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How lethal concentration changes over time : toxicity of cadmium, copper, and lead to the freshwater isopod *Asellus aquaticus*

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1 **Running head:** How LCx changes over time

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**How LCx changes over time: Toxicity of Cd, Cu and Pb to the freshwater
isopod *Asellus aquaticus***

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ABSTRACT

Metal pollution is a serious threat to environmental health. While the aquatic isopod *Asellus aquaticus* L. (Isopoda) is an important decomposer of freshwater ecosystems, very little research has reported its long-term or incipient lethal concentrations for metals. Moreover, the LCxs that can be found in the literature are often based on unmeasured concentrations, which could lead to a severe underestimation of the actual toxicity. In this exploratory study the 1-day, 4-day, 7-day and 14-day LC10, LC20 and LC50 of copper, cadmium and lead for adult *Asellus aquaticus* were determined. LCxs were calculated using the nominal concentrations, the effective concentrations and the free ion activities (FIAs). Incipient lethal values were determined as well. In general, surprisingly lower lethal concentrations than in the literature were found. Furthermore, the present study shows that lethal concentrations based on FIAs were generally much lower than nominal and effective concentrations. Although almost all LC values were higher than the environmental quality standards (EQS), the Cu 14-day LC10 and several (incipient) Pb LC10s and LC20s, all calculated with FIAs, were lower than the EQS. We conclude that lethal or effective concentrations based on FIAs provide a more realistic view, which is vital for environmental policy making.

Key words: Aquatic invertebrates; Environmental toxicology; Speciation; Trace metals; Incipient LCx

INTRODUCTION

Metals endanger aquatic life with anthropogenic pollution playing an important role, introducing metals in nature through, for example, emission at smelting activities, domestic waste water and metal containing pesticides and fertilizers. These trace metals can be divided in two groups. The first group are the non-essential metals, which disrupt normal metabolic processes. Cadmium, for example, is known to compete with zinc and calcium, inhibiting

uptake [1,2,3] whereas lead, on the other hand, disrupts calcium and sodium homeostasis [4]. The second group, the essential metals, are necessary to diverse biological functions (e.g., Cu). However, essential metals can become toxic as well when a certain threshold is exceeded, which varies depending on e.g., the water chemistry, the species and body mass [5,6]. While many proteins are copper-dependent, Cu can also inhibit sodium uptake and influence chloride absorption [5,7]. Therefore, it is important to know when these metals affect the health of aquatic organisms.

The crustacean *Asellus aquaticus* L. (Isopoda) is an epibenthic detritivore and can be found throughout the northern hemisphere [8]. These isopods are an important component of freshwater ecosystems by shredding leaf material and so providing energy for other organisms, but they are also a food source for both invertebrate predators and fish [9,10]. *Asellus aquaticus* has a one year life-cycle and is often used as a test species in *ex situ* and *in situ* metal toxicity studies [8,11,12]. This isopod is considered as relatively tolerant to organically polluted waters [8,13], but can be sensitive to trace metals [12]. Yet, very few papers have investigated long-term mortality effects of metals for *A. aquaticus* [14,15].

Some studies report LC50 values for unmeasured concentrations, which could lead to large deviations from reality. Martin & Holdich for example, calculated LC values based on nominal concentrations [16]. However, they observed significant precipitation of several metals and emphasized to consider this when examining their results. Sometimes measured concentrations were used [17,18], but to our knowledge none have used free ion activities (FIAs) of dissolved metals to determine LC values. Because free ions can bind to biological receptors and therefore represent the chemical reactivity, they are generally considered as one of the best predictors of toxic effects [19]. In fact, the Free Ion Activity Model (FIAM) or the Biotic Ligand model (BLM), an extension of the FIAM, are commonly used for the determination of LC50s [20,21]. The US Environmental Protection Agency has incorporated

the BLM in its aquatic life freshwater quality criteria for copper and is currently investigating its use for other metals [22].

In the present study, we determined the change of LC10s, LC20s and LC50s over time for Cd, Cu and Pb for adult *A. aquaticus* L. The LC values were calculated using the nominal concentrations, the effective concentrations and the FIAs. The aim of this study was to compare these data to LC values found in literature, which are mostly based on nominal values and could therefore lead to an underestimation of the actual impact of these metals. Additionally, neither in the U.S. Environmental Protection Agency ECOTOX knowledgebase [23] nor in the literature can LC10 or LC20 values of any metal be found for *A. aquaticus*. These are more vulnerable endpoints and thus important when chronic effects, such as growth or behaviour, are to be evaluated.

MATERIAL AND METHODS

Test animals

Individuals of *A. aquaticus* were taken from the Laakbeek in Lille, Belgium (51° 13' 51.5" N 4° 50' 52.4" E; Basin of the Scheldt River) with a pondnet (500 µm mesh, 200 x 300 mm frame and 500 mm bag depth) fitted to a 1.5 m handle. Lille is a small town in Flanders, located in a region of extensive agriculture. Metal concentrations of this river were measured in 2014 before the start of this experiment. We found very low concentrations of copper (4.58 ± 0.21 µg/L), cadmium and lead (both <0.1 µg/L). The isopods were acclimated for at least two weeks in 20 L glass aquaria filled with moderately hard water [24] under controlled light conditions (16:8 h light:dark photoperiod) and temperature (15 ± 1 °C) in a climate chamber type WT15'+5DU-WB (Weiss Technik). Exposures occurred in the same chamber under the same conditions. As food for *A. aquaticus*, we dried alder leaves (*Alnus glutinosa*) and

submerged them in a bucket of aerated water from the Laakbeek, where they were ‘conditioned’ for at least 6 days [8]. During acclimation, the organisms were fed *ad libitum*.

Experimental design

During a 14-day laboratory experiment *A. aquaticus* was exposed to five concentrations of Cd, Cu and Pb (Table 1). Exposures occurred in polypropylene containers. Stock solutions were prepared in EPA moderately hard water [24] with analytical grade salts of cadmium chloride hydrate ($\text{CdCl}_2 \cdot \text{H}_2\text{O}$), copper chloride dihydrate ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$) and lead nitrate ($\text{Pb}(\text{NO}_3)_2$). All metals were purchased from Merck. Stock solutions were further diluted to reach the desired concentrations. Next, 500 mL was poured in each polypropylene container and a net (1 mm mesh) was placed on the bottom of each container to ease the removal of the isopods. A control treatment with moderately hard water was added. Experiments were executed in triplicate. The containers were left to equilibrate for 24 h.

Ten individuals of *A. aquaticus* (size range: 7 - 9 mm) were introduced in each container. General water characteristics were monitored daily (240 measurements): $T = 15 \pm 1$ °C; $\text{DO} = 8.68 \pm 0.03$ mg/L; $\text{pH} = 7.72 \pm 0.03$; $\text{EC} = 321 \pm 2$ $\mu\text{S}/\text{cm}$; $\text{DOC} = 5.94 \pm 0.13$ mg/L. Since no food was provided in the containers, the animals were taken out of the containers after seven days using the net and fed for four hours with ‘conditioned’ alder leaves to avoid cannibalism [8]. Every day, mortality was assessed and dead animals (no response to gentle prodding) were removed from the solution.

At day 0, 1, 4, 7 and 14, water samples (50 mL) were taken from all containers with a syringe and filtered through a 0.20 μm filter (Chromafil). At day 0 and day 1, the metal concentrations were determined for only one of the replicates since no large variation was expected. At day 4, 7 and 14, samples of all replicates were analyzed. After taking the water samples, 50 mL of medium of the respective metal concentrations was added again. Dissolved

organic carbon was quantified in the filtered water samples using a TOC-analyzer (TOC-VCPH, Shimadzu Corporation). Next, part of the filtered water was acidified to 1% with trace-metal-grade HNO_3 (69%) to determine the trace metals and major ions with an inductively coupled plasma optic emission spectrometer (ICP-OES; Thermo scientific, ICAP 6300 Duo). Standard reference material 1640a (National Institute of Standards and Technology) was used throughout the analyses to assess the instrument performance. Concentrations of the major ions were 16.8 ± 0.1 mg/L Ca; 3.12 ± 0.06 mg/L K; 13.5 ± 0.1 mg/L Mg; and 29.3 ± 0.2 mg/L Na. Water samples were stored at -20°C until measured. As can be seen in Table 1, the copper and lead salts caused solubility issues which led to lower than expected effective concentrations. The free ion activities (FIAs) for the different metals were computed using the Windermere Humic Aqueous Model 6.0.13 (Natural Environment Research Council) in which 100% of the DOC was entered as fulvic acids [25]. Part of the DOC, however, may have been present as humic acids and other hydrophobic or hydrophilic fractions. Therefore, this might not incorporate all effects of DOC composition on metal toxicity [26].

Mortality data were corrected according to the following formula [27]:
$$\text{Corrected \%} = ((\% \text{ mortality in treated asellids} - \% \text{ mortality in control asellids}) / (100 - \% \text{ mortality in control asellids})) \times 100.$$
LC10s, LC20s and LC50s were calculated for the nominal, effective and free ion concentrations of the metals using the package drc [28] of the statistical program R 3.1.2 (R Development Core Team). We used the concentrations measured on day 0, 1, 4, 7 and 14 to determine their respective LC values. The changes of all LCxs were plotted in time. These toxicity curves approached asymptotes (parallel to the time axis) which are called the incipient LC values. This is the concentration below which 100-x% would live indefinitely relative to the lethal effects of the toxicant [29] and can be used, for instance, to determine the ideal exposure duration of these isopods. The incipient LCxs were determined

using the one phase decay equation in GraphPad Prism 7.00 for Windows (GraphPad Software).

RESULTS

No isopods of the control treatment died during the first 96 h. Mortality reached 6.67% after 7 days and 13.3% after 14 days. Due to the long exposure duration, this can be considered a chronic toxicity study, which is only invalid if mortality in the control treatments exceeds 20% [30]. Cumulative survival data of the different exposure treatments are shown in **Figure 1**. While several treatments of Cd and Cu were lethal, for Pb only the highest concentration caused severe mortality. This could be explained by the low effective concentrations and FIAs, compared to the nominal concentrations (Table 1). Consequently, estimations of LC values for Pb were less reliable and no standard error could be calculated in some cases.

The LC10 vs. time profile of the three metals shows a steep decline until day 4, after which the LC10s slowly decrease until a plateau is reached (**Figure 2**). The 1-day, 4-day, 7-day and 14-day LC10s for Cd were 177 µg/L, 6.64 µg/L, 3.49 µg/L and 0.91 µg/L for the nominal concentrations; 183 µg/L, 5.83 µg/L, 3.43 µg/L and 0.80 µg/L for the effective concentrations; and 131 µg/L, 4.87 µg/L, 4.99 µg/L and 1.31 µg/L for the FIAs. The 1-, 4-, 7- and 14-day LC10s of copper were 10914 µg/L, 932 µg/L, 773 µg/L and 332 µg/L for the nominal concentrations; 4070 µg/L, 593 µg/L, 512 µg/L and 271 µg/L for the effective concentrations; and 1788 µg/L, 71.4 µg/L, 44.8 µg/L and 6.16 µg/L for the FIAs. Lastly, we calculated the following 1-, 4-, 7- and 14-day LC10s for Pb: 159157 µg/L, 820 µg/L, 932 µg/L and 677 µg/L for the nominal concentrations; 31887 µg/L, 54.6 µg/L, 97.4 µg/L and 49.7 µg/L for the effective concentrations; and 11556 µg/L, 0.06 µg/L, 0.07 µg/L and 0.04 µg/L for the FIAs. The LC20s and LC50s for Cd, Cu and Pb, all followed the same decline as can be seen for the LC10s (**Table 2**). A clear difference was found between the nominal concentrations of Cu and

Pb resulting in the highest LCxs, while the FIAs produced the lowest LCxs. For example, the Cu 14-day LC50 of 853 µg/L based on the nominal concentrations is more than 30 times higher than the LC50 for the FIAs. For Cd on the other hand, the LCxs of nominal concentrations, effective concentrations, and even the FIAs after day 1, were similar. Estimations of the incipient LC values were determined and again, calculating the incipient concentrations of Pb proved difficult (Table 3). In general, incipient LC values calculated with the FIAs were lower. Almost all incipient concentrations were reached at 14 days. Only the incipient Cd LC50 of 5.27 µg/L calculated with FIAs was not reached at day 14, but was very similar to the LC50 of 6.38 µg/L.

DISCUSSION

This study was conducted to establish more accurate LC values for *A. aquaticus*. No papers could be found that calculated LC50 values based on FIAs. Moreover, both LC10 and LC20 values are not available in the literature, making it difficult to infer metal concentrations for chronic toxicity testing.

Since no LC10s or LC20s were found, only LC50 values can be compared (Table 4). Cadmium LC50s in the present study are generally much lower than values found in the literature. Braginsky and Shcherban found a more than tenfold higher 1-day LC50 (78450 µg/L) [31]. The 4-day LC50 determined by Martin and Holdich was 1320 µg/L [16]. This is three times higher than the one we calculated based on the effective concentrations. However, the metal concentrations in both studies were not measured. Green et al. used measured concentrations and found a 4-day LC50 of 600 µg/L similar to the 4-day LC50 of this study [32]. A study by Van Ginneken et al., set in the same conditions as the present study except for a higher exposure temperature, found a 10-day LC50 of 34.9 µg/L, comparable to our 7-day LC50 [17]. The 15-day LC50 of 61 µg/L reported by Ham et al. on the contrary, is most similar

to the 7-day LC50 of the present study, even though Cd concentrations were also measured [18]. For Cu, Martin and Holdich found a 4-day LC50 of 9210 µg/L, that is two times higher than the nominal 4-day LC50 of this study and seven times higher than the 4-day LC50 based on effective concentrations [16]. De Nicola Giudici et al. found a very similar 7-day LC50 of 1000 µg/L, although this was established for juveniles, known to be generally more sensitive than adults to metals [12,15,33]. For adult females however, they found a 14-day LC50 value of 5000 µg/L, which is fivefold higher than the nominal 14-day LC50 of this study. Yet again, metal concentrations of these studies were unmeasured. However, the 10-day LC50 of 2195 µg/L calculated by Van Ginneken et al. is also four times higher than the 14-day LC50 of the present study, even though both values were calculated using measured concentrations [17]. Lead LC50s for *A. aquaticus* in the literature are scarce. Van Ginneken et al. found a 10-day LC50 of 443 µg/L calculated with the measured concentrations, which is comparable to the 14-day LC50 in this study (677 µg/L for the effective concentrations) [17]. The Pb 4-day LC50 of the effective concentrations is similar to the 4-day LC50 of 64100 µg/L determined by Martin & Holdich [16].

The LC50s of this study were generally lower than the values found in the literature. This could be explained by interpopulation differences in sensitivity. It is possible that the animals from some studies were sampled from (heavily) metal-contaminated sites, therefore being less sensitive than the isopods used in the present study due to genetic adaptation [34,35]. Still, the LC50 values of these studies differed greatly from the present study and are not likely explained by the former reason alone. We also observed that many of the discussed studies used unmeasured metal concentrations. It is very likely that these were higher than the actual concentrations due to complex forming and precipitation. A similar pattern can be seen for the data of Cu and Pb in the present study. When examining the lethal concentrations based on FIAs, we found even lower values than for the nominal or measured concentrations, again

especially for Cu and Pb. This can be explained by WHAM 6 which assumes a larger affinity of Cu and Pb to dissolved organic matter than Cd [36]. The results in the present study demonstrate that, although often described as a tolerant species, these isopods are more sensitive than would be expected from the available LC50 data in the literature.

In the present study mortality was used as an endpoint, but this requires high contaminant concentrations. In the natural environment exposure to lower concentrations of metals will already result in the manifestation of various sublethal effects, which can have large implications for the health of the entire ecosystem. Many studies, for example, report an inhibition of feeding rate due to metal exposure [37,38]. This could, for example, lead to a reduced growth, lower reproduction rates and reductions in detritus processing rates [39]. Further study is required to investigate the sublethal effects of these LC values. Other limitations of this exploratory study need to be acknowledged, most notably that no metal body concentrations or metabolically available metals were measured [40,41]. Because part of the accumulated metals is biologically detoxified by metallothioneins and metal-rich granules, metabolically available metal could be considered as a more reliable indicator of metal toxicity than body concentrations or FIAs. A better understanding of subcellular partitioning is thus imperative to improve predictions of metal toxicity in nature [42].

Incipient LC values were determined as well. As these are not influenced by time of exposure, they predict the effects of long-term exposure more accurately than short-term LCxs [43]. Furthermore, the time at which acute lethality ceases can differ between different species and even contaminants. Therefore, knowing the incipient values allows for a meaningful comparison [44]. Others, on the other hand, argue that these can only be compared to similar LC values and that ecological significance in terms of population effects is often unclear [29,45]. Despite these valid arguments, the incipient concentrations of the present study are still useful for the design of future experiments, e.g. to explore to which concentrations these

isopods can be exposed when examining chronic effects or to investigate when a longer exposure time will not lead to any more discernible changes in effect, thus determining the optimal duration for future experiments. Because almost all incipient concentrations were reached at 14 days, we established that *A. aquaticus* should be exposed to Cu, Cd and Pb for longer than 7 days and preferably 14 days.

All incipient values were higher than the environmental quality standards (EQS) except for the Pb LC10 and LC20 of the FIAs, which were lower than its EQS (for Flanders, Belgium: Cu = 7 µg/L [46]; for Europe: Cd = 0.15 µg/L [47] and for Pb = 1.2 µg/L [48]). The Cu 14-day LC10 calculated with FIAs exceeded its EQS as well. It is important to note that the EQS for Cu and Cd are based on dissolved metal concentrations. Although for cadmium water hardness was taken into account, other factors such as pH or dissolved organic matter, were not considered. For some metals like Pb and Ni however, an EQS_{bioavailable} was developed, which refers to the bioavailable fraction of the dissolved concentration as determined by the physico-chemical characteristics of the water [48]. While the Pb 4-, 7- and 14-day LC10 and 14-day LC20 were also lower than the EQS, these values must be interpreted carefully since mortality in most treatments was very low.

This study shows that lethal or effective concentrations based on FIAs could be considerably lower than values calculated using theoretical concentrations. While free ion activity plays an important role, it is not the sole factor determining toxicity. Interactions with the biotic ligand, the site of toxic action, should also be considered. Some improvements to the EQS for a few metals were already made by the development of an EQS_{bioavailable} [48]. However, it is imperative that further effort is made to incorporate the BLM concept or other bioavailability models for more contaminants in environmental policy making.

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FIGURE LEGENDS

Figure 1: Survival of *A. aquaticus* as a function of days of metal exposure. Observed mortalities were corrected with the Schneider-Orelli formula (N = 480).

Figure 2: Comparison of LC10s ($\pm$ SE) calculated with the nominal concentrations, effective concentrations and free ion activities (FIAs) for Cd, Cu and Pb (N = 4 time points).

DATA ACCESSIBILITY

Data not shown in the manuscript (e.g., dose-response curves) can be made available upon request. Please contact the corresponding author: marjolein.vanginneken@uantwerpen.be.

471 **Table 1: Overview of nominal concentrations, effective concentrations and free ion activities (FIAs) on day 0; control values are the means of 3 determinations ($\pm$ SE).**

Treatment	Nominal ($\mu\text{g/L}$)	Effective ($\mu\text{g/L}$)			FIAs ($\mu\text{g/L}$)		
	Metal	Cd	Cu	Pb	Cd	Cu	Pb
Control	<0.1	<0.1	1.74 $\pm$ 0.21	<0.1	<0.1	<0.1	<0.1
Cd A	0.1	<0.1	2.21	<0.1	<0.1	<0.1	<0.1
Cd B	1	1.02	1.70	<0.1	0.33	<0.1	<0.1
Cd C	10	11.3	2.00	<0.1	4.41	<0.1	<0.1
Cd D	60	69.9	1.44	<0.1	34.9	<0.1	<0.1
Cd E	180	182	1.41	<0.1	103	<0.1	<0.1
Cu A	50	<0.1	17.8	<0.1	<0.1	<0.1	<0.1
Cu B	500	<0.1	360	<0.1	<0.1	44.7	<0.1
Cu C	1000	<0.1	816	<0.1	<0.1	213	<0.1
Cu D	5000	<0.1	2496	<0.1	<0.1	877	<0.1
Cu E	50 000	<0.1	20350	<0.1	<0.1	8790	<0.1
Pb A	20	<0.1	1.78	0.71	<0.1	<0.1	<0.1
Pb B	200	<0.1	1.91	25.6	<0.1	<0.1	<0.1
Pb C	2000	<0.1	1.70	110	<0.1	<0.1	0.36
Pb D	20 000	<0.1	15.0	358	<0.1	<0.1	4.67
Pb E	200 000	<0.1	17.8	37616	<0.1	6.94	18982

472 **Table 2: Overview of the LC20s and LC50s for each exposure time ($\pm$ SE), calculated with the nominal concentrations, effective concentrations and free ion activities**
 473 **(FIAs) (N = 480). NA = “Not Available”, could not be calculated by R.**

Metal	Day	Nominal concentrations ($\mu\text{g/L}$)		Effective concentrations ($\mu\text{g/L}$)		FIAs ($\mu\text{g/L}$)	
		LC20	LC50	LC20	LC50	LC20	LC50
Cd	1	634 $\pm$ 386	5617 $\pm$ 4880	670 $\pm$ 395	6149 $\pm$ 5076	256 $\pm$ 233	803 $\pm$ 1288
	4	29.6 $\pm$ 13.4	380 $\pm$ 256	28.3 $\pm$ 13.3	420 $\pm$ 314	17.4 $\pm$ 7.3	153 $\pm$ 93
	7	9.83 $\pm$ 3.81	57.6 $\pm$ 16.9	10.1 $\pm$ 4.2	64.1 $\pm$ 19.3	9.88 $\pm$ 3.07	31.8 $\pm$ 6.7
	14	2.25 $\pm$ 0.85	10.5 $\pm$ 2.9	2.06 $\pm$ 0.85	10.4 $\pm$ 2.9	2.35 $\pm$ 0.72	6.38 $\pm$ 1.35
Cu	1	21174 $\pm$ 6341	65744 $\pm$ 6341	6853 $\pm$ 1655	16703 $\pm$ 4334	3751 $\pm$ 1281	13319 $\pm$ 4985
	4	1613 $\pm$ 357	4123 $\pm$ 825	800 $\pm$ 94	1333 $\pm$ 148	120 $\pm$ 24	291 $\pm$ 57
	7	1124 $\pm$ 194	2133 $\pm$ 338	662 $\pm$ 82	1026 $\pm$ 84	69.6 $\pm$ 14.9	147 $\pm$ 21
	14	471 $\pm$ 80	853 $\pm$ 103	351 $\pm$ 49	547 $\pm$ 48	10.6 $\pm$ 3.2	26.5 $\pm$ 4.8
Pb	1	191276 $\pm$ 47506	261902 $\pm$ NA	36246 $\pm$ NA	45122 $\pm$ NA	23686 $\pm$ 10418	80785 $\pm$ NA
	4	10538 $\pm$ 7379	829640 $\pm$ 646300	767 $\pm$ 556	70263 $\pm$ 62668	2.76 $\pm$ 3.30	2002 $\pm$ 1218
	7	7504 $\pm$ 4604	265170 $\pm$ 206020	602 $\pm$ 326	13562 $\pm$ 9330	1.93 $\pm$ 2.02	600 $\pm$ 383
	14	2733 $\pm$ 1320	29679 $\pm$ 12252	130 $\pm$ 43	677 $\pm$ 255	0.31 $\pm$ 0.22	9.13 $\pm$ 7.39

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478 **Table 3: Overview of the incipient LC10s, LC20s and LC50s ($\pm$ SE), calculated with the nominal concentrations, effective concentrations and free ion activities (FIAs)**
479 **(N = 4 time points). NA = “Not Available”, could not be calculated by R.**

Metal	Nominal concentrations ($\mu\text{g/L}$)			Effective concentrations ($\mu\text{g/L}$)			FIAs ($\mu\text{g/L}$)		
	LC10	LC20	LC50	LC10	LC20	LC50	LC10	LC20	LC50
Cd	2.11 ± 1.26	5.43 ± 3.46	21.7 ± 12.9	2.05 ± 1.31	5.56 ± 3.76	23.4 ± 15.0	3.11 ± 1.85	5.65 ± 3.66	5.27 ± 0.93
Cu	536 ± 222	765 ± 322	1408 ± 608	377 ± 122	491 ± 156	767 ± 238	24.3 ± 19.2	38.5 ± 29.3	84.4 ± 59.8
Pb	805 ± 128	4959 ± 2347	NA	$67.21 \pm \text{NA}$	361 ± 236	NA	0.05 ± 0.01	1.12 ± 0.81	280 ± 283

492 **Table 4: Summary of LC50s and experimental details: measured metal concentrations, length of *Asellus aquaticus*, temperature, pH & water hardness.**

Metal	Endpoint	LC (µg/L)	Measured	Length (mm)	T°	pH	Hardness	References
Cd	4-day LC50	1320	No	7	13	6.75	Soft	[16]
	10-day LC50	34.9	Yes	9.43 ± 0.17	20	8.04	Moderately hard	[17]
	15-day LC50	61	Yes	4 - 6	16	6.9	Moderately hard	[18]
	1-day LC50	78450	No	-	15	-	-	[31]
	4-day LC50	600	Yes	8 - 10	11	7.83	Moderately hard	[32]
Cu	7-day LC50	1000	No	1	18	7.2	Very hard	[15]
	14-day LC50	5000	No	6 - 8	18	7.2	Very hard	[15]
	4-day LC50	9210	No	7	13	6.75	Soft	[16]
	10-day LC50	2195	Yes	9.43 ± 0.17	20	8.04	Moderately hard	[17]
Pb	4-day LC50	64100	No	7	13	6.75	Soft	[16]
	10-day LC50	443	Yes	9.43 ± 0.17	20	8.04	Moderately hard	[17]

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Figure 1: Survival of *A. aquaticus* as a function of days of metal exposure. Observed mortalities were corrected with the Schneider-Orelli formula (N = 480).

Figure 2: Comparison of LC10s ($\pm$ SE) calculated with the nominal concentrations, effective concentrations and free ion activities (FIAs) for Cd, Cu and Pb (N = 4 time points).