

Annex V: Environmental fate and ecotoxicity data for UVCB constituents

This annex contains the environmental fate data and PNECs for each of the driving constituents considered in the assessment of the refinable substances. Data access has been provided to the relevant sections of the constituent IUCLID dossiers and this information is presented here without further editing.

Table of contents

4. ENVIRONMENTAL FATE PROPERTIES	5
4.0 General discussion of environmental fate and pathways	5
4.0.1 Cadmium	5
4.0.2 Copper	6
4.0.3 Diarsenic trioxide	12
4.0.4 Lead	12
4.0.5 Nickel	13
4.0.6 Silver	13
4.0.7 Zinc	13
4.2. Environmental distribution	14
4.2.1. Adsorption/desorption	14
4.2.1.1 Cadmium	14
4.2.1.2 Copper	14
4.2.1.3 Diarsenic trioxide	15
4.2.1.4 Lead	17
4.2.1.5 Nickel	20
4.2.1.6 Silver	23
4.2.1.7 Zinc	30
4.2.4. Summary and discussion of environmental distribution	32
4.2.4.1 Cadmium	32
4.2.4.2 Copper	32
4.2.4.3 Diarsenic trioxide	32
4.2.4.4 Lead	32
4.2.4.5 Nickel	32
4.2.4.6 Silver	32
4.2.4.7 Zinc	32
4.3. Bioaccumulation	34
4.3.0.1. Cadmium	34
4.3.0.2 Copper	34
4.3.0.3 Diarsenic trioxide	34
4.3.0.4 Lead	34
4.3.0.5 Nickel	34
4.3.0.6 Silver	34
4.3.0.7 Zinc	35
4.3.3. Summary and discussion of bioaccumulation	35
4.3.3.1. Aquatic bioaccumulation	35
4.3.3.1.1 Cadmium	35
4.3.3.1.2 Copper	36
4.3.3.1.3 Diarsenic trioxide	38
4.3.3.1.4 Lead	39
4.3.3.1.5 Nickel	40
4.3.3.1.6 Silver	40
4.3.3.1.7 Zinc	43
4.3.3.2. Terrestrial bioaccumulation	43
4.3.3.2.1 Cadmium	43
4.3.3.2.2 Copper	44
4.3.3.2.3 Diarsenic trioxide	45
4.3.3.2.4 Lead	45
4.3.3.2.5 Nickel	46
4.3.3.2.6 Silver	46
4.3.3.2.7 Zinc	47
7 Environmental hazard assessment	48
7.6. PNEC derivation and other hazard conclusions	48
7.6.1 Cadmium	48
7.6.2. Copper	52
7.6.3 Diarsenic trioxide	59
7.6.4 Lead	63
7.6.5 Nickel	65

7.6.6 Silver.....	71
7.6.7 Zinc.....	77

Table of tables

Table 1. Summary of the transformation/dissolution results, relevant to copper powders	8
Table 2. Partition coefficients for soil, sediments and suspended matter	15
Table 3. Reported log KD, SPM values for Pb in freshwaters in Europe	17
Table 4. Reported KD, SPM values for Pb in estuarine surface waters.	17
Table 5. Reported log KD, SPM values for Pb in marine surface water	18
Table 6. Regression models of KD as a function of soil parameters.	19
Table 7. Reported log Kp, suspended particulate matter (SPM) values for Ni in freshwater surface waters in Europe	20
Table 8. Overview of Kp-values on sediment	21
Table 9. Mean Kp values for nickel in river sediments (Gunn et al. 1992)	21
Table 10. Partitioning coefficients Kp in Danish brackish coastal water:	22
Table 11. Partition coefficients Kp for STP:	23
Table 12. The range of bioaccumulation factor (BAF in L/kg ww) of Pb in freshwater organisms	39
Table 13. The range of bioaccumulation factor (BAF in L/kgww) of Pb in the mixed diet.	39
Table 14. Bioaccumulation factors between soil or decomposed leaf litter and isopods. The Pb concentrations in the biota are the product of BAF and soil Pb concentration.	45
Table 15. Hazard assessment conclusion for the environment for cadmium	48
Table 16. Hazard assessment conclusion for the environment for copper	52
Table 18. Hazard assessment conclusion for the environment for arsenic	59
Table 19. Hazard assessment conclusion for the environment for lead	63
Table 20. Hazard assessment conclusion for the environment for nickel	65
Table 21. Hazard assessment conclusion for the environment for silver	71
Table 22. Hazard assessment conclusion for the environment for zinc	78

4. ENVIRONMENTAL FATE PROPERTIES

Environmental fate endpoints for the inorganic UVCB are assessed by assessing constituents' transport and distribution, bioaccumulation potential and secondary poisoning. For these endpoints, data access has been provided to the IUCLID dossiers for the relevant constituents. These data are provided in the following sections. Constituent data are not required for other endpoints, such as biodegradation and hydrolysis, as these endpoints are not relevant for inorganic substances.

4.0 General discussion of environmental fate and pathways

4.0.1 Cadmium

Stability

Waived: Inorganic substance: According to Annex IX of REACH Regulation, information on hydrolysis is not required for inorganics.

Also the other endpoints under 5.1. e.g., phototransformation in air, water and soil, are not applicable to Cadmium metal

Biodegradation

Biodegradation is not applicable to metals/inorganic substances. Tests are not to be conducted if the substance is inorganic (Annex VII of REACH regulation)

Bioaccumulation in water/sediment

BCF's for cadmium are highest in algae and lowest in fish; High BCF in algae does not necessarily reflect high bioconcentration, because a significant part of the Cd is absorbed to the outer side of the organisms, and not taken up. Another factor of error is the lack of gut clearance in invertebrates. Organs (kidney, liver) contain most Cd.

Main influencing factors for Cd BCF are hardness and Cd concentration in the water. Increasing water hardness reduces Cd uptake. BCF is also inversely related to Cd concentration in water.

McGeer et al (2003) recently extensively reviewed the evidence on bioconcentration and bioaccumulation of cadmium as a function of exposure concentration in a number of taxonomic groups (algae, molluscs, arthropods, annelids, salmonid fish, cyprinid fish, and other fish). The data clearly illustrated that there is a significant degree of control on internal cadmium content. In general, BCF data showed an inverse relationship to exposure concentrations. In all cases, the relationship of BCF to exposure was significant and negative. The slopes of the BCF/BAF – exposure relations were: algae: -0.72, insects: -0.32, arthropods: -0.61, molluscs: -0.50, salmonids: -0.87, Centrarchids: -0.47, Killifish: -0.05, other fish: -0.72. Overall, species mean slope was -0.49 +/- 0.04 (McGeer et al 2003. *Environm. Toxicology & Chemistry*, vol. 22, nr. 5, 1017 -1037).

Bioaccumulation in soil

The EU Risk assessment (ECB 2008) presents BAF values that were calculated from the soil: biota concentration ratios. Most organisms are earthworms and the Cd levels were expressed on dry or wet weight basis. All the data on earthworms were obtained from specimens with guts voided prior to analysis.

Cadmium is concentrated from the soil into earthworms organisms (BAF values are all higher than 1). Most important factors affecting the bioaccumulation of Cd by earthworms are the Cd concentration of the soil, soil type, pH, soil organic matter and CEC.

The influence of the Cd content of the soil on the bioaccumulation of Cd is illustrated in most of the studies. Cadmium concentrations in earthworms increase with increasing Cd levels in a non-proportional way. As a result, the BAF observed in Cd-contaminated or Cd-enriched soils is lower than the BAF observed in control soils.

Transport and distribution

For metals, the transport and distribution over the different environmental compartments e. g. the water (dissolved fraction, fraction bound to suspended matter), soil (fraction bound or complexed to the soil particles, fraction in the soil pore water...) is described and quantified by the metal partition coefficients between these different fractions. The information on these partition coefficients is given under 5.6.

Partition coefficients for cadmium in freshwater have been reviewed in the EU risk assessment report (ECB 2008). Based on this experimental evidence, a partition coefficient for the distribution between solid particulate matter and water (K_{psusp}) of $130 \times 10^3 \text{ l/kg}$ has been defined for EU waters and was used throughout the RAR.

The K_p for the distribution between sediment and water ($K_{p_{sed}}$) was estimated in the RAR from the ratio of the average sediment (1.32 mg/kg dw) to average water Cd concentrations ($0.14 \mu\text{g/l}$). This "best fit" K_d yields 10000 L/kg dw (ECB 2008).

These partition coefficients have been used since then in other legislative processes in the EU (e. g. the water framework directive) and will also be used for REACH.

For the marine water, a partition coefficient water/suspended matter of 617 l/kg has been derived.

4.0.2 Copper

General summary of the information on environmental fate and pathways

Copper is a natural element and transition metal with more than one oxidation state. Copper in its metallic form (Cu^0) is not available. Copper needs to be transformed to its ionic forms to become available for uptake by living organisms.

The classic standard testing protocols on hydrolysis and photo-transformation are not applicable to copper.

This was recognized in the Guidance to Regulation (EC) No 1272/2008 Classification, Labelling and Packaging of substances and mixtures (metal annex): "Environmental transformation of one species of a metal to another species of the same does not constitute degradation as applied to organic compounds and may increase or decrease the availability and bioavailability of the toxic species. However as a result of naturally occurring geochemical processes metal ions can partition from the water column. Data on water column residence time, the processes involved at the water – sediment interface (i.e. deposition and re-mobilisation) are fairly extensive, but have not been integrated into a meaningful database. Nevertheless, using the principles and assumptions discussed above in Section IV.1, it may be possible to incorporate this approach into classification."

Relevant fate aspects for copper in the environment have been included in the section "additional information on fate and pathways" and are summarized below.

As outlined in the guidance (2009 & 2012), the understanding of the transformation of copper into more or less bioavailable species is relevant to the environmental hazard assessments and this is described below.

Transformation/dissolution of Cu^0

Copper in its metallic form (Cu^0) needs to be transformed to its ionic forms to become available for uptake by living organisms and therefore the rate and extend of the transformation/dissolution of copper in its massive and powder forms have been assessed from transformation/dissolution tests (in accordance to the OECD guidance, Annex 10 of the GHS).

The results of the transformation/dissolution tests are described in details in "additional information fate and pathways" and are summarized as follows

Massive copper materials

For massive copper materials, seven days transformation/dissolution tests were carried out at pH 6, 7 and 8, in accordance to the OECD guidelines (Annex 10 of GHS) using different surface loadings (Rodriguez et al, 2007,

2011 and 2012) – record in section “additional information on environmental fate and pathways”). The results were used to derive the release of copper-ions from 1 mm particles at loadings of 1, 10 and 100mg/L.

Rodriguez et al., 2007 assessed the need for classification of pure massive copper materials and tested, during 7 and 28 days T/D tests, copper releases from copper wire pieces (99.9% purity) with varying diameters. The tests were carried out with various mass loadings (1-100 mg/l), surface area loadings (1-281 mm²/L) and covering pH 6-8.

The data demonstrated that

- Copper releases during the transformation/dissolution tests were dependent on the stirring rate. At high stirring rates (>50 rpm), copper release increases exponentially and high intra- and inter-vessel variability in measured dissolved copper concentrations were observed. Considering that this increased release and variability was related to particle abrasion, and particle abrasion should be prevented (GHS rev. 4, 2011, Annex 10, A10.2.3.1), only release rates from tests at 50 rpm were further considered.
- Copper releases during transformation/dissolution tests are pH dependent and 6 times higher releases were measured at pH 6 compared to pH 7 and 8.
- Copper releases during transformation/dissolution tests are related to the surface loading tested (mm²/l). The average surface-specific copper release (µg dissolved Cu /mm²exposed) for respectively 7 (acute) and 28 (chronic) dissolution/transformation tests are:
 - 1.15 µg Cu/ mm²after 7 days T/D tests at pH 6
 - 0.13 µg Cu/mm²after 7 days T/D tests at pH 7
 - 0.19 µg Cu/mm²after 7 days T/D tests at pH 8.
 - 4.2 µg Cu/mm²after 28 days T/D test at pH 6 (excluding one outlier).

Since the GHS and CLP agreed on a default value of 1 mm particles for the massive classification, the surface loading applicable to a 1mm particle at various mass loadings (1-100 mg/l) was calculated. Following this methodology, for copper with a density is 8.9 g/cm³, at a loading of 1 mg/L, the surface loading for a 1 mm particle corresponds to 0.67 mm²/L. The surface loading approach avoids issues associated with the selection of a given particle type or preparation of particles specifically for classification purposes (non-commercial particles) and allows easy and precise measurements of the surface area.

To ensure consistency with the GHS guidance (stirring at 100 rpm, without causing abrasion of particles), Rodriguez *et al.*, 2011 and 2012 avoided abrasion of the particles to the test vessels by applying distinct non-abrasion devices: copper wires mounted in polypropylene disks and copper massive samples mounted in epoxy and polished. The lowest coefficient of variations were obtained for copper massive samples mounted in epoxy and polished and the results from these tests were retained for classification purposes. The non-abrasion device (epoxy mounted) resulted in the following surface-specific copper releases:

- 0.41 µg Cu/mm² after 7 days T/D tests at 47 mm²/L, pH 6 (Rodriguez et al., 2011)
- 1.5 µg Cu/mm² after 7 days T/D tests at 0.67 mm²/L, pH 6 (Rodriguez et al., 2012)
- 5.0 µg Cu/mm²after 28 days T/D test at 0.67 mm²/L, pH 6 (Rodriguez et al., 2012)

The results at a surface loading of 0.67 mm²/L (Rodriguez *et al.*, 2012) are retained for the classification of copper massive (particles of 1 mm at loading of 1mg/L corresponds to a surface loading of 0.67 mm²/L):

- 1 µg dissolved Cu/L released after 7 days T/D tests at 1mg/L, 0.67 mm²/L, pH 6 (copper release rate of 1.5 µg Cu/7 days. mm²)
- 3.4 µg dissolved Cu/L released after 28 days T/D test at 1mg/L, 0.67 mm²/L, pH 6 (copper release rate of 5.0 µg Cu/28 days. mm²)

Copper powders

For Copper powders, relevant records on transformation/dissolution tests are reported in section “additional information on environmental fate and pathways”.

The surface areas of copper powders (10 µm – 1 mm), assumed to be spheres, theoretically range between 67.5 and 0.67mm²/mg. The surface area of a fine representative powder was measured as 48 mm²/mg (measured by sieving) and 107 mm²/mg (measured by sieving and BET) (Skeaff and Hardy, 2005).

Following the surface loading approach, copper release rates for reasonable worst case powder surface loadings

of 67.5 and 107 mm²/L is used for the classification of copper powders at 1 mg/L mass loading.

Skeaff and Hardy, 2005 (available from VRAR, 2008) performed T/D tests (7 days at pH6) on the fine representative copper powder sample (48 mm²/mg (measured by sieving) and 107 mm²/mg (measured by sieving and BET)) at loadings of 1 and 100 mg/l. The data can be summarized as:

- The measured release of copper to the aqueous medium at 7 days for the 1 mg/L loading at pH 6 was 82 µg/L. The ratio of dissolved copper to solid copper at 7 days (1 mg/L; pH6) was 0.079.
- The 1 and 100 mg/L mass loadings correspond to surface area loadings, A_{calc} , of 50 and 4800 mm²/L. The 7 days data for the five tests with the copper powders corresponded to the non-linear equation:
- The author mentioned that part of the observed release may have been related to abrasion of the particles

The surface area-specific release rate measured by Rodriguez et al. 2007 and 2011 are considered as relevant for read-across to copper powder

7 days transformation/dissolution

- The results from the 7 days non-abrasive T/D test set-up, at pH 6 and loading of 47 mm²/L (Rodriguez et al., 2011) are retained for read-across to powders T/D at loadings of 1 mg/L (67-107 mm²/L). The obtained surface specific release rate of 0.41 µg Cu/mm² results in a copper release from copper powders at a mass loading of 1 mg/L and corresponding surface loading of 67.5 and 107 mm²/L of 0.028 – 0.044 mg Cu/L (pH 6). Comparison of the release at 107 mm²/L with the release measured by Skeaff and Hardy, 2005 indicates that abrasion during the T/D test performed by Skeaff and Hardy did influence the data (factor 2 difference).
- At pH 7 and 8, the results from the 7 days T/D test data from Rodriguez et al., 2007 can be used as a worst case (with abrasion). The obtained surface specific release rate of 0.19 µg Cu/mm² (pH 7) and 0.13 µg Cu/mm² (pH 8) results in a copper release from copper powders at a mass loading of 1 mg/L and corresponding surface loading of 67.5 and 107 mm²/L of respectively 0.013-0.021 mg Cu/L (pH 7) and 0.009-0.014 mg Cu/L (pH 8)

28 days transformation/dissolution

- The release rates after 28 days T/D were calculated following linear extrapolation of the above retained 7 days transformation/dissolution test results: 0.112-0.176 mg Cu/L (pH 6), 0.051-0.084 mg Cu/L (pH 7) and 0.035-0.056 mg Cu/L (pH 8). The release data at pH 7 and 8 include particle abrasion.

The Table below summarizes the available transformation/ dissolution data.

The following copper releases are retained for classification:

- 7 days, pH 6: non abrasion data from Rodriguez et al., 2011: a copper release at 1mg/l of 0.028 – 0.044 mg Cu/L.
- 7 days, pH7: Rodriguez et al, 2007 : a copper release at 1mg/l of 0.013-0.021 mg Cu/L (includes abrasion)
- 7 days, pH 8: Rodriguez et al, 2007 : a copper release at 1mg/l of 0.009-0.014 mg Cu/L (includes abrasion)
- 28 days T/D were calculated following linear extrapolation of the above retained 7 days transformation/ dissolution test results: 0.112-0.176 mg Cu/L (pH 6), 0.051-0.084 mg Cu/L (pH 7) and 0.035-0.056 mg Cu/L (pH 8). The release data at pH 7 and 8 include particle abrasion.

Table 1. Summary of the transformation/dissolution results, relevant to copper powders

The underlined values are retained for the classification

Transformation/dissolution of fine copper powders						
loading (mg/L)	loading (mm ² /L)	Time (Days)	mg dissolved Cu/L			comment
			At pH 6	At pH 7	At pH 8	
1	47-107	7	0.082	-	-	Direct results of T/D of fine copper powder [1] - from Skeaff and Hardy, 2005.
1	67.5	7	0.098	-	-	Calculated T/D of 10 µm powder(1) from = 8.9246 (Skeaff and Hardy, 2005) See discussion below. *

Transformation/dissolution of fine copper powders						
1	67.5- 107	7	0.078-0.123	0.013-0.021	0.009-0.014	Calculated T/D of 10 µm powder ³ from surface area-specific releases (Rodriguez et al., 2007).
1	67.5-107	7	<u>0.028-0.044</u>	-	-	Calculated T/D of 10 µm powder from non-abrasive T/D tests at 47 mm ² /L, pH 6, 0.41 µg Cu/mm ² (Rodriguez et al., 2011)
1	67.5-107	28	<u>0.112-0.176*</u>	<u>0.051-0.084**</u> <u>1)</u>	<u>0.035-0.056**</u> <u>1)</u>	Linear extrapolation between 7 and 28 days): * from Rodriguez et al., 2011; ** from Rodriguez et al., 2007

[1]Includes abrasion of the particles observed

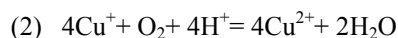
Transformation of Cu-ions released in the environment - Copper speciation

Once released to the environment, copper ions have more than one oxidation state and copper is thus characterized as transition metal. The principal ionic forms are cuprous (Cu(I), Cu⁺) and cupric (Cu(II), Cu²⁺). The trivalent form (Cu(III), Cu³⁺) occurs but is relatively unimportant in physical and biological systems. Cu⁺ is unstable in aqueous media and soluble Cu¹⁺ compounds readily transforms into soluble Cu²⁺ ions, compounds and/or insoluble Cu²⁺ ions, compounds (e.g. copper sulphides) that precipitate. This transformation of Cu⁺ to Cu²⁺ is a result of a redox reaction initiated through atmospheric water vapour as well as in aqueous solution. However, monovalent copper cations are only susceptible to such transformation when they are not chemically bound in insoluble compounds or stabilised in complexed forms.

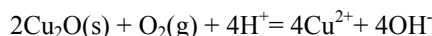
The transformation of Cu(I) to Cu (II) can be described by:



and



Both sub-reactions are summarised as:



Among the copper species released/transformed, Cu (II) is thus the most environmental relevant species. It is further recognised that Cu (II) ions - commonly named free cupric ions- are the most active copper species and that total Cu or Cu(II) concentrations are usually not directly related to ecological effects since exposure of biota may be limited by processes that render Cu unavailable for uptake (ICPS, 1998). Assessing the species of Cu (II) therefore has ecotoxicological relevance. After being released into the environment, the Cu(II) ions typically bind to inorganic and organic ligands contained within water, soil, and sediments. In water Cu(II) binds to dissolved organic matter (e. g., humic or fulvic acids). The Cu(II) ion forms stable complexes with -NH₂, -SH, and, to a lesser extent, -OH groups in these organic acids. Cu(II) will also bind with varying affinities to inorganic and organic components in sediments and soils. For example, Cu(II) binds strongly to hydrous manganese and iron oxides in clay and to humic acids, but much less strongly to aluminosilicates in sand. In all environmental compartments (water, sediment, soil), the binding affinities of Cu(II) with inorganic and organic matter is dependent on pH, the oxidation-reduction potential in the local environment, and the presence of competing metal ions and inorganic anions.

Some key papers on copper speciation in freshwater, marine waters, sediments and soils are provided in the section "additional information on environmental fate"

Copper attenuation, removal from water column, geochemical cycling- Quantitative assessment

As described above, after the release of Cu(II) in the environment, further transformations occur thereby changing the potential for toxicity, induced by the free cupric ions. The concentrations of "active" cupric ions, that remains available for uptake by biota depends on different processes: precipitation, dissolution, adsorption, desorption, complexation and competition for biological adsorption sites (ligands). These processes are critical for the fate of copper in the environment. This was recognized in the Guidance to Regulation (EC) No 1272/2008 Classification, Labelling and Packaging of substances and mixtures (metal annex):

“Environmental transformation of one species of a metal to another species of the same does not constitute degradation as applied to organic compounds and may increase or decrease the availability and bioavailability of the toxic species. However as a result of naturally occurring geochemical processes metal ions can partition from the water column. Data on water column residence time, the processes involved at the water – sediment interface (i.e. deposition and re-mobilisation) are fairly extensive, but have not been integrated into a meaningful database. Nevertheless, using the principles and assumptions discussed above in Section IV.1, it may be possible to incorporate this approach into classification. “

The use of laboratory mesocosm and/or field tests for evaluating removal of soluble metal species through precipitation/partitioning processes over a range of environmentally relevant conditions are described in the guidance (2009) and for copper, such laboratory/mesocosm and/or field tests have therefore been assessed.

Water compartment

In the water compartment, removal of soluble copper species through precipitation/partitioning processes over a range of environmentally relevant conditions, was assessed in Rader K., 2013 and described in the section “additional information on environmental fate and pathways”.

The assessment relies on modelling simulations, based on the Tableau Input Coupled Kinetics Equilibrium Transport (TICKET) model (Farley et al., 2008). The numerical engine of the model is a screening level model used to assess the fate and effects of chemicals through simultaneous consideration of chemical partitioning, transport, reactivity, and bioavailability (MacKay TICKET-UWM). The software includes metal-specific binding to inorganic ligands, and POC (using information from metal speciation models such as WHAM) and average-annual cycling of organic matter and sulfide production in the lake.

The model was applied to a standard lake environment (EUSES characteristics), complemented with a sensitivity analysis on model parameters such as pH. The validity of the model outcome (removal rate) was assessed from mesocosm and field data. The main conclusions are formulated as follows:

- For a standard lake environment consisting of the EUSES model lake parameters and the K_d derived in the copper RA (Log K_d : 4.48), copper removal from the water column satisfies the criterion of rapid removal of 70% dissolved copper removal in 28 days;
- For a standard lake environment consisting of the EUSES model lake parameters but with pH varying between 6 and 8 (K_d estimated from the model), copper removal from the water column satisfies the criterion of rapid removal of 70% dissolved copper removal in 28 days;
- For an experimental freshwater mesocosm study, carried out with a range of copper loadings (Schaefer et al, 2003), the measured data demonstrate a half life of 4 days and thus satisfy the criterion of rapid removal of copper (i.e. greater than 70% in 28 days);
- For the whole-lake spike addition studies (Lake Courtille and Saint Germain les Belles Reservoir), TICKET-UWM results, in concert with the measured data, indicate rapid removal of copper (i.e. greater than 70% in 28 days) for both lake systems;
- Hypothetical TICKET-UWM simulations modelling the removal of copper in the MELIMEX limno-coralls following termination of copper loading demonstrate copper removal that does not meet the rapid removal benchmark because of a low settling velocity, low distribution coefficient, and low suspended solids concentration.

Considering that the MILIMEX system is the only scenario that could not demonstrate “rapid removal” it is critical to assess the environmental relevance of the MILIMEX system. The MILIMEX System was characterised by a settling velocity that is 10 times lower than the one in the EUSES system (0.2 versus 2.5 m/d) and a suspended solid concentration that is almost 3 times lower than the EUSES system (5.9 versus 16 mg/L). It is therefore concluded that the MILIMEX study was carried out under extreme conditions.

From Rader K., 2013, it can therefore be concluded that under “environmental relevant” conditions, copper-ions are rapidly removed from the water-column.

This information is relevant to the environmental classification.

Sediment compartment

In the sediment compartment, copper binds to the sediment organic carbon (particulate and dissolved) and to the anaerobic sulphides, resulting in the formation of CuS . CuS has a very low stability constants/solubility limit

(LogK=-41 (Di Toro et al.,1990) – see section adsorption/desorption) and therefore the “insoluble” CuS keeps copper in the anaerobic sediment layers, limiting the potential for remobilization of Cu-ions into the water column.

Simpson et al (1998) and Sundelin and Erikson (2001) (see section adsorption/desorption) provide field evidence on the stability of the CuS binding:

- Simpson et al (1998) investigated the oxidation rates of model metal sulfide phases to provide mechanistic information for interpreting the observations on natural sediments. CuS phases were kinetically stable over periods of several hours.
- Sundelin and Erikson (2001) provide further evidence that, after long term oxygenation of sediment cores (3 to 7 months) Cu remains comparatively unavailable.

Last but not least, the assessment of 2 field experiments with intermittent copper dosing (Lake Courtille and the Saint Germain les Belles Reservoir lakes, yearly dosed with copper), assessed in Rader K., 2013, provides further support for the absence of re-mobilization. Since both waterbodies are shallow, polymictic lakes, wind-driven resuspension is expected to play a role in copper dynamics in the water column. Nevertheless, even if long-term resuspension does in fact occur, for both waterbodies, > 70% removal in less than 28 days was observed. The information therefore validates the results from the model simulations and absence of remobilization from the water column (Rader K., 2013).

Soil compartment

In soils, decreases in copper solubility and in copper bio-availability are observed following copper spiking in the laboratory and from long-term field copper exposure experiments. Short term attenuation and long term ageing of copper, spiked in soluble forms to soils was demonstrated from laboratory and field experiments (Ma et al., 2006a and 2006b) and reported in the section “adsorption/desorption”.

The soil environmental factors governing short term attenuation and ageing rates are soil pH, organic matter content, incubation time and temperature with soil pH being the key factor for ageing of Cu added to soils. From a range of laboratory and field experiments an ageing factor of 2 was derived as a reasonable worst case when considering field exposure data. This information is relevant to the soil PNEC derivation.

Transport and distribution

Relevant partitioning coefficients are available from literature.

Aquatic compartment

Partition coefficient in freshwater suspended matter (50 th percentile)	$K_{p_{susp}} = 30,246 \text{ l/kg}$ (log Kp (pm/w) = 4.48)
Partition coefficient in freshwater sediment (50 th percentile)	$K_{psed} = 24,409 \text{ l/kg}$ (log Kp(sed/w) = 4.39)
Partition coefficient in estuarine suspended matter (50 th percentile)	$K_{p_{susp}} = 56,234 \text{ l/kg}$ (log Kp (pm/w) = 4.75)
Partition coefficient in marine suspended matter (50 th percentile)	$K_{p_{susp}} = 131,826 \text{ l/kg}$ (log Kp (pm/w) = 5.12)

Terrestrial compartment

Partitioning coefficient (50 th percentile)	Kd value soil: 2120 L/kg(log Kp (pm/w) = 3.33)
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Bioaccumulation

Because copper is an essential nutrient, all living organisms have well developed mechanisms for regulating copper intake, copper elimination and internal copper binding. The information in the accumulation section demonstrates that copper is well regulated in all living organisms and that highest/ BAF values are noted when copper concentrations in water, sediments and soils are low and for organisms/ life stages with high nutritional

needs. The/ BAF values therefore have no ecotoxicological meaning. It should be mentioned that the non-applicability of BCFs for metal and especially for essential metals was already recognized in the regulatory framework of aquatic hazard classification (OECD,2001).

Importantly, the literature review demonstrates that copper is not biomagnified in aquatic or terrestrial ecosystems.

The section further includes critical data related to (1) the accumulation of copper on critical target tissues (e.g. gills in aquatic organisms); (2) the influence of environmental parameters (e.g. Organic Carbon, pH, Cationic Exchange Capacity) as well as food intake on the accumulation of copper.

This information is relevant to the understanding of the accumulation as well as the mechanism of actions, described in the section ecotoxicological information

More detailed summaries on respectively aquatic and terrestrial bioaccumulation are available from the aquatic and terrestrial bioaccumulation summary sections

The information relevant to assessing copper toxicity from dietary exposure - of relevance to secondary poisoning assessments is included in the section "ecotoxicological information". The summary record "ecotoxicological information" further provides an overall summary of the rationale for the absence of bioaccumulation and no-concern for secondary poisoning.

4.0.3 Diarsenic trioxide

This section was not completed in the constituent IUCLID file.

4.0.4 Lead

In assessing the ecotoxicity of metals in the various environmental compartments (aquatic, terrestrial and sediment), it is assumed that toxicity is not controlled by the total concentration of a metal, but by the bioavailable form. For metals, this bioavailable form is generally accepted to be the free metal-ion in solution. In the absence of speciation data and as a conservative approximation, it can also be assumed that the total soluble lead pool is bioavailable. All reliable data on ecotoxicity and environmental fate and behaviour of lead and lead substances were therefore selected based on soluble Pb salts or measured (dissolved) Pb concentration.

The reliable data selected for the environmental fate and behaviour of lead are all based on either monitoring data of prevailing lead concentrations in water, soil, sediment, suspended matter and organisms or on experimental results with lead (di) nitrate and lead chloride. All reliable data are expressed based on elemental Pb concentrations and grouped together in a read-across approach and will be used for all lead substances because upon dissolution of lead substances, the Pb-ion is the controlling adsorption and bioaccumulation.

Lead attenuation, removal from water column, geochemical cycling

As described above, after the release of Pb(II) in the environment, further transformations occur thereby changing the potential for toxicity, induced by the free Pb(II) ions. The concentrations of "active" Pb(II) ions, that remains available for uptake by biota depends on different processes: precipitation, dissolution, adsorption, desorption, complexation and competition for biological adsorption sites (ligands). These processes are critical for the fate of Lead in the environment. This was recognized in the Guidance to Regulation (EC) No 1272/2008 Classification, Labelling and Packaging of substances and mixtures (metal annex):

"Environmental transformation of one species of a metal to another species of the same does not constitute degradation as applied to organic compounds and may increase or decrease the availability and bioavailability of the toxic species. However as a result of naturally occurring geochemical processes metal ions can partition from the water column. Data on water column residence time, the processes involved at the water – sediment interface (i.e. deposition and re-mobilisation) are fairly extensive, but have not been integrated into a meaningful database. Nevertheless, using the principles and assumptions discussed above in Section IV.1, it may be possible to incorporate this approach into classification. "

The use of the Tableau Input Coupled Kinetics Equilibrium Transport (TICKET) model – Unit World Model (UWM) for evaluating removal of soluble metal species through precipitation/partitioning processes over a range of environmentally relevant conditions have been assessed for evaluating the removal of soluble lead

species. The information is reported in the section "*additional information on environmental fate*" and summarized below

Water compartment

In the water compartment, lead is rapidly and strongly bound to the suspended solids of the water column. This binding and subsequent settling to the sediment allows for rapid metal removal of lead from the water column as demonstrated by a decrease in soluble lead concentrations by >70% in 28 days in a range of simulations according to the TICKET-UWM calculations (Rader et al., 2010). This information is reported in the section: additional information on environmental fate and is relevant to the environmental classification.

Sediment compartment

In the sediment compartment, lead binds to the anaerobic sulphides resulting in the formation of PbS. The analysis was based on an AVS concentration between 1 and 9 µmol AVS/g. The results show that "insoluble" PbS keeps lead in the anaerobic sediment layers, limiting the potential for remobilization of Pb-ions into the water column. The potential for remobilization from oxic sediments, quantified by comparing water column lead concentrations resulting from sediment feedback to the 70% removal concentration was also insignificant. For conditions favouring remobilization, water column dissolved lead concentrations at pseudo steady state were more than 100 times less than the 70% removal concentration. All these results therefore suggest that Pb is relatively insignificant and provide evidence that supports the removal of the "persistent" criterion for soluble lead salts. The relevant information is reported in the section "additional information on environmental fate" and is relevant to the environmental classification.

4.0.5 Nickel

Relevant data for Bioaccumulation endpoints and Adsorption/Desorption endpoints are available. Stability and Biodegradation are not applicable endpoints for nickel, as reported in the 2008/2009 European Union Risk Assessment for Nickel.

4.0.6 Silver

Silver metal and silver-containing compounds undergo dissolution in environmental media (to a greater or lesser extent) and release silver ions. Analytical methods do not usually differentiate between silver ions and other silver containing compounds, and so the environmental fate section will therefore discuss the fate of silver in general. As silver is an element it, cannot undergo degradation but may be transformed between different phases, chemical species and oxidation states.

4.0.7 Zinc

Zinc is a natural element, natural part of the earth's crust and present in natural background concentrations in all environmental compartments, incl. Air, water, sediment, soil, biomass.

Moreover, zinc is an essential element that is needed by all living organisms for optimal growth and development.

Because zinc and its compounds are inorganic, the endpoint degradation is not applicable.

Zinc does not bioaccumulate in water or soil.

For metals, the transport and distribution over the different environmental compartments e. g. the water (dissolved fraction, fraction bound to suspended matter), soil (fraction bound or complexed to the soil particles, fraction in the soil pore water...) is described and quantified by the metal partition coefficients between these different fractions.

Some recent monitoring data on zinc concentrations in the environment are reported in IUCLID section 5.5.1. In evaluating measured levels of zinc in the environment, it is important to consider the natural background.

4.2. Environmental distribution

4.2.1. Adsorption/desorption

4.2.1.1 Cadmium

For metals, the transport and distribution over the different environmental compartments e. g. the water (dissolved fraction, fraction bound to suspended matter), soil (fraction bound or complexed to the soil particles, fraction in the soil pore water...) is described and quantified by the metal partition coefficients between these different fractions.

Partition coefficients for cadmium in freshwater have been reviewed in the EU risk assessment report (ECB 2008). Based on this experimental evidence, a partition coefficient for the distribution between solid particulate matter and water (K_{psusp}) of 130×10^3 l/kg has been defined for EU waters and was used throughout the RAR.

The K_p for the distribution between sediment and water (K_{psed}) was estimated in the RAR from the ratio of the average sediment (1.32mg/kg dw) to average water Cd concentrations (0.14µg/l). This "best fit" K_d yields 10000L/kg dw (ECB 2008).

These partition coefficients have been used since then in other legislative processes in the EU (e. g. the water framework directive) and will also be used for REACH.

The marine K_d was derived based on data from several marine waters. the geomean value for cadmium in seawater is 617 l/kg.

The information on these partition coefficients is given under 5.6.

The following information is taken into account for any environmental exposure assessment:

K_p for solid particulate matter and water (K_{psusp}): 130000 l/kg (log value: 5.1)

K_p for water and sediment (K_{psed}); 10000 l/kg (log value: 4)

K_d for marine waters is 617 l/kg (log value: 2.79)

4.2.1.2 Copper

Relevant partitioning coefficients are available from literature.

- For the aquatic compartment, the summaries from Heijerick and Van Sprang (2005 and 2008) have been agreed under the copper RAR (2008) and are used the risk characterization.
- For the terrestrial compartments, in the RA report, preference has been given to the Sauvé et al dataset as it covers the widest range of soil conditions relevant for the risk assessment and these have been used for the risk characterization under the copper RAR (2008).

The partitioning coefficients retained are summarised as:

Aquatic compartment

Partition coefficient in freshwater suspended matter (50 th percentile)	$K_{psusp} = 30,246$ l/kg (log K_p (pm/w) = 4.48)
Partition coefficient in freshwater sediment (50th percentile)	$K_{psed} = 24,409$ l/kg (log K_p (sed/w) = 4.39)
Partition coefficient in estuarine suspended matter (50 th percentile)	$K_{psusp} = 56,234$ l/kg (log K_p (pm/w) = 4.75)
Partition coefficient in marine suspended matter (50 th percentile)	$K_{psusp} = 131,826$ l/kg (log K_p (pm/w) = 5.12)

Terrestrial compartment

Partitioning coefficient
(50th percentile)

Kd value soil: 2120 L/kg(log Kp (pm/w) = 3.33)

Information on short term and long term attenuation of copper in soils as a function of soil chemistry was assessed by Ma et al., 2006a & 2006b. The soil and environmental factors governing short term attenuation and ageing rates are soil pH, organic matter content, incubation time and temperature with soil pH being the key factor for ageing of Cu added to soils.

From the field experiments and a mechanistic understanding of the decrease in bioavailable copper as a function of time following exposures, an ageing factor of 2 was derived as a reasonable worst case when considering field exposure data. This information is relevant to the soil PNEC derivation.

The following information is taken into account for any environmental exposure assessment:

Aquatic compartment

Partition coefficient in freshwater suspended matter Kpsusp= 30,246 l/kg (log Kp (pm/w) = 4.48) (50th percentile)

Partition coefficient in freshwater sediment Kpsed = 24,409 l/kg (log Kp(sed/w) = 4.39) (50th percentile)

Partition coefficient in estuarine suspended matter Kpsusp= 56,234 l/kg (log Kp (pm/w) = 4.75) (50th percentile)

Partition coefficient in marine suspended matter Kpsusp= 131,826 l/kg (log Kp (pm/w) = 5.12) (50th percentile)

Terrestrial compartment

Partitioning coefficient Kd value soil: 2120 L/kg(log Kp (pm/w) = 3.33) (50th percentile)

4.2.1.3 Diarsenic trioxide

Data from the following publications, studies and review documents were summarised to quantify partitioning in soils, sediments and suspended matter:

1. Allison & Allison(2005)
2. World Health Organization (2001)
3. Crommentuijn et al (1997)
4. Sauve et al (2000)

Table 2. Partition coefficients for soil, sediments and suspended matter

Medium	Median log10	Mean log10	SD log10	Min log10	Max log10	n	Review
Soil	3.4	3.2	0.7	0.3	4.3	21	Allison & Allison, 2005
Soil				1.88	3.08	15	WHO, 2001
Soil				2.28	3.61		Crommentuijn et al., 1997
Soil	*	*	*	0.2	5.72	66	Sauve et al., 2000
Sediment	2.2	2.4	0.7	1.6	4.3		Allison & Allison, 2005
Sediment					4.07		Crommentuijn et al., 1997
Sediment (marine)				3.99			Crommentuijn et al., 1997
Suspended matter	4.0	3.9	0.5	2.0	6.0	25	Allison & Allison, 2005
Suspended matter				4.0	4.08		Crommentuijn et al., 1997
Suspended matter (marine)				3.45	3.85		Crommentuijn et al., 1997

* As Sauve et al. (2000) provided statistics (Mean, SD, Median) based on untransformed Kp values, these values were not tabulated.

Soil

- According to Allison and Allison (2005), 21 log Kp values for soils range from 0.3 to 4.3 without differentiating between As(III) and As(V) and thus possibly involving both oxidation states. The median,

- mean and standard deviation assuming a log-normal distribution are 3.4, 3.2 and 0.7, respectively.
- WHO (2001) cited a study by Sakata (1987) reporting log Kp values for arsenite in 15 subsurface soils from different sites in Japan with log Kp values ranging from 1.88 to 3.08 and being significantly correlated with the extractable iron content of the soils.
 - In addition, Crommentuijn et al. (1997) reported log Kp values of 2.28 and 3.61 based on batch experiments by Bockting et al (1992) and field-based data of a polluted site reported by Janssen et al. (1996), respectively.
 - Sauve et al. (2000) summarized 66 log Kp values, including studies using metal-spiked soils, ranging from 0.2 to 5.72. However, as the provided statistics (Mean, SD, Median) were computed using untransformed Kp values, these values are not comparable to the statistics provided by Allison & Allison (2005).

Values summarized by Crommentuijn et al. (1997), Sauve et al. (2000) and WHO (2001) are in the range of Kp values as reported for soils by Allison & Allison (2005) except for the maximum reported by Sauve et al. (2000), who included studies using metal-spiked soils. The maximum reported by Sauve et al. (2000) may not be representative for metal partitioning at ambient As concentrations in the soil as it may not be advisable to use Kp values derived from highly contaminated soils and apply them for risk assessment of ambient conditions. Thus, the median of 3.4 reported by Allison & Allison (2005) was selected for the chemical safety assessment.

Sediment

- According to Allison & Allison (2005), log Kp values for sediments range from 1.6 to 4.3 without differentiating between As(III) and As(V) and thus possibly involving both oxidation states. The median, mean and standard deviation based on a log-normal distribution are 2.2, 2.4 and 0.7, respectively.
- Crommentuijn et al. (1997) reported log Kp values of 4.07 and 3.99 based on monitoring data in freshwater sediments from 3 different locations in the Netherlands during different time intervals by Venema (1996) and monitoring data in marine surface water and sediments at different locations in the North Sea and Wadden Sea by Yland (1996), respectively.

Values summarized by Crommentuijn et al. (1997) are in the range of Kp values as reported for sediments by Allison & Allison (2005). Thus, the median log Kp value of 2.2 reported by Allison & Allison (2005) was selected for the chemical safety assessment.

Suspended matter

- According to Allison and Allison (2005), 25 log Kp values for suspended matter range from 2.0 to 6.0 without differentiating between As(III) and As(V) and thus possibly involving both oxidation states. The median, mean and standard deviation assuming a log-normal distribution are 4.0, 3.9 and 0.5, respectively.
- Crommentuijn et al. (1997) reported log Kp values of 4.00 and 4.08 based on freshwater monitoring data from 4 different locations in the Netherlands in 1983-1986 by Stortelder et al. (1989) and monitoring data from 7 different locations in the Netherlands in 1988-1992 by Venema (1994), respectively.
- Crommentuijn et al. (1997) furthermore reported log Kp values of 3.45 and 3.85 based on marine monitoring data from different locations in the North Sea and Wadden Sea in 1995 by Yland (1996) and Yland and Smedes (1996), respectively.

Values summarized by Crommentuijn et al. (1997) are in the range of log Kp values as reported for suspended matter by Allison & Allison (2005). Thus, the median of 4.0 reported by Allison & Allison (2005) was selected for the chemical safety assessment.

The following information is taken into account for any environmental exposure assessment:

Soil: According to Allison & Allison (2005), 21 log Kp values for soils range from 0.3 to 4.3 without differentiating between As(III) and As(V) and thus possibly involving both oxidation states. As other relevant published log Kp values are in agreement with Allison & Allison (2005), the median of 3.4 was selected for the chemical safety assessment.

Sediment: According to Allison & Allison (2005), log Kp values for sediments range from 1.6 to 4.3 without differentiating between As(III) and As(V) and thus possibly involving both oxidation states. As other relevant published log Kp values are in agreement with Allison & Allison (2005), the median of 2.2 was selected for the chemical safety assessment.

Suspended matter: According to Allison & Allison (2005), 25 log Kp values for sediments range from 2.0 to 6.0 without differentiating between As(III) and As(V) and thus possibly involving both oxidation states. As

other relevant published log K_p values are in agreement with Allison & Allison (2005), the median of 4.0 was selected for the chemical safety assessment.

4.2.1.4 Lead

Freshwater environment

Partitioning coefficient for Pb between the freshwater and suspended particulate matter (SPM) are summarized in the voluntary risk assessment of lead (LDAI, 2008). An overview of the selected data is provided in the Table below.

Table 3. Reported log K_D , SPM values for Pb in freshwaters in Europe.

Location	Log K_D (L/kg)	Remarks	Reference
Four Dutch Lakes	6.0	average	Koelmans and Radovanovic, 1998
Calder River, UK	4.45 - 5.98	min-max range	Lofts and Tipping, 2000
Nidd River, UK	4.69 - 6.25	min-max range	
Swale River, UK	4.58 - 6.20	min-max range	
Trent River, UK	4.61 - 6.06	min-max range	
All rivers	5.41	observed mean	
All rivers	5.71	predicted mean	
Scheldt, Belgium	5.3	salinity of 1.5 ppm	Nolting et al., 1999
Po River, Italy	5.5	median value	Pettine et al., 1994
Dutch freshwater	5.81	mean	Stortelder et al., 1989; in Crommentuyn et al., 1997
Upland-influenced river water, UK	4.6	modelled value	Tipping et al., 1998
Low-salinity water, UK	5.5	modelled value	
7 freshwater locations in The Netherlands	5.93		Venema, 1994; in Crommentuyn et al., 1997
54 Czech rivers / 119 locations	5.44	median K_D	Vesely et al., 2001
	5.18	median $K_A(1)$	
RANGE	4.45 – 6.25		

⁽¹⁾ K_A : based on the acid soluble concentration

For the calculation of local and regional exposure concentrations the median log $K_{D, SPM}$ value of 5.47 is selected. This value corresponds with a $K_{D, SPM}$ of 295,121 l/kg.

For freshwater sediments, the selected K_D value was 153,848 L/kg (Log K_D : 5.19).

Estuarine environment

The next table summarizes the $K_{D, SPM}$ values for suspended particulate matter that were determined in estuarine water bodies. The lowest reported log K_D (3.8) was found in a Texan estuary (Benoit et al, 1994). Other values are generally situated between 5.8 and 6.5 with a maximum value of 7.46 (Zhou et al, 2003). However, this value was calculated based on values of suspended particles derived from a graph. Also dissolved Pb was around or beneath detection level, the detection limit was used to calculate the partition coefficient. K_D values for this study are thus unreliable estimates. These values were therefore not taken into account while calculating the probability distribution. The maximum value can be found in North Australian estuaries with a K_D of 7.2 (Munksgaard and Parry, 2001).

Table 4. Reported K_D , SPM values for Pb in estuarine surface waters.

Location	Log K_D (L/kg)	Remarks	Reference
Seine estuary	6.1-6.3	Min-max range	Chiffolleau et al, 1994
Rhine estuary	5.85-6.26	Min-max value	Golimowski et al, 1990

Location	Log KD (L/kg)	Remarks	Reference
Weser estuary, Germany	5.87-6.27	Different metal extraction methods used	Turner et al, 1992
Mersey estuary, UK	4.7-5.0	Estimated KD for river water	Turner et al, 2002
Scheldt estuary, Belgium	6.0-6.51	Min-max value	Valenta et al, 1986
Scheldt estuary, Belgium	5.4-6.0	Min-max value	Baeyens, 1998
Penze estuary, France	5.5-6.8	Min-max value	Waeles et al, 2007
Mersey estuary, UK	5.0-5.48	Min-max value with increasing salinity	Hartnett & Berry, 2010
Conway estuary, Wales	5.62-7.46 ¹	Min-max range	Zhou et al, 2003
S. Baltic Sea	6.67	Median value	Sokolowski et al, 2001
N. Australia	5.5-7.2	Min-max value	Munksgaard and Parry, 2001
Lena estuary, Russia	6.42	Median value	Martin et al, 1993
Texas	3.8-6.8	Min-max value	Benoit et al, 1994
Danshuei estuary, Taiwan	5.2-6.5	Min-max value	Jiann et al, 2005
Nile estuary	5.2-5.3	Min-max value	Abdel-Moati, 1990
RANGE	3.8-7.46		

¹ Values were calculated based on reported dissolved and particulate Pb-concentrations

For the calculation of local and regional exposure concentrations in estuarine environments, a median log $K_{D, SPM}$ value of 5.83 should be used. This value corresponds with a $K_{D, SPM}$ of 677,954 L/kg.

Marine environment

A median $K_{D, SPM}$ for Pb was calculated for suspended particulate matter in the marine environment, using the data given below. Four reported marine log $K_{D, SPM}$ values were below 5.0 and were representative for the Atlantic Ocean, the Adriatic Sea, the Greek coast near Lesbos and the Scheldt estuary (4.7, 4.8, 4.1 and 4.9, respectively). Log $K_{D, SPM}$ values for the North Sea are situated between 5.0 and 7.25. The maximum value is situated in the Adriatic Sea (log $K_{D, SPM}$ of 7.8). All reported log $K_{D, SPM}$ for other marine water bodies were situated between 5.0 and 7.8.

From a distribution (Triangular) that is fitted through all these data points a median log $K_{D, SPM}$ of 6.18 for Pb in the marine environment is derived. This corresponds with a $K_{D, SPM}$ of 1,518,099 L/kg.

Table 5. Reported log KD, SPM values for Pb in marine surface water

Location	Log KD (L/kg)	Remarks	Reference
Belgian coastal waters	5.30-5.60	Min-max range	Baeyens et al, 1987
North Sea coastal waters	5.0-7.0	Min-max range	Balls, 1989
Scottish Sea Loch	6.47	Average value of 3 sampling stations	Hall et al
Southern North Sea	5.9-7.1 ²	Min-max range, NSP-data	McManus and Prandle, 1996
Dover strait	5.71 ²	Summer/winter value	
Northern North Sea	6.68 ²	Late summer	
Humber/Wash, UK	6.53 ²	Winter/spring	
Humber/Wash, UK	7.24 ²	Summer	
Scheldt, Belgium	4.9	Salinity of 30 ppm	Nolting et al, 1999
Baltic Sea	5.78 ² 6.49 ² 7.10 ²	10 th percentile 50 th percentile 90 th percentile	Pohl and Hennings, 1999

Location	Log KD (L/kg)	Remarks	Reference
North Sea	5.51 ² 6.30 ² 7.25 ²	10 th percentile 50 th percentile 90 th percentile	Tappin et al, 1995
Seawater, UK	6.2	Modeled value	Tipping et al, 1998
Oceans	6.3-6.5	Min-max range	Valenta et al., 1986
Mytilene, Greek coast	4.1	Calculated value	Angelidis et al, 2003
Adriatic Sea	4.8-7.8	Min-max range	Tankéré et al, 2001
Black Sea	5.9-6.6	Min-max range	Tankéré et al, 2001
Atlantic Ocean	4.7-6.4	Min-max range	Helmers, 1996
RANGE	4.1-7.8		

²Values and/or percentiles were calculated based on reported dissolved and particulate Pb-concentrations

For the marine environment, only one study has reported Pb partition coefficients between the aqueous phase (overlying water) and sediment (Yland et al, 1996). They reported for sediments from the North Sea and Wadden Sea a log K_D of 5.66.

Terrestrial environment

The best estimates of K_{DS} to assess leaching losses of Pb from the soil are made by models based on *in situ* pore water concentrations. Adsorption K_{DS} do not take into account ageing reactions while K_{DS} measured in dilute salt extracts tend to underestimate the Pb concentrations in pore water as the ionic strength is usually lower in the extracts than in the pore water. Measurement of Pb concentrations in pore water overcomes these shortcomings. There are only two studies available where K_{DS} are measured based on pore water Pb concentrations (de Groot et al., 1998; Smolders et al., 2000). If the regression models are applied to predict the K_D of a “typical” soil with pH 6.5, 2 % organic matter content and 27.4 mg Pb/kg soil, the model of de Groot predicts a K_D of 19 10³L/kg (Al-ox = 34 mmol kg⁻¹, %fraction 2-38 µm = 12) while the model of Smolders predicts a K_D of 1.8 10³L/kg. If the pH decreases to 3.5 the K_{DS} decrease to 6.9 10² and 2.2 10² L/kg respectively, if the pH increases to 7.5 the K_{DS} increase to 58 10³ and 3.5 10³ L/kg respectively. It is difficult to derive one “typical” realistic K_D based on these two equations. Therefore the average of the median *measured* K_{DS} (see Table below) by de Groot et al. (1998) and Smolders et al (2000), i. e. 6,400 L/kg, can be used as a realistic K_D to calculate Pb leaching losses. A realistic low K_D is 6.0 10² L/kg (10th percentile of the combined K_D datasets of de Groot and Smolders), a realistic high K_D is 43 10³ L/kg (90th percentile of the combined K_D datasets).

Table 6. Regression models of KD as a function of soil parameters.

KD regression model	Notes	Reference
logKD= -0.13 + 0.48 pH +0.16 log(%fraction 2-38 µm) + 0.73 log(Al-ox)	r ² = 0.84, in situ KD, n=47, contaminated and uncontaminated soils from NL, B and D	de Groot et al., 1998
logKD= 0.22 + 0.75 log[Pb]tot+0.30 pH	r ² = 0.94, in situ KD, n=13, contaminated soils from B	Smolders et al., 2000
logKD= 0.28 pH + log(%OM) + 1.0	r ² = 0.38, adsorption KD in 0.005N salts, n=33, contaminated and uncontaminated soils from NL, UK and F	Gerritse & Van Driel, 1984
log[Pb]s= -0.34 – 0.15 pH + 0.61 log(%OM*10)	r ² = 0.37, metal concentration in water extract, n=31, contaminated and uncontaminated soils from NL, UK and F	McBride et al., 1997
log[Pb]tot- 0.988 log[Pb]s= 1.30 + 0.55 pH	metal concentration in 0.005 M CaCl ₂ + 0.005 M Ca(NO ₃) ₂ extract, n=100, contaminated soils from UK	Jopony & Young, 1994
logKD= 0.37 pH + 0.44 log[Pb]tot+ 1.19	r ² = 0.56, compilation of >70 studies, n=204	Sauvé et al., 2000

%OM: percentage organic matter in the soil; $[Pb]_s$: Pb concentration in solution; $[Pb]_{tot}$: total Pb concentration in the soil, Al-ox: amount of aluminium extracted by ammonium oxalate/oxalic acid, %OC: percentage organic carbon content.

The following information is taken into account for any environmental exposure assessment:

From the literature overview, the following partitioning coefficients have been derived for Pb:

Aquatic compartment

- Partition coefficient in freshwater suspended matter: $Kd_{susp} = 295,121 \text{ L/kg}$
- Partition coefficient in freshwater sediment: $Kd_{sed, fw} = 153,848 \text{ L/kg}$
- Partition coefficient in marine sediment: $Kd_{sed, mar} = 457,088 \text{ L/kg}$
- Partition coefficient in estuarine suspended matter: $Kd_{susp} = 667,954 \text{ L/kg}$
- Partition coefficient in marine suspended matter: $Kd_{susp} = 1,518,099 \text{ L/kg}$

Soil compartment

- Partitioning coefficient: Kd value soil: 6,400 L/kg

4.2.1.5 Nickel

Kp- freshwater suspended particulate matter (SPM)

Reported Kp-values between water and suspended particulate matter range from 692 to 309,030 l/kg (Log Kd: 2.84-5.49). The data are extracted from studies reporting water/suspended matter partitioning coefficients for the Netherlands, UK, France, Czech Republic, and Italy.

Table 7. Reported log Kp, suspended particulate matter (SPM) values for Ni in freshwater surface waters in Europe

Location	Log Kp (L/kg)	Remarks	Reference
Four Dutch lakes			
• Dintel	3.91	Median	Koelmans and Radovanovic 1998
• Lake Hollandsch Diep	4.20	Median	
• Lake Volkerak	3.98	Median	
• Lake Zoom	3.94	Median	
Trent river, UK	3.8	Modelled value	Tipping et al. 1998
Upper Swale river, UK	4.9	Modelled value	
Calder river, UK	4.00 - 5.09	min-max range	Lofts and Tipping 2000
Nidd river, UK	3.69 - 5.32	min-max range	
Swale river, UK	4.24 - 5.49	min-max	
Trent river, UK	2.84 - 4.45	min-max range	
Rhône, France	4.51	NA	Elbaz-Poulichet et al. 1996
Humber river, UK	3.8	NA	Comber et al. (1995; in Tipping et al., 1998)
Po river, Italy	4.87	median value	Pettine et al., 1994
Czech lakes (n=119)	4.26	Median value	Vesely et al., 2001
Conwy river, UK	4.33	NA	Zhou et al., 2003
Scheldt (salinity: 1.5 g/L)	3.9	Single value	Nolting et al., 1999
Tweed – Teviot	4.26	median value	Neal and Robson, 2000
Tweed – Boleside	4.55	median value	
Tweed – Norham	4.36	median value	
Wear	4.74	median value	
Swale – Catterick	4.96	median value	
Swale – Manor	4.78	median value	
Nidd	4.65	median value	
Ure	4.72	median value	
Ouse –	4.69	median value	

Location	Log Kp (L/kg)	Remarks	Reference
Ouse – Acaster	4.64	median value	
Derwent	4.95	median value	
Wharf	4.80	median value	
Aire	4.38	median value	
Calder	4.53	median value	
Don	4.15	median value	
Trent	3.93	median value	
Great Ouse	4.29	median value	
Thames	4.33	median value	
Dutch freshwater	3.90	mean	Crommentuijn et al., 1997
RANGE	2.84 – 5.49		

NA: not available

The partitioning coefficients between water and SPM was fitted to a cumulative distribution function (CDF) (Heijerick and P. Van Sprang, 2004; European Nickel Risk Assessment). The 10th, 50th, and 90th percentiles of the CDF were selected as final suspended solids partitioning coefficients for the local/regional exposure analysis:

- Kpsusp = 26,303 l/kg (*log Kp susp* = **4.42 L/kg**) (50th percentile)
- Kpsusp, min = 5,754 (log Kpsusp min = 3.76 L/kg) (10th percentile)
- Kpsusp, max = 117,490 (log Kp susp max = 5.07 L/kg) (90th percentile)

The 50th percentile value of the distribution function represents a typical suspended matter partition coefficient for EU waters and will be used for the derivation of local and typical regional.

Kp-freshwater sediment

Reported Kp-values between water and sediment are presented in the first table below (data extracted from studies reporting water/sediment partitioning coefficients for the land) and in the second table below (river sediment data compiled by Gunn et al., 1992). The Kp values reported by Gunn et al. (1992) represent the individual or mean value of British - German rivers. Individual data that were used for the determination of mean data were not reported. The observed values range from 770 to 24,300 l/kg (Log Kp: 2.89-4.39).

Table 8. Overview of Kp-values on sediment

Water body location	log Kp (L/kg)	Remarks	Reference
Mersey river	3.71	Modelled value	Turner et al. 2002
Dutch freshwater	3.72	Modelled value	Stortelder et al. 1989; in Crommentuyn et al. 1997
Dutch freshwaters:			Van Der Kooij et al. 1991
• Nieuwe Merwede	3.79	Mean value	
• Rijn - Hagestijn	3.86	Mean value	
• Nieuwe Waterweg	3.88	Mean value	
• Oude Maas	3.88	Mean value	
• Waal	3.89	Mean value	
• Rijn – Lobith	3.92	Mean value	
• Ketelmeer	3.96	Mean value	
• Maas- Eijsden	4.04	Mean value	
• IJsselmeer	4.06	Mean value	
• Haringvliet	4.34	Mean value	
Range	3.71-4.34		

Table 9. Mean Kp values for nickel in river sediments (Gunn et al. 1992)

Site – river / estuarine sediments	Number of measurements	Mean Kp value (L/kg)	Log Kp sed	Reference
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Site – river / estuarine sediments	Number of measurements	Mean Kp value (L/kg)	Log Kp sed	Reference
Trent (Althorpe)	3	2750	3.44	Comber et al. 1995
Mersey(Howley weir)	1	7690	3.89	Comber et al. 1995
Rhine (Lauterbourg, Fr/Ger border)	8	24300	4.39	Kernet al. 1998*
Ythan (Scotland)	1	770	2.89	Comber et al. 1995
Ouse (Selby)	1	1000	3.0	Comber et al. 1995
Mean		7300	3.9	

The partitioning coefficients between water and sediment were fitted to a cumulative distribution function (CDF) (Heijerick and P. Van Sprang, 2004; European Nickel Risk Assessment). The 10th, 50th, and 90th percentiles of the CDF were selected as final sediment partitioning coefficients for the local/regional exposure analysis:

Kpsed = 7,079 l/kg (log Kpsed = **3.85**) (50th percentile)

Kpsed, min = 2,138 l/kg (log Kpsed min = 3.33) (10th percentile)

Kpsed, max = 16,982 l/kg (log Kpsed max = 4.23) (90th percentile)

The 50th percentile value of the distribution function represents a typical suspended matter partition coefficient for EU waters and will be used for the derivation of local and typical regional.

It should be noted that the distribution in the above tables is based on data that, with the exception of one value, only represent surface waters located in The Netherlands and the United Kingdom. The representativity of this distribution for the general case is evaluated using the distribution functions of ambient Ni-concentrations in water and sediments that were generated using extensive EU-monitoring data sets (European Union Nickel Risk Assessment, 2008/2009). Based on the 5th/95th, 50th/50th, and 95th/5th percentiles of the distributions of Ni in water/sediment, respectively, a range of theoretical Kp-values was defined, with a median log Kp of 4.07 and an estimated 10/90th percentile range of 3.62-4.56. These percentiles are in line with the values given and indicate that the natural variability of Kp sediment is well covered in this distribution. Studies conducted after the completion of the EU RA were not included in this analysis. Data and analysis from the conclusion i) sediment program will be included after the evaluation and subsequent discussions are completed by DEPA.

Kp-Marine (SPM and Sediment)

Marine partitioning coefficients for sediment and SPM are based on the study by Stuer-Lauridsen et al (1996). The following table represents the data used to derive the marine partitioning coefficients:

Table 10. Partitioning coefficients Kp in Danish brackish coastal water:

Location (1995)	Sediment	Suspended matter	Total water	Filtrate	Pore water	Sediment/ total water	SPM/ filtrate	Sediment/ porewater
	Mg Ni/kg dw	Mg Ni/kg dw	µg Ni/L	µg Ni/L	µg Ni/L	Kd, L/kg	Kd, L/kg	Kd, L/kg
Roskildefjord I	16.40	2.97	0.52	0.79	26.47	15710	3070	620
Roskildefjord II	15.80	5.60	0.66	0.45	2.04	23910	8840	7680
Øresund	18.07	7.40	2.56	1.06	0.53	7070	6960	34260
Mean						15563 (log 4.2)	6290 (log 3.8)	14190 (log 4.2)

In Danish coastal waters (Table above) three locations Kpsusp ranged 3070 to 8840 L/kg with a mean value 6290, i.e. log**Kpsusp. marine 3.8**.

In the Danish study on coastal water, the range from three locations had Kd values from 7070 to 23910 L/kg with the average 15560 (Table above), i.e. log**Kpsed. marine 4.2**

Kp- aquatic to STP:

Partitioning coefficients for aquatic to STP are based on the studies by Moriyama et al. (1998), Comber and Gunn (1992, 1994) and Gould and Genetelli (1992). The table below represents the data used to derive the aquatic to STP partitioning coefficient of **LogKp= 3.4**

Table 11. Partition coefficients Kp for STP:

STP	Coefficient	Range, L/kg	Value, L/kg	Log value	Reference
Kprs	solids-water in raw sewage sludge (m ³ /m ³)	1720 – 2569	2333 ***	3.4	Moriyama et al. 1989, Comber and Gunn 1994
Kpa	solids-water in activated sewage sludge (m ³ /m ³)	126 – 1259 453 – 1935	1000	3	Gould and Genetelli 1982 Comber and Gunn 1992

*** No published data available on Kp for raw sewage – data obtained from combination of total Ni in raw sewage and Ni in faeces, assuming 100 mg/l SS

Nickel removal rates used for determining the STP removal parameter were limited to those reported after 2000 because removal rates increased with time for Denmark and the Netherlands. Ni removal rates in sewage treatment plants from recent years (2000-2002) are situated between 41% (DEPA, 2002) and 50% (CBS, 2004). Based on the available data, the value 40% removal represents a reasonable worst case removal of Ni in STP in the EU.

Kp- Soil:

The variation observed in the American log Kp soil values may relate to the variation in pH values. The log Kp average was 2.08 in 11 soils with a pH range 4.3 to 4.85 (Buchter et al 1989) whereas the log Kp average was 0.77 in 15 soil with a pH range 4.2 to 6.5 (King 1988). Anderson et al. (1988) reported log Kp values in spiked Danish agricultural soils varying between 1.0 and 3.0. As the equilibration times in the Buchter et al. (1989) and King (1988) studies were relatively short, the studies performed by Janssen et al. (1997), De Groot et al. (1998) and Sauvé et al. (2000), describing field-derived partitioning coefficients, are more relevant to the risk assessment. The Janssen et al. (1997) study on 20 contaminated Dutch soils found a median value log Kp 3.17. This value is close to the log Kp value of 2.86 (Aqua regia digestion) found by De Groot et al. (1998). Sauvé et al. (2000) reported a median log Kp value of 3.37 for a wide range of soil types. However, since it is clear that the Kd levels derived in this study are high compared to other studies and it is unclear what type of extraction techniques exactly are used, it is assumed that stronger digestion methods such as HF extraction or X-ray fluorescence -determining the metal fraction built into the crystal structure of the soil minerals, and hence not relevant for RA purposes- may be incorporated in these results. As the De Groot et al. (1998) study covered 46 European soils, although mainly Dutch soils, and aqua regia extraction was used as digestion method, this value is preferred for the risk assessment. The partition coefficient determined by aqua regia extraction is selected since for environmental purposes, the absolutely maximum fraction that may be released in time-determined through aqua regia digestion- is of interest (FOREGS Geochemical Baseline Programme – Analytical Manual, 2001). This digestion method is harmonised as an International Standard (ISO 11466) and is applied in most EU-countries. This extraction method is also applied in the ongoing scientific research program on Ni toxicity and bioavailability in soils. The log Kpsoil of 2.86 (Aqua regia extraction) is used as input value for the exposure modelling, i.e. **log Kpsoil 2.86**

4.2.1.6 Silver

Transport of metals between aqueous phase and soil/sediment/suspended matter should be described on the basis of measured soil/water, sediment/water and suspended matter/water equilibrium partition coefficients (Kd), instead of using common mathematical relationships based on, for example, octanol-water partition coefficients, as is usually done for organic chemicals (TGD, 1996).

In order to reduce the influence of a particular study or sampling site where a large number of Kd determinations have been made, often within a limited geographic area, a single Kd value was identified for each individual study. In some cases where Kd values (or the data from which they could be calculated) were not stated explicitly in the studies these values were estimated from graphs: in these cases, the selected values are used as supporting information only. In other cases, the values were calculated as the mean log Kd value across sampling sites in the study. Mean values are calculated where log transformed Kd values are reported: where Kd values (not log transformed) are summarised the median value is used. In cases where multiple

determinations of K_d were made at each sampling site, the mean $\log_{10} K_d$ value for each individual site was used as the input for the overall $\log K_d$ calculation. A distribution of K_d values is therefore derived for each partitioning phase (suspended sediment, settled sediment), with equal weighting attributed to each study. For soils, the results of a single large European study are used.

The K_d values for soil were derived from partition coefficient information collected within the GEMAS project (Janik et al. 2010). This project used agricultural soils collected from throughout Europe and is a consistent and comprehensive study of silver partitioning in European soils. A weight of evidence approach has been taken to determine the $\log K_d$ values for soil, suspended matter and sediment. Available data on the partitioning of silver in the environment show that a wide range of partition coefficients are observed, depending upon the local conditions. The values for the partition coefficients used in the assessment are median values based on measured data.

The $\log K_d$ values that will be used in the assessment are: $\log K_d$ soil 3.60 (K_d 4023), $\log K_d$ suspended matter 5.28 (K_d 190546) and $\log K_d$ sediment 4.05 (K_d 11092).

The partitioning of silver during sewage treatment has also been addressed empirically based on measured data in STP influent and effluent from several studies. Due to the extremely low volatility of silver, and the fact that it cannot be degraded, all silver which is removed from wastewater is assumed to be partitioned to the sewage sludge. The partitioning of silver in STP is based on the geometric mean of measurements reported for 14 separate STPs in three separate studies and is expressed as the fraction of influent silver in the final effluent (0.199) and the sludge (0.801) – removal efficiency of 80.1 %. Mean and median removal efficiencies were 82.9 and 87.5, respectively.

Summary of available data for uncoated and coated nanosilver

The available studies of the environmental fate of nanosilver particles fall into three categories:

- Aggregation and dissolution behaviour of nanosilver in solution;
- Partitioning of nanosilver in environmental media; and
- Fate of nanosilver during sewage treatment.

None of the studies have followed standard methods and a variety of different nanosilver materials have been tested. Many of the studies have used nanosilver materials which were prepared in-house and a variety of different surface coatings have been employed. These issues make detailed comparisons of the observations made in the different studies difficult, although several consistent patterns of behaviour have been observed.

Aggregation and dissolution behaviour of nanosilver in environmental media

There have been numerous studies of the aggregation behaviour of nanosilver particles in aqueous media. These studies have included both studies in a variety of synthetic freshwaters as well as several natural waters. The influences of ionic strength, cation composition, pH and natural organic matter on the aggregation behaviour of nanosilver have all been studied over a range of conditions. Many of these studies have used nanosilver particles which were laboratory prepared, following a variety of different methods, although some studies have used commercially available nanosilver materials. Consequently, the materials studied exhibit a variety of different sizes and coating materials (including studies on uncoated materials), although considerable consistency between the behaviour of the different materials has been observed.

A potentially important distinction can be made between different types of nanoparticle aggregation. These may be defined as homoaggregation, where nanoparticles interact with each other, and heteroaggregation, where nanoparticles interact with other materials such as naturally occurring colloids or environmental particles. In many cases the studies have not been able to distinguish between these different types of aggregation, although homoaggregation is likely to dominate in relatively concentrated solutions and in the absence of appreciable concentrations of environmental particles, whereas heteroaggregation is likely to be dominant where nanosilver particles are present at low concentrations and environmental particles are present. Cumberland and Lead (2009) studied the effects of pH, ionic composition and the presence of natural organic macromolecules (humic substances) on laboratory prepared nanosilver particles using flow field-flow fractionation. The nanoparticles were prepared in citrate, although some of them were treated in order to remove excess citrate from the materials prior to use (referred to as cleaned particles). The size of the materials was determined both by transmission electron microscopy (after drying) as 13.7 and 13.6 nm for the uncleaned and cleaned particles, respectively and also by dynamic light scattering (in solution) as 25 and 22 nm for the uncleaned and cleaned

particles, respectively.

pH was found to have a relatively limited effect on the size distribution of the particles, although increases in ionic strength (at the same ionic composition based on monovalent cations) resulted in increased aggregation of the particles. Experiments at the same ionic strength, but in a medium based on divalent cations (Ca rather than Na), resulted in very rapid aggregation and precipitation of the particles. The addition of humic substances to the media resulted in greater stability of the nanoparticles, which is likely to be as a result of the formation of surface films on the nanoparticles. The cleaning procedure to remove excess citrate after production of the particles did not have a significant effect on the behaviour of the particles.

Delay et al. (2011) studied the stability of nanosilver particles in solution, using particles which were prepared in-house, either in ultrapure water, humic rich lake water, or a mixture of the two. Nanoparticles prepared in the presence of natural organic matter (from lake water) were larger than those prepared in its absence and had a lower net charge. It is unlikely that these particles are representative of those supplied commercially within Europe, although their behaviour, in terms of the influence of ionic strength and organic matter on their aggregation properties, was comparable to that observed in other studies. Increased ionic strength and concentrations of divalent ions (e. g. Ca) both resulted in an increased tendency for aggregation of the particles and the presence of natural organic matter increased the solution stability of the particles.

El Badawy et al. (2010) also studied the effects of pH, ionic strength and ionic composition on the stability of nanosilver suspensions. The studies were performed on five different nanosilver materials, four of which were prepared in-house and one of which was commercially produced. Consequently, this is one of the few studies which compare the effect of different coating types on the behaviour of the nanomaterials. The materials prepared in-house were prepared using hydrogen reduction (25 nm), citrate reduction (10 nm), sodium borohydride reduction (11 nm), and one which was stabilised using branched polyethyleneimine (11 nm). The commercially produced material was stabilised with polyvinylpyrrolidone (PVP: 200 nm). These resulted in particles with a variety of different hydrodynamic diameters, as determined by dynamic light scattering.

Whilst an increase in ionic strength resulted in aggregation of some of the forms of nanoparticles, it did not have an effect on the commercial polyvinylpyrrolidone stabilised particles. This was considered to be due to the fact that the coating results in steric stabilisation for this material, whereas the other nanosilver materials were either not stabilised (hydrogen reduced), electrostatically stabilised (citrate and sodium borohydride stabilised), or electrosterically stabilised (branched polyethyleneimine stabilised). A large effect of both ionic strength and pH was observed for the branched polyethyleneimine stabilised material and it was not possible to isolate the effect of a single variable.

The type of anions in the electrolyte solution (Cl⁻ or NO₃⁻) also had a significant effect on both the stability of the nanosilver materials in solution and their surface charges. Solutions containing chloride resulted in the formation of stable silver chloride precipitates, which appeared to result in increased stability of some of the nanosilver materials. It also suggests that free silver ions are present in some of the nanosilver suspensions. As has been observed in other studies, solutions containing divalent cations (e. g. Ca) tended to result in increased aggregation of nanosilver materials. The PVP stabilised material, however, was not affected by these changes to the ionic composition of the solutions.

Gondikas et al. (2012) studied the effect of a thiol containing amino acid (cysteine) on nanosilver and found that it increased the rate of release of silver from the particles, although very high cysteine concentrations were used, which may not be environmentally relevant, with the possible exception of during sewage treatment. The addition of cysteine also had an effect on the surface properties and aggregation rates. Citrate coated nanosilver released less silver into solution than polyvinylpyrrolidone coated nanosilver.

Numerous other studies (Chinnapongse et al. 2011, Huynh and Chen 2011, Jin et al. 2010, Li and Lenhart 2012) made similar findings to the studies summarised above. A more limited number of other studies resulted in alternative findings regarding the solution behaviour of nanosilver particles. Piccapitera et al. (2012) found no influence of humic substances on the solution behaviour of nanosilver at concentrations of between 5 and 20 mg/L, although carbonate coated particles were used for this study which may explain why these effects were observed. Prathna et al. (2011) compared nanosilver particles with chemical and biological coatings and, whilst the chemically treated materials behaved similarly to those in the other studies, the biologically coated materials (which may not be representative of commercially produced nanosilver materials) showed opposite effects. Scheckel et al. (2010) studied the effect of aging nanosilver particles in a kaolin suspension and found no significant effects after 12 months of aging.

There are several factors which affect the aggregation behaviour of nanosilver in solution, including the nature of any surface coatings, the concentrations of divalent cations (such as Ca^{2+} and Mg^{2+}), the ionic strength, and the presence of natural organic matter (such as humic substances). Conditions which cause an increase in the aggregation of nanosilver particles, such as elevated concentrations of divalent cations, are likely to result in an increase in the removal of nanosilver from solution, whereas conditions which enhance or maintain the stability of nanosilver particles, such as some types of surface coatings including natural organic macromolecules, are likely to result in a decrease in the rate of removal of nanosilver from solution. Nanosilver materials can also release dissolved silver into solution, although the conditions which promote the dissolution of silver from nanosilver are not well understood. Dissolved silver can make up a significant proportion of the total silver concentration in some stable nanosilver dispersions.

Dobias and Berier-Latmani (2013) evaluate the release of dissolved silver from nanosilver particles of various sizes and surface coatings embedded in gels deployed in natural river and lake waters (in Switzerland). Deployments were up to four months in duration. A considerable difference in dissolution was observed between 5, 10 and 50 nm materials. Small particles dissolve rapidly and almost completely (>80%), while larger materials (50 nm) persisted for longer (<50% reduction in mass over the period of dissolution observed). However, the difference between materials was reduced when dissolution was normalised to surface area, highlighting the importance of this property to nanosilver fate in the environment. In addition, PVP and tannic acid coated materials released relatively greater amounts of silver ions compared with citrate coated nanosilver materials. Release of dissolved silver was characterised by an initial period of rapid dissolution, followed by a period of much slower dissolution. The initial peak of dissolution was considered to be associated with the release of chemisorbed Ag^+ from the surface of particles. The subsequent sustained period of slower release was considered to be consistent with an oxidative dissolution mechanism. Rates of oxidative dissolution may be greater in waters with lower pH than those used in this study (pH of lake and river water in this study were 7.9 – 8.4).

Partitioning of nanosilver in environmental media

Relatively few studies on the fate and partitioning of nanosilver materials in environmental media have been performed and the majority of those which have been performed have studied the retention of the nanosilver particles in soils.

Cornelis et al. (2010) studied the fate and partitioning of added silver to soil samples; a comparison was made between ionic silver, nanosilver and bulk silver (assumed to be metal powder). Retention coefficients (K_r) were calculated, which take account of the fact that metal dissolution from the nanomaterials may occur, in order to distinguish them from partition coefficients which are commonly calculated for chemicals interacting with solid phases. The nanosilver particles had sizes ranging from 20 to 100 nm, although the specified nominal size according to the manufacturer was 10 nm. No mention was made of the nature of any surface treatment of the nanosilver particles, which limits the usefulness of this study given the importance of the coating material in determining the aggregation and solution behaviour of nanosilver particles.

Some soluble silver was released from nanosilver additions but most of the silver was found to be present as nanosilver particles. Differences were observed between nanosilver, ionic silver and bulk silver partitioning in the soils. K_r values were calculated for nanosilver in five soils, which showed a considerable range of partitioning behaviours. The K_r values calculated were 77, 68, 76, 541, 2165 L/kg. The greatest degree of retention was observed in the soil with the highest pH (pH 6.4), although the lowest retention was observed in a soil with a pH which was only slightly lower (pH 6.1). The highest retention coefficients were observed for the soils with the highest clay contents and soil texture appears to be the dominant factor in controlling the mobility of nanosilver particles in soils.

Cornelis et al. (2012) studied the retention of polyvinylpyrrolidone coated nanosilver particles in 16 Australian soils. The authors observed dissolution of silver from nanoparticles in some cases, although in other cases this dissolution was probably not apparent due to the binding of free silver to the soils. The dissolution of silver from nanoparticles was found to be greater in soils than in solutions, probably due to the removal of dissolved silver by chloride complexation or adsorption to soil surfaces. Different retention coefficients were observed for different loading rates of nanosilver applied to the soils, although only minimum, median, and maximum values were reported. At low loading rates a median retention coefficient of 589 L/kg was observed (range 50 to 2511 L/kg), and at high loading rates a median retention coefficient of 9420 L/kg was observed (range 2459 to 2051761 L/kg). Clay content was found to be the key factor in controlling the retention of nanosilver particles in the soils.

Coutris et al. (2012b) studied the effect of aging of nanosilver particles in soils on their mobility. The study employed two types of nanosilver materials, one of which was citrate coated (4.7 nm) and the other was uncoated (19 nm), with ionic silver (silver nitrate) also used for comparative purposes. Treated soils were allowed to age for two hours, two days, five weeks and 10 weeks before they were subjected to a sequential extraction procedure. Silver added as silver nitrate was predominantly associated with the oxidisable (i.e. organic matter) and strong acid digestible fractions after aging, which are not considered to be bioaccessible. After a short aging period of two hours, approximately 50% of the added silver was found to be bioaccessible, although this fraction decreased to 35% after 2 days, 10% after 35 days and 4% after 70 days. A smaller fraction of silver added as citrate stabilised nanosilver showed a smaller bioaccessible fraction: 24% after two hours, 4.5% after two days and 1% after 35 days. Conversely, the bioaccessible fraction of uncoated nanosilver was initially very low, but increased over time to 7% after 70 days.

This study, and others which use similar extraction techniques to those developed for assessing metal availability (e. g. Tessier et al. 1979), may be problematic when addressing the fate of nanosilver particles. This is due to the possibility of oxidising metallic silver to Ag^+ , which could occur due to some of the extractants used for the oxidation of organic matter. Similarly, reduction of Ag^+ to metallic silver could be caused by the reducing agents used to release metals from iron oxide precipitates. Given these concerns, the results of this study must be treated with caution. The association of silver between different soil fractions differed depending on both the form of silver added to the soil, and on the soil type. Two possibilities for the different behaviour observed for the uncoated nanosilver suggested by the authors were saturation of silver binding sites in the soil and also slower release of dissolved silver from larger aggregates of uncoated nanoparticles. Effects of the extractant solutions used on the form of silver were not considered.

Cleveland et al. (2012) studied the fate of ionic silver, two commercial nanosilver materials and three consumer products containing nanosilver in a tidal marine mesocosm study (natural seawater filtered and diluted to 20%). The commercial nanosilver materials had sizes of 20 nm and 80 nm, although no mention was made of any surface coatings. The consumer products were an antimicrobial wound dressing (Acticoat #20601, Smith and Nephew Medical Limited, Hull, UK), a sock reported to be manufactured using silver-coated nylon fibres (Style 2115, Fox River Mills Inc, Osage, IA) and a stuffed toy made with nanosilver coated foam stuffing (Benny the Bear, Pure Plushy, Oak Brook, IL). All experiments employed only a single addition of silver and the consumer products were placed intact on the surface of the water in the mesocosm and left for the duration of the study.

The maximum total silver concentrations measured in the seawater of the mesocosms, and times at which they were observed, increased in the order: ionic silver (0.1 $\mu\text{g/L}$, 6 to 24 hours), 20 nm nanosilver particles (0.6 $\mu\text{g/L}$, 2 hours), 80 nm nanosilver particles (0.8 $\mu\text{g/L}$, 2 hours), sock (5.3 $\mu\text{g/L}$, 12 hours), dressing (5.7 $\mu\text{g/L}$, 12 hours), stuffed toy (14 $\mu\text{g/L}$, 24 hours). Leaching of nanosilver from the consumer products began after 2 to 12 hours and continued for about 30 days. Silver did not accumulate in the intertidal sediment of the mesocosms treated with ionic silver or commercial nanosilver particles but did in mesocosms treated with consumer products. This was considered to be due to the relatively short residence time of the ionic silver and commercial nanosilver materials in the experimental system, compared with the longer term leaching of silver from the consumer products containing nanosilver. The study did not specifically measure the leaching of nanosilver, only total silver.

Lin et al. (2011) studied the adsorption behaviour of uncoated silver nanoparticles (12 nm) in a porous medium of glass beads, either uncoated or coated with haematite. Increased adsorption was observed at both low pH and at high ionic strength. A difference between the degree of adsorption to the particle surfaces was similar for both the haematite coated and uncoated beads at pH 8.3 and at ionic strengths up to 20 mM but was considerably higher to haematite coated beads at pH 5.0. Sagee et al. (2012) studied the transport of citrate coated nanosilver particles in soils and found that their retention was greatest in soils with finer particles and lowest in coarser soils. This is consistent with other findings (Cornelis et al. 2010, Cornelis et al. 2012), and similar to the behaviour of many other substances, and was interpreted as being due to mechanical straining rather than complexation or adsorption.

Unrine et al. (2012) studied the behaviour of nanosilver particles with two different types of surface coatings (gum arabic and polyvinylpyrrolidone) in aquatic mesocosms with and without sediments and plants present. Organic exudates from the plants were found to have an effect on the particles, although the release of exudates by the plants appeared to be stimulated by the release of silver from the nanoparticles. The interactions with the plants appeared to stabilise the polyvinylpyrrolidone coated particles but appeared to destabilise the gum arabic coated particles either through deposition or dissolution. Silver in the particulate phase of the mesocosms was found to be predominantly (>85%) present as Ag_0 , although cysteine complexes were also present in the systems containing plants.

The retention of nanosilver in soils appears to be largely governed by physical processes, rather than chemical processes. Any dissolved silver released from the nanosilver materials would be expected to partition to soil organic matter or be precipitated as silver sulfides. It is possible, however, that the fate of nanosilver particles in soils is governed by chemical interactions such as silver sulfide formation or the release of Ag^+ which would interact strongly with soil particles and organic matter. Current studies are able to observe the overall degree of retention of nanosilver by soils but have been unable to assess the fate of the retained nanoparticles in the soils. Soil texture appears to be the dominant factor in controlling the potential for nanosilver to leach from soils, with fine textured soils (i.e. those predominantly composed of relatively small particles) being most likely to retain nanosilver particles.

Fate of nanosilver during sewage treatment

A number of studies of the fate of nanosilver during sewage treatment have been performed and there are also several studies concerned with the effects of nanosilver on sewage treatment bacteria which also include some information on the fate of nanosilver during sewage treatment. These studies have all been performed on simulated sewage treatment systems on a laboratory scale, using different experimental setups and different types of nanosilver materials. Despite the differences between the various studies, a considerable degree of consistency has been observed in terms of the fate of nanosilver during sewage treatment.

Hou et al. (2012) studied the fate of nanosilver particles during simulated wastewater treatment processes using citrate coated particles with an average size of 23 nm. Minimal removal was observed during primary settling, although virtually all (over 90%) of the nanosilver was removed to the sludge during the biological treatment phase.

Kaegi et al. (2011) studied the fate of polyoxyethylene fatty acid ester coated nanosilver particles during simulated sewage treatment. Nanosilver particles were only found in the effluent shortly after the initial spiking and effluent concentrations of silver were found to be three orders of magnitude lower than the concentrations in the reactor (99.9% removal). The silver present in the sludge was predominantly in the form of Ag_2S and transformation of nanosilver to Ag_2S appeared to take approximately two hours, although transformation to Ag_2S was only 90% complete.

Tiede et al. (2010) studied the fate of ethylene glycol coated nanosilver in sewage sludge supernatant using batch sorption studies. The majority (90%) of the nanoparticles were found to partition to the sludge phase within six hours, although some of the remaining silver in the supernatant was found to be in the nanoparticle form.

In a study of the toxicity of nanosilver to nitrifying bacteria in sewage treatment (Choi et al. 2009), the majority of the nanoparticles added were found to react with sulfide to form silver sulfide complexes or precipitates. The silver sulfides formed were not found to be oxidised during 18 hours of aeration. This suggests that the silver sulfide formed is unlikely to be oxidised during sewage treatment processes. A further study (Choi and Hu 2009) confirmed that sulfide is an important complexant in the fate of nanosilver during sewage treatment.

Liang et al. (2010) also found that the majority of nanosilver added to a simulated sewage treatment system was associated with the sludge phase. This study also derived a partition coefficient for silver in the reactor of 7100 L/kg and identified that all of the silver in the effluent was in soluble forms using ultrafiltration. This finding differs from some of the other studies (e. g. Tiede et al. 2010) which found that a proportion of the silver in the effluent was present as nanoparticles.

Reinsch et al. (2012) studied the tendency of nanosilver particles to undergo sulfidation (reaction with HS^-) and its effect on bacterial toxicity. Two types of nanosilver particles were examined: commercially available polyvinyl alcohol coated particles, which were polydisperse and had a tendency to aggregate, and laboratory prepared polyvinyl alcohol coated particles, which were monodisperse. Both types of particles had similar surface charges. The tendency of the nanoparticles to aggregate affected their tendency to undergo reactions with sulphur species, with the larger, aggregated, particles reacting more slowly with sulphur species than smaller, dispersed, particles. The tendency of silver nanoparticles to aggregate, which is affected by both solution conditions and surface coating, may therefore be an important factor affecting the fate of nanosilver during sewage treatment.

A study of the effect of nanosilver on nitrogen removal processes during simulated sewage treatment (Jeong et al. 2012) using laboratory prepared polyvinylpyrrolidone coated particles found a lower degree of removal of nanosilver than other studies. Up to 30% of the nanosilver was found to be present in the supernatant. The

degree of removal of nanosilver was found to greatest (90%) at a low loading rate of 1 mg/L, decreased to 67.5% at 10 mg/L, and increased to 85.5% at a high loading rate of 100 mg/L. The greater removal at the highest loading rate was considered to be due to the settling of aggregates, although the loading rates used are unlikely to be representative of the exposure observed in real sewage treatment plants.

Lombi et al. (2013) used bench-scale anaerobic digesters to investigate the influence of nanoparticle surface functionality (i.e. coating material, nanoparticle core structure and the nature of post-processing) on the speciation of silver in sewage sludges and biosolids. Various types of nanosilver materials were investigated and neither surface coating (citrate [6.4 nm], polyvinyl sulphonate [10.7 nm] or mercaptosuccinic acid [8.9 nm]) nor core structure prevented the formation of silver sulphides during waste water treatment. Unlike some other metals, the silver sulfides present in sewage sludge were stable over a six month period of simulated composting/stockpiling.

Overall, the available studies indicate that typically 10% of added nanosilver may be present in the effluents of sewage treatment plants, although there is some uncertainty as to the form of the silver in the effluent, which may be ionic silver associated with organic ligands, dispersed particles of nanosilver (in zero-valent form), or dispersed nanoparticles of silver sulfide. Some studies have observed that under some conditions the proportion of silver released in the effluent may be greater than 10%, although the relevance of the conditions to real sewage treatment plants may be questionable. A number of studies have identified that the silver which is associated with the sludge is present as stable silver sulfide precipitates, rather than as intact particles of nanosilver.

Comparison between ionic silver and nanosilver

The behaviour of nanosilver in the environment appears to be largely controlled by physical processes, particularly the tendency for both homo- and hetero-aggregation of nanosilver particles, which may cause them to settle out of suspension, and their tendency to be retained in soils due to either physical straining or sorption processes. The possibility that nanosilver in soils undergoes partitioning reactions which are comparable to those of other chemicals cannot be discounted, due to the approaches which are used to assess this behaviour, although there is no direct evidence to support this. Nanosilver particles do, however, appear to undergo chemical reactions during sewage treatment to form silver sulfide precipitates and it is possible, although presently unconfirmed, that ionic forms of silver may also undergo similar processes during sewage treatment.

The fate and behaviour of ionic, or dissolved, forms of silver in the environment are often controlled to some extent by associations with sulphur containing ligands such as thiols, due to the very high affinity of silver for these groups. Sulphur containing ligands (such as cysteine) have also been shown to promote the dissolution of silver from nanosilver particles, although this does not necessarily imply a similarity between the behaviour of nanosilver and ionic silver. The ability of nanosilver particles to release dissolved (i.e. ionic) silver is the most likely explanation of these phenomena.

Despite the possible differences in the fate and behaviour of nanosilver when compared to ionic silver in the environment studies, the retention of nanosilver in soils which have reported retention coefficients (which are considered to be conceptually comparable to the partition coefficients derived for chemical adsorption interactions) have resulted in values which are broadly comparable to those determined for ionic silver in soils (median KD 4023 L/kg). The retention coefficients derived for nanosilver in soil showed a clear dependency on the loading rate, with greater retention observed at higher loading rates. There is a dependency on concentration for the partition coefficients derived for ionic silver in soil (Daniels and Rao 1983, Jones et al. 1986), although given that the partitioning of ionic silver in soils is controlled by the availability of specific binding sites and ligands, a reduction in the partition coefficient is observed at higher silver concentrations due to saturation of the available binding sites and ligands present in the soils. This suggests that, despite the apparent similarities in the partitioning and retention behaviour of nanosilver and ionic silver in soils, this may not result from the same processes.

There is also considerable similarity in the overall removal efficiencies observed for nanosilver in simulated sewage treatment systems when compared to the removal efficiencies observed for total silver in operating sewage treatment plants. The typical removal efficiency observed for total silver (assumed to be ionic) is 82.9% and the typical removal efficiency observed for nanosilver is 90%. Considerable variability was observed for the removal efficiencies of operating sewage treatment plants, with between 33 and 99% removal being observed. The differences in the removal efficiencies could be due to the operating conditions of the plants and the concentrations of dissolved silver binding ligands in the treated effluents, which could act to maintain silver in solution (although in a complexed form).

Several studies have identified that silver sulfides are formed from nanosilver in the sludge phase of simulated sewage treatment plants, although the form of silver in sewage sludge has been less well studied. If ionic silver is complexed by ligands during sewage treatment then this could reduce the formation of silver sulfides from ionic silver, although ionic silver is usually considered to be present as silver sulfides in sewage sludge (Lytle 1984). It is possible, therefore, that the behaviour of nanosilver and ionic silver is very similar under the conditions of a sewage treatment plant. This may be due to the presence of relatively high concentrations of sulphur containing ligands, which are able to increase the dissolution of ionic silver from the nanosilver particles (Gondikas et al. 2012). Although direct sulfidation of nanosilver has also been observed (Liu 2011) this is only expected to be a dominant process at high sulfide concentrations. The size of aggregates of nanosilver particles, and the nature of their surface coatings, may mean that some forms of nanosilver are converted into ionic silver more or less readily during sewage treatment than other forms.

It is likely that in practice the tendency for silver which is added to the environment as nanosilver to behave in the same way as ionic silver is dependent upon the rate at which dissolved silver is released from the nanosilver particles, although during sewage treatment the extent and rate of formation of silver sulfides may be more important. If the rate of release is slow then the material will tend to behave predominantly as nanosilver; if the rate of release is fast the material will tend to behave predominantly as ionic silver. Understanding the factors which control the rate of dissolution of nanosilver in the environment is, therefore, likely to be important in understanding the overall fate of nanosilver in the receiving environment.

The reaction of nanosilver to produce silver sulfides during sewage treatment is likely to mean that much of the total emission of nanosilver enters the environment in the form of silver sulfides in sewage sludge if the sludge is spread to land.

The following information is taken into account for any environmental exposure assessment:

Read-across from the dissolved silver ion is also applied to fulfil information requirements for uncoated and coated nanosilver. Supporting information for this read-across is summarised in endpoint summaries and in further detail in the appended read-across summary/justification document.

Soil

The results of the GEMAS survey (Janik et al. 2010) are used to derive the K_d value for soil. 497 K_d values for silver were measured in agricultural soils sampled from throughout Europe. The median value is log K_d 3.60 (K_d 4023), 10th and 90th values percentile are log K_d 3.08 and >3.67 (K_d 1200 and >4,700). The value of 4,700 is the upper limit of detection and so is both the maximum value and 90th percentile.

Suspended sediment

The selected value, the mean of four log K_d values reported from separate studies, is log K_d 5.28 (K_d 190546).

Sediment

The selected value, the mean of two log K_d values reported from separate studies, is log K_d 4.05 (K_d 11092).

STP

The partitioning of silver in an STP is expressed in terms of the fraction of influent silver in the final effluent (0.199) and the sludge (0.801). The fraction partitioning to sludge is derived as the geometric mean of 14 measurements reported from three separate studies.

4.2.1.7 Zinc

For metals, adsorption/desorption translates in the distribution of the metals between the different fractions of the environmental compartment, e. g. the water (dissolved fraction, fraction bound to suspended matter), soil (fraction bound or complexed to the soil particles, fraction in the soil pore water...). This distribution between the different compartments is translated in the partition coefficients between these different fractions. Study records on partition coefficients are given under 5.6.

Partition coefficients for zinc in freshwater has been reviewed in the RAR (ECB 2008). Based on the extensive experimental evidence, a partition coefficient for the distribution between solid particulate matter and water (K_{psusp}) of 5.04 (log value) has been defined for EU waters and used throughout the RAR.

The K_p for the distribution between sediment and water (K_{psed}) was estimated in the RAR from that for particulate matter, as follows: $K_{psed} = K_{psusp} / 1.5$, based on the average difference in concentrations of zinc and other metals in both media. For zinc this results in a K_{psed} of 73,000 l/kg. (ECB 2008)

The marine K_d was derived based on data from several marine waters. the geomean value for zinc in seawater is 6010 l/kg

For soil, a solids-water partitioning coefficient of 158.5 l/kg (log value 2.2) was determined experimentally on 11 American soils. This value was used in the RA Zinc.

The following information is taken into account for any environmental exposure assessment:

K_p for solid particulate matter and water (K_{psusp}): 110000 l/kg (log value: 5.04) (ECB 2008)

K_p for water and sediment (K_{psed}); 73000l/kg (log value:4.86) (ECB 2008)

K_d for marine waters is 6010 l/kg (log value: 3.78)

K_d for solids-water in soil is 158.5 l/kg (log value: 2.2)

4.2.4. Summary and discussion of environmental distribution

4.2.4.1 Cadmium

For metals, the transport and distribution over the different environmental compartments e. g. the water (dissolved fraction, fraction bound to suspended matter), soil (fraction bound or complexed to the soil particles, fraction in the soil pore water...) is described and quantified by the metal partition coefficients between these different fractions. The information on these partition coefficients is given under 5.6.

Partition coefficients for cadmium in freshwater have been reviewed in the EU risk assessment report (ECB 2008). Based on this experimental evidence, a partition coefficient for the distribution between solid particulate matter and water (K_{psusp}) of 130×10^3 l/kg has been defined for EU waters and was used throughout the RAR.

The K_p for the distribution between sediment and water ($K_{p_{sed}}$) was estimated in the RAR from the ratio of the average sediment (1.32mg/kg dw) to average water Cd concentrations (0.14µg/l). This "best fit" K_d yields 10000L/kg dw (ECB 2008).

These partition coefficients have been used since then in other legislative processes in the EU (e. g. the water framework directive) and will also be used for REACH.

For the marine water, a partition coefficient water/suspended matter of 617 l/kg has been derived.

4.2.4.2 Copper

This section was not completed in the constituent IUCLID file.

4.2.4.3 Diarsenic trioxide

This section was not completed in the constituent IUCLID file.

4.2.4.4 Lead

This section was not completed in the constituent IUCLID file.

4.2.4.5 Nickel

This section was not completed in the constituent IUCLID file.

4.2.4.6 Silver

This section was not completed in the constituent IUCLID file.

4.2.4.7 Zinc

For metals, the transport and distribution over the different environmental compartments e. g. the water (dissolved fraction, fraction bound to suspended matter), soil (fraction bound or complexed to the soil particles, fraction in the soil pore water...) is described and quantified by the metal partition coefficients between these different fractions. Information on these partition coefficients is given under 5.6.

Partition coefficients for zinc in freshwater have been reviewed in the RAR (ECB 2008). Based on this experimental evidence, a partition coefficient for the distribution between solid particulate matter and water (K_{psusp}) of 5.04 (log value) has been defined for EU waters and was used throughout the RAR.

The K_p for the distribution between sediment and water ($K_{p_{sed}}$) was estimated in the RAR from that for particulate matter, as follows: $K_{p_{sed}} = K_{p_{susp}} / 1.5$, based on the average difference in concentrations of zinc and other metals in both media. For zinc this results in a $K_{p_{sed}}$ of 73,000 L/kg. (ECB 2008)

These partition coefficients have been used since then in other legislative processes in the EU (e. g. the water framework directive) and will also be used for REACH.

For the marine water, a partition coefficient water/suspended matter of 6010 l/kg has been derived.

For soil, a solids-water partitioning coefficient of 158.5 l/kg (log value 2.2) was determined experimentally on 11 American soils. This value was used in the RA Zinc.

4.3. Bioaccumulation

4.3.0.1. Cadmium

This section was not completed in the constituent IUCLID file.

4.3.0.2 Copper

This section was not completed in the constituent IUCLID file.

4.3.0.3 Diarsenic trioxide

This section was not completed in the constituent IUCLID file.

4.3.0.4 Lead

From the literature overview, the following bioaccumulation/bioconcentration factors have been derived for Pb:

Aquatic compartment

Bioaccumulation/bioconcentration factors in freshwater: 1,553 L/kg

Soil compartment

Bioaccumulation/bioconcentration factors in soil: 0.39 kg/kg (dry weight)

4.3.0.5 Nickel

This section was not completed in the constituent IUCLID file.

4.3.0.6 Silver

There are many studies in the literature relevant to the bioaccumulation of silver. However, many of these studies do not report sufficient information for BCF values to be calculated. In addition, many of the data were obtained from studies of short duration which did not achieve steady-state conditions. The available reliable studies suggest low BCF values for fish, although studies of the accumulation of silver by molluscs, such as Hedouin et al. (2006), indicate the potential for higher BAF values for these organisms.

As data on bioaccumulation for fish are preferentially selected for this information requirement under REACH, the key study selected for this endpoint is Baudin et al. (1993) in which carp were exposed to silver in water for a six-week period, followed by a depuration phase. A steady state concentration in fish was achieved after 30 days exposure and a BCF value of 70 was calculated for the whole fish on a wet weight basis.

Recent ECHA guidance on aquatic bioaccumulation assessment for REACH registration observes that it is not possible to make log K_{ow} or solubility based estimates of nanomaterial bioaccumulation as nanomaterials within test systems are “dispersed” and not in solution. As such, measured BCF values are required to fulfil data requirements under REACH.

The guidance also states that it is also of vital importance to consider the influence of aggregation/agglomeration as well as dissolution on bioaccumulation. If possible, information on bioaccumulation of nanomaterials should be supported with information on the form of the substance present in the animal tissue (i. e. are nanoparticles of silver bioaccumulated or just ionic silver released from nanomaterials).

Handy et al. (2012) outline several problems with the performance of conventional bioaccumulation tests using nanomaterials. Critically, they question the founding assumption of the “steady-state” required for BCF measurements from aqueous exposures as colloidal dispersions (of nanomaterials) are dynamic systems which do not achieve steady equilibrium state (Handy et al. 2008). Equally, uptake by endocytosis (a potential mechanism of accumulation of nanoparticles) may also confound the use of standard kinetic relationships employed in bioaccumulation tests that are based on diffusion (i. e. the Fick equations). Handy et al. (2012) warn against the application of bioaccumulation tests without an appreciation of the underlying mechanism of uptake and kinetics. Handy et al. (2012) also discuss the use of diet borne studies. However, limited potential for

the verification of particle size distribution of nanomaterials when incorporated into food (as per studies conducted in soils) are considered to restrict the usefulness of diet-based bioaccumulation tests with nanomaterials.

Read-across from the dissolved silver ion is also applied to fulfil information requirements for silver and silver-based (coated) nanomaterials. Supporting information for this read-across is summarised in endpoint summaries and in further detail in the appended nanosilver read-across summary/justification document.

4.3.0.7 Zinc

Due to homeostatic control mechanisms, bioaccumulation is not relevant to essential elements in general and to like zinc in particular.

In experimental work, high BCF factors are observed at the lowest zinc exposure levels, due to the fact that organisms will concentrate zinc to satisfy internal physiological needs for the essential element. For the same reason of homeostasis, the BCF will strongly decrease when exposure concentrations increase. This results in a general negative relationship between BCF and exposure (McGeer et al 2003).

On bioaccumulation, the EU risk assessment report (ECB 2008) concludes that *“it is concluded that secondary poisoning is considered to be not relevant in the effect assessment of zinc. Major decision points for this conclusion are the following. The accumulation of zinc, an essential element, is regulated in animals of several taxonomic groups, for example in molluscs, crustaceans, fish and mammals. In mammals, one of the two target species for secondary poisoning, both the absorption of zinc from the diet and the excretion of zinc, are regulated. This allows mammals, within certain limits, to maintain their total body zinc level (whole body homeostasis) and to maintain physiologically required levels of zinc in their various tissues, both at low and high dietary zinc intakes. The results of field studies, in which relatively small differences were found in the zinc levels of small mammals from control and polluted sites, are in accordance with the homeostatic mechanism. These data indicate that the bioaccumulation potential of zinc in both herbivorous and carnivorous mammals will be low.”*

4.3.3. Summary and discussion of bioaccumulation

4.3.3.1. Aquatic bioaccumulation

4.3.3.1.1 Cadmium

BCF's for cadmium are highest in algae and lowest in fish; High BCF in algae does not necessarily reflect high bioconcentration, because a significant part of the Cd is absorbed to the outer side of the organisms, and not taken up. Another factor of error is the lack of gut clearance in invertebrates. Organs (kidney, liver) contain most Cd.

Main influencing factors for Cd BCF are hardness and Cd concentration in the water. Increasing water hardness reduces Cd uptake. BCF is also inversely related to Cd concentration in water.

McGeer et al (2003) recently extensively reviewed the evidence on bioconcentration and bioaccumulation of cadmium as a function of exposure concentration in a number of taxonomic groups (algae, molluscs, arthropods, annelids, salmonid fish, cyprinid fish, and other fish). The data clearly illustrated that there is a significant degree of control on internal cadmium content. In general, BCF data showed an inverse relationship to exposure concentrations. In all cases, the relationship of BCF to exposure was significant and negative. The slopes of the BCF/BAF – exposure relations were: algae: -0.72, insects: -0.32, arthropods: -0.61, molluscs: -0.50, salmonids: -0.87, Centrarchids: -0.47, Killifish: -0.05, other fish: -0.72. Overall, species mean slope was -0.49 +/- 0.04 (McGeer et al 2003. Environm. Toxicology & Chemistry, vol. 22, nr. 5, 1017 -1037).

The following information is taken into account for any hazard / risk / bioaccumulation assessment:

In general, BCF and BAF data show an inverse relationship to exposure concentrations.

BCF's for cadmium are highest in algae and lowest in fish. In algae, external adsorption of Cd is one of the reasons for high BCF. Hardness and Cd concentration in the water are inversely related to BCF.

Median BCF (per dry weight) reported in the EU risk assessment (ECB 2008) are: 115116 (algae), 5000 (invertebrates), 233 (vertebrates)

The risk assessment mentions a median BAF for vertebrates of 167. Highest BAF is observed for the invertebrate *Hyalella* (170000), at a very low Cd concentration.

4.3.3.1.2 Copper

Bioconcentration factors (BCF) and bioaccumulation factors (BAF)

There is a considerable amount of copper accumulation data available, that could potentially be used to calculate bioconcentration factors (BCF) and bioaccumulation factors (BAF) and assess the corresponding potential risks in aquatic food chains. However due to the homeostatic regulation of copper (and other metals), the BAF are not independent of exposure concentration (Review papers of Adams et al., 2003; Mc Geer et al., 2003; supported by many papers from many authors (see IUCLID supportive record-summaries). Increase/decreased copper intake/eliminations, lead to BCFs, BAFs that are inversely related to exposure concentration (i. e., decreasing/BAFs with increasing exposure concentration (water and diet). Particular to copper, this inverse relationship was clearly demonstrated for BCFs, BAFs and biota-sediment accumulation factors (BSAFs). The observed inverse relationship has been explained by homeostatic regulations of internal tissue concentrations: at low metal concentrations organisms are actively accumulating metals in order to meet their metabolic requirements while at high ambient metal concentration, organisms are able to excrete excess metals or limit uptake.

A more mechanistic understanding of copper regulations of accumulations as well as internal copper binding mechanisms and sequestrations are provided by e.g. Borgmann, 1993 and Rainbow (1980, 1985, 1989).

Additionally, different BCFs for different species, life stages and seasons have been observed, depending on the organism's metabolic need (in e.g. Cu-enzymes). Resulting different copper levels are found in tissues from different strains, species, life stages and species. Moreover, aquatic invertebrates such as gastropods, crustacea and bivalves, relying on phaeocyanin as respiratory pigment have typically higher copper levels (and thus higher BCFs) than invertebrates relying on haemoglobin as respiratory pigment (e.g. Timmermans, 1989; Amiard et al., 1985).

Field data further show that copper concentrations in tissues of marine mammals and coastal seabirds, regardless of species, except brain, tend to decrease with increasing age (Eisler 1984, Lock et al., 1992). Neonatal marine mammals have higher copper levels compared to the mothers (Law et al., 1992).

As a result, use of a simple ratio $C_{\text{biota}}/C_{\text{water}}$ or $C_{\text{biota}}/C_{\text{sediments}}$ as an overall approach for estimating copper bioconcentration factors or copper body burdens is not appropriate. Useful to mention that the non-applicability of BCFs for metal and especially for essential metals was already recognized in the regulatory framework of aquatic hazard classification (OECD, 2001).

The section further includes critical data related to the accumulation of copper to the critical target tissue for copper (e.g. gills in aquatic organisms) and on the influence of dissolved organic matter, calcium and sodium on the accumulation of copper.

- Benoit, 1975, Perez, 1991 and Kaland 1993, described the importance of copper target accumulation to the gills
- Playle, 1993a demonstrated that copper concentrations in the target organ (gills) correlates to the free copper concentration, not to the total copper concentration in the test water. The study provides a mechanistic understanding of the biotic ligand model by determining the Metal- Gill stability constant and thereby predicting metal accumulation on gills and therefore toxicity to fish.
- McGeer et al, 2002, demonstrated that the addition of dissolved organic matter (administered as humic acids) decreased Cu accumulation in gills and liver.
- Playle 2003b provides a mechanistic understanding of the protective effect of dissolved organic matter for copper toxicity to fish because: copper levels in gills decreased with increasing. of origin or molecular size fraction or did not influence Cu binding to gills, while DO concentration did.
- The section further includes some critical data of relevance to secondary poisoning
- Kamunde et al, 2005 demonstrated interaction between Cu uptake from water and diet: from detailed copper uptake experiments, they demonstrated that elevated dietary NaCl modulates Na^+ and Cl^- homeostasis and reduces accumulation and toxicity of waterborne Cu.
- (2000) provided evidence on the interaction between water and food for the homeostasis of copper: The data suggest that the availability of food prevents growth inhibition and initial ion (Na) losses that usually result from waterborne Cu exposure. The data further demonstrate copper acclimation: a 2 fold increase in

LC50 after pre-exposure of the fish to copper.

- Kamunde, 2001 observed that dietary copper pre-exposure decreased the uptake of Cu across the gills providing further evidence of homeostatic interaction between the two routes of uptake. Rainbow trout regulated dietary Cu at the level of the gut by increasing clearance to other tissues, at the liver by increasing biliary Cu excretion, and at the gill by reducing waterborne Cu uptake in response to dietary exposure. The modest morphological changes in the intestinal tract suggested high cell and organelle turnover and local regulation of Cu. In spite of possible increased energy demand for regulation and tissue repair, there was no significant growth inhibitory effect following dietary exposure.
- Hansen et al (2004), performed a metal exposure study on growth performance in rainbow trout fed a live diet pre-exposed to metal contaminated sediments. The study indicates the absence of copper toxicity at high dietary copper levels.
- Allinson (2002) investigates the bioaccumulation of copper through a simple food chain (*Lemna minor* – *C. destructor*) and observed regulation of copper by the crayfish, *C. destructor*, with the gills being the main site for absorption and depuration of copper to and from the water column. *C. destructor* does not appear to be sensitive to dietary copper.
- Nott, 1994 showed that copper, detoxified by the snails are unavailable to the crabs and they pass straight through the gut and appear in the faecal pellets.

Additional information of relevance to the absence of secondary poisoning is available from a well designed study from De Schampelaere et al, 2004, clearly relating copper toxicity to waterborne and not dietary exposure route (see the section “ecotoxicological information”)

Importantly, the copper mesocosm study from Roussel (2007) reported in the section "additional ecotoxicological information" demonstrated a low sensitivity of the predating fish and did not show a concern from secondary poisoning. Also the freshwater pond mesocosm (Schaefer et al, 2002) and the marine pond mesocosm (Foekema et al 2010) (both reported in the section "additional ecotoxicological information") did not show a concern from secondary poisoning.

Last but not least secondary poisoning of birds and mammals via fish or mussels was investigated for metals, including copper, by RIVM (Smit et al, 2000) (also in section additional ecotoxicological information) who concluded that for copper it was not necessary to integrate secondary poisoning aspects into the copper aquatic quality criteria

Biomagnification factors (BMF)

The absence of copper biomagnification, with consistent BMFs<1, was shown from several papers:

- Barwick and Maher (2003), compared trace metal levels in a contaminated seagrass ecosystem in Lake Macquarie, the largest estuary in New South Wales (Australia). The structure of the estuarine food web was studied in details and all organisms (algae, invertebrates, fish) were categorised as autotrophs, herbivores, planktivores, detritivores, omnivores and carnivores. The results of the analysis showed the absence of copper biomagnification in this estuarine systems. Copper concentrations ranged between 0.27 µg Cu/g dw (Omnivore: *Monacanthus* and 88 µg Cu/g dw (Herbivore: *Bembicium auratum* (gastropod with haemocyanin)). The higher levels (e.g.. *B. auratum*) were associated with species with active accumulation of copper into the respiratory pigment haemocyanin.
- Farag et al., 1998, studied copper concentration in benthic invertebrates that represent various functional groups and sizes from de Coeur d’Alene river, influenced by mining activities. The copper concentrations noted across the trophic chain, demonstrated the absence of biomagnification from the sediment to herbivores, omnivores, detritivores and carnivores
- Weis & Weis (1999) demonstrated the absence of trophic transfer of metals in consumers associated with chromated copper arsenate treated wood panels.
- Wang (2002) noted the biotransformation of metals in the classical marine planktonic food chain (phytoplankton to copepods to fish) and explained the phenomenon as the result of the effective efflux of metals by copepods and the low assimilation of metals by marine fish.
- Quinn et al., 2003, evaluated trophic chain transfer of metals in insects (35 species) from a stream food web influenced by acid mine drainage with copper levels up to 100 µg Cu/L. They demonstrated that metal concentrations were higher in water and insects closer to the mining sites and taxa richness increased with distance away from the site. The relation between the trophic positions, determined from ¹⁵N radio isotope determination, indicated that trophic chain had no effect on copper levels in the insects.

Considering that bio-accumulation has no ecotoxicological meaning for copper, a summary of the relevant

information on copper essentiality, homeostasis, copper mechanism of action, biomagnification and potential for secondary poisoning is provided as annex to the PBT assessment

The following information is taken into account for any hazard / risk / bioaccumulation assessment:

There is a considerable amount of copper accumulation data available. The data have been reviewed by two authors in view of assessing the relation between the Cu BCF/BAF values and the copper concentrations in the water and sediment. Additionally some researchers have assessed the influence of water chemistry (dissolved organic matter), and the physiology of the organisms (species, age, seasons...) on the observed BCF/BAF values.

The information demonstrates that copper is well regulated in all living organisms and that BCF and BAF values have no meaning for a hazard assessment.

The data also demonstrate that waterborne exposure is most the critical exposure route and that copper is not biomagnified in aquatic ecosystems

The key information includes critical data related to (1) the accumulation of copper on critical target tissues (e.g. gills in aquatic organisms); (2) the influence of environmental parameters (e.g. Organic Carbon, pH, Cationic Exchange Capacity) as well as food intake on the accumulation of copper. This information is relevant to the understanding of the accumulation as well as the mechanism of actions, described in the section "ecotoxicological information"

Information relevant to assessing copper toxicity from dietary exposure - of relevance to a secondary poisoning assessment is included in the section "ecotoxicological information".

4.3.3.1.3 Disarsenic trioxide

Summary

Arsenic bioaccumulation depends on various factors, such as environmental setting (marine, estuarine, freshwater), organism type (fish, invertebrate), trophic status within the aquatic food chain, exposure concentrations, and route of uptake. Biomagnification in aquatic food chains does not appear to be significant. Bioconcentration of arsenic occurs in aquatic organisms, primarily in algae and lower invertebrates. An extensive study of the factors affecting bioaccumulation of arsenic in lotic systems found no evidence of biomagnification since arsenic concentrations in organisms tend to decrease with increasing trophic level. Arsenic is mainly accumulated in the exoskeleton of invertebrates and in the livers of fish. No differences were found in the arsenic levels in different species of fish, which included herbivorous, insectivorous, and carnivorous species. The major bioaccumulation transfer is between water and algae, at the base of the food chain and this has a strong impact on the concentration in fish. No differences were found between bottom-feeders and predators in tissue levels of arsenic. BAF values appear to be the highest within the range of ambient arsenic concentrations, and decline steeply to relatively low levels as arsenic concentrations in water increase (according to ATSDR, 2007 and WHO, 2001).

Water:

BAF values are not constant across arsenic concentrations in fresh- and saltwater. BAF values from these studies ranged from 2 to 19,000 and appear to be the highest within the range of ambient arsenic concentrations and decline to relatively low values as arsenic water concentrations increase. Regardless, all BAF values were analysed together in a weight-of-evidence approach for fresh- and saltwater, respectively. The median of freshwater BAF values assuming a log-normal distribution is 270 L/kg wet weight (n=91), and this value was selected for the chemical safety assessment. The median of saltwater BAF values assuming a log-normal distribution is 5866 L/kg wet weight (n=4), and this value was considered for the chemical safety assessment. Biomagnification of arsenic in aquatic food chains does not appear to be significant. On the contrary, the highest BAF values were measured at the lower trophic levels (Tyler et al., 2003).

Sediment:

55 field-measured bioaccumulation data points (BSAFs) were summarised (Anonymous, 1998). The median BSAF value on a dry weight basis for invertebrates was 0.143, indicating that invertebrates in most of the assessed studies did not accumulate arsenic to levels greater than those measured in the associated sediments. The overall median of freshwater BSAF values based on a log-normal distribution is 0.143 dry weight (n=55) and was considered for the chemical safety assessment.

The following information is taken into account for any hazard / risk / bioaccumulation assessment:

BAF values are not constant across arsenic concentrations in fresh- and saltwater. BAF values from these studies ranged from 2 to 19,000 and appear to be the highest within the range of ambient arsenic concentrations and decline to relatively low values as arsenic water concentrations increase. The median of freshwater BAF values assuming a log-normal distribution is 270 L/kg wet weight (n=91), and this value was selected for the chemical safety assessment.

Value used for CSA: BCF: 270 L/kg ww

4.3.3.1.4 Lead

Bioconcentration (BCFs) and bioaccumulation factors (BAFs) for Pb from water to aquatic invertebrates and fish were summarized in the voluntary risk assessment of lead, VRAL (LDAI, 2008).

Within typical environmental concentration range, the gathered BAFs for fish ranged between 11 and 143 L/kg_{ww} (10 – 90th%) with a median value of 23 L/kg_{ww} while the BAFs for molluscs ranged between 18 and 3,850 L/kg_{ww} (median value of 675 L/kg_{ww}), for insects between 968 and 4,740 L/kg_{ww} (median value of 1,830 L/kg_{ww}) and for crustaceans between 1,583 and 11,260 L/kg_{ww} (median value of 3,440 L/kg_{ww}).

Table 12. The range of bioaccumulation factor (BAF in L/kg ww) of Pb in freshwater organisms

Diet	Variable	10 th %	50 th %	90 th %	n
Crustaceans	All exposures	1,187	3,159	10,570	8
	0.18-15 µg/L	1,583	3,440	11,260	7
Molluscs	All exposures	11	473	3,535	14
	0.18-15 µg/L	18	675	3,850	11
Annelids	All exposures	1,620	1,620	1,620	1
	0.18-15 µg/L	1,620	1,620	1,620	1
Acarides	All exposures	1,730	1,730	1,730	1
	0.18-15 µg/L	1,730	1,730	1,730	1
Insects	All exposures	968	1,830	4,740	7
	0.18-15 µg/L	968	1,830	4,740	7
Fish	All exposures	11	24	245	16
	0.18-15 µg/L	11	23	143	16

According to the VRAL (2008) a mixed diet scenario is assumed, considering that birds/mammals consume equal proportion of the different food types. Based on significant high bioaccumulation in molluscs, secondary poisoning was also considered for a “mollusc food chain”. The range of bioaccumulation factor (BAF in L/kg_{ww}) of Pb in the mixed and mollusc food diet is presented below.

Table 13. The range of bioaccumulation factor (BAF in L/kg_{ww}) of Pb in the mixed diet.

Diet	variable	10 th %	50 th %	90 th %	n
Mixed food diet	All exposures	921	1,472	3,740	49
	0.18-15 µg/L	988	1,553	3,890	44
Mollusc food diet	All exposures	11	473	3,535	14
	0.18-15 µg/L	18	675	3,850	11

This table shows that the 50th% of the mixed diet BAF for aquatic organisms is 1,553 L/kg (90th%; 3,890 L/kg) and that the mixed diet scenario is driven by the BAF values retrieved from the invertebrates. The 50th% BAF of the mollusc food diet is somewhat lower, i. e. 675 l/kg (90th%; 3,850 L/kg). The BAF value of 1,553 L/kg is further used for the assessment of the secondary poisoning in the aquatic environment.

The following information is taken into account for any hazard / risk / bioaccumulation assessment:

Bioaccumulation/bioconcentration factors in freshwater: 1,553 L/kg

4.3.3.1.5 Nickel

BAF (aquatic) endpoint summary:

BCFs for fish and phytoplankton are generally less than 100, whereas a few BCF for crustacea were between 100 and 360 and around 500 in a brown macroalgae. In bivalves bioaccumulation factors are generally higher. For marine bivalves, i. e., blue mussel and eastern oyster the reported bioaccumulation factors are between 100 and 350 whereas it seems that for another bivalve (the sediment burrowing bivalve *Cerastoderma edule*) significantly higher factors between 3000 and 60,000 are reported. Only one *C. edule* BAF for Ni is based on measured tissue concentrations and parallel Ni seawater concentrations. This value, reported by Boyden (1975), was 1631 L/kg. This value was used in the EU RAR for situations where *C. edule* was a relevant prey item for marine food chains.

An in-depth review of the bioconcentration literature (laboratory tests) has been performed by Brix and DeForest (2002). The investigators concluded that many aquatic animals regulate metals to a varying degree, based on the observation of an inverse relationship between water concentration and the corresponding BCF within a certain concentration range of metal ion in

the aqueous phase (1 to 100 µg/l, Brix and DeForest 2000). This inverse relationship has been observed for Ni in fish and molluscs. A negative relationship between Ni BCF and Ni exposure concentrations was observed by McGeer et al. (2003) when they aggregated whole fish tissue data published by Lind et al. (1978) and Blaylock and Frank (1979). Using a generic seawater concentration of 1.2 µg Ni/L, a BCF of 270 L/kg was calculated from this linear relationship. The BCF of 270 L/kg was considered to be a cautious estimate, as under conditions where dissolved Ni concentrations are > 1.2 µg Ni/L, the BCF will be < 270 L/kg. In the absence of other data, this value was used to estimate tissue Ni concentrations for all aquatic organisms (e. g., bivalves, fish, and cephalopods) with the exception of *C. edule*.

4.3.3.1.6 Silver

Several studies report the bioaccumulation potential of silver in fish. The key study selected for this endpoint is Baudin et al. (1993) in which carp were exposed to approximately 0.2 µg Ag/L for a six-week period. A steady state concentration in fish was achieved after 30 days and a BCF value of 70 was calculated for the whole fish on a wet weight basis. Various other bioaccumulation studies are available, including Galvez (2002) and Webb et al. (2000).

Galvez (2002) exposed juvenile rainbow trout *Oncorhynchus mykiss* to a two day radioactive pulse of 110mAg at 11.9 µg/L followed by a 19 day post-tracer exposure to non-radioactive Ag(I). A whole body BCF of 2.5 was reported after days one and eight. Garnier et al. (1990) reported a whole body BCF of 2.7 for brown trout *Salmo trutta* fingerlings over 57 days. In both studies, the greatest accumulation was observed in the liver.

Webb et al. (2000) report BCF for a range of marine species based on studies employing OECD guideline 305. The data available for most of the species was not fully reported and did not provide sufficient information for assessment. The steady state BCF for silver in the liver of *Oligocottus maculosus* was calculated using data presented within the study to be 9.2 - 15.1 L/Kg at a salinity of 30 ppt (normal seawater) and 44.3 to 79.4 L/Kg at a salinity of 18 ppt.

In a non-guideline, but otherwise reliable study, Wood et al. (2010) investigated bioaccumulation of dissolved silver in the marine teleost *Opsanus beta* (gulf toadfish). BCF values for fed and fasted fish after 22-23 day exposure were calculated from data presented in the paper as 0.43 and 0.58, respectively.

Summary of available data for uncoated and coated nanosilver

Bioaccumulation in fish

Data relevant to the bioaccumulation of uncoated and coated nanosilver are available from 12 studies. However, whilst none of these studies are consistent with standard assessments of bioaccumulation as outlined in REACH endpoint specific guidance, i. e. studies conducted according to the principles outlined in OECD guidance document 305 (exposure up to 28 days followed by 14 days depuration with measurement of whole body concentrations), the study by Griffitt et al. (2013) provides useful and relevant information for the comparative assessment of the relative bioaccumulation potential of ionic and nanosilver obtained from a study that most

closely resembles a standard method.

Griffitt et al. (2013) undertook a comparative assessment of the accumulation of silver in zebra fish (*Danio rerio*), alongside concurrent effects on gene expression, after exposure to either ionic or nanosilver. Adult zebra fish were subject to a 32 day flow-through exposure (0-28 days with silver) to four concentrations of commercially obtained nanosilver (5, 15, 25 and 50 µg/L) and a single concentration of silver nitrate (5 µg/L). Each treatment comprised four replicates of 20 fish. Nanosilver was characterised in stock solutions to have a particle size of 3.1 ± 2.23 nm or 1.7 nm by TEM and DLS methods, respectively. Measured silver concentrations in exposure media stock solutions were 40-50 % of nominal concentrations. Accumulation of silver was measured in gills and carcass tissue at days 7, 14, 21 and 28. Mean BCF values (with 95% confidence intervals) for silver after exposure to nanosilver and ionic silver were calculated using data on individual carcass burden (µg/L wet weight) versus water concentrations presented in the paper. The mean BCF value for nanosilver was 6.61 (4.68 – 9.12), whilst the mean BCF value for ionic silver was 95.50 (66.07-138.04). BCF was significantly greater in ionic silver exposures than in nanosilver exposures ($p < 0.0001$). The maximum BCF value reported for nanosilver based on an individual measurement of carcass burden versus water concentration was 46.82.

In addition, three studies (Scown et al. 2010, Gaiser et al. 2012, Schäfers and Weil 2013) contain sufficient information to calculate tissue-specific BCF values for silver nanomaterials, which can be used as “worst-case” surrogates for comparison with whole-body BCF data available for ionic silver.

Scown et al. (2012) investigated the fate and effects of three sizes of commercially available silver particles (including two nanoparticles: 10 and 35 nm) and ionic silver (as silver nitrate) using 10 day exposures with *Oncorhynchus mykiss* (rainbow trout). Uptake into the gills, liver and kidneys was quantified by inductively coupled plasma-optical emission spectrometry (ICP-OES). Maximum tissue-specific BCF values for nanomaterials in gills were 17.2 (10 nm) and 27.7 (35 nm), whilst maximum tissue-specific BCF values in liver were 26 (10 nm) and 42 (35 nm). Concentrations of silver in kidneys were below the analytical limit of detection, which precluded calculation of tissue-specific BCF values.

Gaiser et al. (2012) investigated the accumulation and effects of nanosilver and bulk silver (35 and 600–1,600 nm) in 21 day sub-chronic exposures with *Cyprinus carpio*. The authors report that an equivalent mass dose of silver nanoparticles was more toxic than larger micro-sized material. In addition, silver was found to accumulate in the liver, gallbladder and gills of carp exposed via the water. Greater accumulation was observed in fish exposed to 0.1 mg/L than 0.01 mg/L. Maximum tissue-specific BCF values for the 35 nm nanosilver particles were calculated as 6.18 and 2.92 for liver and gills, respectively. These measurements are based on total silver and no characterisation of silver nanoparticles in these tissues is reported. As described by Scown et al. (2010), silver was not detected above the analytical limit of detection in kidneys. Silver was also detected in the intestines of *C. carpio*, resulting in a maximum tissue-specific BCF value of 997. However, no depuration phase was reported in the study and it is not clear if intestine samples would include material present within the gut lumen, rather than accumulated material in mesentery. The former scenario would result in the calculation of greatly enhanced BCF values that are not consistent with true accumulation.

In an supplementary study to a conventional early life-stage toxicity test with nanosilver, Schäfers and Weil (2013) exposed juvenile zebra fish (*Danio rerio*) to 25 and 100 µg/L NM-300K nanosilver for 21 days (semi-static exposure regime with renewal of test dispersions on days seven and 14) and determined differential tissue uptake of silver in: 1) head, skin and gills; 2) intestines and stomach; and 3) remaining fish (fillet, organs, bones). Tissue-specific BCF values in head, skin and gills were 8.76 and 8.31 after exposure to 15.3 and 67.3 µg/L NM-300K, respectively. Tissue-specific BCF values in intestines and stomach were 327.0 and 302.0 after exposure to 15.3 and 67.3 µg/L NM-300K, respectively. Tissue-specific BCF values in the remainder of the fish were 7.58 and 6.17 after exposure to 15.3 and 67.3 µg/L NM-300K, respectively. The values for head, gills and skin and the remainder of the fish agree well with the BCF values for nanosilver calculated from the data presented in Griffitt et al. (2013). However, as observed in Gaiser et al. (2012), BCF values in intestines and stomach were higher than in other tissues. As discussed above, it is not clear if intestine samples would include material present within the gut lumen, rather than accumulated material in mesentery, which would confound BCF value calculation.

In addition, several additional studies describe the behaviour of nanosilver relevant to an assessment of bioaccumulation but which do not report BCF data or data from which either whole-body or tissue-specific BCF values can be calculated.

Kwok et al. (2012) report that hyperspectral imaging of Japanese medaka embryos exposed to nanosilver for 21

days revealed the presence of nanosilver and their aggregates in tissues of fish. Gill distribution was ubiquitous, while small amounts were also found in the liver and brain. No nanosilver particles were detected in/on the epidermis, spine, skeletal muscle, kidney or gonad of fish. Nanosilver particles were observed in the gut lumen but rarely in mural elements or mesentery. The authors conclude that gills were the principal sites of nanosilver uptake, although ingestion was common.

Farkas et al. (2011) report the results of a comparative assessment of the uptake of nanosilver and ionic silver in cultured rainbow trout (*Oncorhynchus mykiss*) gill cells. The largest amount of silver was found in gill cells exposed to ionic silver. However, citrate and PVP coated nanosilver were both observed to be taken up by gill cell monolayers, although uptake was significantly less for the slightly smaller PVP coated nanosilver particles. In contrast, once accumulated, PVP coated nanosilver particles were transported across cultured multilayers to a greater extent than citrate coated nanosilver particles and ionic silver, although the authors of the study conclude that additional work is required to confirm these observations.

Lee et al. (2012c/d) undertook studies of the real-time uptake of various sizes of nanosilver particles (41.6 nm and 11.6 nm average diameter) in early developing (cleavage-stage) zebrafish embryos. Single nanosilver particles were observed to passively diffuse into embryos through chorionic pores via random Brownian motion. Dose and size dependent effects on embryonic development were observed. Nanosilver particles were observed in eye (retina), brain, heart and tail. Deformed zebrafish were found to have accumulated higher numbers of larger particles than control fish. In addition, at the same molar concentrations, larger nanosilver particles (41.6 nm) were more toxic than the smaller nanosilver particles (11.6 nm).

Importantly, where silver is accumulated after exposure to nanosilver, the current fish studies are insufficient to distinguish if nanosilver particles are accumulated or if ionic silver has dissolved from a nanosilver particle, which is then accumulated.

With the exception of the BCF value calculated for intestines using the data presented by Gaiser et al. (2012) and Schäfers and Weil (2013), which may be an artefact of experimental design, none of the studies where BCF or tissue-specific BCF values can be calculated report BCF values for nanosilver in excess of the value of 70 used in the CSR for silver, which is based on a whole body BCF calculated after exposure to ionic silver (silver nitrate). Based on these data, fish would appear more susceptible to accumulation of ionic silver rather than nanosilver and an assessment of the bioaccumulative properties of nanosilver based on the properties of ionic silver would be protective.

Bioaccumulation in aquatic invertebrates

Whilst bioaccumulation in fish drives the assessment of bioaccumulation under REACH, several studies that describe the relative bioaccumulation potential of silver from ionic and nanosilver exposures in aquatic invertebrates are available and these data should be included in a comparative assessment of the hazard properties of nanosilver and ionic silver.

Croteau et al. (2011) compared silver bioavailability and toxicity in *Lymnaea stagnalis* (a freshwater gastropod) after exposure to ionic silver and to nanosilver particles coated with either citrate (17 ± 5 nm by TEM in test media) or humic acid (13 ± 3 nm by TEM in test media). *Lymnaea stagnalis* effectively accumulate silver from both ionic and nanoparticulate exposures via aqueous or dietary routes. However, for both routes, uptake rates were faster for ionic silver than for nanosilver particles. Whilst uptake was slower for nanosilver particles, loss rates of silver after waterborne exposure were also faster for nanosilver particles than dissolved silver. The authors conclude that ingestion of silver associated with particulate materials appears to be the most important vector of uptake.

In a related study, Khan et al. (2012) undertook waterborne exposures with the estuarine snail *Peringia ulvae* and concluded that dissolved silver is twice as bioavailable as nanosilver (citrate coated 16.5 ± 4.5 nm by TEM). Uptake rates in saline waters were slower than those reported for *Lymnaea stagnalis* by Croteau et al. (2011). Aggregation of nanosilver particles was observed to occur in estuarine (17 ppt salinity = 79 ± 13 nm) and marine (33 ppt salinity = 162 ± 21 nm) media. The authors hypothesise that aggregation of nanosilver particles reduces, but does not eliminate, the bioavailability of silver from nanosilver particles.

Buffet et al. 2013 investigated the biochemical and behavioural responses of the endobenthic bivalve *Scrobicularia plana* to nanosilver (40-45 nm diameter, obtained from JRC stabilised in lactate) and ionic silver in seawater and microalgal food after a 14 day semi-static exposure. Measured bioaccumulation in the whole soft tissue after waterborne exposure to ionic silver (10 µg/L) was significantly higher (992 ± 382 ng/g ww) than

in controls (483 ± 253 ng/g ww). However, there was no significant difference between bioaccumulation after exposure to 10 µg/L nanosilver (753 ± 467 ng/g ww) and the control. There was no significant difference between soft tissue concentrations of silver after dietary exposure to either ionic silver or nanosilver. Waterborne exposure to either form of silver did not affect feeding rate. Dietary exposure to either ionic silver or nanosilver resulted in reduced feeding rate after 10 days exposure.

The following information is taken into account for any hazard / risk / bioaccumulation assessment:

A BCF of 70 for *Cyprinus carpio* based on wet weight of whole fish is selected as the key study.

4.3.3.1.7 Zinc

Bioaccumulation is not considered relevant for essential elements because of the general presence of homeostatic control mechanisms.

McGeer et al (2003) recently extensively reviewed the evidence on bioconcentration and bioaccumulation of zinc as a function of exposure concentration in a number of taxonomic groups (algae, molluscs, arthropods, annelids, salmonid fish, cyprinid fish, and other fish). The data clearly illustrated that internal zinc content is well regulated. All eight species taxonomic groups investigated exhibited very slight increases in whole body concentration over a dramatic increase in exposure concentration. In fact, most species did not show significant increases in zinc accumulation when exposure levels increased, even when exposure concentrations reached those that would be predicted to cause chronic effects. This suggests that adverse effects related to Zn exposure are independent of whole body accumulation. Due to the general lack of increased whole body and tissue concentrations at higher exposure levels, the zinc BCF data showed an inverse relationship to exposure concentrations. In all cases, the relationship of BCF to exposure was significant and negative. The slopes of the BCF/BAF – exposure relations were: algae: -1.0, insects: -0.79, arthropods: -0.73, molluscs: -0.83, salmonids: -0.92, Centrarchids: -0.80, Killifish: -0.84, other fish: -0.87. Overall, species mean slope was -0.85 ± 0.03 (McGeer et al 2003).

The physiological basis for the inverse relationship of BCF to zinc exposure concentration arises from Zn uptake and control mechanisms. At low environmental zinc levels, organisms are able to sequester and retain Zn in tissues for essential functions. When Zn exposure is more elevated, aquatic organisms are able to control uptake. There is clear evidence that many species actively regulate their body Zn concentrations, including crustaceae, oligochaetes, mussels, gastropods, fish, amphipods, chironomids by different mechanisms (McGeer et al 2003).

The following information is taken into account for any hazard / risk / bioaccumulation assessment:

Zinc is an essential element which is actively regulated by organisms, so bioconcentration/bioaccumulation is not considered relevant.

4.3.3.2. Terrestrial bioaccumulation

4.3.3.2.1 Cadmium

The EU Risk assessment (ECB 2008) presents BAF values that were calculated from the soil: biota concentration ratios. Most organisms are earthworms and the Cd levels were expressed on dry or wet weight basis. All the data on earthworms were obtained from specimens with guts voided prior to analysis.

Cadmium is concentrated from the soil into earthworms organisms (BAF values are all higher than 1). Most important factors affecting the bioaccumulation of Cd by earthworms are the Cd concentration of the soil, soil type, pH, soil organic matter and CEC.

The influence of the Cd content of the soil on the bioaccumulation of Cd is illustrated in most of the studies. Cadmium concentrations in earthworms increase with increasing Cd levels in a non-proportional way. As a result, the BAF observed in Cd-contaminated or Cd-enriched soils is lower than the BAF observed in control soils.

The following information is taken into account for any hazard / risk / bioaccumulation assessment:

Median BAF observed on earthworms is 15 (expressed on a dry weight basis). For arthropoda, the median BAF is 1.4 (summary from the EU risk assessment, ECB 2008). The BAF observed in Cd-contaminated or Cd-

enriched soils is lower than the BAF observed in control soils. In a field study, the BAF observed under contaminated conditions on *Lumbricus rubellus* was 12-123, under control conditions it was 190.

4.3.3.2.2 Copper

As for the aquatic environment, homeostatic regulation of copper (and other metals) is also relevant to soil organisms.

Inverse relationship between copper soil BCF (concentrations in plants/ concentrations in soils) and copper concentrations in the soils were observed by

- Abrahams and Thornton, 1994 for pasture herbage
- Ginocchio et al (2002) for lettuce, tomato and onions and by Tambasco et al (2000) for lettuce (*Lactuca sativa*)

Inverse relationship between copper soil BAF (concentrations in invertebrates/ concentrations in soils) and copper concentrations in the soils were observed by

- Janssen et al, 1997 for *Eisenia Andrei*
- Heikens et al (2001) for different invertebrate species, collected in the field data
- Svendsen and Weeks (1997) for *Lumbricus sp.*
- Sample et al. (1999), who developed a database of Cu concentrations in soil and earthworm tissue.
- Wong and Cheung, 1986 demonstrated that Caterpillars of the white butterfly (*Pieris conidia*) ingesting large amounts of plant leaf material, do not concentrate metals. Lower Cu contents are found in the organism than in the plant material (BCF of 0.1 to 0.3).

The section further includes some supporting data of relevance to secondary poisoning

- Chaney et al, 1983 introduced the term “Soil-Plant Barrier” for describing the mechanisms behind reduced plant uptake. A “Soil-Plant Barrier” protects the food chain from toxicity of a microelement when one or more of abiotic or biotic processes limit maximum levels of that element in edible plant tissues to levels safe for animals: 1) insolubility of the element in soil prevents uptake; 2) immobility of an element in fibrous roots prevents translocation to edible plant tissues; or 3) phytotoxicity of the element occurs at concentrations of the element in edible plant tissues below that injurious to animals.
- Smit et al (2000) assessed the secondary poisoning for copper and calculated an average BAF of 0.09 for earthworms based on an extensive Dutch database (170 data points) – they concluded that for copper it was not necessary to integrate secondary poisoning aspects into the copper aquatic quality criteria.

Biomagnification factor (BMF)

Lakowski (1991) explored the pattern of biomagnification of Cu in the terrestrial invertebrates' food web. Based on 37 biomagnification factors representing herbivores, carnivores and detritivores, the slope of the linear regression was less than one suggesting regulation of Cu concentration.

Hunter and Johnson (1982) examined the food chain transfer of Cu and Cd in contaminated grassland around a refinery. Metal movement between producers, herbivores and carnivore trophic levels was examined with an emphasis on the small mammal components of the food web. Animal: diet ratios decreased with increasing soil concentrations and were all smaller than 0.2. This illustrates the degree of homeostatic regulation exercised by mammalian systems over body tissue retention of ingested Cu.

On the basis of a literature review, Heikens et al (2001) compared Cu accumulation between different invertebrate species. Metal body concentrations were highest in Isopoda and lowest in Coleoptera. Differences in metal accumulation between taxonomic groups were ascribed to differences in metal kinetics, regulation mechanism and the exposure route. Terrestrial Isopoda are detritivores who live on litter and feed on organic matter. On the other hand Coleoptera are either herbivores or carnivores and have less intensive contact with litter.

Wittassek (1987) came to a similar conclusion when studying the uptake of Cu in vineyard soil organisms adapted to 60 years of continuous use of Cu sulphate fungicides. Slugs, Isopods and Diplopods (detritivores) showed the highest accumulation of Cu. Chilopoda and spiders, as predators, had high Cu concentrations only when their prey concentrations were high (they did not bioaccumulate).

The following information is taken into account for any hazard / risk / bioaccumulation assessment:

There is a considerable amount of copper accumulation data available. The data demonstrate an inverse relation between the copper bioaccumulation from soil and the copper concentrations in the soil. The information demonstrates that copper is well regulated in all living organisms and that the BCF and BAF values have no meaning for a hazard assessment. The data also demonstrate that copper is not biomagnified in the terrestrial ecosystems and that there is no issue for secondary poisoning of copper.

4.3.3.2.3 Diarsenic trioxide

This section was not completed in the constituent IUCLID file.

4.3.3.2.4 Lead

Bioaccumulation factors (BAFs) for Pb from soil to earthworms are summarised in the voluntary risk assessment of lead, VRAL(LDAI, 2008). A median BAF for earthworms on a dry weight basis is $0.39 \text{ kg}_{\text{dw}}/\text{kg}_{\text{dw}}$ (median of 101 values) and 10-90th percentiles are 0.13-1.17. On a fresh tissue weight basis, BAF values are $0.10 \text{ kg}_{\text{dw}}/\text{kg}_{\text{ww}}$ (median) and 0.03-0.27 (10-90th percentiles). The influence of soil properties on the BAF of earthworms (*A. calliginosa*) was studied in different soils and the equation describing the BAF as a function of pH reads, with BAF on a wet weight basis ($\text{kg}_{\text{dw}}/\text{kg}_{\text{ww}}$)

$$\text{BAF} = 13.9 * \exp(-0.76 * \text{pH})$$

(Ma, 1982). This equation predicts that the median BAF of the 101 data points above ($\text{BAF} = 0.10 \text{ kg}_{\text{dw}}/\text{kg}_{\text{ww}}$) is found at pH=6.5. At pH 4.5, this BAF is 4-fold larger. There is no significant effect of total soil Pb on the BAFs (LDAI, 2008).

A few literature data are available for bioaccumulation of Pb in isopods from soil or litter, 14 BAFs were collected and listed in Table 12. Values range from 0.001-0.65 $\text{kg}_{\text{dw}}/\text{kg}_{\text{dw}}$. A median BAF for isopods on a dry weight basis is 0.04 (median of 14 values).

Table 14. Bioaccumulation factors between soil or decomposed leaf litter and isopods. The Pb concentrations in the biota are the product of BAF and soil Pb concentration.

Test substance	Organism	Test conditions	Medium	Duration	Soil/litter (mg Pb/kg dw)	BAF (kg dw/kg dw)	Reference
Pb-soil	Porcellio scaber	15 adult specimen were exposed for 14 days to approximately 600 mL of air-dried experimental soil (polluted and remediated with 2.5, 10, 40 and 4 x 40 EDTA, respectively) in plastic vessels with plastic covers.	polluted soil	14 days	4603	0.04	Udovic et al. 2009
			polluted soil leached with 2.5 mmol kg-1 EDTA		4323	0.04	
			polluted soil leached with 10 mmol kg-1 EDTA		2712	0.035	
			polluted soil leached with 40 mmol kg-1 EDTA		2112	0.035	
			polluted soil leached with 4 x 40 mmol kg-1 EDTA		1239	0.025	
PbCl2	Porcellio scaber	Isopods were kept in plastic boxes on a moist gypsum base covered by decomposed leaf	Control	21 days	7.1	0.41	Gräff et al. 1997
			100 mg Pb/L		517	0.14	
			500 mg Pb/L		2777	0.08	
			1000 mg Pb/L		7676	0.03	

Test substance	Organism	Test conditions	Medium	Duration	Soil/litter (mg Pb/kg dw)	BAF (kg dw/kg dw)	Reference
		litter material, i. e. partly decomposed leaf litter material soaked in an aqueous solution of 100, 500 or 1000 mg l ⁻¹ Pb ²⁺ (as PbCl ₂).					
Pb-soil	Trachelipus rathkei	Near a smelting complex a transect of 5 soil sampling sites was taken	0.3 km from the smelting complex	whole life	61946	0.006	Rabitsch 1995
	Porcellio scaber		0.3 km from the smelting complex		61946	0.002	
	Trachelipus ratzeburgi		0.5 km from the smelting complex on the other side of the low hill		1190	0.649	
			1 km from the smelting complex		4618	0.248	
			2.5 km from the smelting complex		516	0.322	

The following information is taken into account for any hazard / risk / bioaccumulation assessment:

Bioaccumulation/bioconcentration factors in soil: 0.39 kg/kg (dry weight)

4.3.3.2.5 Nickel

This section was not completed in the constituent IUCLID file.

4.3.3.2.6 Silver

Coutris et al. (2012) used neutron activated soluble silver (silver nitrate) to assess the uptake, excretion and bio-distribution of silver in the earthworm *Eisenia fetida* exposed in standard OECD artificial soil (pH 5.95) amended with spiked, air dried, horse manure (0.55 ± 0.15 µg/g dissolved silver). A 30 day BAF_{bioavailable} value of 0.62 ± 0.11 was reported.

Summary of available data for uncoated and coated nanosilver

Bioaccumulation in terrestrial invertebrates

Coutris et al. (2012) used neutron activated nanosilver particles (20.2 ± 2.5 nm by TEM) and soluble silver (silver nitrate) to assess the uptake, excretion and bio-distribution of silver in the earthworm *Eisenia fetida* exposed in standard OECD artificial soil (pH 5.95) amended with spiked, air dried, horse manure (0.55 ± 0.15 µg/g dissolved silver; 0.77 ± 0.15 µg/g nanosilver). 30 day BAF_{bioavailable} values of 0.31 ± 0.12 and 0.62 ± 0.11 were reported for nanosilver particles and ionic silver, respectively. Ionic silver was approximately twice as bioavailable as nanosilver. Silver accumulated from soluble salts and nanoparticles was excreted rapidly.

Hunde-Rinke and Klawonn (2013) also measured bioaccumulation of nanosilver (NM-300K, 15 nm particle size) with *Eisenia fetida*. Worms were exposed to silver via either soil or feed in a long-term (28 day) chronic toxicity study. No depuration phase was included and measurements were made at 28 days only. Despite these limitations, a bioaccumulation factor (BAF) of 0.52 can be calculated from silver accumulation observed in the

lowest test concentration (13.43 mg/kg dry weight) of the soil exposure after 28 days (no adverse effects were observed on survival or reproduction at this nanosilver concentration). It is not clear if steady-state concentration was achieved after this exposure. Exposures to higher concentrations of silver in soil (by factors of up to 13), whilst resulting in toxicity, did not result in broadly greater accumulation of silver. It is unclear if the silver was located in the tissues or the gut of the worm. These results compare favourably to those reported by Coutiris et al. (2012). No comparative exposure to ionic silver was conducted.

The following information is taken into account for any hazard / risk / bioaccumulation assessment:

A 30 day BAF bioavailable value of 0.62 ± 0.11 was reported for silver nitrate bioaccumulation in the earthworm *Eisenia fetida*.

4.3.3.2.7 Zinc

Bioaccumulation is not considered relevant for essential elements because of the general presence of homeostatic control mechanisms. the data from a field food chain transfer study indicate that bioconcentration of zinc is indeed very low. It is in all cases also lower in contaminated soil, as compared to control soil.

The following information is taken into account for any hazard / risk / bioaccumulation assessment:

Zinc is an essential element which is actively regulated by organisms, so bioconcentration/bioaccumulation is not considered relevant.

7 Environmental hazard assessment

The assessment of the inorganic UVCB is conducted by assessing the hazard of the individual UVCB constituents. Data access has been provided to the PNECs included in the IUCLID dossiers for the relevant constituents. The PNECs for the relevant constituents are provided in the following sections,

7.6. PNEC derivation and other hazard conclusions

7.6.1 Cadmium

Table 15. Hazard assessment conclusion for the environment for cadmium

Compartment	Hazard conclusion	Remarks/Justification
Freshwater	PNEC aqua (freshwater): 0.19 µg/L	Assessment factor: 2 Extrapolation method: statistical extrapolation Cadmium: The following considerations are made on the uncertainty around the HC5: The chronic freshwater database covers 28 species from 16 different families, including warm and cold water fish, amphibians, crustaceans, insects, algae and higher plants --> Based on this there is no need for an AF • All NOEC data are from real chronic studies; test durations are between 7 days and 3 years except for the unicellular algae, where different life stages are covered -> Based on this there is no need for an AF • The SSD is statistically significant: the species sensitivity distribution (lognormal) is statistically accepted (calculation with the ETX programme) at all levels (0.1-0.01 significance level) following both the Anderson-Darling test ((A-D statistic 0.42) and the Kolmogorov-Smirnov test (K-S statistic 0.69) --> Based on this there is no need for an AF • Many tests included in the SSD database are performed in synthetic water resulting in a lower degree of Cd-complexation than under natural water conditions. This means that the test results were in general conservative, with regard to the aquatic toxicity of Cd in the real environment --> Based on this there is no need for an AF • The RA discussed some data from microcosm model ecosystems: one out of 9 multi-species was found below the HC5 --> this was used as an argument to apply an AF2 on the HC5 to derive the PNEC • In the database containing 168 reliable single species test results, 3 reliable LOECs were below the HC5 --> this was also used as an argument to apply an AF2 on the HC5 to derived the PNEC
Marine water	PNEC aqua (marine water): 1.14 µg/L	Assessment factor: 2 Extrapolation method: statistical extrapolation Cadmium: The following considerations are made on the uncertainty around the HC5: - The chronic NOEC database is very extensive and contains 48 species entries that cover much more than the requirements for taxonomic groups (8) and species (at least 10, preferably more than 15) set out in the guidance; --> no justification to put an AF - Sensitive life stages or long chronic exposure periods (a few months) are represented in each taxonomic group as set out in the guidance; --> no justification to put an AF - The large number of species in the SSD results in a low uncertainty on the HC5 value, as is shown by the small difference between the 50% confidence level and the 95% confidence limits found for the lognormal distribution: less than a factor of 2.5; --> no justification to put an AF - The lognormal distribution that was used for PNEC derivation resulted in an

Compartment	Hazard conclusion	Remarks/Justification
		HC5 of 2.28 µg/l, which is slightly lower than the HC5 value calculated from the log-logistic distribution (2.54 µg/l), which provided the best fit. So the HC5 that is used for the PNEC derivation highlights the conservative character of the log-normal distribution; --> no justification to put an AF - comparing to field data, the HC5 value from the log-normal SSD may not be protective --> justification to put an AF higher than 1 (2) - There is no indication for a particular sensitive group in the SSD. In addition, whenever an SSD includes > 20 data points, the chance of having a value below the HC5 is significant. So, having one or more values below the HC5 is inherent to bigger datasets and is not an issue as such; --> no justification to put an AF Based on these observations, there is a need to divide the HC5 by an assessment factor 2 which results in a PNEC saltwater for cadmium of 1.14 µg/L (HC5/2).
Intermittent releases to water		Cadmium: Intermittent releases is not applicable for cadmium.
Sediments (freshwater)	PNEC sediment (freshwater): 1.8 mg/kg sediment dw	Assessment factor: 1 Extrapolation method: partition coefficient Cadmium: It is emphasized that the reported PNEC for freshwater sediments is an added PNEC, i.e. natural background needs to be taken into account when characterizing the risks from monitored data. The following considerations are made for determining the size of the assessment factor: •The proposed EqP-derived PNEC_{add, sediments} is nearly 700 fold lower than the only long-term chronic NOEC (NOEC growth for <i>C. elegans</i>: 1226.4 mg/kg d.w.) •The AF method presented in this assessment and based on the lowest NOEC from the field colonization study/AF50 yields a PNEC_{sediment} value that is nearly 30% greater than the PNEC_{add, sediment} value derived with the EqP method (115 mg/kg d.w./AF50 = 2.3 mg/kg d.w.) • The AF approach could also be applied to the only long-term single species test identified in the literature. An AF 100 would then be applied which would result in a PNEC of 1226.4 mg/kg d.w. /100 = 12.26 mg/kg d.w. As such, the PNEC_{add, sediment} based on the EqP approach provides adequate protection for benthic organisms in freshwater systems.
Sediments (marine water)	PNEC sediment (marine water): 0.64 mg/kg sediment dw	Assessment factor: 1 Extrapolation method: partition coefficient Cadmium: It is emphasized that the reported PNEC for saltwater sediments is an added PNEC, i.e. natural background needs to be taken into account when characterizing the risks from monitored data. The following considerations are made for determining the size of the assessment factor: • The only single species long-term chronic NOEC found is a thousand times higher than the proposed EqP-derived PNEC (NOEC survival, growth and reproduction for <i>L. plumulosus</i>: 1370 mg/kg d.w.) • The proposed EqP PNEC is considered protective for ecosystems, according to field study (NOEC ecosystem: 169 mg/kg d.w.; Hansen et al. 1996) •AF approach-derived PNEC based on the lowest NOEC from both saltwater and freshwater dataset (1226.4 mg/kg d.w. /AF100 = 12.3 mg/kg d.w.) is 10 to 20 times higher than the recommended EqP-derived PNEC of 0.64 mg/kg d.w.
Sewage treatment plant	PNEC STP: 20 µg/L	Assessment factor: 10 Extrapolation method: assessment factor Cadmium: The PNEC for STP was derived in the EU Cd risk assessment by

Compartment	Hazard conclusion	Remarks/Justification
		applying an assessment factor of 10 on the lowest observed NOEC (200 µg Cd/l) from the two available studies on Cd toxicity to micro-organisms, testing effects on sludge respiration, which yielded a PNECSTP of 20 µg Cd/l. The same PNEC is used for the present exercise.
Soil	PNEC soil: 0.9 mg/kg soil dw	Assessment factor: 1 Extrapolation method: statistical extrapolation Cadmium: A PNECsoil was derived by statistical extrapolation of the extensive soil microflora data, as in the EU risk assessment. This resulted in a HC5 of 2.3 mg Cd/kg d.w. (AF1). However, a PNEC for soil based on secondary poisoning was also derived, effectively suggesting that biotransfer of Cd from soil to higher trophic levels is the most critical pathway for Cd. This PNECsoil secondary poisoning is based on the HC5-50% value of a distribution of soil concentrations leading to critical kidney concentration of 400µg/g d.w. measured in 8 mammal species. This HC5-50% is 0.9 µg Cd/g d.w. soil and, since it is lower than the value derived for microflora sensitivity, is used as the PNECsoil. This approach is in accordance with the one applied in the EU risk assessment on Cd/CdO (ECB 2008).
Air		
Secondary poisoning	PNEC oral: 0.16 mg/kg food	Assessment factor: 10 Cadmium: The EU risk assessment on cadmium identified 4 and 5 good quality feeding studies on birds and mammals, respectively, using Cd-spiked diets. According to the RA, the PNEC oral secondary poisoning is derived from the lowest NOEC of the 9 chronic studies (NOEC for Mallard ducks of 1.6 mg Cd/kg diet; White et al 1978) divided by an AF10.

Environmental classification justification

The classification of Cadmium metal and sparingly soluble Cd-compounds is based on the capacity of these substances to release the Cd⁺⁺ ion in standardized solutions (= "transformation/dissolution test -T/D test - OECD 2001). The Cd⁺⁺ release measured after a given time (7d for acute effects, 28days for chronic effects) is compared to the ecotoxicity reference values for acute, and chronic aquatic effects, i. e.: 18 µg Cd/l and 0.210 µg Cd/l, resp. (ECB 2007).

Classification under Annex I dangerous substances directive 67/548/EEC

a) Cd metal powder

In the EU Risk assessment, Cd metal powder was classified as N, R50/53 (very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment; cfr Annex I dangerous substances directive 67/548/EEC), based on T/D results showing that at 1mg/l loading of the finest powder on the EU market, the release of Cd⁺⁺ ions after 7days (0.192 mg/l) and 28 days (0.198 mg/l) was exceeding the reference values for acute and chronic ecotoxicity, resp. (ECB 2007).

From these results, it followed also that the acute reference value would be reached at a loading rate between 10 and 100µg/l (e. g. at a loading rate of 100 µg Cd powder/l the expected release of Cd⁺⁺ (19.2µg/l) would still exceed the reference value), so the M-factor was 10. This T/D test was performed in pH 8 medium. Although in pH 6 waters, the release of Cd⁺⁺ is anticipated to be higher than at pH8, the increase is expected not to change the conclusion (expected increase pH 6 versus pH 8 based on data on Cd-containing materials: factor 2.2 maximum).

b) Cd metal in massive form

For the massive form of cadmium metal, the EU risk assessment also concluded to a classification as -N, R50/53. This conclusion was based on 7-d T/D data obtained cadmium metal particles, which gave highly variable results. The rate of transformation/dissolution was not related to loading.

On the basis of the single highest Cd⁺⁺ release value, observed in the 10mg/l loading test, (0.138mg/l; average of the 3 replicates was 0.062 mg/l) and considering also that these results were obtained at pH 8, exceedance of the acute ecotoxicity reference value could be anticipated with 1mg/l loading ($0.014 \times 2.2 = 0.031\text{mg/l}$), so this classification seemed to be supported by the limited data.

In the meantime, more refined techniques have been developed for assessing the release of metal ions from massive metal, and analysis is ongoing at pH 6 (where release is highest). While awaiting these results, the revised classification of cadmium metal in massive form is still based on the data of the EU risk assessment.

Revised classification under 2nd Adaptation to Technical Progress (ATP) to the CLP Regulation (2nd ATP CLP)

a) Cd-metal powder

Reconsidering the data mentioned above on Cd-powder, the following classification can be derived based on the 2nd ATP CLP rules:

Acute aquatic effects: at 1mg/l loading of the finest powder on the EU market, the release of Cd⁺⁺ ions after 7days (0.192mg/l) was exceeding the reference values for acute (18µg/l) ecotoxicity (ECB 2007). Therefore Cd powder is classified as acute 1. M-factor: 10, H400 (Very toxic to aquatic life) (see above GHS).

Chronic aquatic effects: at 1mg/l loading of the finest powder on the EU market, the release of Cd⁺⁺ ions after 28 days was 0.198 mg/l. Extrapolating these data to the loading criteria under 2nd ATP CLP, the Cd⁺⁺-release at 100 µg/l and 10 µg/l loading of the Cd-powder can be calculated as being 19.8 µg/l and 1.98 µg/l, resp. These releases exceed the reference value for chronic aquatic effects (0.210µg/l). So, Cd powder is classified chronic 1, in any case.

For defining the M-factor, the “degradability” needs to be determined. Cadmium, like all metals, is an element, and therefore the criterion “degradability” cannot be applied as it is for organic substances. As a surrogate for assessing “degradability”, the concept of “removal from the water column” was developed to assess whether or not a given metal ion would remain present in the water column upon addition (and thus be able to exert a chronic effect) or would be rapidly removed from the water column. In this concept, “rapid removal” (defined as >70% removal within 28 days) is considered as equivalent to “rapidly degradable”. Under section 4.6., the rapid removal of Cadmium from the water column is documented. Consequently, the metal is considered as equivalent to being ‘rapidly degradable’ in the context of classification for chronic aquatic effects.

Considering the above, the following extrapolations from the maximum observed T/D data can be made:

- at 1 mg/L loading transformation/dissolution is 0.198mg Cd⁺⁺/L
- at 10µg/L loading, the extrapolated Cd⁺⁺ release is 1.98 µg/L
- at 1µg/L loading, the extrapolated Cd⁺⁺ release is 0.198 µg/L

The chronic ecotoxicity reference value of 0.21 µg/l would thus not be exceeded by adding 1 µg/l Cd powder (extrapolated Cd release: 0.198µg/l. However, this result was obtained at pH 8. At pH 6, the Cd⁺⁺ release will be higher, factor 2.2 maximum. Consequently, the chronic ecotoxicity reference value is expected to be exceeded at 1µg/l loading. At 0.1µg/l loading, the reference value would not be exceeded. Therefore the classification for Cd metal powder is chronic 1, with M-factor 10, H410 (very toxic to aquatic life with long lasting effects).

b) Cadmium metal in massive form

Acute aquatic effect: the acute ecotoxicity reference value is exceeded at 1mg/l loading (see above: the single highest Cd⁺⁺ release value, observed in the 10mg/l loading test, was 0.138mg/l); considering also that these results were obtained at pH 8, the anticipated T/D at pH 6 can be estimated as $0.014 \times 2.2 = 0.031\text{mg/l}$, which exceeds the acute reference value). It can be further calculated from these data that the reference value is reached at about 550µg/l loading (by extrapolation: 1mg/l loading: 0.031mg/l; so, 0.018mg/l is reached at $1 \times 18/31 = 0.55\text{mg/l}$ loading). In conclusion, Cadmium metal in massive form is classified acute 1, with an M-factor of 1, H400 (Very toxic to aquatic life).

Chronic aquatic effect: no tests were performed for longer than 7 days on Cd massive metal. As indicated above, the results obtained after 7 days were not consistent (e. g. no relationship between loading and Cd-release could be measured). However, in the lowest loading series (10mg/l, giving the highest release!), 2 out of 3

replicas were reaching equilibrium at 7 days (ECB 2007). So these data can be extrapolated to 28 days, with reservation: the transformation/dissolution at 28 days is assumed the same as at 7 days.

Considering Cd further as equivalent to “rapidly degradable” (see above), the reference value for chronic ecotoxicity is exceeded at 10 µg/l loading (1.4 µg Cd/l versus 0.21 µg/l), so the substance is classified chronic 1.

M-factor: the chronic ecotoxicity value is still exceeded at 1 µg/l loading (0.14 µg/l x 2.2 (see above) = 0.31 µg/l), but not at 0.1 µg/l loading. In conclusion, cadmium metal in massive form is classified chronic 1, with an M-factor of 10, H410 (very toxic to aquatic life with long lasting effects).

As indicated, the T/D data on Cd metal in massive form were not consistent and unreliable. Therefore, cadmium in massive form is being re-tested, using the more advanced techniques that have in the meantime been developed for massive metal T/D testing. These tests are ongoing; the classification of cadmium metal in massive form will be reconsidered based on these data; in the meantime however, the analysis as above is proposed.

General discussion

A basic assumption made in this hazard assessment and throughout this CSR, (in accordance to the same assumption made in the EU RA process) is that the ecotoxicity of cadmium and cadmium compounds is due to the Cd⁺⁺ ion. As a consequence, all aquatic, sediment and terrestrial toxicity data in this report are expressed as “cadmium”, not as the test compound as such, because ionic cadmium is considered to be the causative factor for toxicity. A further consequence of this is that all ecotoxicity data obtained on different cadmium compounds, are mutually relevant for each other. For that reason, the available ecotoxicity databases related to cadmium and the different cadmium compounds are combined before calculating the PNECs. The only way cadmium compounds can differ in this respect is in their capacity to release cadmium ions into (environmental) solution. That effect is checked eventually in the transformation/dissolution tests and may result in different classifications.

7.6.2. Copper

Table 16. Hazard assessment conclusion for the environment for copper

Compartment	Hazard conclusion	Remarks/Justification
Freshwater	PNEC aqua (freshwater): 7.8 µg/L	Assessment factor: 1 Extrapolation method: statistical extrapolation Copper: as agreed by the Competent Authorities for Biocides and Existing Substance Regulations. See also PNEC derivations in the CSR
Marine water	PNEC aqua (marine water): 5.2 µg/L	Assessment factor: 1 Extrapolation method: assessment factor Copper: in accordance to the discussions with the Competent Authorities for Biocides and Existing Substance Regulations. See also PNEC derivations in the CSR
Intermittent releases to water		Copper: Not applicable
Sediments (freshwater)	PNEC sediment (freshwater): 87 mg/kg sediment dw	Assessment factor: 1 Copper: as agreed by the Competent Authorities for Biocides and Existing Substance Regulations. See also PNEC derivations in the CSR
Sediments (marine water)	PNEC sediment (marine water): 676 mg/kg sediment dw	Assessment factor: 1 Extrapolation method: partition coefficient Copper: in accordance to the discussions with the Competent Authorities

Compartment	Hazard conclusion	Remarks/Justification
		for Existing Substance Regulations. See also PNEC derivations in the CSR
Sewage treatment plant	PNEC STP: 230 µg/L	Assessment factor: 1 Extrapolation method: statistical extrapolation Copper: as agreed by the Competent Authorities for Biocides and Existing Substance Regulations. See also PNEC derivations in the CSR
Soil	PNEC soil: 65 mg/kg soil dw	Assessment factor: 1 Copper: in accordance to the Competent Authorities for Biocides and Existing Substance Regulations. See also PNEC derivations in the CSR
Air		
Secondary poisoning	No potential for bioaccumulation	Copper: as agreed by the Competent Authorities for Biocides and Existing Substance Regulations. Additional justification below

Environmental classification justification

For the environmental classification of copper metal, the reference effects data obtained with soluble copper compounds (summary in attached- Van Sprang, 2010) are compared to the copper releases as observed from transformation/dissolution tests (section 4. environmental fate).

The detailed comparison is summarized in the attached.

Massive copper materials

For massive copper materials, the results, at a surface loading of 0.67 mm²/L (Rodriguez *et al.*, 2012), are retained for the classification of copper massive (particles of 1 mm at loading of 1mg/L corresponds to a surface loading of 0.67 mm²/L):

- 1 µg dissolved Cu/L released after 7 days T/D tests at 1mg/L, 0.67 mm²/L, pH 6 (copper release rate of 1.5 µg Cu/7 days. mm²)
- 3.4 µg dissolved Cu/L released after 28 days T/D test at 1mg/L, 0.67 mm²/L, pH 6 (copper release rate of 5.0 µg Cu/28 days. mm²)

Conclusions on Acute classification for copper massive

- Worst case release rates are observed at pH 6.
- The ratio between the copper release after 7 days T/D at a loading of 1 mg/L at pH 6 (0.001 mg/L) and the acute ERVs (0.025-0.035 mg dissolved Cu/L) is <1 and does not trigger acute classification

Conclusions on Chronic classification for copper massive

- The ratio between the copper release after 28 days T/D at a loading of 1 mg/L and pH 6 (0.0034mg/L) and the acute ERVs (0.007 - 0.020 mg dissolved Cu/L) is <1 and does not trigger chronic classification.

The data therefore demonstrate that there is no need to classify copper in its massive form.

Copper powders

For Copper powders, transformation/dissolution tests were carried and the copper releases are as summarized in "environmental fate and pathways - summary record". These copper releases are compared to the ERVs defined in Van Sprang, 20103.

The results from the transformation/dissolution tests on powders and massives demonstrated a relation between the releases and the surface area exposed and increased releases due to abrasion of the particles to the test vessels. Rodriguez et al, 2001 demonstrated the importance of particle abrasion and developed a method to avoid this artefact. The surface-specific release (0.041 µg Cu/mm² at 47 mm²/L), using the most reliable non-

abrasion device, as determined by Rodriguez et al., 2011 was therefore preferred for the classification of copper powders at pH 6. For the hazard classification, the following powder specific surface loadings are considered : 67.5 mm²/L (calculated for smooth 10µm sphere, 1 mg/l) and 107 mm²/L (BET measurement from Skeaff and Hardy, 2005 and 1 mg/l) to determine the copper releases (details in section 4 - environmental fate properties).

Conclusions on Acute classification for fine copper powders

- The ratio between the non-abrasive copper release after 7 days T/D at a loading of 1 mg/L at pH 6 (0.028-0.044 mg dissolved Cu/L; from Rodriguez et al., 2011) and the acute ERV (0.025 mg dissolved Cu/L) triggers environmental classification as Acute 1, M factor 1.
- The ratio's between the copper release after 7 days T/D (Rodriguez et al., 2007) at a loading of 1 mg/L at pH 7 & 8 (0.013-0.021 and 0.009-0.0014 mg dissolved Cu/L) and the pH specific acute ERVs (0.035 and 0.030 mg dissolved Cu/L) is <1.

The release data at pH 7 and 8 include particle abrasion. The pH 6 data therefore lead to the highest classification outcome and are retained for the final classification proposal.

Chronic classification for fine copper powders

- The ratio between the estimated copper release after 28 days T/D (conservative linear extrapolation from 7 days T/D test to 28 days) at pH 6 of 1 mg/L (0.112-0.176 mg dissolved Cu/l) and the chronic ERV (0.02 mg dissolved Cu/l) at pH 6 is >1. The ratio is <1 at 0.1 mg/L (linear extrapolation between 1 and 0.1 mg/L).

This triggers an environmental classification as Chronic 3 with "removal from the water-column". Without removal from the water column, a chronic 2 classification would be needed. Rapid copper removal is discussed in Rader et al., 2012 and summarized in the "environmental fate and pathways - summary record"

- The ratio between the copper release after 28 days T/D of 1mg/L, at pH of respectively 7 and 8 (0.051-0.084 and 0.035-0.056 mg dissolved Cu/l), and the pH specific chronic ERVs (0.0074 and 0.011 mg dissolved Cu/l) is >1 at a loading of 1 mg/L. Following linear extrapolation between 1 and 0.1 mg/L, the ratio is <1 at 0.1 mg/l at pH 8 data and around 1 for the pH 7 data. Considering that the pH 7 and pH 8 release data include particle abrasion, the classification derived at pH 6 is retained.

Conclusion copper powder

Acute 1 with M-factor 1 AND Chronic 3: Using the surface-specific release from Rodriguez et al, 2011, the powder critical surface area, below which no acute classification is needed is 60 mm²/mg. The powder critical surface area, below which no chronic classification is needed is 12 mm²/mg.

Conclusion copper flakes

For Coated copper flakes, no specific transformation/dissolution tests are available today.

The surface coating of the flakes results in the formation of high particle-specific surface areas (2080-2900 mm²/mg), being much higher than the surface area that would be predicted from the particle size (135 mm²/mg). No transformation/dissolution data are available for the coated copper flakes.

The main constituent is metallic copper. Metallic copper (Cu⁰) is insoluble and needs to be transformed to solubilise. In absence of measured data, copper release from the flakes are estimated from the surface-specific copper release rates (massives and powders) and the flake-specific surface area:

7 days transformation/dissolution

- Using the surface-specific release of 0.41 µg Cu/mm² from the 7 days non-abrasive T/D test set-up, at pH 6 (Rodriguez et al., 2011) and a loading of 2900 mm²/L (flakes) it can be calculated that all copper would be dissolved 1 mg Cu/L (pH 6)
- Using the non-linear release equation from Skeaff and Hardy, 2005, at pH 6, 84% of all copper would be dissolved at 1 mg/L (0.84 mg/L)

28 days releases

- 100% solubility is estimated at pH 6
- The transformation/dissolution of Cu-ions from metallic copper is pH dependent with higher release rates at lower pH (Rodriguez et al., 2007). Consistent with such pH dependent solubilities observed for copper and well as copper compounds (e.g. CuO and Cu₂O), it is reasonable to assume lower, partial solubility at higher pHs. The ratio between the soluble copper release from T/D test (at 7 and 28 days) and the ERV of the dissolved copper ions result in the following classification outcome:

Conclusions on Acute classification

The ratio between the copper release after 7 days T/D at a loading of 1 mg/L at pH 6 (0.84 to 1 mg Cu/L) and the acute ERV (0.025 mg Cu/L) ranges between 34 and 40. This results in an environmental classification as Acute 1, M factor 10.

Conclusions on Chronic classification

Assuming full solubility at pH 6, the chronic ERV of 0.02 mg dissolved Cu/l results in an environmental classification as Chronic 2 with “removal from the water-column”. If full solubility would be assumed at pH 7, the chronic reference values at pH ranges (0.007), could potentially lead to a classification as chronic 1 (ratio of 1.3). Considering however the pH depending release of copper, and solubilities/ classification of CuO and Cu₂O the pH 6 data are considered as worst case and the chronic 2 classification is retained

Conclusion coated copper flake

Acute 1 with M-factor 10 and Chronic 2

See attachments below "Van Sprang_2010" and " Revised classification of copper (massive, powder, flakes)_2013_01 -13"

General discussion

Justification for absence of bioaccumulation potential and no-concern for secondary poisoning is summarized below.

The copper Risk Assessment Report (2008) and REACH Chemical Safety Report (2010) have provided detailed information on (1) the essentiality of copper; (2) the homeostatic control of copper; (3) the mechanisms of action of copper-ions; (4) the comparison between copper toxicity from dietary versus waterborne exposures.

The data clearly demonstrate that:

- Copper is an essential nutrient for all living organisms
- Copper ions are homeostatically controlled in all organisms and the control efficiencies increase with trophic chain. As a consequence,
 - copper BCF/BAF values decrease with increasing exposure concentrations (water and food), vary depending on nutritional needs (seasonal, life stage, species dependent), vary pending on “internal detoxification” mechanisms
 - Copper BMFs values are < 1
- Water-borne exposure (not diet borne exposure) is the exposure route critical to copper toxicity

Essential trace element

Copper is an essential micronutrient, needed for optimal growth and development of micro-organisms, plants, animals and humans. It plays a vital role in the physiology of animals: for foetal growth and early post-natal development, for haemoglobin synthesis, connective tissue maturation especially in the cardiovascular system and in bones, for proper nerve function and bone development, and inflammatory processes. Copper acts as an active cofactor in over 20 enzymes and proteins, notably the respiratory enzymes haemocyanin and cytochrome oxidase and the anti-oxidant superoxide dismutase (WHO, 1998). Copper deficiency has been observed in intensive cultures of fish, crops and farm animals. The most striking examples of copper deficiency come from farming practices. Insufficient bioavailable copper in soils has been shown to reduce agricultural yields and to produce metabolic copper deficiencies in animals. Copper deficiency was first recognised in Europe in the 1930s and its incidence increased with the intensification of arable farming over the last 50 years. Copper deficiency has also been noted in a wide variety of soils world-wide (IPCS 1998). Depending on the organism's

metabolic need, different copper levels are found in tissues from different strains, species and life stages.

Differences among species and strains:

Aquatic invertebrates such as gastropods, some crustacea and bivalves, relying on haemocyanin as respiratory pigment, have typically higher copper levels than invertebrates relying on haemoglobin as respiratory pigment (e. g. Timmermans et al, 1989). In higher organisms (vertebrates), homeostatic control of copper supply is achieved mainly by storage in the liver and biliary secretion (Underwood and Suttle, 1999). Copper is bound to proteins such as ceruloplasmin and metallothionein, functioning as copper storage and mobilised as needed.

Differences within species:

Of all factors that affect the physiology of animals, body size exerts the major effect and provides an integrated value of all physiological processes (Marsden and Rainbow, 2004). In aquatic environments, several investigators demonstrated an inverse relation between copper tissue levels and the length or weight of the organisms (e. g. Timmermans et al, 1989). Different copper needs are of relevance to both agricultural and medical practices: Copper supplements are provided to piglets and pigs to enhance growth. Considering the high needs during the fast growth stages, copper levels given to piglets are much higher than those given to adult pigs. The copper concentration in the liver of a mammalian and human foetus is much higher during the last term of pregnancy than in an adult. This is because of the high copper need during this period, as well as during the first months after birth, and because breast milk contains little copper. Consequently, the milk formulae for premature babies contain higher copper levels than those for newborns.

In summary, as an essential element, all organisms will naturally accumulate copper without deleterious effects. Different levels of accumulation in tissue reflect differences in nutritional needs.

Homeostatic control, uptake and depuration of copper ions

The natural copper levels, available for plants, micro-organisms and animals, living in a specific environment, depend on the natural geological and physico-chemical characteristics of the water, sediments and soils. Homeostatic regulation of copper allows organisms, within certain limits, to maintain the physiologically required levels of copper in their various tissues, both at low and high copper intakes. The molecular mechanism of copper homeostasis is related to 2 key elements: P-type ATPases that can pump copper across biological membranes in either direction or copper chaperones, important for intracellular copper homeostasis (Odermatt et al, 1992). The latter is considered to be universal as the sequences of copper chaperones are highly conserved between species (Wunderli et al. 1999). Besides these active cellular regulation mechanisms, some groups of organisms have developed additional mechanisms (molecular binding to e. g. metallothioneins and sequestration in granules) to prevent copper excess (Borgmann, 1993 and Rainbow, 1980, 1985, 1989; Marsden and Rainbow, 2004). Vertebrate dietary copper exposure studies (fish, mammals, birds and humans) demonstrate additional organ-related homeostasis. Intestinal adsorption/biliary excretion of copper is regulated with varying dietary intakes (WHO, 1998). Due to the homeostatic regulation of copper (and other metals), BCFs and BAFs are not independent of exposure concentration (e. g. Mc Geer et al., 2003). Increased/decreased copper intakes/eliminations, lead to BCFs and BAFs that are inversely related to exposure concentration (i. e. decreasing/BAFs with increasing exposure concentration (water and diet). For copper, this inverse relationship was clearly demonstrated for BCFs, BAFs and biota-sediment accumulation factors (BSAFs) (Adams et al., 2003). The observed inverse relationship has been explained by homeostatic regulations of internal tissue concentrations. At low metal concentrations, organisms are actively accumulating metals in order to meet their metabolic requirements, while at high ambient metal concentrations, organisms are able to excrete excess metals or limit uptake. Additionally, different BCFs for different species, life stages and seasons have been observed, depending on the organism's metabolic need (in e. g. Cu-enzymes). Further complicating the application of BCF and BAF to metals is that many aquatic organisms store metals in detoxified forms, such as in inorganic granules or bound to metallothionein-like proteins. The use of granules is of particular note in the context of BCFs, because high body burdens are often associated with this storage mechanism, but there is a lack of adverse effects. Using BCF and BAF for essential metals and their compounds to assess ecotoxicity therefore ignores fundamental physicochemical and toxicological properties associated with these substances.

Compared with the diffusional uptake of neutral organics, metal uptake is complex. It includes a diversity of mechanisms, accumulation of both essential and non-essential elements from the natural background, homeostatic control of accumulation, as well as internal detoxification, storage and elimination. [1]

Mechanism of action of copper toxicity/deficiency

Freshwater

From the copper risk assessment, it was clearly concluded that the most sensitive uptake route for acute and chronic copper toxicity is directly from the water with free Cu-ions as most potent Cu-species. The key indicator of copper toxicity is disturbance of the sodium homeostasis (e. g. Paquin et al., 2002; De Schamphelaere and Janssen, 2003; Kamunde et al., 2001 & 2005). The key target tissue for copper toxicity is therefore the water/organism interface, with cell wall and gill-like surfaces acting as target biotic ligands in all species investigated. The importance of water-borne exposure was confirmed from the freshwater chronic ecotoxicity database, demonstrating:

- The influence of water chemistry on chronic copper toxicities (influence of DOC, pH... on chronic NOECs)
- The small inter-species variability in observed NOECs (after BLM normalisation) (max/min NOEC ratio of 23 for 27 species),
- Small acute to chronic ratios (typically a factor of 1 to 3)
- Higher sensitivity of smaller compared to larger organisms (Grosel et al., 2007).
- No concern of secondary poisoning from copper mesocosm studies:
- Roussel (2007) reported for a lentic mesocosm study a low sensitivity of the predating fish compared to the invertebrates and algae.
- The freshwater pond mesocosms (Schaefer et al, 2002 and Roussel (2007) and the marine pond mesocosm (Foekema et al 2010) did not show a concern from copper secondary poisoning.

Marine

Freshwater and marine organisms face very different ion- and osmo-regulatory problems related to living in either a very dilute or concentrated salt environment. These differences in ion- and osmo-regulatory physiology may also lead to differences in metal accumulation and metal toxicity (Prosser, 1991; Wright 1995; Rainbow, 2002). Marine organisms are, as freshwater organisms, also exposed via the gills. But in addition, they take in water via the gut exposing an additional series of epithelial structures to the metals (Wang and Fisher 1998; Glover et al 2003; Mouneyrac et al 2003). Both the epithelia of gills and gut are thus important and potentially sensitive targets because they provide a variety of essential physiological functions such as the energy dependent transport of nutrients across the interface and the maintenance of homeostatic balance. Despite these apparent physiological differences, it has been shown that marine fish also suffer from osmo-regulatory disturbances under metal exposure. The importance of waterborne exposure was confirmed from marine ecotoxicity databases, demonstrating:

- The mitigating effect of DOC on the marine NOECs/EC10s.
- The absence of a higher copper sensitivity with increasing trophic chain level.
- For the bivalve *Mytilus edulis*, the short term (48 hrs) early life stage NOEC was similar to the 10 days growth inhibition NOEC. The 83 days marine mesocosm study (Foekema et al., 2010), furthermore showed that the safe level in the mesocosm could be predicted from the single-species SSD and DOC correction, developed for water-only exposure.

The interaction between free copper-ions and “gill-like structures” induce osmo-regulatory stress. Osmo-regulatory disturbance from waterborne exposure is recognised as the primary symptom of copper toxicity to aquatic organisms.

Copper toxicity from dietary versus waterborne exposures

Invertebrates:

A few key studies are available:

- De Schamphelaere and Janssen (2003) demonstrated the influence of water characteristics on the chronic toxicity of *D. magna* and showed that, for *D. magna*, waterborne copper and not dietary copper uptake is responsible for copper toxicity (De Schamphelaere and Janssen, 2004).
- Similarly, Allinson (2002) investigated the bioaccumulation of copper through a simple food chain (*Lemna minor* – *C. destructor*) and observed regulation of copper by the crayfish, *C. destructor*, with the gills being the main site for absorption and depuration of copper to and from the water column. *C. destructor* does not appear to be sensitive to dietary copper.

Fish:

- Kamunde, 2001 observed that dietary copper pre-exposure decreased the uptake of copper across the gills, providing further evidence of homeostatic interaction between the two routes of uptake. Rainbow trout regulated dietary copper at the level of the gut by increasing clearance to other tissues, at the liver by increasing biliary copper excretion, and at the gill by reducing waterborne copper uptake in response to dietary exposure. The modest morphological changes in the intestinal tract suggested high cell and organelle turnover and local regulation of copper. In spite of possible increased energy demand for regulation and tissue repair, there was no significant growth inhibitory effect following dietary exposure.
- Blust et al., 2007, reviewed the literature on copper toxicity after dietary copper exposures of fish and compared waterborne versus diet borne toxicity of copper to fish. After detailed evaluation of the Clearwater et al., 2002 review paper, Blust derived critical diet borne toxicity effects value for Atlantic salmon and Rainbow trout of respectively 15.5 and 44 mg Cu/kg fresh weight/day. Blust et al, 2007 further assessed if the waterborne exposures, PNEC of 7.8 µg Cu/l, as derived in the copper risk assessment, would result in a dietary copper dose or copper food concentration exceeding a critical level. The concentrations in food were calculated from the regressions presented in McGeer et al. (2003) which allow the estimation of the whole body copper concentration, for different types of aquatic organisms, as a function of the copper waterborne concentration. The results of the simulations show that aquatic invertebrates exposed to 8 µg Cu/l waterborne copper reach mean Cu body levels of 53-84 mg Cu/kg dry weight (depending on the diet). The resulting daily uptake by fish at 7.8 µg Cu/L was < 4.20 mg Cu/kg fresh weight/day. These results lead to the conclusion that the copper concentrations in food items and daily dietary copper dose in fish are unlikely to cause negative effects at the threshold waterborne copper concentration of 7.8 µg/l.

Comparison of the dietary copper levels and “normal” background Cu levels in live food items

Cu is a naturally occurring element and is essential to all living organisms. Naturally, a background Cu burden is present in all organisms to fulfil their biochemical requirements. Table 1 presents a summary of a few ‘background’ Cu concentrations in freshwater biota that may serve as food items for fish.

Table 17. Background Cu burdens of selected freshwater biota that may be considered food items for fish. Species

Species	Cu _{H₂O} (µg/L)	Cu _{food} (mg/kg dry wt)	Reference
<i>Daphnia magna</i> (adults)	1	10.2 - 22.1	Bossuyt et al. (2005a, b) a
<i>Daphnia magna</i> (juveniles)	1	20 - 120	Bossuyt et al. (2005a, b) a
<i>Hyalella azteca</i>	3.5	79	Borgmann et al. (1993)

^aFifth or 6th generation daphnids taken from a multi-generation exposure; 1 µg/L was sufficient to avoid deficiency

Bossuyt et al. (2005a, b) reported background body burdens of 40d-old *D. magna* at 1 µg Cu/L between 10.2 and 22.1 mg Cu · kg⁻¹ dry wt. Juvenile daphnids of up to 2 days old seemed to have higher copper body burdens between 20 and 120 mg Cu · kg⁻¹ dry wt.

Borgmann et al. (1993) report a Cu burden in *Hyalella azteca* of 79 mg Cu · kg⁻¹ dry wt in organisms exposed to control conditions, i.e. 3.5 µg Cu/L.

The background copper burdens (10 - 120 mg Cu/kg dry weight) as determined above, furthermore encompass the simulated Cu body levels of 53 - 84 mg Cu/kg dry weight calculated for aquatic invertebrates exposed to 8 µg Cu/l waterborne copper and therefore provide additional evidence that the Cu concentrations in food items and daily dietary Cu dose in fish are unlikely to cause negative effects at the threshold waterborne Cu concentration of 8 µg/L.

Waterborne Cu is therefore recognised as the critical copper exposure route for invertebrates and fish.

Critical papers of relevance to bio-magnification

The absence of copper bio-magnification, with consistent BMFs < 1, was shown from several papers:

- Barwick and Maher (2003), compared trace metal levels in a contaminated seagrass ecosystem in Lake Macquarie, the largest estuary in New South Wales (Australia). The structure of the estuarine food web was studied in detail and all organisms (algae, invertebrates, fish) were categorised as autotrophs, herbivores,

planktivores, detritivores, omnivores and carnivores. The results of the analysis showed the absence of copper bio-magnification in this estuarine system. Copper concentrations ranged between 0.27 µg Cu/g dw (Omnivore: *Monacanthus* and 88 µg Cu/g dw (Herbivore: *Bembicium auratum* (gastropod with haemocyanin)). The higher levels (e. g. *B. auratum*) were associated with species with active accumulation of copper into the respiratory pigment haemocyanin.

- Farag et al., 1998, studied copper concentration in benthic invertebrates that represent various functional groups and sizes from de Coeur d'Alene river, influenced by mining activities. The copper concentrations noted across the trophic chain, demonstrated the absence of bio-magnification from the sediment to herbivores, omnivores, detritivores and carnivores.
- Wang (2002) noted the bio-diminution of metals in the classical marine planktonic food chain (phytoplankton to copepods to fish) and explained the phenomenon as the result of the effective efflux of metals by copepods and the low assimilation of metals by marine fish.
- Quinn et al., 2003, evaluated trophic chain transfer of metals in insects (35 species) from a stream food web influenced by acid mine drainage with copper levels up to 100 µg Cu/L. They demonstrated that metal concentrations were higher in water and insects closer to the mining sites and taxa richness increased with distance away from the site. The relation between the trophic positions, determined from $\delta^{15}N$ radio isotope determination, indicated that the trophic chain had no effect on copper levels in the insects.

Copper is therefore not bio-magnified across the trophic chain.

Conclusion

There is a substantial amount of information available on copper. The data clearly demonstrate that:

- Copper is an essential nutrient for all living organisms
- Copper ions are homeostatically controlled in all organisms and the control efficiencies increase with trophic chain. As a consequence, copper BCF/BAF values decrease with increasing exposure concentrations (water and food), vary depending on the nutritional needs (seasonal, life stage, species dependent), vary depending on "internal detoxification" mechanisms
- Copper BMFs values are < 1
- Copper waterborne exposure (and not diet borne exposure) is the exposure route critical to copper toxicity [1]Mc Geer et al, 2010. From: <http://www.icmm.com/page/1321/environmental-fact-sheet-8-the-use-of-bioaccumulation-criteria-for-hazard-identification-of-metals>

See CSR Annex 4 - PNEC Summary Report

7.6.3 Diarsenic trioxide

Table 18. Hazard assessment conclusion for the environment for arsenic

Compartment	Hazard conclusion	Remarks/Justification
Freshwater	PNEC aqua (freshwater): 17.1 µg/L	Assessment factor: 50 Extrapolation method: assessment factor Disarsenic trioxide: Reliable long-term toxicity data are available for 4 freshwater organisms covering two trophic levels (primary consumers and secondary consumers): aquatic invertebrates (<i>Daphnia magna</i>), fish (<i>Jordanella floridae</i> , <i>Pimephales promelas</i>) and amphibian (<i>Rana pipiens</i>). Chronic NOEC or EC10-values based on dissolved arsenic concentration range from 0.633 mg As/L to 2.13 mg As/L. The lowest chronic NOEC value of 0.633 mg As/L was observed in a reproduction test of <i>Daphnia magna</i> (Lima et al, 1984). According to REACH Guidance on information requirements and chemical safety assessment (Table R.10-4), an assessment factor of 50 should be applied to the lowest endpoint of long-term toxicity data representing two trophic levels. This results in a freshwater PNEC _{add} of 0.013 mg As/L (corresponding to 0.0171 mg As ₂ O ₃ / L).
Marine water	PNEC aqua	Assessment factor: 50

Compartment	Hazard conclusion	Remarks/Justification
	(marine water): 1.2 µg/L	Extrapolation method: assessment factor Disarsenic trioxide: In addition to long-term toxicity data to freshwater organisms covering two trophic levels, reliable long-term toxicity data are available for the effect of arsenic on survival of the marine invertebrate <i>Americamysis bahia</i> and growth and reproduction of two marine macroalgae <i>Champia parvula</i> and <i>Macrocyctis pyrifera</i>, respectively. The NOEC values based on dissolved arsenic range from 0.04 mg/L to 0.631 mg As/L with the lowest value observed for the 42-h reproduction test of <i>Macrocyctis pyrifera</i>. According to the REACH Guidance on information requirements and chemical safety assessment (Table R.10-5), an assessment factor of 50 should be applied to the lowest two long-term results from freshwater or saltwater species representing two trophic levels and one long-term result from an additional marine taxonomic group. The lowest chronic NOEC reported for aquatic organisms (freshwater + marine water) is a NOEC of 0.04 mg/L for reproduction-related endpoints of <i>Macrocyctis pyrifera</i>, resulting in a marine water PNECadd of 0.9 µg As/L (corresponding to 1.2 µg As2O3 / L).
Intermittent releases to water	PNEC aqua (intermittent releases): 1.2 µg/L	Assessment factor: 100 Extrapolation method: assessment factor Disarsenic trioxide: Reliable short-term toxicity data are available for 7 freshwater organisms, representing the three trophic levels: aquatic invertebrates (<i>Bosmina longirostris</i>, <i>Ceriodaphnia dubia</i>, <i>Gammarus pseudolimnaeus</i>, <i>Hyalella azteca</i>), fish (<i>Oreochromis mossambicus</i>, <i>Pimephales promelas</i>) and aquatic plants (<i>Lemna minor</i>). Additionally, short-term toxicity data are available for marine fish (<i>Oreochromis mossambicus</i>), the marine crustacean (<i>Americamysis bahia</i>) and the marine macroalgae <i>Macrocyctis pyrifera</i>. Acute L(E)C50 values were based on dissolved arsenic concentrations and range from 0.08 mg As/L to 15.98 mg As/L. The lowest L(E)C50 value was observed in a 42-h test of the marine macroalga (<i>Macrocyctis pyrifera</i>) (Garman et al, 1994). According to REACH Guidance on information requirements and chemical safety assessment (Chapter R.10.3.3), the PNECwater, intermittent is derived by application of an assessment factor of 100 to the lowest L(E)C50 of at least 3 short-term tests from 3 trophic levels. This results in a PNECwater, intermittent (add) of 0.9 µg As/L (corresponding to 1.2 µg As2O3 / L). It has to be noted that this PNECwater, intermittent is based on saltwater data, is lower than the PNEC for freshwater and is therefore over-protective and not used in the chemical safety assessment.
Sediments (freshwater)	PNEC sediment (freshwater): 171.1 mg/kg sediment dw	Extrapolation method: partition coefficient Disarsenic trioxide: Reliable studies (Klimisch 1 or 2) on toxicity of arsenic substances to sediment organisms are not available. According to ECHA Guidance on information requirements and chemical safety assessment (Chapter R.10.5.2), in the absence of ecotoxicological data for sediment-dwelling organisms, the PNEC for freshwater sediment can be derived from the PNECfreshwater and the partitioning coefficient (Kp) for solids-water in suspended matter using the equilibrium partitioning method: $PNEC_{freshwater\ sediment} (mg\ As/kg\ dw) = PNEC_{freshwater} (0.013\ mg\ As/L) * Kp\ suspended\ matter (L/kg\ dw)$. A log Kp of 4.0 was selected for suspended matter in freshwater and marine systems based on the 50th percentile of log Kp values for 25 water systems (log Kp values range from 2.0 to 6.0). This results in a PNECfreshwater sediment (add) of 130 mg As/kg dw (corresponding to 171.1 mg As2O3 / L).. This PNEC for freshwater sediment is protective as it is well below toxic As concentrations in laboratory-spiked freshwater sediments. In a laboratory study (Visviki et al, 2006; Klimisch 4), the LC 50 for 10-d survival

Compartment	Hazard conclusion	Remarks/Justification
		of <i>L. plumulosus</i> in spiked natural sediments is 800 µg As/L. Applying a log Kp value (solids-water in suspended matter) of 4.0, this LC50 corresponds to 8000 mg As /kg dw sediment.
Sediments (marine water)	PNEC sediment (marine water): 12 mg/kg sediment dw	Extrapolation method: partition coefficient Disarsenic trioxide: Reliable studies (Klimisch 1 or 2) on toxicity of arsenic substances to sediment organisms are not available. According to ECHA Guidance on information requirements and chemical safety assessment (Chapter R.10.5.2), in the absence of ecotoxicological data for sediment-dwelling organisms, the PNEC for marine sediment can be derived from the PNECmarine and the partitioning coefficient (Kp) for solids-water in suspended matter using the equilibrium partitioning method: PNECmarine sediment (mg As/kg dw) = PNECmarine (0.9 µg As/L) * Kp suspended matter (L/kg dw) A log Kp of 4.0 was selected for suspended matter in freshwater and marine systems based on the 50th percentile of log Kp values for 25 water systems (log Kp values range from 2.0 to 6.0). This results in a PNECmarine sediment of 9 mg As/kg dw (corresponding to 12 g As2O3 / L).
Sewage treatment plant	PNEC STP: 80.3 µg/L	Assessment factor: 100 Extrapolation method: assessment factor Disarsenic trioxide: An activated sludge respiration inhibition test resulted in an EC50 value of 6.1 mg As/L. No further data are available to assess the toxicity of arsenic substances to microorganisms. According to the ECHA Guidance on information requirements and chemical safety assessment (Table R.10-6), an assessment factor of 100 should be applied to an EC50 of a sludge respiration test. Thus, the resulting PNEC value (add) for microorganisms in STPs is 0.061 mg As/L (corresponding to 0.0803 mg As2O3/L).
Soil	PNEC soil: 0.7 mg/kg soil dw	Assessment factor: 10 Extrapolation method: assessment factor Disarsenic trioxide: Studies are available on the long-term effect of arsenic to terrestrial organisms covering three trophic levels: microbes, plants and invertebrates. Chronic NOEC/EC10 values are based on added arsenic concentrations. Soil invertebrates appear to be most sensitive, and the lowest reported NOEC value of 5 mg/kg dw (added As) for the reproduction of <i>Folsomia candida</i> (Lock and Janssen, 2002) is considered for the chemicals safety assessment. According to ECHA Guidance on information requirements and chemical safety assessment (Table R.10-6), an assessment factor of 10 should be applied to NOECs for additional long-term toxicity tests for three species of three trophic levels, resulting in a PNECsoil (add) of 0.5 mg As/ g dw soil (corresponding to 0.7 mg As2O3 / kg dw soil).
Air		
Secondary poisoning	PNEC oral: 1.31 mg/kg food	Assessment factor: 30 Disarsenic trioxide: Reliable studies are available regarding the avian toxicity of arsenic covering survival, body weight or reproduction of the two species <i>Gallus gallus domesticus</i> and <i>Anas platyrhynchos</i>. Based on a NOEC of 30 mg As/kg diet, the NOAELs vary between 1.49 and 2.24 mg As/kg bw/d, respectively. According to ECHA Guidance on information requirements and chemical safety assessment (Table R.10-13), an assessment factor (AF) of 30 should to be applied to the lowest chronic NOEC for birds, resulting in a PNECoral of 1 mg As/kg diet. The lowest NOAEL for mammals (1.04 mg As/kg diet) was observed in a 183-days study with female beagle dogs exposed to arsenic via food (Neiger and Osweiler, 1989). Applying a conversion factor

Compartment	Hazard conclusion	Remarks/Justification
		of 40 (g bw/g diet per day), this NOAEL corresponds to a NOEC of 41.6 mg As/kg diet. According to the ECHA Guidance on information requirements and chemical safety assessment (Table R.10-13), an assessment factor of 30 should be applied to a chronic NOEC value for mammals, resulting in a PNEC _{oral} of 1.39 mg As/kg diet. If data are available for birds and mammals, the lower of the resulting PNECs should be used in the risk assessment. Therefore, the PNEC for birds of 1 mg As/kg diet (corresponding to 1.31 mg As ₂ O ₃ / kg diet) was selected for the secondary poisoning assessment.

Environmental classification justification

Acute and chronic reference values for environmental classification are based on standard test as laid down in Council Regulation (EC) No 440/2008 on “Test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)”.

Acute toxicity data of arsenic are available for three trophic levels. The lowest L(E) C50 for freshwater fish, crustacean or algae/plant growth is a 7-d LC50 of 0.43 mg As/L (based on dissolved As concentration), observed in a test with *Hyalella azteca* (Borgman et al, 2004). The lowest L(E) C50 for marine fish, crustacean or algae/plant growth is a 42-h EC50 in the range of 0.09 - 0.11 mg As/L for standardized reproduction-related endpoints, i. e. tube growth, of the macro-alga *Macrocystis pyrifera* based on a graphical display of test data by Garman et al, 1994. However, as the later value has not been derived using appropriate statistical tools and as this EC50 value may be above the cutoff value of 0.1 mg/L, this endpoint is equivocal for the purpose of classification and labelling according to Regulation (EC) No 1272/2008 and subsequent adaptations. Thus, considering also the 7-d LC50 of 0.43 mg As/L observed in a test with *Hyalella azteca* (Borgman et al, 2004), it is assumed that acute LC/EC50 values are above 0.1 mg As/L.

Chronic toxicity data of arsenic are also available for three trophic levels. The lowest chronic NOEC/EC10 value for freshwater fish, invertebrates or algae is a 28-d NOEC of 0.633 mg As/L (based on dissolved As concentration) observed in a reproduction test with *Daphnia magna* (Lima et al, 1984). The lowest chronic NOEC/EC10 value for marine fish, crustacean or algae/plant growth, i. e. a 42-h NOEC value of 0.04 mg As/L, is reported for germ tube growth of a macro-alga (Brown alga, *Macrocystis pyrifera*), a standardised effect in this assay, by Garman et al. (1994). This value is taken forward as the critical threshold concentration.

Acute and chronic reference values of 0.43 mg As/L and 0.04 mg As/L are based on dissolved elemental As concentrations. Because arsenic is an inorganic element, there is no potential for degradation and there is not sufficient evidence for removal from the water column. For the classification of diarsenic trioxide, the acute and chronic reference values based on mg As/L must be corrected for the molecular weight of diarsenic trioxide (75.7% As) resulting in reference values of 0.57 mg As₂O₃/L and 0.05 mg As₂O₃/L, respectively. Based on these reference values, the classification for diarsenic trioxide is “Aquatic Acute Category 1” and “Aquatic Chronic Category 1” (acute reference value ≤ 1 mg/L and chronic reference value ≤ 0.1 mg/L). The resulting acute M-Factor is 1 as the L(E) C50 is in the range 0.1 – 1 mg/L. The resulting chronic M-Factor is also 1 since the NOEC value is in the range 0.01 – 0.1 mg/L and As₂O₃ is non-rapidly degradable.

General discussion

Read across approach

A read-across approach based on data available for inorganic arsenic compounds is applied for the ecotoxicity assessment of diarsenic trioxide. This grouping of arsenic substances for estimating the aquatic hazard properties is based on the assumption that these properties are similar as the specific environmental conditions predominantly affect speciation and toxicity of arsenic substances and not the inorganic arsenic source. For most of the metal-containing substances, it is the metal ion that becomes available upon contact with and dissolution in water and that is predominantly of ecotoxicological concern. This assumption holds when i) differences in solubility of different As compounds do not affect the ecotoxicity, ii) there are not any important differences in the speciation of inorganic arsenic substances in the environment.

Arsenic is present naturally in the aquatic and terrestrial environments from weathering and erosion of rock and

soil. Because of its reactivity and mobility, however, arsenic can cycle extensively through both biotic and abiotic components of local aquatic and terrestrial systems, where it can undergo a variety of chemical and biochemical transformations. Three major modes of biotransformation of arsenic species have been found to occur in the environment: redox transformation between arsenite and arsenate, the reduction and methylation of arsenic, and the biosynthesis of organoarsenic compounds.

When diarsenic trioxide is deposited directly into aerobic surface waters, it forms As(III) species, i.e. arsenite. Arsenite is thermodynamically unstable, and therefore tends to oxidize to dissolved As(V) species, i.e. arsenates. This oxidation can be accelerated by oxidizing agents such as manganese and iron oxyhydroxides which are fairly abundant in natural environments or by the action of certain bacteria. Some As(III) and As(V) species can interchange oxidation states depending on Eh, pH and biological processes. The ratio between oxidized and reduced species appears to be significantly influenced by the presence of iron and manganese oxides. However, the predominant arsenic species in oxidizing environments is the thermodynamically stable form, i. e. arsenate. Arsenite is present in amounts exceeding those of arsenate only in reduced, oxygen-free micro- and macro-environments.

Information about the redox speciation of arsenic compounds during the various tests was not available, but the reliable data for the various endpoints do not appear to differ significantly between the different arsenic substances tested. Thus, all reliable ecotoxicity data of arsenic were considered based on measured dissolved arsenic concentrations or the fact that the respective As substance is soluble. For the ecotoxicity assessment of metals in different environmental compartments (aquatic, soil and sediment), it is typically assumed that the toxicity is not controlled by the total concentration of a metal, but rather by the bioavailable form in the respective medium. Regarding metals, this bioavailable form is typically accepted to be the free metal-ion or the oxy-anion in solution. In the absence of speciation data and as conservative assessment, it was assumed that i) all dissolved arsenic is bioavailable when dissolved concentrations are provided, and that ii) in the absence of information about dissolved levels, all of the applied arsenic is dissolved and bioavailable.

Reliable ecotoxicity results selected for read-across from different arsenic substances are based on tri- and pentavalent As substances (diarsenic trioxide, diarsenic pentaoxide, sodium dioxoarsenate, monosodium arsenate, aluminium arsenate, calcium arsenate, disodium hydrogenarsenate, potassium dihydrogenarsenate). Respective counter-ions (Al^{3+} , Ca^{2+} , K^{+} and Na^{+}) are abundant in natural environments and are not expected to cause any toxic effect at the concentration tested. Thus, the hazard assessment based on dissolved arsenic levels is considered conservative.

7.6.4 Lead

Table 19. Hazard assessment conclusion for the environment for lead

Compartment	Hazard conclusion	Remarks/Justification
Freshwater	PNEC aqua (freshwater): 6.5 µg/L	Assessment factor: 3 Extrapolation method: statistical extrapolation
Marine water	PNEC aqua (marine water): 3.4 µg/L	Assessment factor: 3 Extrapolation method: statistical extrapolation
Intermittent releases to water		Lead: There is no indication that the environmental exposure is not constant (i.e., not the result of a frequent release). Derivation of an intermittent PNEC is only required when discharges are limited in time (e.g., emissions from batch productions)
Sediments (freshwater)	PNEC sediment (freshwater): 174 mg/kg sediment dw	Assessment factor: 3 Extrapolation method: statistical extrapolation
Sediments (marine water)	PNEC sediment (marine water): 164 mg/kg sediment dw	Assessment factor: 3 Extrapolation method: statistical extrapolation

Compartment	Hazard conclusion	Remarks/Justification
Sewage treatment plant	PNEC STP: 100 µg/L	Assessment factor: 10 Extrapolation method: assessment factor
Soil	PNEC soil: 147 mg/kg soil dw	Assessment factor: 2 Extrapolation method: statistical extrapolation
Air		
Secondary poisoning	PNEC oral: 10.9 mg/kg food	Assessment factor: 6

Environmental classification justification

Massive Lead - Environment

Transformation Dissolution data are available for two forms of massive Lead metal corresponding with aged and non-aged material. The metal can for the environmental endpoint be clearly differently classified from the Lead powder form given that all CLP criteria to allow a distinctive classification are fulfilled:

- 1). both are produced in a separate way
- 2). both have different dissolution kinetics and equilibrium
- 3). both result in different environmental classification categories

The solubility levels in the Transformation Dissolution for the aged and the non aged sample are comparable and low:

- 7d TDP at pH 6 corresponds respectively with 567 or 428 µg/l at 100mg/L dosing and 56,7 or 43,2 µg/L at 10 mg/L while the ATR is 73,6 µg/L at pH6, and
- 15 or 14,2 µg/L for the 1 mg/L dosing at 28 days while the CTR is 17,2 µg/L at pH 6.

°extrapolated value

The TICKET UWM model was successfully applied to demonstrate rapid degradation equivalent (rapid elimination from the water column) for the soluble Pb ion. Indeed 70 % elimination from the water column is already reached in 2 hours while also the binding to sediments is assured (no net release) being it slower than the elimination rate in water. The REACH Registration file further concludes no bioaccumulation levels that could lead to a concern for classification.

All this evidence for Pb massive metals leads to the following conclusions:

Under CLP: No environmental classification for both forms

In line with annex 4 chapter IV.5.3 of the CLP, Metals must be classified by comparing Transformation Dissolution data with toxicity data for the soluble metal ion. The availability of toxicity information on the soluble ion (developed under the Lead metal registration file) makes the requirement for aquatic ecotoxicity tests on Lead metal redundant but also irrelevant since requiring nominal testing, which is not allowed for metals.

Lead powder - environment

24h TDP screening information in accordance with the CLP guidance is available for Lead metal powder. Lead metal powder with an aerodynamic diameter of 75 µm fails the 24h screening test because of the high solubility (> 3000 µg/L) compared to the acute toxicity reference value of 73,6 µg/l) and should therefore be classified as the soluble Pb ion being:

- Under DSD: R50/53, with an M factor of 10
- Under CLP: Acute 1 – Chronic 1, with an M factor of 10

- Under GHS 3rd rev: Acute 1 – Chronic 1, with an acute M factor of 10 and a chronic M factor (tbd)

This environmental classification recommendation holds for mixtures composed of > 25 % of Lead powder

In line with annex 4 chapter IV.5.3 of the CLP, Metals must be classified by comparing Transformation Dissolution data with toxicity data for the soluble metal ion. The availability of toxicity information on the soluble ion (developed under the Lead metal registration file) makes the requirement for aquatic ecotoxicity tests on Lead metal redundant but also irrelevant since requiring nominal testing, which is not allowed for metals.

Lead with arsenic – environment

See above for the justifications for no classification of massive lead metal. The classification of the arsenic containing grade of massive lead metal has been derived by applying the CLP rules for classifying mixtures.

General discussion

In assessing the ecotoxicity of metals in the various environmental compartments (aquatic, terrestrial and sediment), it is assumed that toxicity is not controlled by the total concentration of a metal, but by the bioavailable form. For metals, this bioavailable form is generally accepted to be the free metal-ion in solution. In the absence of speciation data and as a conservative approximation, it can also be assumed that the total soluble lead pool is bioavailable. All reliable data on ecotoxicity and environmental fate and behaviour of lead and lead substances were therefore selected based on soluble Pb salts or measured (dissolved) Pb concentration.

7.6.5 Nickel

Table 20. Hazard assessment conclusion for the environment for nickel

Compartment	Hazard conclusion	Remarks/Justification
Freshwater	PNEC Freshwater: 3.6 µg dissolved Ni/L	Nickel: PNEC Freshwater: 3.6 µg dissolved Ni/L Ni bioavailability and toxicity to aquatic organisms is influenced by abiotic parameters such as pH, hardness, and dissolved organic carbon (DOC), the distribution of which varies considerably among EU surface waters. PNEC values can also vary considerably from site to site and from region to region, which raises the need to develop a bioavailability normalization process. Briefly, the bioavailability normalization process normalizes the ecotoxicity data to sets of standard physicochemical conditions for important abiotic factors (i.e., pH, hardness, and dissolved organic carbon (DOC)). This approach allows for the comparison of intrinsic toxicity among organisms on an equal basis. For the aquatic compartment, Biotic Ligand Models (BLMs) were used to normalize the aquatic toxicity database. BLMs were developed and validated for two invertebrates (<i>Ceriodaphnia dubia</i> and <i>Daphnia magna</i>), an alga (<i>Pseudokirchneriella subcapitata</i>), and a fish species (<i>Oncorhynchus mykiss</i>). The BLMs should only be used to normalize toxicity data for which the relevant abiotic parameters (pH and hardness) fall within the boundaries of these parameters that were used to develop and validate the models. In general, the BLMs cover between 10 and 90% of the pH, hardness, and DOC observed in EU surface waters (Table 1). Table 1: Ranges of pH and hardness used for data selection Test organism pH range Hardness range (mg/l CaCO ₃) Algae – <i>P. subcapitata</i> 5.7-8.2 (20-480) Higher plants – <i>H. vulgare</i> 4.1-7.5 NA Invertebrates – <i>D. magna</i> 5.9-8.2 6-320 Invertebrates – <i>C. dubia</i> 6.5-8.2 6-320 Fish – <i>O. mykiss</i> 5.4-8.5 20-310 Discussion continued in attached document...
Marine water	PNEC marine water: 8.6 µg dissolved Ni/L	Nickel: PNEC marine water: 8.6 µg dissolved Ni/L Bioavailability: No incorporation of potential modifying effect of abiotic factors was included in this analysis. Most of the factors known to affect Ni bioavailability and toxicity in freshwater, e.g., Ca ²⁺ , Mg ²⁺ , and H ⁺ , are relatively constant in marine waters, and therefore the utility of bioavailability correction is limited. DOC can vary considerably in coastal marine waters, and has been shown to

Compartment	Hazard conclusion	Remarks/Justification
		be an important factor controlling the toxicity of nickel in the freshwater environment. However, there is no information on the effect of DOC on the toxicity of Ni to marine organisms. HC5 and PNEC derivation: A range of statistical options was explored for determining the HC5(50%) from the marine ecotoxicity data using the species sensitivity distribution (SSD) approach. Alternatives to the preferred approach of using the lognormal distribution were required because this distribution was rejected based on Goodness of Fit tests. The alternative approaches included 1) alternative non-rejected parametric frequency distributions (all data points) 2) log-normal distribution using a reduced data base (with exclusion of fish and dinoflagellate data based on a mechanistically based hypothesis); and, 3) a non-parametric approach called “flexible kernel density estimation” (all data points). It was concluded that each of the above approaches had advantages and disadvantages (pros and cons) and that no approach could be said to be superior. A weight-of-evidence approach was used to evaluate the options, and it was agreed to take the mean from the most cautious approach of option 1 (i.e. the arithmetic mean HC5(50%) value of 19.9 µg/L for all statistically valid parametric distributions) and the outcome of option 3 (i.e. the HC5(50%) of 14.5 µg/L of the Kernel Density Estimation approach with optimal band width). Therefore an HC5(50%) value of 17.2 µg/L was used for the PNECmarine... Discussion continued in attached document
Intermittent releases to water		Nickel: Data Waived. The PNEC intermittent releases endpoint is based on emissions from batch productions when environmental exposure is limited in time (ECHA Guidance R.10.3.3). All processes covered are continuous processes and therefore, the PNEC intermittent releases endpoint is not relevant.
Sediments (freshwater)		Nickel: PNEC sediment: Pending outcome of sediment testing program. See section 6.2 and attached document
Sediments (marine water)		Nickel: PNEC sediment: Pending outcome of sediment testing program. See section 6.2 and attached document
Sewage treatment plant	PNEC STP: 0.33 mg Ni/L	Nickel: PNEC STP: 0.33 mg Ni/L The relevant data for aquatic micro-organisms are insufficient to perform statistical extrapolation (i.e., Species Sensitivity Distribution). From the extracted test results, two types of tests were considered relevant for deriving the PNEC for micro-organisms in STPs: (1) tests with a mixed inoculum (e.g. activated sludge) for the endpoint respiration (oxygen uptake rate, OUR), and (2) a test with ciliated protozoa (preferably Tetrahymena) for the endpoint inhibition of the exponential growth rate. The key publications that were selected for Ni-PNECSTP-derivation are Cokgor et al (2007) and Bogaerts et al (1998). According to the TGD (EC, 2003) the PNECmicro-organisms should be derived as follows: - thePNECmicro-organisms is set equal to a NOEC from a test performed with ‘specific bacterial populations’ like nitrifying bacteria or P. putidaor from a (growth) inhibition test performed with ciliated protozoa. An EC50 from such a test is divided by an assessment factor of 10, - A NOEC from other test systems like the respiration inhibition test is divided by a factor of 10 (EC50 by a factor of 100), - The lowest value is selected as the PNECmicro-organisms. The lowest reliable observed EC50-value for respiration (inhibition of oxygen uptake rate) using activated sludge was 33 mg Ni/L reported by Cokgor et al (2007). No indication is given whether this value represents total or dissolved Ni-concentrations. Based on the guidance given in the TGD, an assessment factor of 100 should be used. First, a factor of 10 is applied because respiration is the endpoint. An additional factor of 10 is applied because the effect level is an EC50 instead of an EC10. These two factors of 10 are multiplied to yield an overall 100-fold assessment factor, which when applied to the EC50of 33 mg Ni/L yields a PNECmicro-organism of 0.33 mg Ni/L. Discussion continued in attached document

Compartment	Hazard conclusion	Remarks/Justification
Soil	PNEC soil: 29.9 mg Ni/kg	Nickel: Derivation of soil (terrestrial) PNEC: PNEC soil: 29.9 mg Ni/Kg As for the aquatic compartment, bioavailability models were used to normalize ecotoxicity data to sets of standard physicochemical conditions. For the soil compartment, relationships between cation exchange capacity (CEC) and chronic nickel toxicity were used to normalize the ecotoxicity data. Appropriate use of normalization of chronic toxicity to soil organisms necessitates that the CEC boundaries of the experimentally derived relationships are defined relative to the environmental conditions considered. Hence data from studies that passed the reliability and relevance criteria in general, but that were conducted on soils that fell outside of this CEC range were not maintained for PNEC derivation. Data from studies that did not report CEC or additional information enabling an estimation of CEC were rejected. Bioavailability correction: As clear toxicity differences between various soils were observed, bioavailability correction models (total) were established for 2 plant and 2 invertebrate species, and for 3 microbial processes. The toxicity data from the tested soils showed that among the parameters tested, i.e. CEC, pH, organic material (OM), Clay and Ni background, the CEC alone consistently explained a large fraction of variability for the 7 species and endpoints tested in the 16 soils ($r^2 = 0.60$ to 0.92). The CEC range is from 1.8 to 52.8 cmol/kg, which covers most soils. Accordingly, the intra-species variability in chronic toxicity to nickel may to a large extent be due to differences in soil chemistry. Hence, to reduce soil-type related impact on the determination of an HC5 (and subsequently the PNEC) for a given soil, all data in the nickel soil ecotoxicity database were normalized to a set of standard soil properties, using the established CEC based models. Although models were established for 7 species, more species were present... Discussion continued in attached document
Air		
Secondary poisoning	Aquatic PNECoral: Harbour seal: 4.6 mg/kg, Oystercatcher: 12.3 mg/kg, Bivalve-eating bird: 12.3 mg/kg, European otter: 2.3 mg/kg, Terrestrial PNECoral: Worm eating bird: 8.5 mg/kg, Shrew: 0.12 mg/kg.	Nickel: Aquatic PNECoral: Harbour seal: 4.6 mg/kg, Oystercatcher: 12.3 mg/kg, Bivalve-eating bird: 12.3 mg/kg, European otter: 2.3 mg/kg, Terrestrial PNECoral: Worm eating bird: 8.5 mg/kg, Shrew: 0.12 mg/kg. The PNECoral values represent dietary predicted no effect concentrations, below which food concentrations are not expected to pose a risk to birds or mammals. PNECoral values were identified in two tiers. In Tier 1, derivation of PNECoral values followed the standard guidance in the TGD and did not account for any species-specific differences in food ingestion rates and body weights. Based on the TGD, generic PNECoral values are calculated through the division of the NOEC by an assessment factor of 30 to account for interspecies variation in sensitivity and lab-to-field extrapolation. In Tier 2, PNECoral values were derived based on species specific food ingestion rate-to-body weight ratios for the birds and mammals considered in this evaluation (i.e., oystercatcher, worm-eating bird, harbour seal, shrew). An AF of 10 as oppose to 30 was used for Tier 2. As the interspecies variability among birds and among mammals is relatively low, and the Tier 2 PNEC values account for key interspecies differences (i.e., food ingestion rate-to-body weight ratios), the reduction in the Assessment Factor from 30 to 10 is justified. This was supported by the Technical Committee for New and Existing Substances (TC NES). Birds: Tier 1: Six sub-chronic and chronic feeding studies in which the diet was spiked with a Ni salt were identified (Wilson et al., 2001; Ling and Leach et al., 1979; Oscar et al., 1995; Weber and Reid 1968; Eastin and O'Shea 1981; Cain and Pafford 1981). The most sensitive lowest observed effect concentration (LOEC) from these six studies, focusing on endpoints related to survival, growth, and reproduction... Discussion continued in attached document.

Environmental classification justification

Classification of Nickel Metal Massive and Nickel Metal Powder

Nickel metal massive was assigned no classification for aquatic toxicity based on comparison of the transformation/dissolution data for nickel metal granules with the acute and chronic ecotoxicity reference values (ERVs) for soluble nickel, in accordance with the aquatic toxicity classification schemes for metals and metal compounds described in European Union (EU) and United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (United Nations, 2003).

Since the transformation/dissolution values were less than the acute and chronic ERVs no classification for aquatic toxicity of nickel metal massives was appropriate. This is consistent with the CLP harmonized classification for nickel metal massives for the environment.

For nickel metal powder a position paper entitled “Aquatic Toxicity Classification of Nickel Metal Powder” was prepared by the Nickel Producers Environmental Research Association (NiPERA). The paper was discussed at the European Chemical Bureau’s Commission Working Group on the Classification and Labelling of Dangerous Substances, which took place on April 26-27, 2006. The recommended classification from the DK Rapporteur and the Working Group was the same as that of NiPERA.

The NiPERA position paper presents information that supports the aquatic classification of nickel metal powder (particle size diameter < 1 mm) as Acute III/Chronic III in the International UN GHS system and as N; R52/53 in the EU DSD system. These are the classifications that have been reported in the 1st ATP to the CLP Regulation. Acute III/Chronic III in the International UN GHS system is equivalent to Chronic III in the European Union GHS system (CLP Regulation).

The NiPERA paper specifically includes the following information:

- Ecotoxicity data used to determine acute and chronic Ecotoxicity Reference Values (ERVs) of soluble Ni²⁺;
- Results of Transformation/Dissolution Protocol (T/D P) testing with nickel metal powder;
- Comparison of Ni dissolution from T/D P studies with relevant ERVs to determine the appropriate classification; and,
- The use of the Critical Surface Approach to distinguish between Ni metal powder and Ni metal massive.

Derivation of the Classification for nickel metal powder

The aquatic toxicity classification for nickel metal powder can be derived by comparing the transformation/dissolution data for nickel metal powder with the acute and chronic toxicity reference values for soluble nickel compounds. This approach is outlined in the classification schemes for metals and metal compounds described in European Union (EU) and United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (United Nations, 2003).

Transformation-Dissolution Data

Acute (7 day) and chronic (28 day) transformation/dissolution (T/D) data for nickel metal powder were collected. 7 d T/D P tests included testing at 1, 10, and 100 mg/L loading rates, whereas 28 d T/D P testing was performed at the 1 mg/L loading rate. All duration/loading rates were performed at both pH 6 and 8.

Acute Toxicity Reference Value

In order to assess acute toxicity classification, low and high pH acute toxicity reference values are needed to compare to the existing T/D data at pH 6 and 8, respectively. The acute toxicity reference value chosen for high pH was 0.068 mg Ni/L [same value proposed by the Danish EPA in document ECBI/26/95 Add. 76], which is the geometric mean of the 6 *Ceriodaphnia dubia* ecotoxicity (LC₅₀) values in the pH range of 8.3-8.7 and hardness range of 258-290 mg/L as CaCO₃ (Schubauer-Berigan et al. 1993; Parametrix 2005a; Parametrix 2005b).

The lowest ecotoxicity value at pH 6, which is 0.012 mg Ni/L for the alga *Pseudokirchneriella subcapitata* (Deleebeeck *et al.*, 2004), was chosen as the ecotoxicity reference value for comparison of the T/D data at pH 6.

Chronic Toxicity Reference Value

Currently, the EU DSD classification system uses the most sensitive NOEC/EC₁₀ value available as the chronic Ecotoxicity Reference Value. The chronic toxicity reference value was chosen to be the EC₁₀ of 0.0024 mg Ni/L calculated by Parametrix (2004) [same value proposed by the Danish EPA in document ECBI/26/95 Add. 76] since: this value was derived at a pH slightly higher (pH 8.4), but similar to that of the high pH T/D data (pH 8), the pH of the study was approximately at the high pH limit (pH 8.5) for chronic T/D testing, the hardness conditions are similar to that of the T/D medium at pH 8, and a calculated EC₁₀ is appropriate to use when the NOEC value was not measured (a less than value).

Aquatic Toxicity Classification

Aquatic toxicity classification can be assigned to a nickel sample by comparing the transformation/dissolution (T/D) results with the acute and chronic toxicity reference values for similar conditions using the EU and GHS classification schemes for metals and metal compounds.

For 7 day Ni metal powder T/D P data, the only loading rate at which dissolved Ni concentrations exceeded the acute ERVs was at 100 mg/L. Dissolution of 100 mg/L @ pH 6 was 0.35 mg/L, which exceeded the pH 6 ERV of 0.12 mg/L (dissolution of 10 mg/L @ pH 6 was 0.024 mg/L). Likewise, dissolution of 100 mg/L @ pH 8 was 0.28 mg/L, which exceeded the pH 8 ERV of 0.068 mg/L (dissolution of 10 mg/L @ pH 8 was 0.029 mg/L).

For the 28 d test using 1 mg/L loading, dissolution was 0.0023 mg/L at pH 6, and 0.0035 mg/L at pH 8. The pH 8 dissolution of 0.0035 mg/L exceeded the chronic ERV of 0.0024 mg/L.

Based on these results, the appropriate classification is R52/53 in the EU DSD system, Acute III/Chronic III in the International GHS system and Chronic III in the EU GHS system.

Critical Surface Area (CSA) Approach

An alternative approach to deriving the classification of R52-53 for nickel metal powder is the Critical Surface Area (CSA) Approach, described by Skeaff *et al.* (2000) and the MERAG (Metal Environmental Risk Assessment Guideline) fact sheet on classification (MERAG 2007), as well as referred to in the GHS under paragraph A8.7.5.4.4 (United Nations, 2003). The CSA Approach utilizes both the transformation/dissolution data and toxicity reference values to determine acute and chronic classification, as does the current EU and GHS classification systems, but the CSA Approach enables determination of classification based on surface area and equivalent spherical particle diameters.

Acute Toxicity

The CSA Approach can be used to assign an acute classification to nickel metal powder based on measured surface area using the measured surface area of 0.43 m²/g for the smallest representative size powder on the EU market. Since this surface area is greater than 0.1 m²/g but less than 1 m²/g, the acute classification for nickel metal powder would be R52. The CSA Approach can also be used to classify nickel metal massive, where the measured surface area of the CERAC granules^[1] (representative of nickel massives) is 0.086 m²/g. This surface area is less than all of the SA_{crit} so there would be no acute classification for nickel metal massive. These acute classification conclusions for nickel metal powder and massive are consistent with the EU and GHS classification approaches described above.

Chronic Toxicity

The CSA Approach can be used to assign a chronic classification to nickel metal powder based on measured surface area using the measured surface area of 0.43 m²/g for the smallest representative size powder on the EU market. Since this surface area is greater than 0.342 m²/g, ***nickel metal powder would be classified as R53.*** The CSA Approach can also be used to classify nickel metal massive, where the measured surface area of the CERAC granules^[1] (representative of nickel massives) is 0.086 m²/g. This surface area is less than the chronic SA_{crit} so there would be ***no chronic classification for nickel metal massive.*** These chronic classification conclusions for nickel metal powder and massive are consistent with the EU and GHS classification approaches described above.

Conclusions

Nickel metal powder should be classified as N; R52-53 using the data presented above in the EU DSD classification system for metals and metal compounds. This is equivalent to a classification of Aquatic Chronic

III in the EU GHS classification system, as classified in the 1st ATP to the CLP Regulation.

Nickel metal powder including a Nickel oxide impurity should also be classified as N; R52-53 in the EU DSD classification system and Aquatic Chronic III in the EU GHS classification system. No change to the Environmental Classification will result from the Nickel oxide impurity because Nickel oxide is classified as Aquatic Chronic IV in the 1st ATP to the CLP Regulation, which is less stringent than the classification for Nickel metal powder.

Nickel metal massive are not classified in the EU DSD or EU GHS aquatic classification systems for metals and metal compounds based on transformation/dissolution data for nickel metal massive being less than the acute and chronic ERVs.

The 2nd ATP to the CLP introduced the chronic (long-term) environmental toxicity endpoint as defined by the 3rd version of the UN-GHS into the EU hazard classification and labelling scheme. The GHS and EU scheme include the concept of degradation whereby rapid degradation from the water column (greater than 70 % removal in 28 days) results in different classification cut-off values and categories. For metals and inorganic metal compounds, the rapid and irreversible removal from the water column is equated to the rapid degradation concept for organics. The current draft guidance on metals includes a proposal to apply the “rapid degradation principle for organics” measured as a 70 % removal rate in 28 days in a comparable way for metals from laboratory and field experiments or by using a recently developed model. A Unit World Model (UWM) has recently been developed specifically for metals, building on previous screening-level calculations that have been developed for organic contaminants, and is capable of assessing the fate and effects of chemicals by the simultaneous consideration of chemical partitioning, transport, reactivity, and bioavailability. With regard to hazard assessment, the UWM is capable of assessing the removal of soluble metals from the water column resulting from sorption to particulate material, settling to the sediment compartment, and subsequent changes in speciation via precipitation by sulfides naturally present in the sediment compartment.

The UWM was used to assess the rapid removal of a group metals (e. g., Ni, Cu, Pb, Zn, As, Al, Co) in a generalized lake environment resulting from metal removal from the water column and sequestration in sediment. To estimate sorption by particulate matter in the water column, the UWM can use empirical, measured distribution coefficients (K_d), or the speciation module within the UWM (the Windermere Humic Aqueous Model, or WHAM) can calculate K_ds. When an empirical K_d of log 4.42 was used, greater than 70% nickel removal was achieved in every loading and pH scenario. WHAM-based K_ds tended to be substantially lower than empirical K_ds, indicating that refinement of the WHAM approach was needed. To this end, the UWM was refined to accommodate an updated version of WHAM (WHAM 7). Additionally, the inorganic thermodynamic database used by WHAM to perform speciation calculations was updated because the previous version was found to be out of date and inaccurate. Analyses using WHAM7 and the revised inorganic thermodynamic database showed that greater than 70% nickel removal was achieved under the three pH scenarios with metal loadings at the acute and chronic ERVs at 28 days. At the upper chronic cutoff value of 1 mg/L, rapid removal was achieved for pH 6 and 8 without oxide binding and for all three pH values with oxide binding. Rapid removal was demonstrated at all pH values when loading was based on acute Ecotoxicity Reference Values (120 µg Ni/L at pH 6 and 68 µg Ni/L at pH 8) and chronic Ecotoxicity Reference Values (2.4 µg Ni/L) using calculated K_d values. Based on these results, nickel metal fulfils the criteria for rapid degradation for the environmental classification scheme in the 2nd ATP to the CLP.

General discussion

The approach for deriving PNEC values was used in the 2008/2009 European Union Existing Substances Risk Assessment of Nickel (EU RAR) (EEC 793/93). The EU RAR was jointly prepared by the Danish Environmental Protection Agency (DEPA), which served as the Rapporteur of the Existing Substances Risk Assessment of Nickel, and the Nickel Producers Environmental Research Association (NiPERA), which represented the Nickel Industry in this process. The complete Environment section of the EU RAR can be found in the pdf linked to the following URL:

http://ecb.jrc.ec.europa.eu/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/nickelreport311.pdf

All of the approaches described were discussed by the Technical Committee for New and Existing Substances (TC NES), and received final approval at the TC NES I meeting in April, 2008.

Common effects assessment basis

The ecotoxicity databases on the effects of soluble nickel compounds to aquatic, soil- and sediment-dwelling organisms are extensive. It should be noted that the effects assessments of Nickel metal is based on the assumption that adverse effects to aquatic, soil- and sediment-dwelling organisms are a consequence of exposure to the bioavailable Ni-ion, as opposed to the parent substances. The result of this assumption is that the ecotoxicology will be similar for all soluble Ni substances used in the ecotoxicity experiments. Therefore, data from soluble nickel substances are used in the derivation of chronic ecotoxicological NOEC and L(E) C10 values. If both NOEC and L(E) C10 data are available for a given species, the L(E) C10 value was used in the effects assessment.

7.6.6 Silver

Table 21. Hazard assessment conclusion for the environment for silver

Compartment	Hazard conclusion	Remarks/Justification
Freshwater	PNEC aqua (freshwater): 0.04 µg/L	Assessment factor: 3 Silver: Extrapolation method: statistical extrapolation Silver: All PNECs have been derived in accordance with Chapter R.10 of the European Chemicals Agency's guidance on information requirements and chemical safety assessment under REACH (ECHA 2008; Characterisation of dose [concentration]-response for environment). For a full report of the calculation of PNECs see appendix attached.
Marine water	PNEC aqua (marine water): 0.86 µg/L	Assessment factor: 10 Extrapolation method: assessment factor Silver: All PNECs have been derived in accordance with Chapter R.10 of the European Chemicals Agency's guidance on information requirements and chemical safety assessment under REACH (ECHA 2008; Characterisation of dose [concentration]-response for environment). For a full report of the calculation of PNECs see appendix attached.
Intermittent releases to water		Silver: A PNEC aqua (intermittent release) has not been calculated for silver as an intermittent exposure pattern is not expected. The PNEC aqua (freshwater) will be used for the risk assessment.
Sediments (freshwater)	PNEC sediment (freshwater): 438.13 mg/kg sediment dw	Assessment factor: 10 Extrapolation method: assessment factor Silver: All PNECs have been derived in accordance with Chapter R.10 of the European Chemicals Agency's guidance on information requirements and chemical safety assessment under REACH (ECHA 2008; Characterisation of dose [concentration]-response for environment). For a full report of the calculation of PNECs see appendix attached. The most sensitive reported sediment NOEC is 12 mg Ag/kg dry sediment for growth of the amphipod <i>Hyaella azteca</i> exposed for 10 days (Call et al. 2006). An assessment factor of 10 was applicable, and the value was normalised to 5 % organic content to give the derived PNEC of 438.13 mg/kg dw.
Sediments (marine water)	PNEC sediment (marine water): 438.13 mg/kg sediment dw	Assessment factor: 10 Extrapolation method: assessment factor Silver: All PNECs have been derived in accordance with Chapter R.10 of the European Chemicals Agency's guidance on information requirements and chemical safety assessment under REACH (ECHA 2008; Characterisation of dose [concentration]-response for environment). For a full report of the calculation of PNECs see appendix attached. The most sensitive reported sediment NOEC is 12 mg Ag/kg dry sediment for growth

Compartment	Hazard conclusion	Remarks/Justification
		of the amphipod <i>Hyaella azteca</i> exposed for 10 days (Call et al. 2006). This was based on freshwater sediment data, which is considered to be conservative for marine. As such as assessment factor of 10 was applicable, and the value was normalised to 5 % organic content to give the derived PNEC of 438.13 mg/kg dw.
Sewage treatment plant	PNEC STP: 0.025 mg/L	Assessment factor: 1 Extrapolation method: assessment factor Silver: All PNECs have been derived in accordance with Chapter R.10 of the European Chemicals Agency's guidance on information requirements and chemical safety assessment under REACH (ECHA 2008; Characterisation of dose [concentration]-response for environment). For a full report of the calculation of PNECs see appendix attached.
Soil	PNEC soil: 1.41 mg/kg soil dw	Assessment factor: 3 Extrapolation method: statistical extrapolation Silver: All PNECs have been derived in accordance with Chapter R.10 of the European Chemicals Agency's guidance on information requirements and chemical safety assessment under REACH (ECHA 2008; Characterisation of dose [concentration]-response for environment). For a full report of the calculation of PNECs see appendix attached.
Air	No hazard identified	Silver and silver compounds are not expected to contribute to ozone depletion, ozone formation, global warming or acidification. Therefore, the evaluation of atmospheric risk is not required.
Secondary poisoning		The TGD BCF trigger of 100 in whole fish is not exceeded by silver so there is no need to derive PNECs for secondary poisoning.

Environmental classification justification

Acute and chronic aquatic toxicity data are available for a range of different freshwater and marine species. For silver and silver compounds, the acute ecotoxicity reference value (ERV) is 0.22 µg Ag/L and the chronic ERV is 0.16 µg Ag/L.

In line with the risk assessment/classification approach adopted for other metals and inorganic metal compounds (ECHA 2013), ecotoxicity data are reported in terms of the concentration of dissolved silver ions from soluble inorganic silver species. Predominantly, these are studies that used silver nitrate (AgNO₃) as the source of dissolved silver ions. Silver nitrate is considered to be the form of silver with the greatest toxicity as it dissociates rapidly and completely in aqueous solution. Where data for silver nitrate was not available, data derived from other inorganic salts (e. g. silver chloride) were used, but only after the exposure conditions were determined to be acceptable (e. g. testing was conducted within the limits of solubility and the Ag⁺ ion was likely to be the dominant dissolved species).

A complete base set of acute ecotoxicity studies is available for soluble inorganic silver species, comprising numerous studies for fish, invertebrates and algae. The lowest reliable acute value is an EC₅₀ of 0.22 µg Ag/L for the invertebrate *Daphnia magna* (Bianchini et al. 2002).

A complete chronic data set is also available for soluble inorganic silver species, with chronic ecotoxicity data available for various species of fish, invertebrates and algae. The lowest reliable chronic value is an EC₁₀ of 0.16 µg Ag/L for the blue-green algae *Nostoc muscorum* (Rai et al. 1990). Additional chronic toxicity data of similar sensitivity are also available for the Brown Trout *Salmo trutta* (217 day EC₁₀ of 0.19 µg Ag/L) and *Oncorhynchus mykiss* (196 day EC₁₀ of 0.17 µg Ag/L) obtained from Davies et al. (1998).

Based on current guidance (ECHA 2013), soluble silver is classified as R50/53 under the DSD, with specific concentration limits of C_n ≥ 0.025% for R50-53, ≥ 0.0025% C_n < 0.025% for R51-53, and ≥ 0.00025% C_n < 0.0025% for R52-53. Soluble silver is also classified as Acute 1 and Chronic 1 under the CLP regulations and

subject to an acute M factor of 1000 and a chronic M factor of 100.

Silver metal- Transformation/Dissolution tests

Massive and powder forms of silver are considered to be poorly/sparingly soluble. The environmental hazard of poorly/sparingly soluble forms of metals is considered to be associated with their potential to release soluble bioavailable metals ions (e. g. Ag⁺ ions) or other metal-bearing species into aqueous media, from where they can elicit adverse effects (ECHA 2013). The potential for aqueous transformation or dissolution of elemental or sparingly soluble forms of metals can be measured using standardised Transformation/Dissolution (T/D) tests.

Transformation/Dissolution tests have been performed on silver metal in both massive and powder forms. The massive form of silver was tested using a procedure which controls the available surface area by encasing the sample in glass and exposing a specified surface area.

Silver in powder form (median particle size >100 nm - <1 mm)

Two sets of full T/D test results are available for silver powder (CIMM 2009, ECTX 2010b). CIMM (2009) report T/D results for a silver powder with a median particle size of 1.9 µm and surface area of 3.0 m²/g after exposure for seven (loading rates of 1, 10 and 100 mg/L) and 28 days (loading rate of 1 mg/L) in OECD media at both pH 6 and pH 8. ECTX (2010b) reports T/D results for a silver flake with a median particle size of 2.6 µm and surface area of 1.17 m²/g after exposure for seven (loading rates of 1, 10 and 100 mg/L) and 28 days (loading rate of 1 mg/L) in OECD media at pH 6 only. The results of the tests performed at pH 6 on the two different silver materials are similar, with average dissolved silver concentrations after seven days of 1.3 and 1.8 µg/L (1 mg/L loading rate) and average dissolved silver concentrations after 28 days of 3.6 and 3.7 µg/L (1 mg/L loading rate) for powder and flake, respectively. Where T/D data are available for the same material under both pH 8 and 6 (CIMM 2009), dissolution of silver powder after 7 days at the highest loading rate (100 mg/L) is reported to be greater at pH 6 (37.36 µg/L) than at pH 8 (26.03 µg/L). However, at the two lower loading rates (1 and 10 mg/L), dissolution is greater at pH 8 (2.55 and 12.14 µg/L, respectively) than pH 6 (1.25 and 8.46 µg/L, respectively). Equally, dissolution of silver powder after 28 days at 1 mg/L was greater at pH 8 (5.71 µg/L) than at pH 6 (3.55 µg/L). The implications of this observation on classification are discussed below.

Discussion on observed effects

At all loading rates the dissolution of silver powder declined throughout the exposure period. Further, at the lower loading rates of 1 and 10 mg/L, the rate of dissolution appeared to be greater for tests performed at pH 8 than for those performed at pH 6, which is not consistent with the behaviour observed with many other metals.

The interpretation of the results observed in the T/D tests for silver powder may be complicated by the elevated chloride concentration in the high pH (pH 8) OECD T/D testing media. The high pH media for T/D tests contains 10 times more chloride than the low pH media. As silver chloride complexes are relatively soluble at concentrations below their solubility limit, the increased dissolution of silver observed at high pH relative to low pH may not be due to test media pH but to the concentration of chloride in the test media. The discrepancy between the dissolution behaviour observed at high and low loading rates is likely to be because the solubility limit of silver chloride complexes is reached in the pH 8 media at high loading rates, limiting dissolved silver concentrations to below those found in the pH 6 media at the highest loading rate due to its lower chloride content.

Bioaccessibility testing has also indicated that the dissolution of silver may be dominated by the solution chloride concentration rather than by the pH. This is supported by chemical speciation calculations performed using VisualMINTEQ that indicate that the formation of soluble silver chloride complexes may be important in silver solubility.

Given that the formation of soluble silver chloride complexes may explain the greater dissolution observed at pH 8, the T/D testing of silver powder at pH 6 may still represent environmentally relevant worst case dissolution of silver. However, classification and the selection of test media for “definitive” T/D testing with massive silver is based on the “worst-case” T/D testing results obtained at pH 8.

Silver in massive form (median particle size >1mm)

Initial studies to determine the T/D behaviour of silver in massive form (ECTX 2010a), which were performed using an epoxy resin carrier to control the exposed surface area of silver during the test, indicated unusual

dissolution/solubility behaviour. Dissolved silver concentrations increased rapidly but then declined to a steady state concentration. Further experimentation suggested that the epoxy resin could act as an adsorbent phase for silver, which would result in the decline in the dissolved silver concentrations observed. It was further hypothesised that, during preparation of the test item, minute particles of silver may have become embedded in the epoxy vehicle, considerably increasing the exposed surface area during the test. Given these complications further experiments were proposed using a quartz glass vehicle.

To address the limitations of the 2010 study, a further “definitive test” on massive silver was performed using a quartz glass vehicle. As it was technically challenging to test massive silver at a nominal loading of 1 mg/L “surface equivalent” loading rates of 3, 9, and 27 mg/L were tested, which were then used with the intention to extrapolate to a conventional loading rate of 1 mg/L (ECTX 2013). The corresponding surface area under the loading rates was 3.14, 9.42 and 28.3 mm², respectively. The surface equivalent loading rate is the mass of material that would result in the same exposed surface area (i.e. the same degree of dissolution). This study was conducted at a worst case pH of 8 only (based on the results of silver powder T/D testing) and used fluorinated ethylene propylene vessels to minimise adsorption of silver to the test system during the course of the 28 day test. The test items were also cleaned ultrasonically prior to the start of the exposure to remove residual particles of silver from the surface of the quartz glass tubes remaining from sample preparation. The average blank corrected dissolved silver concentration in the test medium after seven days exposure was the below the analytical limit of detection of 0.02 µg/L at all loading rates. The average blank corrected dissolved silver concentration in the test medium after 28 days exposure was below the analytical limit of detection of 0.02 µg/L at loading rates of 3 and 9 mg/L and was 0.03 (± 0.07) µg/L at a loading rate of 27 mg/L.

Uncoated and coated nanomaterials (as per EU definition – median particle size <100 nm)

When added to an aqueous solution, nanosilver (a form of elemental silver powder) will initially form a colloidal dispersion before undergoing oxidative dissolution to release soluble silver ions. As such, its classification could be based on the results of T/D testing. However, there are several issues associated with conducting T/D testing on nanosilver as the basis for classification, as follows:

The standard T/D test methodology uses a 0.2 µm (200 nm) filter to distinguish between dissolved (i. e. ionic) and insoluble metal fractions. This methodology will not adequately distinguish soluble metals ions from their parent nanoparticles (<100 nm) within a T/D test system.

Whilst alternative fractionation methods that could be applied within T/D testing have been described in the literature (e. g. ultracentrifugation), their suitability for regulatory purposes has not yet been established. Equally, no standard methodology for T/D testing of nanomaterials is currently available.

The presence and type of any particle coating will influence the stability of the particle dispersion (affecting the tendency of the material to form aggregates and agglomerates), potentially influencing the rate of ionic dissolution. The choice of coating could therefore unduly influence the outcome of the testing.

Because of these limitations, T/D testing has not been conducted on uncoated or coated nanomaterials as a basis for their classification. As an alternative, a precautionary classification for silver and silver-based (coated) nanomaterials has been read across from data for soluble silver (see below). Similarly, appropriate M factors have been based on read across from soluble silver.

Classification

The classification strategy for poorly soluble silver substances has been based on ECHA guidance (ECHA 2013). This guidance describes how to determine the classification and appropriate M factors for poorly soluble substances, such as elemental silver powder and massive silver, from acute and chronic ERV and the results of T/D tests (Annex IV: Metals and Inorganic Metal Compounds). Acute and chronic classifications have been undertaken individually.

Chronic classifications for silver metal and powder have been derived from the chronic ERV and 28 day data from T/D tests. The available 28 day T/D data for silver powder and massive silver metal is restricted (for technical reasons) to a minimum loading rate of 1 mg/L. However, as chronic classification requires T/D data obtained from further loading rates of 0.01 and 0.1 mg/L, data for these theoretical loading rates have been extrapolated, as detailed in the guidance (footnote 108, page 608), from tests conducted at higher loading rates using either empirically derived relationships (i. e. linear regression) or precautionary assumptions (i. e. based on analytical limits of detection). Where the available data on which to base assumptions was limited, an

alternative classification, based on the surrogate approach to chronic classification (section IV.5.2.2.2) using the acute ERV, is also reported for comparative purposes. The most stringent classification has been adopted.

Silver in powder form (median particle size >100 nm - <1 mm)

DSD / DPD classification

Silver in powder form is classified as R50/53 under the DSD, with specific concentration limits of $C_n \geq 0.025\%$ for R50-53, $\geq 0.0025\% C_n < 0.025\%$ for R51-53, and $\geq 0.00025\% C_n < 0.0025\%$ for R52-53 derived using the acute ERV, as set out in Annex III of the DPD.

Acute classification under CLP / GHS

The loading rate of 1 mg/L in full T/D testing at both pH 6 (powder and flake) and pH 8 (powder only) resulted in a seven day dissolved silver concentration above the acute ERV of 0.22 µg/L (Bianchini et al. 2002). Therefore, Ag in powder form is classified as Acute 1 under the CLP regulations.

In addition, according to section IV.5.4 of ECHA guidance, the acute ERV of 0.22 µg/L and the maximum seven day T/D test result at a loading rate of 1 mg/L of 2.6 µg/L (silver powder at pH8) determines that an acute M factor of 10 should be applied to the classification.

Chronic classification under CLP / GHS

The concentration of dissolved silver after 28 days at pH8 in theoretical loading rates of 0.01 and 0.1 mg/L was extrapolated from a linear relationship of dissolution as a function of surface area loading (SAL) derived from a combination of 28 day T/D data at pH 8 for both massive silver and silver powder (calculations after Skeaff et al. 2008), as follows:

$$\text{Log}_{10}[\text{Ag } \mu\text{g/L}] = 1.13 \times \text{log}_{10}(\text{SAL mg/L}) + 3.5953 \text{ (n=2)}$$

This relationship calculated Ag concentrations in 0.01 and 0.1 mg/L silver powder loading after 28 days at pH 8 of 0.03 and 0.41 µg/L, respectively. The concentration at 0.1 mg/L loading rate is greater than the chronic ERV of 0.16 µg/L resulting in a classification of Chronic 1 under the CLP regulation.

As the available data upon which to base the extrapolation to loading rates of 0.01 and 0.1 mg/L was limited to two results, a complimentary chronic classification derived from the results of T/D testing after 7 days exposure at a loading rate of 1 mg/L and acute ERV according to the surrogate approach detailed in ECHA guidance (for application in instances where either chronic EVR or T/D testing data are not available) was also conducted. This approach also results in a classification of Chronic 1 under the CLP regulation.

In addition, according to section IV.5.4 of ECHA guidance, the chronic ecotoxicity reference value of 0.16 µg/L and the maximum 28 day T/D test result of 5.71 µg/L (silver powder pH 8) determine that a chronic M factor of 10 should be applied to the classification.

The chronic classification of metals and metal compounds can incorporate information on the environmental fate of dissolved ions. Where there is evidence of rapid environmental transformation of dissolved ions to less bioavailable forms in the water column and sediments, a less stringent chronic classification can be applied. This concept is similar to the use of data on the degradation of organic chemicals to inform classification. However, according to current ECHA guidance on the application of CLP, there is a lack of scientific consensus on the interpretation of rapid removal of dissolved metal species from the water column in the context of classification. Certain parts of Annex IV of the CLP guidance have therefore been removed until the agreement on the validity of use of the concept of rapid removal for classification purposes has been reached. The chronic classification of silver powder is therefore currently based on an assumption that dissolved silver ions are not rapidly partitioned from the water column. However, dissolved silver ions are known to undergo rapid environmental transformation through a combination of geochemical (partitioning to suspended particulate matter and settling) and speciation processes, such as the formation of metastable organic and inorganic reduced sulfide complexes (Kramer et al. 2002). This behaviour, which is dominated by reaction with sulfides, results in short residence times for the free silver ion in the dissolved phase of aquatic systems compared to other metals (Kramer et al. 2002).

The “Tableau Input Coupled Kinetics Equilibrium Transport Unit World Model for Metals in Lakes” (hereafter referred to as TICKET-UWM and available from <http://blog.unitworldmodel.net>), which was developed to

address the complexities of metal speciation (using WHAM V speciation software) and its influence on the fate and effects of metals in the environment, was applied to explore the removal of silver in a theoretical lake system. Processes considered by the TICKET-UWM include complexation by aqueous inorganic and organic ligands such as dissolved organic carbon (DOC), adsorption to particulate phases such as particulate organic carbon (POC) and iron/manganese oxides and cycling of organic matter and sulfide production in lakes. The version of TICKET-UWM used to assess the fate of silver also incorporated a preliminary appreciation of the role of binding to sulfide containing ligands (measured as Chromium Reducible Sulfide - CRS). Modelling of water column removal and sediment sequestration were performed. Performance against criteria for rapid removal and long-term sequestration was assessed.

Simple linear modelling (using EUSES defaults) of a lake water column using TICKET-UWM based on empirically derived K_d values determines that silver is rapidly removed (within 2-4 days) from the water column based on simple equilibrium partitioning to solid-phases (i. e. suspended particles) and particle settling behaviour.

When CRS was considered within speciation-based non-linear modelling, simulations at pH 7 resulted in rapid removal of silver (defined as > 70% removal achieved in 28 days) from the water column using two out of the three modelling approaches adopted at each of the initial concentrations assessed, including the chronic cut-off concentrations of 0.1 and 1.0 mg/L. In the third modelling approach, the formation of silver chloride precipitates, which sustained concentrations of dissolved silver, prevented rapid removal. Removal under additional pH conditions (i. e. between 6 and 8) were not explored. Modelling that did not incorporate CRS binding did not achieve criteria for rapid removal at the lower chronic cut-off concentration (0.1 mg/L) when default modelling assumptions were used. Equally, in a sensitivity analysis, lake depth and settling velocity was determined to influence the ability to achieve rapid removal of silver.

Modelling of long-term sequestration in sediments (predominantly as Ag_2S) suggests that binding to acid volatile sulfide (AVS) in anoxic sediments will result in low remobilisation potential. However, within oxic sediments not all of the criteria for sequestration were achieved.

Despite this understanding of fate, there is currently limited information on several elements of the fate of silver in the environment that are critical to any decision of classification, such as the reversibility of the formation of silver-sulfide complexes in the water column, fate processes under various environmental conditions (i. e. different pH values or within riverine systems) and the bioavailability (toxicity) of sulfide-bound silver.

Once guidance on the interpretation of information on rapid environmental degradation becomes available, and if relevant information to address the limitations of understanding described above, the current chronic classification of silver powder should be revisited.

Silver in massive form (median particle size >1mm)

DSD / DPD classification

No classification

Acute classification under CLP / GHS

Dissolved silver concentrations in T/D testing after 7 days exposure were below the analytical limit of detection (0.02 $\mu g/L$) in all loading rates (3, 9 and 27 mg/L) and replicates. As the limit of detection is below the acute ERV of 0.22 $\mu g/L$, silver massive, according to ECHA guidance, should not be classified for acute hazard.

Chronic classification under CLP / GHS

Similar to the results of 7 day T/D testing, the results of 28 day T/D testing at loading rates of 3 mg/L and 9 mg/L were also below the analytical limit of detection (0.02 $\mu g/L$). Only a single replicate at the highest equivalent loading rate of 27 mg/L reported concentrations of dissolved silver above the analytical limit of detection. As the analytical limit of detection used in the T/D testing was significantly lower than the chronic ERV of 0.16 $\mu g/L$, it is not considered necessary to classify silver massive for long-term hazard as even a precautionary extrapolation to concentrations of dissolved silver at lower loading rates of 0.01 and 0.1 mg/L (assuming that silver is present at a concentration equivalent to the analytical detection limit of 0.02 $\mu g/L$) would also result in values of dissolved silver lower than the chronic ERV.

This reasoning is supported by a linear relationship of dissolution as a function of surface area loading (SAL)

derived from a combination of 28 day T/D data at pH 8 for both massive silver and silver powder (calculations after Skeaff et al. 2008), as follows:

$$\text{Log}_{10}[\text{Ag } \mu\text{g/L}] = 1.13 \times \text{log}_{10}(\text{SAL m}^2/\text{L}) + 3.5953 \text{ (n=2)}$$

This relationship, whilst based on limited data, results in dissolved silver concentrations in 0.01, 0.1 and 1.0 mg/L silver massive loading after 28 days at pH 8 of 0.000004, 0.00004 and 0.0007 $\mu\text{g/L}$, respectively. The concentration at 1.0 mg/L loading rate is, as expected, less than the chronic ERV of 0.16 $\mu\text{g/L}$, which necessitates no chronic classification is required under the CLP regulation.

Uncoated and coated nanomaterials (as per EU definition – median particle size <100 nm)

The classification of uncoated and coated nanomaterials has been directly read across from soluble forms of silver without recourse to a T/D test (see above). Silver and silver-based nanomaterials would therefore be classified as per soluble silver substances: Acute and Chronic Category 1.

As there is currently no agreed standard methodology for conducting T/D tests on nanomaterials, appropriate M factors have also been read across from soluble forms of silver. This read-across results in acute and chronic M factors of 1000 and 100, respectively, for uncoated and coated nanomaterials.

As the weight of evidence available suggests that the ecotoxicity of uncoated and coated nanomaterials is lower than soluble silver on an equivalent mass basis (see read-across matrix for environmental endpoints), read across from soluble silver in this case is expected to result in a precautionary classification and M-factors for nanosilver.

Equally, as the dissolution rates of uncoated and coated nanomaterials are expected to be variable dependent on morphology, particle size, particle size distribution and coating, this approach encompasses the range of likely properties of nano silver and is consistent with the read across rationale applied to uncoated and coated nanomaterials for environmental hazard endpoints.

For the purposes of this read-across, silver-based (coated) nanomaterials are considered to be those that are primarily based on elemental silver and fulfil the criteria of the Commission Recommendation for the definition of nanomaterials, but which also comprise a surface functionalisation/coating/capping. The scope of the read-across from soluble silver to elemental silver and elemental silver-based (coated) nanomaterials for classification is identical to the scope of the environmental assessment of silver and silver-based (coated) nanomaterials in the REACH chemical safety assessment (CSA), i. e. the read-across is only considered to be applicable where applied to elemental silver nanomaterials or elemental silver-based nanomaterials with additional surface functionalisation/coating/capping that is demonstrated to be non-toxic, biodegradable, “passive” and applied primarily to increase the stability of nanomaterial dispersions in aqueous or non-aqueous solutions (i. e. to prevent aggregation or agglomeration) through steric, electrostatic or comparable mechanism. Surface functionalisation/coating/capping that are specifically designed to interact with biological receptors or which have hazardous properties in their own right (e. g. meet the criteria for SVHC) would not be compatible with this read-across approach for classification. Equally, multi-metal nanomaterials incorporating elemental silver are outside of the scope of this read-across.

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General discussion

See CSR Annex 4 - PNEC Summary Report

7.6.7 Zinc

Table 22. Hazard assessment conclusion for the environment for zinc

Compartment	Hazard conclusion	Remarks/Justification
Freshwater	PNEC aqua (freshwater): 20.6 µg/L	Assessment factor: 1 Extrapolation method: statistical extrapolation Zinc: The following considerations are made on the uncertainty around the HC5 and for determining the size of the assessment factor: •The number of chronic species NOECs (23) covers more than the requirements for taxonomic groups (8) and species (at least 10, preferably more than 15) set out in the guidance. •The large number of species in the SSD resulting in a low uncertainty on the HC5 value •The lognormal distribution giving a more conservative HC5 than the best fitting distribution (extreme values distribution) •The chronic data are from tests performed in a variety of natural freshwaters, properly reflecting the range of abiotic conditions found in the European freshwaters •The general consideration that the bioavailability of metals under real life conditions is lower than the bioavailability in laboratory toxicity tests. •The few specific NOEC values of the database that are below the HC5 can be explained by non-environmentally relevant conditions. •The inclusion of data obtained under very low background conditions into the NOEC database which introduces a significant level of conservatism into the SSD and the PNEC derivation . •Predictions from the chronic Biotic Ligand Model showing that the NOECs predicted for the realistic worst case conditions of EU waters, correspond well with the generic species mean NOEC values observed for the BLM organisms and used in the SSD. •Comparison with reliable Mesocosm data, showing no evidence that the HC5 is not protective. •Evidence on the PNEC from a large scale field survey study, demonstrating that the PNEC for zinc should be in the range 20-27µg Zn dissolved/l The consideration of the elements above does not support the application of an additional assessment factor on the HC5 for setting the PNEC.
Marine water	PNEC aqua (marine water): 6.1 µg/L	Assessment factor: 1 Extrapolation method: statistical extrapolation Zinc: The following considerations are made on the uncertainty around the HC5: -The chronic NOEC database is very extensive and contains 39 species entries that cover much more than the requirements for taxonomic groups (8) and species (at least 10, preferably more than 15) set out in the guidance; - Sensitive life stages or long chronic exposure periods (a few months) are represented in each taxonomic group as set out in the guidance; -The large number of species in the SSD results in a low uncertainty on the HC5 value, as is shown by the small difference between the 50% confidence level and the 95% confidence limits found for the lognormal distribution: less than a factor of 2.5; -The lognormal distribution that was used for PNEC derivation resulted in an HC5 of 6.09 µg/l, which is markedly lower than the HC5 value calculated from the Weibull distribution (8.5 µg/l), which provided the best fit. So the HC5 that is used for the PNEC derivation is a conservative value; - The HC5 value from the log-normal SSD is protective for mesocosm data; - There is no indication for a particular sensitive group in the SSD. In addition, whenever an SSD includes > 20 data points, the chance of having a value below the HC5 is significant. So, having one or more values below the HC5 is inherent to bigger datasets and is not an issue as such; Based on these observations, there is no need for an assessment factor higher than 1. The PNEC saltwater for zinc is thus of 6.09 µg/L.
Intermittent releases to water		Zinc: Intermittent release is not applicable for zinc.

Compartment	Hazard conclusion	Remarks/Justification
Sediments (freshwater)	PNEC sediment (freshwater): 117.8 mg/kg sediment dw	Assessment factor: 1 Extrapolation method: statistical extrapolation Zinc: It is emphasized that the reported PNEC for freshwater sediments is an added PNEC, i.e. natural background needs to be taken into account when characterizing the risks from monitored data. The following considerations are made on the uncertainty around the HC5 and for determining the size of AF: •The 7 available chronic single-species tests are among the most important taxonomic groups for the sediment ecosystems and as such reflect a broader difference in sensitivity among species (7.5 instead of 2.5 in the Zn RAR). •The sediment chronic database covers long term (3 to 8 weeks) lethal and sub-lethal endpoints that are all relevant for potential effects at population level. •The lognormal distribution gives a more conservative HC5 than the best fit distribution (logistic distribution) •The proposed PNECadd, sediment is lower than all other sediment quality guidelines/values proposed in various jurisdictions around the world (see e.g. Chapman et al. 1999, MacDonald et al. 2000) •Field data demonstrate that the proposed PNECadd, sediment is protective for ecosystems. The consideration of the elements above does not support the application of an additional assessment factor on the HC5 for setting the PNEC.
Sediments (marine water)	PNEC sediment (marine water): 56.5 mg/kg sediment dw	Assessment factor: 1 Extrapolation method: partition coefficient Zinc: It is emphasized that the reported PNEC for saltwater sediments is an added PNEC, i.e. natural background needs to be taken into account when characterizing the risks from monitored data. The following considerations are made on the uncertainty around the HC5 and for determining the size of AF: • Lowest reported NOEC value (A. marina; emergence; 207 mg/kg d.w.) is higher than the proposed EqP PNEC • The proposed marine EqP PNEC is lower than all other sediment quality guidelines/values proposed in various jurisdictions around the world (see e.g. Chapman et al. 1999) • The HC5 based on combined freshwater and seawater data derived from the lognormal distribution gives a value of 106 mg/kg d.w, which divided by AF2 gives a PNEC of 53 mg/kg. This value is close to the EqP PNEC • Marine sediment PNEC in the Zn RA is based on the freshwater sediment PNEC of 49 mg/kg which is in the same range • Using the AF approach gives a low PNEC value (14.6 mg/kg d.w.) which is in the lower percentiles of the natural background concentrations for zinc and is therefore not relevant
Sewage treatment plant	PNEC STP: 100 µg/L	Assessment factor: 1 Extrapolation method: assessment factor Zinc: Considering that the nitrification inhibition test is most sensitive of the data available, the PNEC is derived from the NOEC (100 µg Zn/l ; Juliastuti et al. 2003) divided by AF 1 to give the PNEC-STP of 100 µg Zn/l.
Soil	PNEC soil: 35.6 mg/kg soil dw	Assessment factor: 1 Extrapolation method: statistical extrapolation Zinc: The given value is the generic PNECadd, i.e. it has to be added to the natural background concentration of zinc. This generic PNECadd, should as a rule be multiplied with a factor 3 to take into account "lab-to-field" differences in toxicity. As such, the generic corrected PNECadd is 107 mg Zn/kg dw. Soil-specific PNEC values can further be calculated when the characteristics of the soil are documented. The following considerations are

Compartment	Hazard conclusion	Remarks/Justification
		made on the uncertainty around the HC5: i) The chronic NOEC database is very extensive and largely fulfils the requirements for taxonomic groups (8) and species (at least 10, preferably more than 15) set out in the guidance; ii) the database covers ecologically relevant endpoints and full chronic studies ; iii) the NOECs/EC10 were obtained by testing on soils covering the variability of soil characteristics all over the EU; iv) the log-normal distribution was accepted at all significance levels; v) considering all individual NOEC levels, the HC5 is protective; vi) comparison with field /mesocosm studies demonstrates that the HC5 is protective. The consideration of the elements above does not support the application of an additional assessment factor on the HC5 for setting the PNEC.
Air		
Secondary poisoning	No potential for bioaccumulation	Zinc: Zinc is an essential element that is actively regulated within the body of all organisms. Due to the general lack of increased whole body and tissue concentrations at higher exposure levels, the zinc BCF data show generally an inverse relationship to exposure concentrations (McGeer et al 2003). The physiological basis for the inverse relationship of BCF to zinc exposure concentration arises from Zn uptake and control mechanisms. At low environmental zinc levels, organisms are able to sequester and retain Zn in tissues for essential functions. When Zn exposure is higher, aquatic organisms are able to control uptake. There is clear evidence that many species actively regulate their body Zn concentrations, including crustaceans, oligochaetes, mussels, gastropods, fish, amphipods, chironomids by different mechanisms (McGeer et al 2003). The bioaccumulation potential in mammals is also considered low. Based on this, the EU risk assessment concludes that secondary poisoning is considered to be not relevant in the effect assessment for zinc.

Environmental classification justification

In the RA, distinction was made for classification between zinc in powder form and in massive form.

Classification of zinc powder/dust under Annex I dangerous substances directive 67/548/EEC

Zinc powder-zinc dust (pyrophoric and stabilized) was classified N; R50-53 (Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment) according to Annex 1 of Directive 67/548/EEC (ECB 2008).

Revised classification of zinc powder/dust under 2nd Adaptation to Technical Progress (ATP) to the CLP Regulation (2nd ATP CLP)

According to the classification listed in Annex I to Directive 67/548/EEC, the classification of zinc powder was translated as H400 - H410 (very toxic to aquatic life with long lasting effects) according to Annex VI of the EU CLP Regulation (EU 2008).

The M-Factor for the acute aquatic effect of zinc powder is 1, referring to the acute aquatic ecotoxicity values of 136 µg Zn/l and 413 µg Zn/l for the zinc ion at pH 8 and 6 respectively.

The M-factor for chronic aquatic effect of zinc powder/dust is 1, referring to a) the lowest chronic aquatic ecotoxicity value observed for the algae *Pseudokirchneriella subcapitata* (19 µg Zn/L) at neutral pH. This reference value is in any case less than factor 10 different from the criterion for chronic 1 classification for aquatic effect (being 10 µg/l or 100 µg/l depending on whether or not the substance is considered rapidly degradable or not).

In conclusion, zinc powder is classified under CLP as H400, H410. The M-factor for both acute and chronic aquatic effects is 1.

Further considerations on the classification of Zinc powder/dust based on recent information

Taking into account the lowest chronic ecotoxicity value observed on a wide variety of species of different taxonomic groups (19 µg Zn/L), it can be reconsidered what exactly the level of chronic classification of the substance should be:

As was mentioned above the chronic ecotoxicity reference value for the substance is 19 µg Zn/L. This value must be compared with the criteria for chronic classification, also taking into account whether the substance is considered rapidly degradable or not.

The concept of “Degradability” was developed for organic substances and is not applicable to inorganic substances like zinc. As a surrogate approach for assessing “degradability”, the concept of “removal from the water column” was developed to assess whether or not a given metal ion would remain present in the water column upon addition (and thus be able to exert a chronic effect) or would be rapidly removed from the water column. In this concept, “rapid removal” (defined as >70% removal within 28 days) is considered as equivalent to “rapidly degradable”. Under section 4.6., the rapid removal of zinc from the water column is documented. Consequently, zinc is considered as equivalent to being ‘rapidly degradable’ in the context of classification for chronic aquatic effects.

Considering this, in combination with the abovementioned chronic ecotoxicity reference value for zinc of 19 µg/L, the classification of the substance for chronic aquatic effect should be “chronic 2”, rather than the “chronic 1” classification in Annex VI of the CLP

Classification of zinc in massive form under Annex I dangerous substances directive 67/548/EEC

Zinc metal in massive form was not classified under Directive 67/548/EEC, and does not figure in. Annex 1 of Directive 67/548/EEC (ECB 2008).

Classification of zinc metal in massive form under 2nd Adaptation to Technical Progress (ATP) to the CLP Regulation (2nd ATP CLP)

The potential ecotoxicity of metals in massive form is determined by their capacity to release ions in aqueous media. This capacity is assessed in transformation/dissolution (T/D – see section 4.6.) testing. For zinc metal in massive form, this approach is relevant. The assessment below is made on T/D results obtained at pH 6, at which release of zinc ions from metal is maximal.

Acute classification

Under section 4.6., transformation dissolution data were presented that demonstrate that zinc ions are released from massive zinc in a limited way only. It was concluded from the data that at 1mg/l loading, the critical diameter of a sphere resulting in sufficient surface loading to reach the reference value for acute aquatic effects, is 0.082 mm.

In other words, the diameter of a zinc metal sphere of 1mg should be $\leq 0.082\text{mm}$, in order to reach the reference value for acute aquatic effects. This particle size is much smaller than the default particle size distinguishing massive metal from powder/dust (1mm).

Consequently, zinc metal in massive form is not classified for acute aquatic effect.

Chronic classification

In a similar approach as the one described above for acute classification, T/D data were used for chronic classification, too. In this case, however, information on long-term T/D (28days) were used (section 4.6.). The critical diameter of a spherical metal particle, resulting in sufficient surface loading to reach the reference value for chronic aquatic effects at 1mg/l loading of the substance was set at 2.1 mm. Accordingly, the critical diameters of a sphere, resulting in reaching the reference value for chronic aquatic effect at mass loading criteria of 0.1mg/l and 0.01mg/l are determined to be 0.21 mm and 0.021 mm, resp.

The 2.1mm critical diameter for loading at 1mg/l is slightly greater than the default particle size distinguishing massive metal from powder/dust (1mm). However, 2 additional elements can be observed in this respect:

- When the epoxy-mounted zinc surfaces (see section 4.6.) after the test were kept for 3 days in the laboratory

air, and the test was repeated, the zinc amount dissolving was only 50% of the original amount that had been observed (T/D after 7 days at 100 mg/l loading was 2656 µg Zn/l and 1321 µg Zn/l, on freshly polished metal and metal passivated in water, respectively) (Rodriguez 2010). This demonstrates that the freshly polished metal surface had passivated over 7 days in the test solution, and, when re-immersed for checking further dissolution, only released half of the original amount. So passivation in water is occurring rapidly (within 7 days) and is significant. The critical diameter for zinc release of a “passivated” spherical particle be only half the one calculated for the freshly polished surface. This observation is relevant for the dissolution potential of zinc from a metallic zinc surface in aqueous medium. Considering this effect, the critical diameter of a spherical metal particle, resulting in sufficient surface loading to reach the reference value for chronic aquatic effects at 1 mg/l loading of the substance would be equal to the default size for distinguishing powders/dusts and metal in massive form.

An additional remark can be made on the forms and sizes of metallic zinc produced in the EU. The vast majority of zinc in massive form is put on the EU market as large ingots and blocks (>1Tonne). Detailed analysis of the sizes and forms and related tonnages that were put on the market shows that < 0.01% of the massive forms would fall in the category 0.3-1cm; sizes smaller than this range are only observed in the powder/dust range (<1mm) (IZA-Europe 2002). Furthermore, under normal handling and use, zinc in massive form is basically melted by the first line zinc users (e. g; galvanisers, alloyers, manufacturers of semi-finished products such as rolled zinc). The massive zinc is not polished, ground, machined or handled in any other way that may give rise to small (e. g; <1mm) particles. The CLP guidance allows for deviation from the default particle size of 1mm for massive forms for classification, if justified. The elements mentioned under this item demonstrate that 2.1mm size particles are not at all representative for the massive forms of zinc produced and put on the EU market.

In conclusion, considering the elements above, zinc metal in massive form is not classified for chronic aquatic effect.

General discussion

A basic assumption made in this hazard assessment and throughout this CSR, (in accordance to the same assumption made in the EU RA process) is that the ecotoxicity of zinc and zinc compounds is due to the Zn^{++} ion. As a consequence, all aquatic, sediment and terrestrial toxicity data in this report are expressed as “zinc”, not as the test compound as such, because ionic zinc is considered to be the causative factor for toxicity. A further consequence of this is that all ecotoxicity data obtained on different zinc compounds, are mutually relevant for each other. For that reason, the available ecotoxicity databases related to zinc and the different zinc compounds are combined before calculating the PNECs. The only way zinc compounds can differ in this respect is in their capacity to release zinc ions into (environmental) solution. That effect is checked eventually in the transformation/dissolution tests and may result in different classifications.