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EFFECT OF TEMPERATURE ON NICKEL UPTAKE AND ELIMINATION IN *DAPHNIA MAGNA*

Effect of temperature on Ni uptake and elimination

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Abstract: It is well-known that temperature can affect the ecotoxicity of chemicals, including metals, to aquatic organisms. It was recently reported that nickel (Ni), a priority substance under the European Water Framework directive, showed decreasing chronic toxicity to *Daphnia magna* with increasing temperature, between 15°C and 25°C. Here, we performed a toxicokinetic study to contribute to an increased mechanistic understanding of this effect. More specifically, we investigated the effect of temperature on Ni uptake and elimination in *D. magna* (in four clones) using an experimental design that included Ni exposures with different stable isotopic composition and using a one-compartment model for data analysis. Both Ni uptake and elimination were affected by temperature and some clear inter-clonal differences were observed. On average (across all clones), however, a similar pattern of the effect of temperature was observed on both Ni uptake and elimination, i.e. the uptake rate constant (k_u) and elimination rate constant (k_e) during 72 hours of Ni exposure were lower at 25°C than at 19°C, by 2.6-fold and 1.6-fold, respectively, and they were similar at 19 and 15°C. This pattern does not correspond with the effects of temperature on chronic Ni toxicity reported previously, suggesting that Ni compartmentalization and/or toxicodynamics may also be affected by temperature. The data gathered with our specific experimental design also allowed us to infer that (i) the k_u was upregulated over time, i.e. k_u after 2 days of Ni exposure was significantly higher than the initial k_u, by 1.5 to 2.3 fold, and (ii) that the k_e decreased significantly when the external Ni exposure was stopped, by 1.2 to 1.9-fold. These two findings are in contrast with two commonly used assumptions in toxicokinetic models, i.e. that k_u is constant during exposure and that k_e is independent of external exposure. We suggest that future toxicokinetic studies consider this in their experimental designs and data analyses. Overall, our study contributes to the growing body of evidence that temperature affects toxicokinetics of metals (and chemicals in general), but at the same time emphasizes

that knowledge of toxicokinetics alone is not necessarily sufficient to explain or predict temperature effects on (chronic) toxicity.

Key words: Uptake, elimination, temperature, nickel, *Daphnia magna*

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INTRODUCTION

Temperature is a critical factor for aquatic ectothermic organisms as it is well known to affect chemical and biochemical reactions, membrane transport processes, metabolic rates, as well as toxicokinetic processes, including metal uptake and elimination (Heugens et al. 2001; Willmer et al. 2004). Increases in metal uptake rates due to the increase of temperature have been associated with the increase of acute metal toxicity (Heugens et al. 2001; Heugens et al. 2003; Sokolova and Lannig 2008). This is in line with the critical body concentration hypothesis in which the internal concentration determines the toxicity to the organism (Vijver et al. 2004). However, it is the balance of uptake, compartmentalization, and elimination processes that ultimately determines the internally metabolically available metal concentration and therefore the metal toxicity to the organism (Rainbow 2007; Vijver et al. 2004)**.** Toxicokinetic studies can contribute to a better understanding of the mechanisms of metal toxicity and incorporating results of such studies into bioavailability models (e.g. Biotic ligand model) may allow predicting metal toxicity under various exposure regimes (e.g. constant, pulse or time-variable exposures) (Vergauwen et al. 2013; Pavlaki et al. 2017). Hence, the study of metal toxicokinetics as a function of temperature, may contribute to a better understanding of the mechanisms by which temperature affects metal toxicity and, ultimately, to predicting metal toxicity as a function of temperature.

A review by Sokolova et al. (2008) showed that in aquatic ectothermic organisms an increase of temperature increased metal uptake (or accumulation), metal elimination and mortality in 85% (n=45), 26% (n=35) and 80% (n=115) of the studies, respectively. However, the majority of the studies reviewed by Sokolova et al. (2008) reported about acute toxicity and did not include a temperature pre-acclimation phase in their study experimental design. For *Daphnia*, an ectothermic organism and a well-known ecotoxicological model organism, only

a single study is available concerning the effect of temperature on metal toxicokinetics. Heugens et al. (2003) studied the influence of temperature (10, 20, 26ºC) on cadmium (Cd) uptake kinetics in *Daphnia* (0 to 45 h of exposure) and showed that the uptake rate constant (k_u) at 20°C was significantly higher than at 10°C and that the k_u at 26ºC was similar to 20ºC. However, Heugens et al. (2003) did also not acclimate the test organisms to the different temperature treatments prior to metal exposure, whereas an acclimation period is necessary to allow the test organisms to physiologically adjust to the environmental temperature (Williams et al. 2012). Temperature-acclimated *Danio rerio* (zebrafish) and *Enchytraeus crypticus* (a terrestrial worm) exhibited an increase of Cd accumulation with increased temperature, but no corresponding increase of toxicity (Vergauwen et al., 2013; Cedergreen et al., 2013).

Recently, Pereira et al. (2017) showed that temperature had a significant effect on chronic metal toxicity to *Daphnia magna* that were pre-acclimated to the temperature treatment. A chronic, 21-day life table experiment was performed with copper, zinc and nickel (Ni) at 15°C, 20°C and 25°C and survival and reproduction were evaluated (Pereira et al. 2017). Pereira et al. (2017) showed that in comparison with 20°C, which is the standard temperature recommended by the OECD guideline for the *D. magna* reproduction test (OECD 2012), chronic metal toxicity to *D. magna* increased at 15°C and decreased at 25°C. This is in contrast with previous acute toxicity studies (Boeckman and Bidwell 2006; Ferreira et al. 2010; Heugens et al. 2003; Persoone et al. 1989; Vandenbrouck et al. 2011). Furthermore, Pereira et al. (2017) showed that the effect of temperature on chronic Ni toxicity depended on the *D. magna* clone. Nickel is a priority substance in the European water frame directive (Directive 2008/105/EC) and Van Regenmortel et al. (2017) indicated that certain freshwater bodies in Europe (e.g., Dommel river basin, The Netherlands) are potentially at risk due to Ni. The importance of Ni as a priority substance, the demonstrated effects of temperature on chronic Ni toxicity, and the idea that toxicokinetic studies can contribute to a better

understanding of metal toxicity leads to our first and main research objective (part I of the present study), i.e. to investigate the effect of temperature on Ni uptake and elimination in *D. magna* (pre-acclimated, i.e. in homeostatic balance with the external temperature).

In toxicokinetic studies, a simple one-compartment model is most commonly used to analyze experimental data and experiments usually include two phases: an uptake phase in which organisms are exposed to metals via contaminated medium (elimination also takes place) (Heugens et al. 2003; Komjarova and Blust 2008; 2009a; 2009b; Lebrun et al. 2011) and an elimination phase in which organisms are transferred from the contaminated medium to a clean medium (Adam et al. 2015; Guan and Wang 2004; Lam and Wang 2006; Lebrun et al. 2011; Zhao et al. 2009). Two important assumptions are associated with this design. The first assumption is that k_u is constant over the entire exposure duration. Most toxicokinetic studies assume that k_u does not change during the exposure period (Adam et al. 2015; Cedergreen et al. 2013). However, Laskowski et al. (2010) showed that k_u of *Lumbricus terrestris* (earthworm) decreased after about 1.5 d of Ni exposure. The second assumption is that the k_e is the same in the presence and in the absence of the contaminant. This second assumption was tested in *Hydropsyche* larvae using a one-compartment model (Evans et al. 2002; 2006). Cadmium, lead (Pb), zinc (Zn) and copper (Cu) elimination in *Hydropsyche* larvae was similar in the presence vs. the absence of the contaminant. However, to our knowledge, this assumption has never been tested in any other species or with Ni. The second objective of the present study (part II) was therefore to test these two assumptions for Ni in *D. magna*.

The use of stable isotopes is a useful technique to investigate toxicokinetic processes (Balcaen et al. 2008; Evans et al. 2002; 2006; Komjarova and Blust 2009a; Pane et al. 2003). Therefore, our experimental design made use of the stable isotope ^{62}Ni and of Ni with a

natural isotope composition, and the isotope ratio of Ni in the exposure medium was switched during the experiment (see Materials and Methods for details).

MATERIAL AND METHODS

Organism cultures and test medium

The four *D. magna* clones used in the present study were obtained from the KNO17 population. This population was established from ephippia collected from a temporal pond in Knokke, West Flanders, Belgium (for details consult supportive information in Hochmuth et al., 2015). The four clones were maintained under laboratory conditions during three years at 19°C under a controlled light cycle (16 h of light: 8 h of dark) (OECD 2012) and were fed daily with *Pseudokirchneriella subcapitata* with a food density of 2.5 mg $C \cdot L^{-1}$.

In order to reach physiological acclimation in each of the three temperature treatments, the daphnids were acclimated for two generations (Mitchell and Lampert 2000; Williams et al. 2012). More detailed information about the acclimation process can be found in Pereira et al. (2017). Juveniles (5 days old) were used to guarantee the availability of sufficient biomass for whole-body metal analysis and to avoid Ni elimination through the release of offspring during the experiment. Neonates (<24h) collected from the 3rd or 4th brood of the acclimated mothers were collected and cultured in aquaria for 5 days. These 5-6 day old juveniles were then used to start the toxicokinetic experiment. A modified M4 medium was used (for details consult Pereira et al. (2017)).

Experimental design

We investigated Ni uptake and elimination in four *D. magna* clones at 15°C, 20°C and 25°C. The experimental design is shown in Figure 1. Organisms were not fed during the experiment to avoid Ni uptake via food. The experimental setup was divided in two parts. In part I,

organisms were exposed to ^{62}Ni during 72 h. Organisms were sampled at the time points 0, 8, 24, 48, and 72 h. In part II, after 48h of exposure to ^{62}Ni, organisms were transferred to test medium spiked with Ni with a natural isotope ratio ("presence of Ni") or to control medium ("absence of Ni"). Organisms were sampled at the time points 48, 56 and 72 h (i.e. 8 and 24 h after changing the isotope ratio of the exposure medium).

Two Ni concentrations were tested at each temperature (i.e. 56 and 73 µg Ni·L^{-1}, measured dissolved concentration). These Ni concentrations correspond to the 21-d EC50 (estimated based on the average reproduction across all four clones at 15 and 20°C, respectively (Pereira et al. 2017). The treatment 73 µg Ni·L^{-1} also corresponds to the 21-d EC20 at 25°C. A control treatment was also included in the design and *Daphnia* samples were taken at 0 and 72 h. Organisms were exposed in 200 ml of medium in polyethylene cups. Three replicates with 5 organisms per replicate were taken for each clone, for each temperature, and for each Ni concentration at each time point. Part I and part II of the experiment were performed in a single experiment.

The test medium was spiked with Ni three days before the start of the experiments and the test solutions were kept in the dark at 20°C. In part I of the experiment, the stable isotope ^{62}Ni was used and obtained from Cortecnet (France) (Quality certificate: 28-01-62-4303) with the following composition 98.02, 1.02, 0.67, 0.25, 0.04 % of ^{62}Ni, ^{60}Ni, ^{58}Ni, ^{61}Ni and ^{64}Ni, respectively. In part II of the experiment, a natural isotope ratio was used (i.e. 3.63, 26.22, 68.07, 1.13, 0.92 of ^{62}Ni, ^{60}Ni, ^{58}Ni, ^{61}Ni and ^{64}Ni, respectively (Gramlich et al. 1989) in the form of Ni (II) chloride hexahydrate, obtained from EMSURE (Darmstaldt, Germany).

Analysis of Ni content in Daphnia magna

Sampled organisms were transferred to control medium for 10 minutes, after which they were transferred to a Na_2EDTA (5mM) (Sigma-Aldrich) solution , where they remained during 1 minute (Adam et al. 2015). Organisms were then transferred to a sieve were they were quickly rinsed with deionized water. Organisms were then transferred to a small piece of parafilm paper (PARAFILM M, Sigma-Aldrich) and all samples were placed in an oven at 60°C until constant dry weight (about 48h). Finally, organisms were weighed on a Sartorius Digital Micro balance (type 2405, Germany), and transferred to an Eppendorf tube (2 mL).

To each tube, containing dried daphnids, 100 µL HNO_3 (Normaton Ultrapure 69%, Prolabo) was added and the vial was left to stand overnight. Samples were digested in a microwave in 5 steps of 2 min at 90, 5 steps of 2 min at 160 W, and 5 steps of 1 min at 360 W. A cooling interval of 2 min was included at the end of each step. Then, 25 µL H_2O_2 was added to each sample (AnalaR NORMAPUR 30%) and 30 min later samples were digested further in a microwave in 1 step of 2 min at 90W, 1 step of 2 min at 160 W and 1 step of 1 min at 360 W. Samples were diluted with water (ultra-pure, Milli-Q) to 2% HNO_3. As quality control, two reference samples (mussel tissue 2976, NIST) and two procedural blanks were included in each 40-sample rack (Mubiana and Blust 2007). All samples with a total *Daphnia* tissue dry weight less than the quantification limit (i.e.104 µg) were excluded from the data analysis. Daphnid digests were measured with High Resolution Inductively Coupled Plasma - Mass Spectrometry (HR-ICP-MS) (Thermo Scientific Element 2 XR) to determine ^{60}Ni and ^{62}Ni concentrations. The recovery percentage of Ni in the reference samples was 70% of the certified values (10% coefficient of variation). The procedural blanks were all below quantification limit (0.001 µg $^{60}Ni \cdot L^{-1}$, 0.001 µg $^{62}Ni \cdot L^{-1}$). The quantification limits of Ni body concentrations in *Daphnia* (on dry weight basis) were 4 µg $^{60}Ni \cdot g^{-1}$ and 19 µg $^{62}Ni \cdot g^{-1}$.

Analyses of water chemistry

The reference material used in all chemical analyses and the quantification limits of all chemical analysis can be consulted in SI (Table S1). Water samples for metal and organic carbon analyses were taken at all time points, for all test solutions and for each temperature treatment. Samples were taken for analysis of both total and dissolved concentrations. Dissolved concentrations refer to 0.45 µm membrane filtered samples (Acrodisc, PALL Life Sciences). Samples were acidified to a final concentration of 0.14 $mol \cdot L^{-1}$ of HNO_3 (Normaton Ultrapure 69% HNO_3, Prolabo) prior to analysis. Temperature was recorded daily. Dissolved oxygen concentration was measured at the beginning and at the end of the experiment. pH was measured at all time points in all test solutions in all temperature treatments. Concentrations of major cations (Na, K, Ca, and Mg) were measured using iCAP 7000 Series Inductively Coupled Plasma Optical-Emission Spectrometry (ICP-OES) (Thermo Scientific) in water samples collected at 72h.

Concentrations of ^{60}Ni and ^{62}Ni in water samples were measured with HR-ICP-MS (Thermo Scientific Element 2 XR). Samples for dissolved organic carbon analysis were measured with TOC-L CPH (Shimadzu). All data analyses were performed based on the mean measured dissolved concentrations. pH was measured with a pH meter 826 pH mobile (Metrohm). Dissolved oxygen was measured with an Oximeter WTW (probe WTW, cell Ox 325).

Data analysis

A one-compartment model was applied to calculate kinetic uptake and elimination parameters. The accumulation process can be described by the following differential equation:

(1)

$$\frac{dC_{organism}}{dt} = k_u \times \mathrm{C_w} - k_e \times C_{organism}$$

which states that the change in concentration in an organism ($C_{organism}$) (µg·g^{-1}) over time (t) (h) is a function of uptake minus elimination. C_w is the dissolved metal concentration in the exposure medium (µg·L^{-1}), k_u is the uptake rate constant and is expressed as L·g^{-1}·h^{-1} and k_e is the elimination rate constant and is expressed as h^{-1} (Luoma and Rainbow 2008). The k_e can be determined by the integrated version of equation 1

(2)

$$C_{organism} = C_0 \times e^{(-k_e \times t)}$$

and the k_u can be determined by

(3)

$$C_{organism} = C_0 + C_w \times \frac{k_u}{k_e} \times (1 - e^{(-k_e \times t)})$$

where C_0 is the metal concentration in the organism at t = 0.

As a first step in the analysis, the k_e in the presence ($k_{e,presence}$) and in the absence of Ni ($k_{e,absence}$) of the 4 clones were estimated for each temperature treatment using equation 2. To investigate whether the *D. magna* k_e changed when the organisms were transferred to a clean medium, a paired t-test was performed to test significant differences between $k_{e,presence}$ vs. $k_{e,absence}$ (observations on the same clone and at the same temperature are paired). Significant differences were observed between $k_{e,\ presence}$ and $k_{e,\ absence}$ and therefore the $k_{e,presence}$ values were used to estimate k_u according to equation 3. As a second step in the analysis, the k_u values of the 4 clones at each temperature treatment were estimated based on equation 3. The

k_u were estimated with the data from part I of the experiment ($k_{u,I}$). The following function was used

(4)

$$[^{62}\mathrm{Ni}]_{\mathrm{daphnia}} = [^{62}\mathrm{Ni}]_{\mathrm{daphnia,i}} + [^{62}\mathrm{Ni}]_{\mathrm{w}} \times \frac{k_{u,\mathrm{I}}}{k_{e,presence}} \times (1 - e^{(-k_{e,presence} \times t_{0\ to\ 72\ h})})$$

where $[^{62}\mathrm{Ni}]_{\mathrm{daphnia}}$ is the whole body ^{62}Ni concentration in the daphnia ($\mu g \cdot g^{-1}$), $[^{62}\mathrm{Ni}]_{\mathrm{daphnia,i}}$ is the ^{62}Ni concentration in the daphnia at t = 0 h, $[^{62}\mathrm{Ni}]_{\mathrm{w}}$ is the ^{62}Ni concentration in the exposure medium ($\mu g \cdot L^{-1}$). In addition, the initial k_u were estimated with the data points from 0 to 24 h ($k_{u,init}$) from part I of the experiment. The following function was used

(5)

$$[^{62}\mathrm{Ni}]_{\mathrm{daphnia}} = [^{62}\mathrm{Ni}]_{\mathrm{daphnia,i}} + [^{62}\mathrm{Ni}]_{\mathrm{w}} \times \frac{k_{u,init}}{k_{e,presence}} \times \left(1 - e^{(-k_{e,presence} \times t_{0\ to\ 24\ h})}\right)$$

After 2 d of Ni exposure, the k_u were estimated with the data points from 48 to 72 h ($k_{u,48h}$), i.e. part II of the experiment. The following function was used

(6)

$$[^{60}\mathrm{Ni}]_{\mathrm{daphnia}} = [^{60}\mathrm{Ni}]_{\mathrm{daphnia,i}} + [^{60}\mathrm{Ni}]_{\mathrm{w}} \times \frac{k_{u,48h}}{k_{e,presence}} \times (1 - e^{(-k_{e,presence} \times t_{48\ to\ 72\ h})})$$

where $[^{60}\mathrm{Ni}]_{\mathrm{daphnia}}$ is the whole body ^{60}Ni concentration in the daphnia ($\mu g \cdot g^{-1}$), $[^{60}\mathrm{Ni}]_{\mathrm{daphnia,i}}$ is the ^{60}Ni concentration in the daphnia at t = 48h, $[^{60}\mathrm{Ni}]_{\mathrm{w}}$ is the ^{60}Ni concentration in the exposure medium ($\mu g \cdot L^{-1}$).

The uptake and elimination rate constants of each clone in each temperature treatment were estimated using the *nlstool* package in R software (version 3.4.0). To test the effect of

temperature on Ni uptake and on Ni elimination in *D. magna* (objective I), paired t-tests were performed to test significant differences of $k_{u,I}$ values, $k_{u,init}$ and $k_{u,48h}$ values, and $k_{e,presence}$ and $k_{e,absence}$ values between 15°C, 19°C and 25°C (observations on the same clone are paired). The ratio between $k_{u,I}$ and $k_{e,presence}$ (k_u/k_e= bioconcentration factor at equilibrium) was also determined for each clone at each temperature and the mean value over all clones was determined for each temperature. To test if k_u is constant over the exposure duration, a paired t-test was performed to test significant differences between $k_{u,init}$ vs. $k_{u,48h}$ (observations on the same clone and at the same temperature are paired). Similarly, a paired t-test was also conducted to test if $k_{e,presence}$ and $k_{e,absence}$ were different. All statistical analyses were performed in R software (R Core Team 2017).

RESULTS

Water chemistry

Temperatures remained stable during the experiment in all treatments, i.e. 14.9 (14.5-15.2°C), 19.3 (19.0-19.5°C) and 24.9°C (24.5-25.5°C) (average, and average minimum and average maximum temperature are given). The dissolved oxygen concentration (8.7±0.6 $mg \cdot L^{-1}$), the dissolved organic carbon (3.3±0.5 $mg \cdot L^{-1}$), the pH (7.6±0.1) and the major ion concentrations (17.7±0.3 mg $Na \cdot L^{-1}$, 8.8±0.2 mg $Mg \cdot L^{-1}$, 2.9±0.2 mg $K \cdot L^{-1}$, 52.1±1.2 mg $Ca \cdot L^{-1}$) also remained stable (average ± standard deviation) (SI, Table S2).

The total and dissolved ^{60}Ni and ^{62}Ni concentrations remained stable during the toxicokinetic experiment (SI, Table S3). The total Ni concentration remained within 15% of the nominal concentration. In part I of the experiment, the measured dissolved Ni concentrations in water were 0.0±0.0, 56.8±3.4 and 73.7±13.4 $\mu g \cdot L^{-1}$ (average ± standard deviation). In part II of the

experiment (presence of Ni), the measured dissolved Ni concentrations in water were 0.0±0.0, 48.1±10.8, 69.5±19.2 µg·L^{-1} (average ± standard deviation).

Effect of temperature on Ni uptake and elimination in Daphnia magna

The results of the present study indicate that Ni uptake and Ni elimination in *D. magna* were affected by temperature. The same temperature effect patterns were observed on Ni uptake and on Ni elimination. The k_u and k_e were lower at 25°C than at 19°C and they were similar between 19 and 15°C (Figure 2, 3 and 4). The $k_{u,I}$ was significantly higher at 19ºC than at 25ºC by 2.6-fold (paired t-test, n=4, $p<0.05$), but the $k_{u,I}$ at 15ºC was not significantly different from the one at 19ºC and 25ºC (paired t-test: 15 vs. 19°C, n=3, $p>0.05$; 15 vs. 25°C, n=3, $p>0.05$) (Figure 2; SI, Table S4, Figure S1 and S2). The same temperature trend as the one observed for $k_{u,I}$ was also observed for $k_{u,init}$ and $k_{u,48h}$ values. The $k_{u,init}$ and $k_{u,48h}$ values were significantly higher at 19ºC than at 25ºC by 1.5-fold (paired t-test: 19 vs. 25°C n=8, $p<0.05$; 15 vs. 19°C, n=6, $p>0.05$; 15 vs. 25°C, n=6, $p>0.05$) (Figure 3; SI, Table S4, Figure S3 and S4). The $k_{e,\ absence}$ and $k_{e,\ presence}$ values were significantly lower at 25ºC than at 15°C and 19ºC by 1.5-fold and 1.6-fold, respectively (paired t-test: 15 vs. 25°C, n=5, $p<0.05$; 19 vs. 25°C, n=5, $p<0.05$; 19 vs. 15°C, n=5, $p>0.05$) (Figure 4; SI, Table S5). An overview of the fits of the estimated uptake and elimination curves is presented in Figure 1. For certain clones at certain temperatures, the k_e and k_u values could not be estimated because the total dry mass of the collected samples was below the quantification limit.

The ratio $k_{u,I}$ / $k_{e,presence}$ (the bioconcentration factor) was higher at 20°C (3.7 L·g^{-1}) than at 15°C (2.3 L·g^{-1}) and 25°C (1.9 L·g^{-1}). Also, the internal Ni concentration observed after 48h of exposure ($[Ni]_{daphnia,48h}$) was higher at 20°C than at 15°C and 25°C (SI, Figure S1 and S2).

Finally, the effect of temperature on Ni uptake and elimination showed differences among the four *D. magna* clones tested (see Figure 2, Figure 4; and SI, Table S4, S5). For instance, in Clone A, the $k_{u,I}$ values at 15°C and 19°C were similar (1.76, 1.80 $L \cdot g \cdot h^{-1}$, respectively), but they were about 3-fold lower at 25°C (0.56 $L \cdot g \cdot h^{-1}$). In clone D, however, the $k_{u,I}$ value was lowest at 15°C (0.52 $L \cdot g \cdot h^{-1}$), while they were higher at 19°C (0.94 $L \cdot g \cdot h^{-1}$) and 25°C (0.72 $L \cdot g \cdot h^{-1}$) (Figure 2, Table S4). For clone A, the $k_{e,\ presence}$ decreased monotonically with increasing temperature (15°C >20°C >25°C) (2.4-fold, while for clone D, the value at 15°C was 1.3-fold higher than the $k_{e,\ presence}$ values at 20°C and 25°C (Figure 4, Table S5).

Is k_u constant with exposure duration?

Nickel uptake rate constants were up-regulated over time in the four *D. magna* clones. The $k_{u,48h}$ values were significantly higher than the $k_{u,init}$ values by 2.3, 1.5, 1.7-fold at 15°C, 19°C and 25°C, respectively (paired t-test, n=11, $p<0.05$) (Figure 4; SI, Table S4).

Is k_e affected by the presence or absence of nickel?

The Ni k_e in *D. magna* decreased when the external Ni exposure stopped (Figure 3; SI, Table S5). The $k_{e,absence}$ values were significantly lower than the $k_{e,presence}$ values by 1.2, 1.9 and 1.5-fold at 15, 19 and 25°C, respectively (paired t-test, n=10, $p<0.05$)(Figure 3; SI, Table S5, Figure S5, S6, S7 and S8).

DISCUSSION

Effect of temperature on Ni uptake and elimination in Daphnia magna

Ni uptake and Ni elimination in *D. magna* were affected by temperature. The same temperature effect patterns were observed on Ni uptake and on Ni elimination: k_u and k_e were

on average lower at 25°C than at 19°C and they were similar at 19 and 15°C (Figure 2, 3 and 4). Until the present study, to our knowledge, only a single study had assessed the effect of temperature on metal toxicokinetics in *Daphnia*. Heugens et al. (2003) indicated that an increase of temperature increased the Cd k_u (i.e. the Cd k_u at 26ºC and 20ºC were similar, but the Cd k_u at 20ºC was higher than at 10ºC). The results of Heugens et al. (2003) seem to contrast with those in the present study. However, in Heugens et al. (2003) a single clone was tested, the test organisms were not pre-acclimated to the temperature treatments and another metal (Cd) was studied. In the present study, the organisms were pre-acclimated to the temperature treatments, which in ectothermic organisms is essential to allow physiological adjustment to the environmental temperature (Williams et al. 2012). In addition, we used four clones (instead of only one) to guarantee that the observed effects of temperature on Ni toxicokinetics in *D. magna* would be more broadly valid at population-level (rather than for a single clone only). This is important, as our results (including the examples given in the *Results* section) clearly show that conclusions drawn about the effect of temperature on Ni uptake and elimination in *D. magna* could be quite different depending on which clone is tested. Thus, the observed effects of temperature on Ni toxicokinetics in any single clone may not represent the effects on a population, as we have also reported earlier for chronic Ni toxicity in *D. magna* (Pereira et al., 2017). It would be interesting to investigate whether inter-clonal differences in temperature effects on Ni toxicokinetics could be explained by genetic differences in membrane proteins involved in Ni transport.

Effect of temperature on Ni uptake and elimination in relation to chronic Ni toxicity.

Generally, the increase of metal uptake is associated with the increase of acute metal toxicity (Heugens et al. 2001; Heugens et al. 2003; Sokolova and Lannig 2008). However, little information is available concerning the effect of temperature on toxicokinetic processes in pre-acclimated organisms (i.e. organisms in homeostatic balance with their environmental

temperature) and its association with chronic metal toxicity. The effect of temperature on the ratio between k_u and k_e (k_u/k_e= Bioconcentration factor at equilibrium) determines the temperature effect on the internal metal concentration at equilibrium. The critical body concentration hypothesis states that the internal concentration of a chemical determines the toxicity to the organism (Vijver et al. 2004). However, it is the balance of uptake, internal distribution, and elimination processes that ultimately determines the internally metabolically available metal concentration and therefore the metal toxicity to the organism (Rainbow 2007; Vijver et al. 2004).

Our previous study, in which the same *D. magna* clones were tested as in the present study, indicated that chronic Ni toxicity to *D. magna* increased with decreasing temperature (25ºC<20ºC<15ºC) (Pereira et al. 2017). The the present study indicates that the ratio of $k_{u,I}$ and $k_{e,presence}$ (bioconcentration factor) was lower at 25°C than at 19°C by 1.9-fold. Also, the $[Ni]_{daphnia,48h}$ were lower at 25°C than at 19°C (SI, Figure S1 and S2). Thus, the $[Ni]_{daphnia,48h}$ (present study) and the chronic Ni toxicity (Pereira et al. 2017) were both lower at 25°C than at 20°C (19°C in present study), which is in line with the critical body concentration hypothesis (i.e. the internal concentration determine the toxicity to the organism) (Vijver et al. 2004). However, the ratio of $k_{u,I}$ and $k_{e,presence}$ was lower at 15°C than at 19°C by 1.6-fold, and the $[Ni]_{daphnia,48h}$ was lower at 15°C than at 19°C (SI, Figure S1 and S2). In contrast, chronic Ni toxicity was higher at 15ºC than at 19ºC (Pereira et al., 2017). Therefore, other processes than Ni uptake and elimination are likely involved in and contribute to explain the effect of temperature on chronic Ni toxicity to *D. magna* (Luoma and Rainbow 2008; Rainbow 2007; Rainbow and Luoma 2011). The present study supports the findings of Pereira et al. (2018), who showed that at lower temperatures a lower internal Ni concentration in *D. magna* (clone K6) (adults) was needed to induce the same Ni toxicity than at higher temperatures (Pereira et al. 2018). Hence, in Pereira et al. (2018), the internal Ni

concentrations in *D. magna* did also not predict the effect of temperature on chronic Ni toxicity.

Previous studies have also shown that the same metal body burden can have different levels of toxicity to organisms when they are pre-acclimated and exposed to different temperatures Vergauwen et al. (2013) and Cedergreen et al. (2013) showed that in *Danio renio* (zebrafish) and in *Enchytraeus crypticus* (worm), respectively, Cd accumulation was generally higher at higher temperatures, but toxicity was not. The link between the effect of temperature on metal uptake and elimination on the one hand and chronic metal toxicity on the other hand is not always straightforward, because internal and toxicodynamic processes (i.e. metal interaction with toxicological target sites) also play a role in the occurrence of toxic effects compartmentalization (Vijver *et al*. 2004; Rainbow 2007).

Ni kinetics in Daphnia magna. The present study shows fast Ni kinetics in *D. magna* (Figure 2 and 3; SI, Table S4 and S5). Previously, Ni toxicokinetics have been studied in *D. magna* at 20°C (Komjarova and Blust 2009a). They exposed *D. magna* simultaneously to low ecologically relevant concentrations of Ni (18.6 $\mu g \cdot L^{-1}$), Cd (5.3 $\mu g \cdot L^{-1}$), Cu (9.8 $\mu g \cdot L^{-1}$), Pb (10.2 $\mu g \cdot L^{-1}$), and Zn (10.1 $\mu g \cdot L^{-1}$) at 20°C and they reported a Ni k_u of 0.04 $L \cdot g^{-1} \cdot h^{-1}$ (875 $L \cdot kg^{-1} \cdot d^{-1}$). The Ni k_u values at 19°C in the present study (at 19ºC) the $k_{u,I}$ varied from 0.94±0.15 to 1.80±0.13 $L \cdot g^{-1} \cdot h^{-1}$ (mean ± standard error) and are thus more than one order of magnitude higher than in Komjarova and Blust (2009a). However, several differences exist between both studies. Firstly, organisms of different life stages were used, that is Komjarova and Blust (2009a) used adults (15-16 d old) and the present study used juveniles (5d old). Previous studies have suggested that Ni can be taken up via Mg transport pathways, that Ni exposure can disrupt magnesium (Mg) homeostasis in *D. magna*, and that Mg is of crucial importance for development and growth (Deleebeeck et al. 2008; Pane et al. 2003; Wolf and

Cittadini 2003). Therefore, the higher k_u observed in the present study may be due to the juveniles' higher need for Mg. Secondly, in Komjarova and Blust (2009a), Ni uptake was studied in the presence of other metals, which may have influenced the toxicokinetics of Ni. This is not unlikely, as some studies have also shown interactive effects between Ni, Cu and Zn on daphnid reproduction (Nys et al. 2015; Nys et al. 2017). Hence, the Ni k_u estimated in Komjarova and Blust (2009a) may not be representative for the Ni k_u in single exposure. Thirdly, different media with different water hardness were used. The water hardnes of the media used in the present study and in Komjarova and Blust (2009a) were 176 and 231 $mg \cdot L^{-1}$ as $CaCO_3$, respectively. Deleebeeck et al. (2008) showed that the increase of water hardness (Ca and Mg combined) protected *D. magna* against chronic Ni toxicity, suggesting that Ca and Mg cations can compete with the Ni for binding at active sites (Deleebeeck et al. 2008). Thus, the competition between hardness cations (Ca and Mg) and Ni may also partly explain the lower Ni k_u reported in Komjarova and Blust (2009a).

Is k_u constant with exposure duration?

Nickel uptake was up-regulated over time in the four *D. magna* clones (Figure 4; SI, Table S4). A previous study showed that Ni assimilation rates can change along metal exposure (Laskowski et al. 2010). Laskowski et al. (2010) studied Ni toxicokinetics in *Pterostichus oblongopunctatus* (ground beetles) and in *Lumbricus terrestris* (earthworm) and showed that a three-parameter model, in which k_u decreases or even becomes zero after an initial period of exposure, better described metal toxicokinetics over time than the classic two-parameter model (i.e. a one-compartment toxicokinetic model) with constant k_u and k_e. Laskowski et al. (2010) suggested that the physiological status of the organism can change with exposure time in reaction to the contaminant exposure. Previous studies suggest that Ni exposure can disrupt Mg homeostasis in *D. magna* (Deleebeeck et al. 2008; Pane et al. 2003). Nickel and Mg are

chemically similar, and their ions have the same size and water exchange constants (Wolf and Cittadini 2003). Magnesium is an essential ion important for several biological functions including structural stabilization of nucleic acids, cell membranes and to promote specific structural organization of enzymes and ribosomes (Wolf and Cittadini 2003). Therefore, in order to maintain Mg homeostasis, increased Mg uptake may be triggered by Ni exposure and hence Ni k_u may also be up-regulated over time. Future toxicokinetic studies should consider that the k_u may not be constant over the entire exposure period. Our study suggests that exposure duration can have about an equally large effect on Ni k_u as interclonal variation (i.e. about 2-fold).

Is k_e affected by the presence or absence of nickel?

The k_e of Ni in *D. magna* decreased when the external Ni exposure stopped (Figure 3; SI, Table S5). Previously, the studies of Evan et al. (2002, 2006) showed that the elimination rates of Cd, Cu, Pb and Zn in *Hydropsyche* larvae were similar in the presence and in the absence of the contaminant. However, different species from different classes were tested: *Hydropsyche* larvae from the class Insecta and order Tricoptera (ITIS 2018) in Evan et al. (2002, 2006); *D. magna* from the class Branchiopoda and order Diplostraca in the present study (ITIS 2018). Differences among species on uptake and elimination processes can be due to physiological differences (e.g. locomotor and feeding activities) (Luoma and Rainbow 2008). The present study showed that the differences between $k_{e,presence}$ and $k_{e,absence}$ were small but statistically significant. Therefore future toxicokinetic studies should consider that the k_e may be different in the presence and in the absence of the chemical stressor.

CONCLUSION

The present study indicates that Ni uptake and Ni elimination in *D. magna* are affected by temperature. In general, the same patterns of temperature effects were observed on Ni uptake and on Ni elimination, i.e. the k_u and k_e were lower at 25°C than at 19°C and they were similar at 19 and 15°C. These effects do not correspond with effects of temperature on chronic Ni toxicity reported in our previous study (Pereira et al., 2017), suggesting that Ni compartmentalisation and/or toxicodynamics may also be affected by temperature. Furthermore, our results clearly show that the effects of temperature on Ni toxicokinetics are clone-depedent and thus, that any single clone may not represent the effects on a population. In addition, in contrast with the common assumption that k_u and k_e are constant, we showed that the Ni k_u of *D. magna* was up-regulated over time and that the Ni k_e decreased when the external Ni exposure stopped. Thus, we suggest that future toxicokinetic studies of chemicals consider possible violations of these assumptions, in the experimental design and the data analysis. Overall, besides this suggestion for improving future toxicokinetic studies, our study contributes to the growing body of evidence that temperature affects toxicokinetics of metals (and chemicals in general), but at the same time suggests that knowledge of toxicokinetics alone is not necessarily sufficient to predict temperature effects on (chronic) toxicity.

Supplemental data – The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

Tables S1-S5

Figures S1-S8

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Data availability – Data are available on request from the authors (CeciliaManuela.Pereira@ugent.be, Karel.DeSchamphelaere@UGent.be).

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Figure Legends

Figure 1. Overview of the experimental design used to investigate the influence of temperature on Ni uptake and elimination in *Daphnia magna* and of the results obtained. Four *D. magna* clones (5 d old) were exposed to Ni (56 and 73 µg·L^{-1}) at 15°C, 19°C and 25°C. The experimental design was divided in two parts: I) organisms were exposed to ^{62}Ni during 72h; II) after 48h of exposure to ^{62}Ni organisms were transferred to a test medium spiked with $^{\text{Natural isotope ratio}}$ Ni (presence of Ni) (i.e. switching to another isotope ratio) or to a control medium (absence of Ni). The sampling time is indicated. The results shown are of clone A at 19°C exposed to 56 µg Ni·L^{-1}. Marker points represent observations and lines represent the estimated uptake or elimination curves and their respective confidence limits.

The letters specify the parts of the experimental design used and the types of results obtained to answer objectives II a) and II b). Objective II a) was to investigate whether the uptake rates are dependent on the time of exposure. Objective II b) was to investigate whether the *D. magna* elimination rates change when the organisms are transferred to a non-contaminated medium. Dotted grey lines represent the predicted uptake curves after 48h of exposure with the water Ni concentration adjusted to the natural isotope ratio (^{60}Ni:0.26%). Uptake rate constants (k_u) are expressed as $L \cdot g^{-1} \cdot h^{-1}$ and elimination rate constants (k_e) are expressed as h^{-1}.

Figure 2. The effect of temperature on the nickel (Ni) uptake rate constant (k_u) (± standard error) in four *Daphnia magna* clones. The k_u were estimated with the data from part I of the experiment ($k_{u,\,I}$), in which the organisms were exposed to 56 and 73 µg $^{62}Ni \cdot L^{-1}$ during 72h. Clones (A, B, C and D) are represented by different symbols. The averages of the $k_{u,\,I}$ across the four *D. magna* clones at 15, 19 and 25°C were also plotted (Av). For certain clones at certain temperatures, the k_u could not be estimated because the total dry mass of the samples were below the quantification limit.

Figure 3. Uptake rate constants (k_u) (± standard error) estimated at the first day of nickel (Ni) (56 and 73 µg $^{62}Ni \cdot L^{-1}$) exposure (0-24h, initial) ($k_{u,init}$) and after 2 days (48-72h, after 48h) ($k_{u,\,48h}$) (48 and 70 µg $^{Nat}Ni \cdot L^{-1}$) in four *Daphnia magna* clones at 15, 19 and 25ºC. Clones are represented by different symbols. The averages of the k_u across the four *D. magna* clones at 15, 19 and 25°C were also plotted (Av). Nat.: natural isotope ratio. For certain clones at certain temperatures, the k_u could not be estimated because the total dry mass of the samples were below the quantification limit.

Figure 4. Elimination rate constants (k_e) of nickel (Ni) estimated for the four *Daphnia magna* clones at 15, 19 and 25ºC in the presence and absence of Ni. After 48h of exposure to 56 and

73 µg $^{62}Ni \cdot L^{-1}$, *D. magna* were transferred to a medium spiked with Ni with a natural isotope ratio (presence) or to a control medium (absence). Clones are represented by different marker points. The averages of the k_e across the four *D. magna* clones at 15, 19 and 25°C were also plotted (Av). For certain clones at certain temperatures, the k_u could not be estimated because the total dry mass of the samples were below the quantification limit.

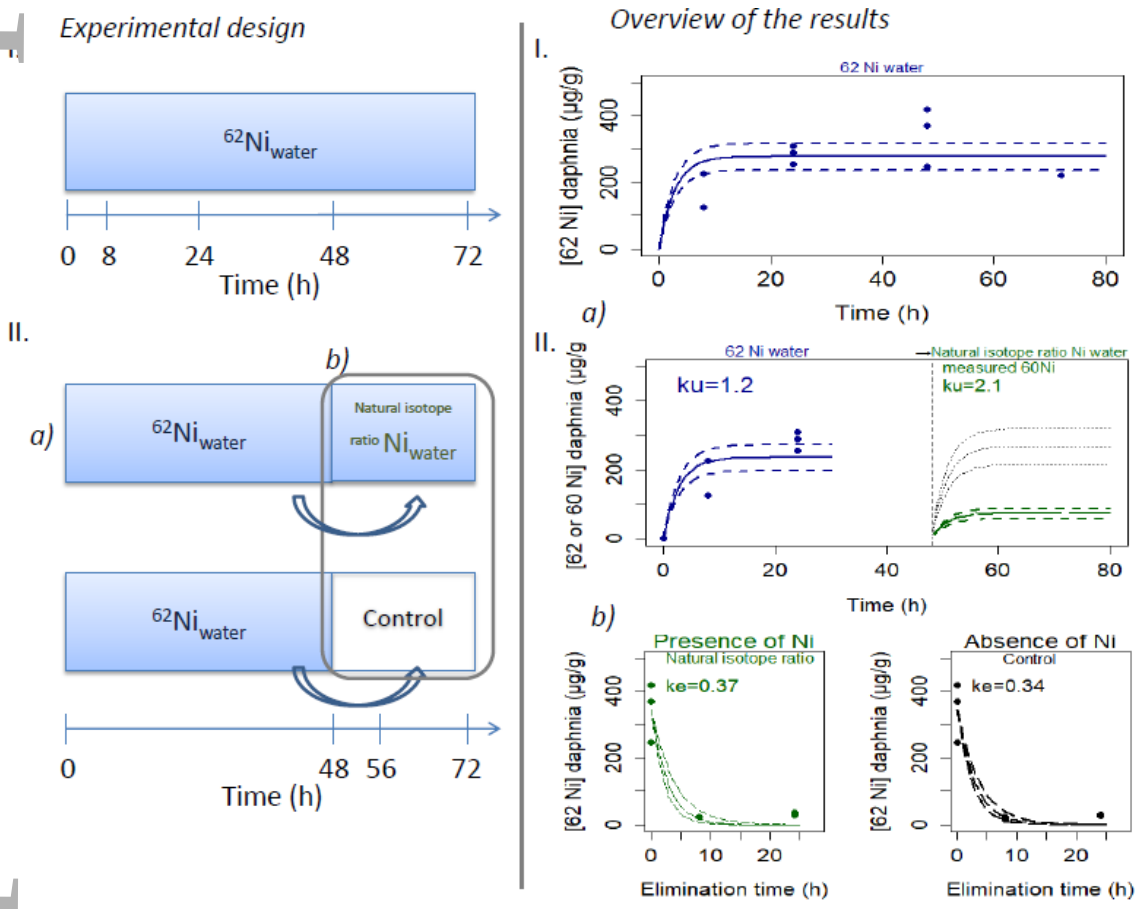

Figure 1

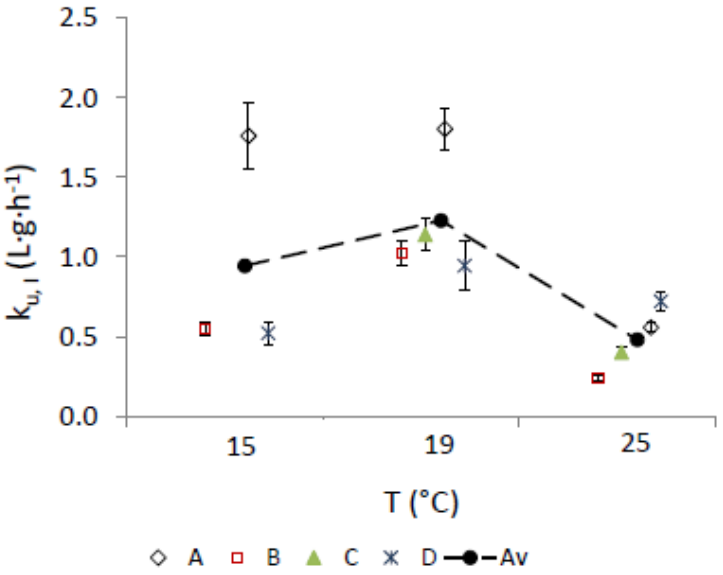

Figure 2

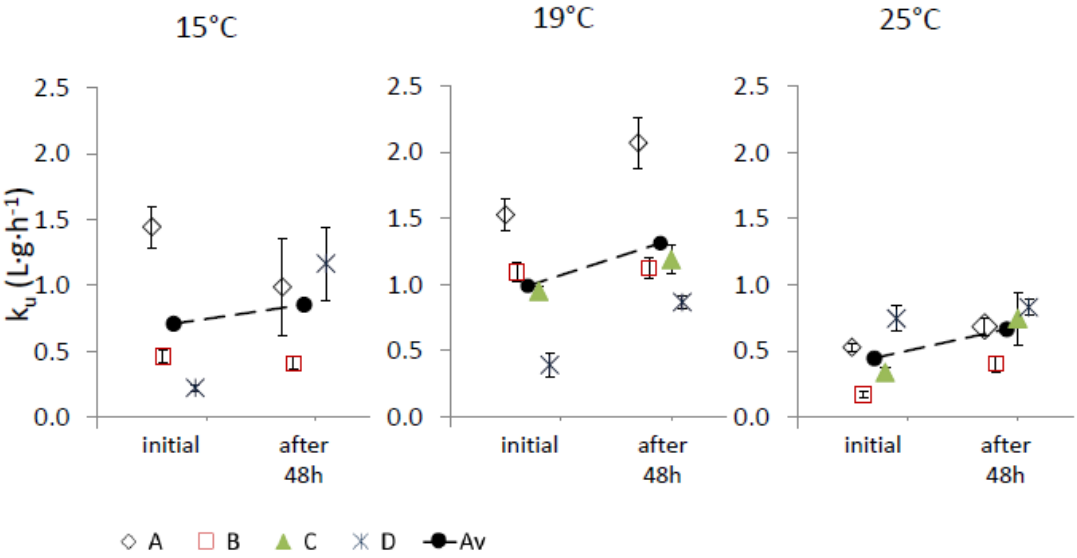

Figure 3

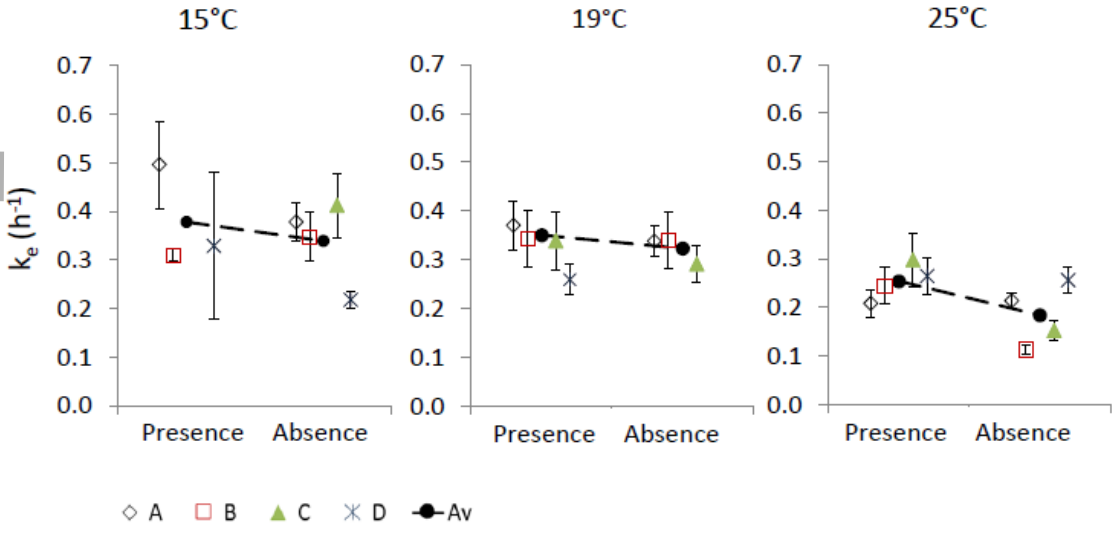

Figure 4