hyperplasia and adenoma) in the prostate was significantly higher in rats exposed to 50 ppm cadmium in both the zinc-adequate group (22.7 %) and the zinc-deficient group (15.4 %), as compared to controls (1.9 %). A clear dose response of the proliferative lesions was not observed.

The incidence of leukaemia (large granular lymphocytes) was significantly higher in rats exposed to 50 and 100 ppm cadmium, but not 200 ppm, in the zinc-adequate group, as compared to controls. In the zinc-deficient group the incidence of leukaemia increased with the dose of cadmium, but the difference in incidence was statistically significant only for rats exposed to 200 ppm, as compared to controls. The incidence of leukaemia in controls was 5.4 %, and the highest incidence observed in a cadmium-exposed group was 28 %.

The incidence of testicular tumours (interstitial cell tumours) was significantly higher in rats exposed to 200 ppm cadmium in the zinc-adequate group (22.2 %), as compared to controls (3.6 %).

#### 4.9.1.2 Carcinogenicity: inhalation

##### Rat

The carcinogenicity of cadmium in male Wistar (TNO/W75) rats after inhalation exposure to cadmium chloride was evaluated in a study by Takenaka et al. (1983). Rats, six weeks of age, were exposed for 23 hours per day, 7 days a week for 18 months to cadmium chloride aerosols with cadmium concentrations of 0, 12.5, 25 and 50  $\mu\text{g}/\text{m}^3$  (40 rats/exposed group and a control group of 41 rats). After the end of the exposure period, the rats were observed for an additional 13 months before the study was ended.

During the course of the study, autopsy of dead or dying rats was carried out as soon as possible after they had been detected. At the end of the study, surviving rats were sacrificed and autopsied. All rats were subjected to histopathological examination.

The incidence of primary lung carcinomas (mostly adenocarcinomas but also epidermoid carcinomas and mucoepidermoid carcinomas) showed an exposure-related increase starting at 20 months of the study. For all exposed groups the difference in incidence was statistically significant, as compared to the incidence in the control group. The incidences were 0 % (0/38), 15 % (6/39), 53 % (20/38) and 71 % (25/35) in the experimental groups exposed to aerosols with cadmium concentrations of 0, 12.5, 25 and 50  $\mu\text{g}/\text{m}^3$ , respectively. Rats with multiple lung tumours were frequently observed, and several tumours showed metastases or were regionally invasive. The incidence of adenomatous hyperplasia was also increased by cadmium exposure.

## ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON CADMIUM HYDROXIDE

In a study of similar design as the study by Takenaka *et al.* (1983) above, Glaser *et al.* (1990) evaluated the carcinogenicity of cadmium in male and female Wistar (BOR-WISW, formerly called TNO/W75) rats after inhalation exposure to cadmium chloride, cadmium sulphate, cadmium sulphide, cadmium oxide dust and cadmium oxide fume. Rats, nine weeks of age, were exposed for 22 hours per day, 7 days a week for up to 18 months to aerosols of the cadmium compounds with cadmium concentrations as shown in Table 14. A few experimental groups were exposed discontinuously for 40 hours a week for 6 months. These groups are not included in this study summary. After the end of the exposure period, groups of 20-40 rats were kept and observed until death or up to an additional 13 months, at which time the study was ended. The exposure and observation periods were terminated if mortality during these periods reached 25 % and 75 %, respectively. All rats were subjected to histopathological examination.

Table 14 Incidence of primary lung tumours in rats exposed to cadmium (adapted from Glaser *et al.* 1990)

Experimental group	Cadmium concentration in aerosol ( $\mu\text{g}/\text{m}^3$ )	Exposure period (months)	Observation period (months)	Animals with lung tumours/animals examined	Animals with lung tumours (%)
<b>Males</b>					
Control	0	0	31	0/40	0
Cadmium chloride	30	18	30	15/20	75
	90	6	30	11/20	55
	90	14	31	11/20	55
Cadmium sulphide	90	18	30	17/20	85
	270	16	30	14/20	70
	810	7	30	11/20	55
Cadmium oxide dust	2430	4	30	7/16	44
	30	18	31	28/39	72
	90	7	31	12/39	31
Cadmium oxide fume	10	18	31	0/40	0
	30	18	31	8/38	21
<b>Females</b>					
Control	0	0	31	0/20	0
Cadmium chloride	30	18	31	13/18	72
	90	6	29	3/18	17
Cadmium sulphate	90	18	29	18/20	90
Cadmium sulphide	90	18	31	15/20	75
	270	16	30	16/19	84
	810	10	29	13/20	65



	2430	3	31	6/19	32
Cadmium oxide dust	30	18	31	15/20	75
	90	11	31	14/19	74

All cadmium compounds increased the incidence of primary lung tumours (mostly adenomas and adenocarcinomas but bronchioalveolar adenomas and squamous-cell carcinomas were also observed in a few rats) above that in controls (see Table 14). The highest incidence (90 %) was observed in females exposed to cadmium sulphate (90  $\mu\text{g}/\text{m}^3$ ). Generally, there was no difference between males and females.

### Mouse

The carcinogenicity of cadmium in female Han:NMRI mice after inhalation exposure to cadmium chloride, cadmium sulphate, cadmium sulphide, cadmium oxide dust and cadmium oxide fume was evaluated in a study by Heinrich et al. (1989). Groups of 48 mice, age unspecified, were exposed for 9 or 18 hours per day, 5 days a week for 6 to 69 weeks to aerosols of the cadmium compounds with cadmium concentrations of 30 and 90  $\mu\text{g}/\text{m}^3$  (cadmium chloride), 30 and 90  $\mu\text{g}/\text{m}^3$  (cadmium sulphate), 90, 270 and 1000  $\mu\text{g}/\text{m}^3$  (cadmium sulphide), 10, 30, 90 and 270  $\mu\text{g}/\text{m}^3$  (cadmium oxide dust), and 10, 30 and 90  $\mu\text{g}/\text{m}^3$  (cadmium oxide fume). The total duration of experiments was 71-107 weeks. In some experimental groups the exposure was terminated when the mortality started to increase. All mice were subjected to histopathological examination.

Statistically significant increases in the incidence of lung tumours (histopathological types not reported) above that in the control group was observed in the experimental groups exposed to cadmium as cadmium oxide fumes at 30  $\mu\text{g}/\text{m}^3$  (29.6 % versus 20.0 % tumour bearing mice in the control group) and 90  $\mu\text{g}/\text{m}^3$  (34.0 % versus 14.6 % tumour bearing mice in the control group), and in the experimental group exposed to cadmium as cadmium oxide dust at 10  $\mu\text{g}/\text{m}^3$  (26.1 % versus 14.6 % tumour bearing mice in the control group).

### Syrian hamster

The carcinogenicity of cadmium in male and female Hoe:SYHK Syrian golden hamsters after inhalation exposure to cadmium chloride, cadmium sulphate, cadmium sulphide, cadmium oxide dust and cadmium oxide fume was evaluated in a study by Heinrich et al. (1989). Groups of 24 male and 24 female Syrian hamsters, age unspecified, were exposed for 19 or 8 hours per day, 5 days a week for 13 to 65 weeks to aerosols of the cadmium compounds with cadmium concentrations of 30 and 90  $\mu\text{g}/\text{m}^3$  (cadmium chloride), 30 and 90  $\mu\text{g}/\text{m}^3$  (cadmium sulphate), 90, 270 and 1000  $\mu\text{g}/\text{m}^3$  (cadmium sulphide), 10, 30, 90 and 270  $\mu\text{g}/\text{m}^3$  (cadmium oxide dust), and 10, 30 and 90  $\mu\text{g}/\text{m}^3$  (cadmium oxide fume). The total duration of experiments was 60-113 weeks for males and 61-87 weeks for females. In some experimental groups the exposure was terminated when the mortality started to increase. All mice were subjected to histopathological examination.

In none of the exposed groups an increase in the incidence of lung tumours was observed. In only six of the exposed groups (males and females combined) was there one or, in one case, two animals with a papilloma or a polypoid adenoma of the trachea. One papilloma was also found in the control group.

#### **4.9.1.3 Carcinogenicity: dermal**

No studies were located regarding carcinogenicity after dermal exposure to cadmium compounds.

#### 4.9.1.4 Carcinogenicity: other routes

##### Subcutaneous administration

The carcinogenicity of cadmium in DBA/2NCr and NFS/NCr mice after subcutaneous administration of cadmium chloride was evaluated in a study by Waalkes and Rehm (1994). Groups of 30-40 mice, 8 weeks old, were administered a dose of 0 or 40 µmol/kg bw once, or once per week for 16 weeks. Statistically significant increases in lymphomas, lung tumours and injection-site sarcomas were observed.

The carcinogenicity of cadmium in male Wistar [CrI:(WI)BR] rats after subcutaneous administration of cadmium chloride was evaluated in a study by Waalkes *et al.* (1988). Groups of 30 rats, 6 weeks old, were administered a single dose of 0, 10, 2.5, 5.0, 10.0, 20.0 or 40.0 µmol/kg bw. Statistically significant increases in injection-site sarcomas, testis (interstitial cell) tumours and prostate tumours in rats were observed.

#### 4.9.2 Human information

To investigate the carcinogenic potential of cadmium in humans, several occupational cohort studies have been performed, involving workers in cadmium recovery plants, copper-cadmium alloys plants, nickel-cadmium batteries plants and cadmium processing plants. In some of the studies increases in lung cancer risk for the workers were observed. However, most of the studies were afflicted with shortcomings limiting their value as evidence for a causal relationship between exposure to cadmium and cancer, as concluded both in the EU RAR (2007) and the IARC Monograph Volume 100 (C) (2012). In particular, (i) lack of historical data on exposure to cadmium and the inability to classify workers according to a quantitative index of cumulative exposure, (ii) confounding effect of occupational exposure to other potential carcinogens (e.g. nickel, arsenic, welding fumes, mineral oils, lead, zinc, beryllium and other metals), and (iii) insufficient control of non-occupational cancer-risk factors, of which the most important is tobacco smoking, constrains the assessment of cancer risk from exposure to cadmium in the studies. The dossier submitter agrees with the view presented in the EU RAR (2007) and the IARC Monograph Volume 100 (C) (2012) regarding the imperfect quality of the studies and therefore concludes that the data of the studies would not be sufficient for classification with Carc. 1A. Accordingly, summaries of the occupational cohort studies would not add information decisive for the classification, and they have therefore not been included in the present CLH report.

Epidemiological studies on cadmium carcinogenicity assessing the risk for cancer in the prostate (occupational cohort studies, studies of people residing in cadmium-contaminated areas and case-control studies of individuals with prostate cancer), the kidney, the bladder, the breast and the endometrium (case-control studies) are reported in the EU RAR (2007) and the IARC Monograph Volume 100 (C) (2012). In some of the studies increases in cancer risks were observed that may be the result of an association with cadmium exposure. However, the studies suffer from shortcomings regarding study designs, exposure assessments or consideration of confounding factors, and therefore the data of the studies would not be sufficient for the purpose of classification. Accordingly, summaries of the studies would not add information decisive for the classification, and they have therefore not been included in the present CLH report.

Despite the shortcomings of the studies highlighted in the IARC Monograph Volume 100 (C) (2012), it is concluded in the monograph that there is sufficient evidence in humans for the carcinogenicity of cadmium and cadmium compounds. Notwithstanding this conclusion, the dossier submitter is of the opinion that the data of the available studies in humans would not be sufficient

for classification with Carc. 1A according to CLP, since the criteria for this classification require that it is largely based on human evidence from studies establishing a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen). The view of the dossier submitter is that this requirement has not been met, since it cannot be ruled out with reasonable confidence that the positive association between the exposure to cadmium and cancer observed in some of the studies is a result of chance, bias or confounding.

### 4.9.3 Other relevant information

### 4.9.4 Summary and discussion of carcinogenicity

The carcinogenic potential of cadmium has been investigated in rats, mice and Syrian hamsters. To investigate this potential in humans, numerous epidemiological studies have been performed. The studies in humans were generally afflicted with shortcomings limiting their value as evidence for a causal relationship between exposure to cadmium and cancer. Hence, the data would not be sufficient for the purpose of classification according to CLP and, therefore, the studies have not been evaluated in the present CLH report.

In rats, oral exposure to cadmium chloride induced proliferative lesions (hyperplasia and adenoma) in the prostate, leukaemia (large granular lymphocytes) and testicular tumours (interstitial cell tumours) (Waalkes and Rehm, 1992).

In rats, inhalation exposure to cadmium chloride aerosols induced primary lung carcinomas (mostly adenocarcinomas but also epidermoid carcinomas and mucoepidermoid carcinomas) and adenomatous hyperplasia (Takenaka *et al.*, 1983).

In rats, cadmium chloride, cadmium sulphate, cadmium sulphide, cadmium oxide dust and cadmium oxide fume all induced primary lung tumours (mostly adenomas and adenocarcinomas but bronchioalveolar adenomas and squamous-cell carcinomas were also observed in a few rats) after inhalation exposure (Glaser *et al.*, 1990).

In mice, cadmium oxide dust and cadmium oxide fume, but not cadmium chloride, cadmium sulphate, cadmium sulphide, induced lung tumours (histopathological types not reported) after inhalation exposure (Heinrich *et al.*, 1989).

In Syrian hamsters, cadmium chloride, cadmium sulphate, cadmium sulphide, cadmium oxide dust and cadmium oxide fume did not induce tumours after inhalation exposure (Heinrich *et al.*, 1989).

In mice, subcutaneous injection of cadmium chloride induced lymphomas, lung tumours and injection-site sarcomas (Waalkes and Rehm, 1994).

In rats, subcutaneous injection of cadmium chloride induced injection-site sarcomas, testis (interstitial cell) tumours and prostate tumours (Waalkes *et al.*, 1988).

The dossier submitter considers that there is sufficient evidence to conclude that cadmium is carcinogenic after oral and inhalation exposure in rats, and after inhalation exposure in mice. In mammalian toxicity, the toxicity of cadmium salts is regarded to result from the intrinsic properties of the Cd<sup>2+</sup> ion. As outlined in Section 4, it is reasonable to assume that the Cd<sup>2+</sup> ion is bioavailable after oral and inhalation exposure of cadmium hydroxide. Hence, the carcinogenic effects of other cadmium salts observed in the studies summarised above are relevant also for cadmium hydroxide. Accordingly, it is concluded that cadmium hydroxide is carcinogenic in experimental animals.

#### 4.9.5 Comparison with criteria

According to the criteria in the CLP Regulation, Annex I: 3.6.2.1, classification in Category 1A is reserved for substances known to have carcinogenic potential for humans. Since the available studies in humans were generally afflicted with shortcomings limiting their value as evidence for a causal relationship between exposure to cadmium and cancer, the data are not of sufficient quality for the purpose of classification. Therefore, classification in Category 1A is not justified.

Where no evidence from human studies is available, classification in Category 1B or 2 is based on the strength of evidence from animal studies. Classification in Category 1B is for substances for which there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen), and Category 2 is for substances for which there is limited evidence of carcinogenicity in animal studies (suspected human carcinogen). On consideration of the animal data referred to in this CLH report, they are considered to demonstrate sufficient evidence of carcinogenicity in animals. This is based on the facts that treatment related tumours were observed in two species (rat and mouse), in three different studies in one species (rat), in both sexes of one species (rat), and that tumours occurred at multiple sites and/or were of different types.

In view of these considerations, the evidence referred to is deemed to match the criteria for classification of cadmium hydroxide as a Category 1B carcinogen. There is no ground to specify any specific route of exposure for the classification.

#### 4.9.6 Conclusions on classification and labelling

Cadmium hydroxide should be classified in Carc. 1B (H350).

#### **RAC evaluation of carcinogenicity**

##### **Summary of the Dossier submitter's proposal**

The DS proposed a Carc. 1B; H350 classification for cadmium hydroxide.

Human epidemiological studies were generally affected by shortcomings limiting their value as evidence for a causal relationship between exposure to cadmium and cancer. Hence, the data would not be sufficient for the purpose of classification according to CLP and, therefore, the studies were not evaluated in the CLH report.

The carcinogenic potential of cadmium has been investigated in rats, mice and Syrian hamsters.

In rats, oral exposure to cadmium chloride induced proliferative lesions (hyperplasia and adenoma) in the prostate, leukaemia (large granular lymphocytes) and testicular tumours (interstitial cell tumours). Inhalation exposure to cadmium chloride aerosols induced primary lung carcinomas (mostly adenocarcinomas but also epidermoid carcinomas and mucoepidermoid carcinomas) and adenomatous hyperplasia.

In another rat study, inhalation of cadmium chloride, cadmium sulphate, cadmium sulphide, cadmium oxide dust or cadmium oxide fume induced primary lung tumours (mostly adenomas and adenocarcinomas but bronchioalveolar adenomas and squamous-cell carcinomas were also observed in a few rats) after inhalation exposure.

In mice, cadmium oxide dust and cadmium oxide fume, but not cadmium chloride, cadmium sulphate, cadmium sulphide, induced lung tumours (histopathological types not reported) after inhalation exposure.

In Syrian hamsters, cadmium chloride, cadmium sulphate, cadmium sulphide, cadmium oxide

dust and cadmium oxide fume did not induce tumours after inhalation exposure.

In other studies in which cadmium chloride was administered by subcutaneous injection, it induced lymphomas, lung tumours and injection-site sarcomas in mice and testicular and prostate tumours together with injection-site sarcomas in rats.

The DS concluded that these studies demonstrated the carcinogenicity of the Cd<sup>2+</sup> ion in both rats and mice. As it was apparent that Cd<sup>2+</sup> would be bioavailable after oral and inhalational exposure to cadmium hydroxide, these data were also considered of relevance to this substance.

Based on the observations that treatment-related tumours were observed in two species (rat and mouse), in three different studies in one species (rat), in both sexes of one species (rat), and that tumours occurred at multiple sites and/or were of different types, the DS concluded that there was sufficient evidence to demonstrate the carcinogenicity of Cd<sup>2+</sup> in animals, and therefore that cadmium hydroxide meets the criteria for a Category 1B carcinogen.

The DS did not provide a comprehensive assessment of the available epidemiological data relating to the carcinogenicity of cadmium compounds (and thus Cd<sup>2+</sup>) in humans. Briefly, the DS commented that several occupational cohort studies have reported increases in lung cancer risk for exposed workers but reviews under the EU Existing Substances Regulation and by IARC identified significant shortcomings that prevented definitive conclusions about the cadmium carcinogenicity being made from these studies. The DS presented the conclusion about epidemiological studies that had assessed links between cadmium exposure and cancer of the prostate, kidney, bladder, breast and endometrium.

Although the DS acknowledged that IARC (2012) had concluded there is sufficient evidence in humans for the carcinogenicity of cadmium and cadmium compounds, the DS concluded that the available studies in humans did not provide sufficient evidence for a Cat. 1A classification under CLP. The justification for this position was that the criteria require human evidence from studies establishing a causal relationship between human exposure to a substance and the development of cancer. The DS was not able to rule out with reasonable confidence that the positive association between exposure to cadmium and cancer observed in some of the studies was a result of chance, bias or confounding factors.

### **Comments received during public consultation**

The International Cadmium Association supports the proposed classification for cadmium hydroxide as Carc. 1B; H350.

One MS has suggested that Carc. 1A may be a more appropriate classification for cadmium hydroxide based on the fact that in 2012, IARC considered that there was sufficient evidence available in humans to demonstrate carcinogenicity of cadmium compounds.

In response to the comment from the MS, the DS explained that the IARC also considered that the assessment of human studies was constrained by various flaws or that results of different studies are inconsistent.

One MS also asked why the DS had not proposed a specific concentration limit for the carcinogenicity classification of cadmium hydroxide, given that a limit of 0.01% was in place for cadmium chloride. The DS explained that they considered it inappropriate to extrapolate estimates of potency from one substance to another, even when they may have a comparable inherent hazard.

### **Assessment and comparison with the classification criteria**

RAC acknowledges that the highly water soluble salts cadmium chloride, cadmium sulphate

and cadmium fluoride are all classified as category 1B carcinogens in Annex VI of the CLP Regulation on the basis that they possess common hazards related to the ready bioavailability of the  $\text{Cd}^{2+}$  ion. Notably, evidence that cadmium chloride induces cancer in rats following oral and inhalational exposure, together with the mutagenicity of this substance in somatic tissues, justified this classification.

On re-assessment of the available information, RAC observed that there were only increased lung tumours in rats following inhalation exposure to inorganic cadmium compounds. Exposure to the more highly soluble chloride and sulphate salts might be expected to have led to higher uptake of  $\text{Cd}^{2+}$  ions, but there was no evidence of an increased tumour response when compared to rats exposed to the less soluble cadmium compounds. However, the increased lung tumours seen with all these compounds strongly supports their classification in category 1B for carcinogenicity. Although it has not been tested, RAC considers that cadmium hydroxide would also present a lung cancer hazard if inhaled by rats. The mixed findings in cancer studies with mice and hamsters do not detract from this conclusion. Although not described in any detail by the DS, IARC noted that poor study design (e.g. lack of complete histopathological examinations) and reporting inadequacies limited the conclusions that could be made for these animal species. The mechanisms by which the various different inorganic compounds induce lung cancer have not been elucidated, although a direct genotoxic activity cannot be ruled out.

Only very limited information is available about the carcinogenicity of the inorganic cadmium compounds to animals exposed orally. In male rats fed 0, 25, 50, 100 or 200 ppm cadmium chloride for 77 weeks, there were statistically significant increases in leukemia at 50 and 100 ppm. Evidently general toxicity may have prevented an increase being seen in the top dose group. The relatively short exposure period may have limited the sensitivity of this carcinogenicity test. Increased focal atypical hyperplasia and adenomas were seen in the ventral prostate but a clear dose-response was lacking and no similar lesions were seen in the dorso-lateral prostate. No carcinomas were seen in the prostate of these animals. There was an increased incidence of interstitial cell tumours of the testes in the highest dose group. Subsequent studies (reviewed by IARC but not by the DS) have shown that cadmium perturbs the hypothalamic-pituitary-testes axis in rats and that the increased testicular tumours were likely related to cadmium-induced testicular function and reduced circulating testosterone levels.

Given the increased lung cancer incidences seen in rats exposed to inorganic cadmium compounds of high, moderate and poor water solubility, RAC considers that repeated inhalation of cadmium hydroxide can reasonably be assumed to present such a carcinogenic hazard and that it should be classified in the same way as these other cadmium compounds.

The DS described a study which showed 87% solubility of cadmium carbonate (which has a low water solubility similar to that of cadmium hydroxide) in artificial gastric juice (pH 1.47) during a 2 hour incubation period at 37°C. This suggests that  $\text{Cd}^{2+}$  species can evolve in the highly acidic regions of the gastro-intestinal tract following oral exposure to cadmium compounds with low water solubilities, and be available for systemic absorption. Therefore, RAC is of the opinion that the data relating to the possible carcinogenicity of water soluble cadmium chloride following oral treatment of rats is also relevant for cadmium hydroxide.

During the public consultation, one MS queried whether a category 1A classification might be more appropriate for cadmium hydroxide, citing the recent review of IARC on cadmium and its compounds. . The DS did not provide an assessment of the relevant data and, had a category 1A classification have been proposed, it would have been inconsistent with the existing 1B classification for the other cadmium compounds. Taking this into consideration, RAC concluded that it would be inappropriate to initiate an independent review of its own on the human carcinogenicity of cadmium hydroxide in the absence of a proposal from the DS.

In conclusion, RAC agrees with the proposal of the DS to classify cadmium hydroxide as a category 1B carcinogen in line with the existing harmonised classification of other inorganic

cadmium compounds. The carcinogenic findings in the lungs of rats especially are of relevance to humans.

RAC notes the response of the DS to the query from one MS about the setting of a specific concentration limit for this endpoint and agrees that it would be inappropriate to extrapolate estimates of potency from the very soluble cadmium compounds to the less soluble cadmium hydroxide, despite the inherent hazards being comparable.

#### **4.10 Toxicity for reproduction**

Not evaluated in this report.

#### **4.11 Other effects**

Not evaluated in this report.

### **5 ENVIRONMENTAL HAZARD ASSESSMENT**

Environmental effects are not evaluated in this report.

### **6 OTHER INFORMATION**

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## ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON CADMIUM HYDROXIDE

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