# Counter-ion effects in ecotoxicity testing of inorganic substances

## 1. Introduction

The effect of a metal or metalloid to aquatic, terrestrial or sediment test organisms is in most cases assessed by exposing the organism(s) to increasing doses of a soluble salt of the metal of interest, preferably in standard toxicity tests. Depending on the assay, the test medium can be a natural or reconstituted/artificial water, soil or sediment or a specifically prepared test medium (e.g. OECD 201 algal growth medium; OECD; 2011).

The metal salt will dissolve in the aquatic test medium or the water phase of the soil or sediment. During this process, the metal ion (which can be anionic or cationic) and the counter-ion separate. The test organism(s) will thus simultaneously be exposed to the metal ion, the counter-ion, and some proportion of the undissociated complex, and the observed effect will be the combination of the effects exerted by all.

It is widely acknowledged that the bioavailability and hence the toxicity of undissociated metal complexes is generally lower than this of the dissolved metal ions (e.g. Giller et al., 1998) and hence such substances are not considered for testing metal toxicity in the environment. This document therefore focuses on soluble metal salts that completely dissociate into its constituent ions upon dissolution.

Typical inorganic counter-ions are sodium, potassium, calcium, ammonium or magnesium for anionic metal salts (like antimonate, stannate, platinate or vanadate) and chloride, sulphate, hydrogen carbonate or nitrate for cationic metal salts (like silver, copper, zinc, lead, cobalt or nickel). Typical organic counter-ions are acetate, citrate, stearate or propionate. These counter-ions will be covered in the current manuscript. In case other counter-ions are used for ecotoxicity testing, a similar assessment needs to be performed and documented.

This document assesses under what conditions the counter-ion may contribute to the observed toxic effects in the aquatic, terrestrial or sediment compartment.

This document does not cover organometallics, i.e. compounds that contain at least one metal (like aluminium or cobalt) or metalloid (like antimony, arsenic, boron, germanium, silicon or selenium) covalently bonded to a carbon. For regulatory purposes, the definition of organometallics also includes other compounds identified as coordination complexes where the metal or metalloid has covalent-character bonds with oxygen, nitrogen, sulphur and/or phosphorus belonging to an organic moiety (OECD series on Testing and Assessment 2012 (OECD, 2017)), unless rapid and complete dissociation can be experimentally demonstrated using the 24-hours TDp screening test (OECD TG29 (OECD, 2002)).

## 2. Presence of counter-ions in environmental compartments

Similar to metal ions, the considered inorganic counter-ions often occur naturally in the environment. Their background concentrations vary depending on the site-specific geological, climatological and/or physicochemical conditions. In the below, an overview is given of typical background concentrations of these counter-ions in the aquatic, terrestrial and sediment compartment.

The organic counter-ions considered are also present in natural environments as part of the natural nutrient cycling. Their concentrations vary widely in time and with location, and are related to for instance to climatological conditions (like temperature or rainfall) and biotic activity (active and/or passive excretion by plants or micro-organisms) (Badri and Vivanco, 2009; Marschner et al., 2011).

## a. Natural waters and aquatic test media

Most inorganic counter-ions are naturally occurring in waters in significant concentrations. This is well illustrated in the FOREGS Database (Salminen et al., 2005), where an overview is given of the chemical composition of more than 800 European natural waters. A summary of the information for some typical inorganic counter-ions is provided in Table 1. As illustrated in that table, the concentrations for common inorganic counter-ions vary 20 to 1000-fold in European waters.

***Table 1: Ionic concentrations in European natural waters and selected test media.***

| **Element** | **Natural waters (taken from FOREGS) Ionic concentrations in mg/L*** | | | **Test media (ionic concentrations in mg/L*)** | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | **OECD201 - algae** | | **OECD202 - daphnids** | | **OECD29 - T/D test at pH6** |
| | **10th percentile** | **Median** | **90th percentile** | **USEPA AAP** | **OECD201 /ISO8692** | **ISO 6341** | **Elendt M4** | **10x diluted ISO6341** |
| Ca | 2.8 | 40.2 | 119 | 1.2 | 4.9 | 80.8 | 80.7 | 8.1 |
| K | 0.3 | 1.6 | 6.83 | 0.5 | 0.4 | 3.0 | 3.2 | 0.3 |
| Mg | 0.8 | 6.02 | 27.3 | 2.9 | 2.9 | 12.2 | 12.2 | 1.2 |
| Na | 1.3 | 6.58 | 25.7 | 11.0 | 13.7 | 17.7 | 19.4 | 1.8 |
| $NH_4$ | *NR* | *NR* | *NR* | *na* | 5 | *na* | *na* | *na* |
| $SO_4$ | 1.8 | 16.1 | 103 | 5.7 | 5.8 | 48.0 | 48.0 | 4.8 |
| Cl | 0.8 | 8.81 | 43.6 | 6.5 | 22.9 | 145.6 | 145.5 | 14.6 |
| $NO_3$ | <0.04 | 2.82 | 28.2 | 18.6 | *na* | *na* | 0.2 | *na* |
| $HCO_3^{\$}$ | *9.7* | *126* | *339* | 10.9 | 36.3 | 47.0 | 47.1 | 4.7 |
| $PO_4$ | *NR* | *NR* | *NR* | 0.6 | 0.9 | *na* | 0.2 | *na* |
| pH | 6.4 | 7.7 | 8.3 | 7.5 | 8.1 | 6-9 | 6-9 | 6 |

**except pH*

*$^{\$}$reported as alkalinity*

*NR = not reported, na = not added to the medium*

Selected test media compositions (OECD, 2002; OECD, 2004; OECD, 2011) are also included in table 1. The composition of test media, as defined in internationally accepted test guidelines, typically mimic the composition of the natural habitat of the test organism considered. The variation in ionic concentrations between the different test media relates to the specific ionic conditions often choosen for optimising test organism performance, and are within the expected variability of natural background concentrations. The chloride concentrations in the ISO6341 and Elendt M4 medium are higher than the reported typical Cl-concentrations in natural waters.

*In some experimental studies, ionic conditions are intentionally outside the natural concentrations or recommended ranges. Examples hereof are studies to develop metal bioavailability models. However, for most studies, ionic conditions are within the ranges of natural waters or comply with the test guidelines.*

With regard to ammonia as a counter-ion, the actual concentration and speciation should be determined as both unionized ($NH_3$) and ionized ($NH_4^+$) forms are present in aqueous solutions. Firstly, ammonia in water volatilizes, and this process is highly pH-dependent. Secondly, also the speciation of ammonia in water is pH dependent ($pK_a$=9.25). The dominant form of ammonia at environmental relevant pH-levels (6.5 – 8) is $NH_4^+$.

Similarly, phosphates can be present in aquatic solution as $H_3PO_4$, $H_2(PO_4)^-$, $H(PO_4)^{2-}$ and $PO_4^{3-}$ depending on pH. However, at environmental relevant pH-levels (6.5 – 8), $H_2(PO_4)^-$ and $H(PO_4)^{2-}$ are the dominant species ($pK_a$=7.2). Although salts of a metal with the various phosphate species exist (example: $NaH_2(PO_4)$, $Na_2H(PO_4)$ and $Na_3(PO_4)$), the released phosphate-anion upon dissolution will rapidly respeciate to a similar phosphate-species depending on the solution pH.

The organic counter-ions considered in this document are not added to aquatic test media. Nevertheless, they can be present at detectable levels as a result of the biotic activity of the test organisms (for instance algal exudates). If available, they might affect metal speciation hence toxicity.In natural waters, the organic matter concentrations vary widely. Reported concentrations for Europe are:

-0.25-75 mg C/L in upstream rivers,

-0.6-47 mg C/L in rivers, and

-0.7-45 mg C/L in lakes (Mostafa et al., 2015).

The concentration and composition vary widely depending on for instance geography, geology, climate and biotic activity (Mostafa et al., 2015). The organic counter-ions considered in this docuement will thus also be present at varying concentrations depending on for instance location and timing of the sampling. However, all organic counter-ions are considered to degrade rapidly, irrespective of their presence as organic acid or deprotonised anion, as illustrated in the REACH dossiers of their Na, K, Mg or Ca salts.

## b. Terrestial compartment

Inorganic counter-ions occur naturally in soils. Many of these ions are also applied to soil via fertilizers.

The natural background concentrations of Ca, Mg, K and Na in approximately 2000 arable European soils are summarised in table 2 (data taken from GEMAS http://gemas.geolba.ac.at). However, it is generally accepted that ions in *soil solution* are the main species available to most common test organisms (Giller et al., 1998).

Typical background ranges of ionic concentrations in *soil solution* (taken from 13 European soils covering a wide range of soil physico-chemical properties) are included in table 2 (taken from Thakali et al., 2006). Concentrations in *soil solution* are only a fraction of the total concentration of the ion in soil, and are linked to the distribution of ions between the solid and solution phase. This is mainly governed by the mineral constituents, particle size and pH of the soil.

***Table 2: Ionic concentrations in European arable soils (data taken from GEMAS, http://gemas.geolba.ac.at) and soil solutions (data taken from Thakali et al., 2006).***

| **Element** | **Total concentrations in mg/kg dw (except for pH)** | | | ***Soil solution* concentration in mg/L (except for pH)** |
|---|---|---|---|---|
| | **10th percentile** | **Median** | **90th percentile** | **Median (range as min – max; n=13)** |
| Ca | 906 | 3035 | 71146 | 52 (2.7 – 870) |
| K | 342 | 1250 | 3137 | 26 (9.4 – 239) |
| Mg | 614 | 2860 | 9040 | 12 (1.1 – 105) |
| Na | 17 | 48 | 144 | 15 (7.5 – 39) |
| pH | 4.4 | 5.8 | 7.4 | 5.5 (3.9 - 8.0) |

The concentrations of inorganic anions in European soils have not been determined systematically or are not publically available. As a proxy, concentrations of these anions in 1785 bottled waters (groundwaters) has been reported in table 3 (data taken from Reimann and Birke, 2010). The composition of the *soil solution* will in most cases differ from the underlying groundwater due to the specific biogeochemical conditions (like interactions with the soil solid phase or gas phase or soil biological activities) at the surface compared to deeper layers (where groundwater is typically located) (Nieminen et al., 2013) . Movement of water from the surface (*soil solution*) towards deeper layers (*groundwater*) or vice versa will as such imply different biological and physicochemical conditions, affecting its composition. Despite these differences, the data for bottled waters give a good impression of typical concentration ranges and variability that exist between soils and regions.

***Table 3: Ionic concentrations in 1785 European bottled waters (data taken from Reimann and Birke, 2010).***

| **Element** | **Total concentrations in mg/L*** | | | |
|---|---|---|---|---|
| | **10th percentile** | **Median** | **90th percentile** | **Max** |
| Cl | 2.4 | 19 | 180 | 3627 |
| $NO_3$ | <1 | 1.3 | 9.2 | 995 |
| $SO_4$ | 3.4 | 30 | 447 | 20342 |
| $HCO_3^{\$}$ | 1.4 | 4.7 | 17 | 264 |
| $PO_4$ (as P) | <6.5 | 45 | 93 | 2824 |

*except $HCO_3^-$ - reported as alkalinity [in mEq/L]

Similar to the inorganic counter-ions, also organic counter-ions are present in *soil solution* at a wide concentration range. Thakali et al. (2006) reported total dissolved organic carbon (‘DOC’) concentrations in *soil solution*s of 13 European soils of 7.2 - 1085 mg C/L. In a more detailed fractionation analysis of organic carbon in *soil solution*s, concentrations up to:

- 1 mM for monocarboxylic acids like acetic or propionic acid or
- 50 µM for di/tricarboxylic like citric acid

were reported by Adeleke et al. (2017). Concentrations of individual organic acids differ largely between soils and sampling times, as they depend on a complex interaction of for instance geology, climate, land use and biotic activity. On top, all these organic molecules degrade rapidly as part of the natural nutrient cycling process (cfr. REACH dossiers) and natural degradation of one organic counter-ion implies the formation of another organic molecule. One example is the degradation of citric acid whereby acetic acid can be released.

## c. Sediment compartment

The counter-ions of most commonly used metal salts occur naturally in sediments. The natural background concentrations of Ca, Mg, K and Na in approximately 850 stream sediments are summarised in table 4 (FOREGS, http://weppi.gtk.fi/publ/foregsatlas/articles/Statistics.pdf).

***Table 4: Ionic concentrations in European streamwater sediments (data taken from FOREGS, http://weppi.gtk.fi/publ/foregsatlas/articles/Statistics.pdf).***

| **Element** | **Total concentrations in mg/kg dw** | | |
|---|---|---|---|
| | **10th percentile** | **Median** | **90th percentile** |
| Ca | 357 | 1665 | 11935 |
| K | 888 | 1669 | 2665 |
| Mg | 30 | 724 | 1990 |
| Na | 223 | 668 | 1855 |

In another study, physico-chemical properties of 84 European stream sediments have been determined. The pore water pH varied between 5.3 - 8.2 with a median value of 7.3 (Burton et al., 2009). As far as we are aware, there are no further systematic screening data for typical sediment pore water concentrations available. However, it is reasonable to assume that, comparable to soils, the concentrations of the anionic and cationic counter-ions are a fraction of the total concentrations in the sediment and that concentrations vary with for instance geological conditions, hydrology, climatological conditions, biotic activity and the overlying water.

Total organic carbon content in 84 European stream sediments was 0.13 – 17% with a median value of 1.9 (Burton et al., 2007). A monitoring study of organic acids in pore water in four European showed mean concentrations of

-acetic acid: 0.006 - 7.4 mg C/L,

-citric acid: 0.7 - 29 mg C/L, and

-propionic acid: 0.03 - 5 mg C/L (Ciskova et al., 1999).

Again, all these organic molecules degrade rapidly, irrespective of their presence as organic acid or deprotonised anion, as part of the natural nutrient cycling process. This has been included in the REACH dossiers of their Na, K, Mg or Ca salts.

# 3. Toxicity of counter-ions in natural compartments

## a. Toxicity of counter-ions in the aquatic environment

There are several peer-reviewed publications reporting the acute and chronic toxicity of typical inorganic counter-ions towards algae, invertebrates or fish. Examples are the studies of Elphick et al. (2011), Erickson et al. (2017), Mount et al. (1997,2016) and Simmons (2012). These authors show that acute and chronic toxic thresholds of typical inorganic counter-ions are in the mg/L-range, for some even in the g/L-range. As illustration, the toxicity data of Mount et al. (1997) are summarised in table 5.

***Table 5: Mean LC50 values for selected test organisms (in mg/L; data taken from Mount et al., 1997)***[1]

| | ***Ceriodaphnia*** | ***Daphnia magna*** | **Fathead minnow** |
|---|---|---|---|
| NaCl | 1960 | 4770 | 6390 |
| $Na_2SO_4$ | 3080 | 4580 | 7960 |
| $NaHCO_3$ | 1020 | 1640 | <850 |
| KCl | 630 | 660 | 880 |
| $K_2SO_4$ | <680 | 720 | 680 |
| $KHCO_3$ | 630 | 650 | <510 |
| $CaCl_2$ | 1830 | 2770 | 4630 |
| $CaSO_4$ | >1910 | >1970 | >1970 |
| $MgCl_2$ | 880 | 1330 | 2120 |
| $MgSO_4$ | 1770 | 1820 | 2820 |

The low toxicity of typical inorganic counter-ions (reported in table 5) is supported by the information that is provided in the REACH registration dossiers for simple inorganic salts like sulphates, chlorides, carbonates or nitrates. In table 6, the Ecotoxicity Reference Values (ERV) for freshwater are reported. These ERVs are recalculated from the reported PNEC values in freshwater and the assessment factor (AF) applied to calculate this PNEC. ERV values are preferred to PNEC values, to ensure a level playing field between data-rich metals (with typically Species Sensitivity Distribution (‚SSD')-type approaches implying a low AF) and data-poor metals (where an AF up to 1000 might be required for PNEC dervation).

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[1] This study is included to illustrate the ‚low' toxicity of salts of typical inorganic counter-ions. The data are supported/confirmed by a series of other publications listed in this paper. These data are however not considered for the final assessment of potential counter-ion effects, and therefore included without elaborated discussion.

***Table 6: ERV values in freshwater (in mg/L) for salts of major cations with inorganic and organic counter-ions, derived as reported PNEC values in freshwater x assessment factor (values in bold). Assessment Factor ('AF') is added between brackets. CAS number per source substance is added, plus the source data underlying the ERV specified as test species (fish ('F'), Invertebrates ('I) or algae ('A')) and test duration (acute ('a') or chronic ('c') (source: ECHA Dissemination website dd March 2020).***

| | Inorganic counter-ions | | | | Organic counter-ions | | | |
|---|---|---|---|---|---|---|---|---|
| | **Chloride** | **Sulphate** | **Hydrogen carbonate** | **Nitrate** | **Acetate** | **Citrate** | **Stearate** | **Propionate** |
| **Na** | **250** (AF50) | **1109** (AF100) | NHI | NHI | **100** (AF1000) | **440** (AF1000) | NR | NHI |
| | *CAS 7647-14-5* | *CAS 7757-82-6* | *CAS 144-55-8* | *CAS 7631-99-4* | *CAS 127-09-3* | *CAS 68-04-2* | | *CAS 137-40-6* |
| | F / c | I / c | | | F / a | F / a | | |
| **K** | **100** (AF1000) | **680** (AF1000) | NHI | NHI | **460** (AF1000) | **440** (AF1000) | NR | * |
| | *CAS 7447-40-7* | *CAS 7778-80-5* | *CAS 298-14-6* | *CAS 7757-79-1* | *CAS 127-08-2* | *CAS 866-84-2* | | |
| | A / c | F / a | | | I / a | F / a | | |
| **Ca** | NHI | NHI | NR** | NHI | **960** (AF1000) | **440** (AF1000) | NHI | NHI |
| | *CAS 10043-52-4* | *CAS 7778-18-9* | *CAS 471-34-1* | *CAS 10124-37-5* | *CAS 62-54-4* | *CAS 813-94-5* | *CAS 1592-23-0* | *CAS 4075-81-4* |
| | | | | | F / a | F / a | | |
| **Mg** | **321** (AF100) | **680** (AF1000) | NR** | NHI | **87** (AF1000) | **440** (AF1000) | NR | NR |
| | *CAS 7786-30-3* | *CAS 7487-88-9* | *CAS 546-93-0* | *CAS 10377-60-3* | *CAS 142-72-3* | *CAS 3344-18-1* | | |
| | I / c | F / a | | | F / a | F / a | | |
| **$NH_4$** | **2.5** (AF10) | **3.1** (AF10) | **3.7** (AF10) | NHI | **154** (AF50) | NHI<br>*CAS* | NR | NHI |
| | *CAS 12125-02-9* | *CAS 7783-20-2* | *CAS 1066-33-7* | *CAS6484-52-2* | *CAS 631-61-8* | *3458-72-8* | | *CAS 17496-08-1* |
| | I / c | I / c | I / c | | F / c | | | |

*NR: Not Registered*

*NHI: No Hazard Identified; $PNEC_{fw}$ not derived/reported under REACH as no aquatic hazard has been identified from experimental data. The dessimination website refers in most cases that data do not indicate an effect at concentrations below 100 mg/L, and that no environmental classification and labelling is triggered.*

** no PNEC or justification identified on ECHA Dissemination Website*

*** Calcium carbonate and magnesium carbonate are registered (the hydrogencarbonate salts are not). Both are registered as being "not hazardous to the aquatic environment" and no PNEC has been derived. A similar conclusion will likely apply to the hydrogen carbonate salt considering the chemistry and expected behaviour/effects of both salts*

The inorganic salts covered in the tables 5 and 6 can be considered of relatively low toxicity to typical freshwater test species, as shown by the natural water concentrations (cfr. table 1) compared to:

- the experimental test data of Mount et al. (1997 – cfr. table 5) as well as
- the Ecotoxicity Reference Values (ERV) recalculated as ERV = PNEC x AF using the reported PNEC and AF in the EU REACH registration dossiers (cfr. table 6).

The ERVs s for the inorganic metal salts vary between 100 mg/L (for KCl) and 1100 mg/L (for $Na_2SO_4$).

*The ERVs mentioned for each source substance in above table 6 can be considered as chronic ERV since they are based on chronic data, or are used as surrogate for chronic ERV in absence of chronic tox data.*

With regard to ammonia, $NH_4^+$ is the most abundant species at ecologically relevant pH values whereas $NH_3$ is considered as the most toxic form. As the relative abundance of $NH_3$ increases with pH, also the toxicity of total ammonia (i.e. $NH_3$ and $NH_4^+$ combined) will increase with pH. Reported acute toxicity ($LC_{50}$) of un-ionised $NH_3$ for different invertebrates ranges between 1 and 10 mg/L and for saltwater and freshwater fish these values are in the 0.3 to 3 mg/L range (data taken from ECHA dissemination website). Total ammonia concentrations (i.e. $NH_3$ and $NH_4^+$ combined) however, would be (much) higher than these values as also ionised $NH_4^+$ needs to be added.

Metal phosphates are often poorly soluble in aquatic media at ecologically relevant pH. There is however always a fraction of the phosphate salt that dissociates. Dissolution might vary with pH, and a rapid respeciation between the various phosphate ionic species ($PO_4^{3-}$ … $H_3PO_4$) will occur depending on solution pH. The dissolved phosphate concentration might thus be orders of magnitude below the total P concentration. The toxicity of a metal phosphate salt has been shown to be comparable between phosphate ionic species with the same cation (e.g. $M_a(PO_4)_b$ vs $M_c(HPO_4)_d$ vs $M_e(H_2PO_4)_f$; Kim et al., 2013; OECD, 2007). Phosphate is a nutrient for biota and excessive concentrations in solution might give rise to algal blooms and consequent eutrophication and oxygen depletion of the water body.

By comparing these toxic thresholds with the ionic concentrations in natural waters or test media, it can safely be assumed that the concentrations of inorganic counter-ions in the aquatic test media have negligible contributions to toxicity for the organisms typically tested in ecotoxicity tests (except for some studies where extreme conditions are intentionally generated).

Some metals are tested as organic metal salts (such as citrate, acetate, propionate or stearate salts). These metal salts dissociate rapidly and completely in the metal ion and the organic counter-ion when dissolved in the test medium (*in contrast to organometallic salts, which are not covered in this assessment*). As indicated in Section 2a, these organic counter-ions are typically present in natural waters at variable but generally very low concentrations (because of rapid biodegradation), and are not added to artificial test media. The aquatic toxic concentrations reported in the REACH dossiers (calculated as PNEC x AF) are again in the high mg/L-range varying from 87 mg/L (for Mg acetate) to 960 mg/L (for Ca acetate). For propionate and stearate, a comparable low aquatic toxicity is expected, based on information from other assessments (OECD, 2007; OECD, 2014; ECCC, 2017; ECCC 2018). Therefore, also for the organic counter-ions, it can safely be assumed that the concentrations of organic counter-ions in the aquatic test media have negligible contributions to toxicity.

## b. Soil compartment

The standard approach in convential toxicity testing is to spike soils with soluble metal salts (cfr. risk assessments for several metals under the Existing Substances Regulation (EEC) No 793/93). However, together with the metal content of the soil, the ionic strength of the *soil solution* increases and the soil pH decreases. This is explained by the addition of the counter-ions and the displacement of protons and other ions from the binding sites of the soil constituents. These changes in pH and ionic strength can affect the biological response either directly or indirectly (through their effect on metal bioavailability) as shown for metal cations such as Zn and Pb (Speir et al., 1999; Stevens et al., 2003; Bongers et al., 2004) and metal oxyanions such as antimonate and molybdate (Oorts and Smolders, 2009; Buekers et al., 2010). The effect of counter-ions cannot easily be distinguished from the effect of ions displaced from the solid phase by the added metal since they both contribute to the increase in salinity of the soil pore water.

Anionic counter-ions such as $Cl^-$, $NO_3^-$, $HCO_3^-$ and $SO_4^{2-}$ poorly adsorb to solid phases and hence are rather mobile in soil. Moreover, nitrogen, sulfur and carbon species can be readily transformed by soil microorganisms as part of the natural element cycles.

Effects of changing pH and ionic strength with increasing metal concentration are not observed in field contaminated soils because of the natural leaching of the excess cations and anions. For laboratory testing, artificial leaching of metal salt amended soils before toxicity testing results in clear reduction of toxicity (Oorts et al. 2006,2007; van Gestel et al., 2012, Smolders et al., 2015) and removes potential bias of metal toxicity due to the counter-ions (Bongers et al., 2004).

For a number of metals, correction factors have been derived to directly account for these confounding effects on metal toxicity following spiking with soluble salts (Smolders et al., 2009, OECD 2017). These so-called ‘Leaching-Ageing factors’ are determined at

- 1 for cadmium,
- 1-3 for nickel and 1.1-3.5 for cobalt (increasing as function of pH),
- 2 for copper,
- 3 for zinc and
- 4.2 for lead.

Toxicity data to soil organisms are scarce for most common counter-ions due to their low hazard profile. A study on comparative effects of chloride and sulphate salts of sodium, potassium, calcium, and magnesium on ammonification and nitrification in soil only observed effects above 0.25% (i.e. 2500 mg/kg; Sindhu and Cornfield, 1967). The poor availability of soil ecotoxicity data for these counter-ions also reflects on the reported ERV values for soil (recalculated from the reported PNEC values in soil and the assessment factor (AF) applied to calculate this PNEC) in the REACH registration dossiers for simple inorganic salts (table 7).

***Table 7: ERV values in soil (in mg/kg) for salts of major cations with inorganic and organic counter-ions, derived as reported PNEC values in soil x reported assessment factor, <u>or</u> the values derived using an Equilibrium-Partitioning approach (EqP; ECHA Dissemination website) (Values in bold). Assessment Factor (‘AF’) per source substance is added between brackets. For CAS number per source substance, reference is made to Table 6. Per source substance, the source data underlying the ERV specified as test species (plant (‘P’)) and test duration (chronic (‘c’)) is added (source: ECHA Dissemination website dd March 2020). If an Equilibrium Partitioning method (‘EqP’) is used, the underlying data are those mentioned in table 6***.:

| | Inorganic counter-ions | | | | Organic counter-ions | | | |
|---|---|---|---|---|---|---|---|---|
| | **Chloride** | **Sulphate** | **Hydrogen carbonate** | **Nitrate** | **Acetate** | **Citrate** | **Stearate** | **Propionate** |
| **Na** | 243 (AF50) | 1.54 (EqP) | NHI | NHI | NHI (*value of 0 reported (EqP)*) | 33.1 (EqP) | NR | NHI |
| | P / c | | | | | | | |
| **K** | * | Insufficient data | NHI | NHI | 0.002 (EqP) | 33.1 (EqP) | NR | * |
| | | | | | | | | |
| **Ca** | NHI | NHI | NHI** | NHI | 0.154 (EqP) | 33.1 (EqP) | NHI | NHI |
| | | | | | | | | |
| **Mg** | 662.77 (EqP) | Insufficient data | NHI** | NHI | 0.0004 (EqP) | 33.1 (EqP) | NR | NR |
| | | | | | | | | |
| **NH$_4$** | 507 (AF10) | 626 (AF10) | 749 (AF10) | NHI | 0.72 (EqP) | NHI | NR | * |
| | P / c | P / c | P / c | | | | | |

*NR: Not Registered*
*NHI: No Hazard Identified; PNEC not derived/reported under REACH as no aquatic hazard has been identified from experimental data for aquatic organisms.*
** no PNEC/justification provided under REACH*
*** Calcium carbonate and magnesium carbonate are registered (and not the hydrogencarbonate salts), and both are registered as being not hazardous to the aquatic environment and no PNEC has been derived. A similar conclusion will likely apply to the hydrogen carbonate salt considering the chemistry and expected behaviour/effects of both salts*

Based on the identified soil testing data, it is concluded that the toxicity of typical inorganic counter-ions varies between 250 mg/kg (for sodium chloride) and 750 mg/kg (for ammonium hydrogen carbonate). If no soil test data are available, an Equilibrium Partitioning (‘EqP’)-based approach can be used as alternative to estimate the effects in soil. This EqP approach is based on freshwater toxicity data and a conservative soil:water distribution coefficient (Kd).

*The ERVs mentioned for each source substance in above table 7 can be considered as chronic ERV since they are based on chronic data, or are used as surrogate for chronic ERV in absence of chronic tox data.*

Using EqP-based approaches, a value of 1.54 mg/kg is derived for sodium sulphate. However, for this substance the aquatic toxicity thresholds are above 1000 mg/L, and the reported value by Sindu and Cornfield (1967) is in the g/kg-range. As such, there is no reason to assume that soil biota would be extremely sensitive to sodium sulphate considering its ubiquous presence in the environment and essentiality for biota. A similar conclusion was made in the OECD HPV assessment for this substance (OECD, 2005). Moreover, the soil specific toxic thresholds for sodium chloride (as alternative sodium salt) and ammonium sulphate (as alternative sulphate salt) are 250 and 630 mg/kg, respectively. Considering that all these salts rapidly and completely dissociate in water or *soil solution* under normal environmental conditions, it is concluded that the reported $PNEC_{soil}$ value for sodium sulphate is overconservative, and will likely be >250 mg/kg.

For the effects of the nitrate anion, reference is made to the OECD HPV (High Production Volumes) Chemicals-program where several nitrates have been assessed simultaneously. The conclusion was that none of the nitrate salts assessed were of concern to the environment (OECD, 2007)

For none of the organic counter-ions, reliable soil test data have been identified in the REACH dossiers. Instead, the PNEC values are derived by the EqP approach. Also these salts dissociate rapidly and completely when added to water or *soil solution*. Looking at the data of inorganic Na, K, Mg, Ca and $NH_4^+$ salts, it was concluded that these cations had a low toxicity to soil biota (cfr. table 7). The organic counter-ions, included in table 7, are ubiquously present in soil as part of natural nutrient cycling and are rapidly degrading in soil. The relevance of these EqP-based values as $PNEC_{soil}$ value is low because they are generally based on the poor information on the distribution coefficients (for instance data for undissociated organic acids are used for their salts). Looking at alternative assessments, citric acid (OECD, 2001), acetic acid (included in assessment of Ethyltriacetoxysilane; OECD, 2012), propionic acid (OECD 2007) and stearic acid (OECD, 2014) were all concluded as being of low toxicity to the environment with aquatic threshold values for the three trophic levels in the mg/L to g/L range. Taken together, it can be confidently concluded that the reported $PNEC_{soil}$ values for the organic counter-ion are too low due to the limitations of the EqP method, and will most likely be orders of magnitude higher when being generated via soil specific testing.

## c. Sediments compartment

Similar to soils, also sediment spiking procedures can alter sediment characteristics significantly. Consequently, the added metal may not behave in an environmentally realistic manner for reasons such as the contribution of excess cations in pore water to toxicity due to acidification reactions (Doig and Liber, 2006; risk assessments for several metals under the Existing Substances Regulation (EEC) No 793/93)). In order to mitigate these pH effects, pH adjustments are quite often performed by adding base or acid, which might give rise to a further build up of ionic strength in the porewater ('salt effect'; Brumbaugh et al., 2013). These effects of increased salinity and ion displacements can be minimized if precautionary steps are taken in the spiking protocol to achieve more stable and environmentally realistic partitioning of metals in spiked sediments. For example, a two stage spiking method can be used where a pH adjusted 'super-spike' is equilbrated for 4 weeks before it is further diluted using increased proportions of unspiked sediment (Brumbaugh et al., 2013). However, spiking methods alone cannot be expected to produce environmentally realistic metal partitioning when spiking levels approach the limits of the sediment binding capacity. To effectively minimize the "excess" spiked metal (and sodium ions from pH adjustment), a high frequency of overlying water volume additions per day and a 6 to 7 day pre-test equilibration for sediment toxicity testing may further reduce any bias from counter-ion toxicity.

Due to their low hazard profile (as reflected in the soil and aquatic ecotox dataset) and the above reported difficulties associated with sediment testing, toxicity data for common inorganic and organic salts to sediment organisms are scarce. This also reflects on the reported ERV values for sediment in the REACH registration dossiers, which are mostly derived by an EqP-based approach from freshwater toxicity data (table 8).

***Table 8: ERV values in sediment (in mg/kg) for salts of major cations with inorganic and organic counter-ions, derived as reported PNEC values in sediment x reported assessment factor, or the values derived using an Equilibrium-Partitioning approach (EqP; ECHA Dissemination website) (values in bold). Assessment Factor ('AF') per source substance is added between brackets. For CAS number per source substance, reference is made to Table 6. Per source substance, the source data underlying the ERV specified as test species (invertebrates ('I')) and test duration (chronic ('c')) is added (source: ECHA Dissemination website dd March 2020). If an Equilibrium Partitioning method ('EqP') is used, the underlying data are those mentioned in table 6***.

| | **Inorganic counter-ions** | | | | **Organic counter-ions** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Chloride** | **Sulphate** | **Hydrogen carbonate** | **Nitrate** | **Acetate** | **Citrate** | **Stearate** | **Propionate** |
| **Na** | NE | 40.2 (EqP) | NHI | NHI | NHI (*value of 0 reported (EqP)*)) | 34.6 (EqP) | NR | NHI |
| | | | | | | | | |
| **K** | * | Insufficient data | NHI | NHI | 0.002 (EqP) | 34.6 (EqP) | NR | * |
| | | | | | | | | |
| **Ca** | NHI | NHI | NHI** | NHI | 0.726 (EqP) | 34.6 (EqP) | NHI | NHI |
| | | | | | | | | |
| **Mg** | 288.9 (EqP) | Insufficient data | NHI** | NHI | 0.0004 (EqP) | 34.6 (EqP) | NR | NR |
| | | | | | | | | |
| **$NH_4$** | NHI | 6.3 (AF100) | 13.3 (AF100) | NHI | 2.51 (EqP) | NHI | NR | * |
| | | I / c | I / c | | | | | |

*NR: Not Registered*

*NE: No Exposure of sediment expected*

*NHI: No Hazard Identified; $PNEC_{fw}$ not derived/reported under REACH as no aquatic hazard has been identified from experimental data.*

** no PNEC/justification provided under REACH*

*** Calcium carbonate and magnesium carbonate are registered (and not the hydrogencarbonate salts), and both are registered as being not hazardous to the aquatic environment and no PNEC has been derived. A similar conclusion will likely apply to the hydrogen carbonate salt considering the chemistry and expected behaviour/effects of both salts.*

*I = Invertebrates, c = chronic*

Sediment test data are only reported for ammonia sulphate and ammonia hydrogen carbonate. The toxic thresholds are 6.3 and 13.3 mg/kg, respectively. For none of the other counter-ions assessed in table 8, sediment specific test data are available. The $PNEC_{sediment}$ values of these counter-ions are derived using an EqP-based approach. Considering the values and argumentation in the aquatic and soil assessments of these counter-ions, there is no reason to assume that any of these would be significantly more toxic than ammonia sulphate or hydrogen carbonate. Therefore, also for the sediment compartment, it is concluded that the EqP-based values for $PNEC_{sediment}$ are not reliable, as they suffer from the same weaknesses as in the soil compartment assessment.

*The ERVs mentioned for each source substance in above table 8 can be considered as chronic ERV since they are based on chronic data, or are used as surrogate for chronic ERV in absence of chronic tox data.*

# 4. Conclusion

The concentrations of typical common inorganic counter-ions in natural waters and aquatic test media are in the mg/L-ranges. These concentration ranges are much lower than the toxic thresholds of these counter-ions. At the same time, these concentrations are much higher than the toxic concentrations for soluble metal ions that express toxicity (tables 1, 4 and 5), which are typically in the µg/l-concentration range.

In soils and sediments, the total concentrations of typical inorganic counter-ions are in the mg/kg-ranges, but the (bioavailable) concentrations in solution are only a fraction of the total concentration (typically in the mg/L for most cations and anions) and largely variable between soils, even with similar total ion concentration in soil or sediment.

For the purpose of assassing metal toxicity, a readily soluble metal salt is mostly applied to the compartment of interest at a proper dosing level. This dosing level depends on the toxicity of the metal, and can be from the µg/L or µg/kg-range for the most toxic metals to the high mg/L or mg/kg-range for the least toxic metals. Together with the metal ion, also the corresponding inorganic or organic counter-ion is added and it can reasonably be assumed that the likelihood of a (partial) contribution of the counter-ion to the observed effect increases with increasing metal salt dosing.

**Relative contribution of the counter-ion**

In order to estimate the contribution of the counter-ion to the observed effect, the following approach is proposed. Assuming additivity of the toxicity of the metal ion and the counter-ion, the corresponding Toxic Units (‘TU’) for the metal and the counter-ion are calculated as:

$$\mathrm{TU}i = \frac{[\,]i}{\mathrm{TT}i}$$

with $TU_i$ = Toxic Unit for ion *i*, $[\,]_i$ = concentration of ion *i* and $TT_i$ = Toxic Threshold (like ECx, NOEC…) for ion *i*.

The $TU_{(counter\text{-}ion)}$ needs to be considered with care, especially for the soil and sediment compartment, as reliable soil and sediment toxicity data are often limited (or even completely lacking). In these cases, metal toxicity data obtained using test methods that account for or minimize counter-ion effects (e.g. leaching, ageing, or salt control treamtents) may provide useful additional context.

The effect of the added counter-ion can be assumed negligible for the most toxic metals. In these cases, the $TU_{metal\text{-}ion} >> TU_{counter\text{-}ion}$ at concentrations where effects occur, and the contribution of the added counter-ions to the observed toxic response can be ignored.

In contrast, for metals with lower toxicity, $TU_{metal\text{-}ion}$ can be comparable to or lower than $TU_{counter\text{-}ion}$ at concentrations where effects occur. In this case, the effect of the added counter-ions to the observed toxicity should be investigated and addressed in the assessment.

The Multimetallic Database (MMDB) is a platform, developed under the supervision of Eurometaux, where multiple metal consortia share relevant information used for compliance with EU REACH. The MMDB contains as such also information of relevance for the purpose of the document, like the ERVs in the various environmental compartments. This information can be consulted to conclude on the relative toxicity of the metal of interest compared to the counter-ions.

**In general:**

**- the effect of the added counter-ion is assumed to be negligible if $\frac{\mathrm{TU(metal-ion)}}{\mathrm{TU(counter-ion)}} > 10$. In this case, the observed effect can be solely related to the metal ion.**

**- a more in-depth investigation is required if $\frac{\mathrm{TU(metal-ion)}}{\mathrm{TU(counter-ion)}} \leq 10$. In this case, the added counter-ion could significantly contribute to the observed effects, and further investigation of potential counter-ion effects is required.**

**As a reminder organo metals are excluded from this counter-ion guidance.**

**ILLUSTRATIVE EXAMPLES:**

Hypothetical metal X: ERV(freshwater) = 10 µg X/L (tested as XCl) , MW(X)=100
Hypothetical metal Y: ERV(freshwater) = 10 mg Y/L (tested as $YCl_3$), MW(Y)= 150
Chloride counter-ion: ERV(freshwater) = 47.5 mg Cl/L

(*recalculated from ERV(KCl);*
*MW(KCl) = 74.55 g/mol; MW(Cl) = 35.45 g/mol*
*ERV = 100 mg KCl/L (cfr. table 6) = (100/74.55) mM = 1.34 mM KCl*
*Upon complete dissolution, 1 mol KCl releases 1 mol Cl counterion.*
*As such, released amount of Cl at ERV(KCl)1.34 mM equaling (1.34 x 35.45) mg/L = 47.5 mg/L)*

Next, the **TU for both the metal ion and the counter-ion are calculated at ERV(metal)-level** as TU = concentration/ERV.

***Take care to express 'concentration' and 'ERV' for TU calculation of each ion in similar units, either weight (like g/L) or molar (like mol/L) based!***

For Metal X:
TU(X) = 10 / 10 = 1
Test compound was XCl. At ERV(X) level, equal amount of Cl to X has been released into the test medium.
Therefore: 10 µg X/L corresponds to (10 / 100 =) 0.1 **µ**M X. The same amount of Cl has been released in the test medium. The corresponding TU(Cl) = 0.1 µM / 1340 µM = $7.7 \times 10^{-5}$

Finally, the ratio of TU(X) / TU(Cl) is calculated as 1 / $7.7 \times 10^{-5}$ = 13400 (>> 10!).
***It is concluded that the observed effect at ERV level can be attributed to X only.***

For metal Y:
TU(Y) = 10/10 = 1
Test compound was YCl3. At ERV(Y) level, three times the amount of Cl to Y has been released into the test medium.
Therefore: 10 mg Y/L corresponds to (10 / 150=) 0.067 mM Y. Added amount of Cl at this level of Y equals (3 x 0.067 =) 0.2 mM Cl. The corresponding TU(Cl) = 0.2 mM / 1.34 mM = 0.15.

Finally, the ratio of TU(Y) / TU(Cl) is calculated as 1 / 0.15 = 6.7 (< 10!).
***It is concluded that the observed effect at ERV level CAN NOT be attributed to Y only, and that a further investigation of the potential effects of Cl is needed.***

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