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Temperature-Dependent Effects of Cadmium on *Daphnia magna*: Accumulation versus Sensitivity

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Standard toxicity tests are performed at one constant, optimal temperature (usually 20 °C), while in the field variable and suboptimal temperatures may occur. Lack of knowledge on the interactions between chemicals and temperature hampers the extrapolation of laboratory toxicity data to ecosystems. Therefore, the aim of this study was to analyze the effects of temperature on cadmium toxicity to the waterflea *Daphnia magna* and to address possible processes responsible for temperature-dependent toxicity. This was investigated by performing standard toxicity tests with *D. magna* under a wide temperature range. Thermal effects on accumulation kinetics were determined by estimating uptake and elimination rates from accumulation experiments. To study temperature dependency of the intrinsic sensitivity of the daphnids to cadmium, the DEBtox model was used to estimate internal threshold concentrations (ITCs) and killing rates from the toxicity and accumulation data. The results revealed that increasing temperature lowered the ITC and increased the killing rate and the uptake rate of the metal. Enhanced sensitivity of *D. magna* was shown to be the primary factor for temperature-dependent toxicity. Since temperature has such a major impact on toxicity, a temperature correction may be necessary when translating toxicity data from the laboratory to the field.

Introduction

Standard toxicity tests performed in the laboratory are used extensively to predict the effects of chemicals in ecosystems. These toxicity tests are mostly performed at a constant and favorable temperature, usually 20 °C, while in the field, temperature is highly variable because of season and climate. As most aquatic organisms are ectotherms, temperature is an important factor having a high impact on the rate of most physiological processes. This may have great effects on the exposure of organisms to toxicants. For instance, differences in the ambient temperature may affect uptake, elimination, and detoxication rates because of changes in metabolic, locomotory, and feeding activity of organisms (*1*−*4*). Besides alterations in exposure, the sensitivity of organisms to chemical compounds may be modified by changes in the physiological condition, for example, by the induction of cold- or heat-protective proteins. Furthermore, close to the thermal tolerance limits, temperature stress may enlarge adverse effects of toxicants (*1*, *2*, *5*).

A number of reviews considered the joint effects of contaminants and temperature (*1*, *5*, *6*) and revealed that temperature is of major importance for the outcome of toxicity tests. Although many authors cited in these reviews proposed underlying mechanisms responsible for the observed interactions between temperature and chemicals, such as altered accumulation kinetics and sensitivity of the organisms, few studies have actually tested these hypotheses. The aim of this study was therefore to analyze the influence of temperature on the acute toxicity of metals and to address the processes responsible for a possible temperature-dependent toxicity. With this purpose, the waterflea *Daphnia magna* was exposed to a range of water temperatures, and thermal effects on toxicity and accumulation kinetics of cadmium were determined. The influence of temperature on the intrinsic sensitivity of the daphnids to cadmium was evaluated by relating tissue cadmium concentrations to toxic effects in time, assuming that the tissue concentration determines the effect. It is hypothesized here that, if temperature only affects accumulation kinetics of chemicals, then the intrinsic sensitivity should be the same for all temperature regimes. This hypothesis was investigated with the mathematical model DEBtox (*7*). DEBtox is able to describe time-dependent toxicity data, which contains information about the dynamic aspect of the occurrence of effects. The model was adapted to fit the data from the toxicity and accumulation experiments simultaneously to reveal if thermal effects on cadmium toxicity resulted from changes in accumulation kinetics or intrinsic sensitivity of the daphnids or both.

Materials and Methods

Culture Conditions. The *D. magna* population used in the present study was obtained from the Institute for Inland Water Management and Waste Water Treatment (RIZA, Lelystad, The Netherlands), where it was cultured for several years. The culture consisted of cohorts with a density of 20 daphnids/L. A cohort was kept for 4−5 weeks, whereafter a new cohort was started with at least third-brood neonates (<24 h). Artificial Elendt M7 medium was used for culturing (*8*). The medium was renewed three times a week, and juveniles were removed. The culture was maintained under a light−dark regime of 16:8 h and at a temperature of 20 °C. On working days, the daphnids were fed with 2 mg of C L^{-1} of a concentrated suspension of *Selenastrum capricornutum*. The algae were cultured in a chemostat in Woods Hole medium (*9*). Every week, algae were harvested and centrifuged at 3000 rpm for 10 min. The supernatant was removed, and the algae were resuspended in Elendt M7 medium, whereafter the total organic carbon concentration of the suspension was measured with a total carbon analyzer

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(College Station, TX). The suspension was stored at 4 °C in a dark room until used for feeding.

Experimental Design. To study temperature-dependent toxicity and to distinguish between thermal effects on accumulation kinetics and sensitivity of the daphnids, the following experiments were conducted:

Influence of Temperature on Toxicity. To study temperature effects on cadmium toxicity to the daphnids, acute toxicity tests at a temperature range of 10−35 °C were performed.

Influence of Temperature on Accumulation Kinetics. To examine how temperature affects accumulation kinetics, short-term accumulation experiments were executed at 10−26 °C. This temperature range was chosen because at these temperatures no control mortality occurred because of temperature stress during the test period.

Influence of Temperature on Sensitivity. (a) To detect if the sensitivity of the daphnids is altered by temperature, time-dependent toxicity tests at 10−26 °C were performed. In these tests, the survival of the daphnids was measured at several exposure times, in contrast to the toxicity experiments at 10−35 °C described above.

(b) The results of (a) were combined with the outcomes of the accumulation experiments and analyzed with the DEBtox model. To ensure that the cadmium accumulation pattern was independent of the exposure concentration, a short-term accumulation experiment was performed at 20 °C in which cadmium accumulation was studied at exposure concentrations used in the time-dependent toxicity tests.

The daphnids were not acclimated to the test temperature prior to exposure because acclimation may result in differentiation between individuals. During the acclimation period, the daphnids at the higher temperatures will reach a larger body size than those at the lower temperatures, leading to variation in the initial body size of the daphnids, which hampers the interpretation of the test results. Furthermore, acclimation of animals to temperatures in the higher temperature range may prove to be useless because animals exposed to these stressful temperatures are likely to die within the acclimation period.

Toxicity Tests. *Acute Toxicity Tests at 10−35 °C.* Acute toxicity tests were performed in accordance with standard protocols (*10*), except where noted. Test animals used were at least third-brood neonates. Groups of five daphnids (<24 h) were assigned to 60-mL polypropylene tubes containing 50 mL of test medium without food. Per temperature, at least six cadmium concentrations were tested, obtained by diluting a solution of cadmium chloride (Titrisol, Merck) in Elendt M7 medium. The test tubes were placed at 10, 13, 16, 20, 23, and 26 °C (two replicates per treatment) and at 29, 32, and 35 °C (three replicates per treatment). A light−dark regime of 16:8 h was applied. After 24 and 48 h, the number of animals not responding to gentle stimulation with a pipet was scored. Those animals were considered to be dead. To determine the actual cadmium concentrations in the water, 1-mL water samples of each treatment were taken in duplicate after 1, 24, and 48 h. The samples were acidified with 20 μL of 65% nitric acid (Merck, p.a.) and analyzed by air−acetylene flame (Perkin-Elmer 1100B) or graphite furnace atomic absorption spectrometry (AAS) (Perkin-Elmer 5100PC/HGA600/AS60), depending on the metal concentration in the samples. The oxygen concentration in the test solutions during the experiment was at least 86% of the air saturation value at the temperature used.

Time-Dependent Toxicity Tests at 10−26 °C. Toxicity tests were performed at 10, 20, and 26 °C following the procedure described above. However, in these experiments, the survival of the daphnids was scored twice a day during 96 h of exposure (three replicates per temperature and cadmium treatment). Each day, water samples were taken in duplicate for measurement of the actual cadmium concentration.

Accumulation Experiments. *Short-Term Accumulation Experiments at 10−26 °C.* The influence of temperature on cadmium accumulation was studied in neonates (<24 h) descending from at least the third brood. Three groups of 60 and three groups of approximately 200 daphnids were randomly transferred to 600-mL glass beakers containing 500 mL of Elendt M7 medium (control) and test medium with an actual concentration of 101 ± 0.24 (SE) μg of Cd L^{-1} Elendt M7 medium, respectively. One control and one cadmium-containing beaker were placed at each of the selected temperatures: 10, 20, and 26 °C. After 2, 5, 8, 24, and 45 h, 8−15 cadmium-exposed daphnids were collected from each temperature treatment. Control animals were gathered at the start of the experiment and when a treatment was ended. Only daphnids that were not killed or immobilized were used. Therefore, the number of animals gathered at the end of the experiment could be smaller than at the beginning, but enough animals survived the treatment to ensure a reliable analysis of the cadmium concentrations. Depending on the number of daphnids available, the number of replicates per exposure time and temperature was two or three. The collected daphnids were kept in a beaker containing clean, double-distilled water for 10 min, pooled in 2-mL polyethylene tubes, lyophilized, weighed, and digested in 65% nitric acid (J. T. Baker, Ultrex) and 30% hydrogen peroxide (Fluka, purum p.a.) using the micro-destruction method described in ref *11*. The concentrated samples were diluted with 500 μL of acidified analytical grade water (5 mL of 65% nitric acid L^{-1} (J. T. Baker, Ultrex)) and analyzed by graphite furnace AAS (Perkin-Elmer 5100PC/HGA600/AS60). Water samples (1 mL, in triplicate) were taken after 1, 24, and 45 h. After acidification with 20 μL of 65% nitric acid (Merck, p.a.), the actual cadmium concentration in the samples was analyzed by air−acetylene flame AAS (Perkin-Elmer 1100B).

Short-Term Accumulation Experiments at 20 °C. The accumulation experiments at 10−26 °C described above focused on the accumulation pattern of cadmium at only one exposure concentration (101 μg of Cd L^{-1}). To verify if the uptake and elimination rate constants were independent of the cadmium concentration, a simplified accumulation experiment at 20 °C was performed. The same procedure as was described for the accumulation experiments at 10−26 °C was used, with the following exceptions. One group of 90 and three groups of 270 daphnids (<24 h) were allocated to 600-mL glass beakers containing 500 mL of Elendt M7 medium (control) and test media with actual concentrations of 217 ± 0.39, 452 ± 0.59, and 1112 ± 2.2 μg of Cd L^{-1} Elendt M7 medium, respectively. All beakers were placed at a temperature of 20 °C. After 0 and 72 h (control); 24, 48, and 72 h (217 μg of Cd L^{-1}); 5, 24, and 48 h (452 μg of Cd L^{-1}); and 5, 10, and 24 h (1112 μg of Cd L^{-1}), 15 mobile animals were collected in triplicate and analyzed for cadmium tissue concentration following the method described above. The choice for these exposure times was based on the outcomes of the toxicity tests: the effects of cadmium on survival of the daphnids started between the first and the last sampling time. Water samples (1 mL) were taken in triplicate daily for measurement of the actual cadmium concentration.

Data Analysis. *Acute Toxicity Tests at 10−35 °C.* Thermal effects on acute cadmium toxicity were assessed by calculating 24- and 48-h LC_{50} values for all temperatures by means of nonlinear regression, using the logistic response model (*12*):

$$Y = \frac{S_0}{1 + e^{b(X-a)}} \quad (1)$$

where Y and S_0 (%) represent survival in cadmium and control

treatments, respectively, a is the log LC_{50} (LC_{50} in μg of Cd L^{-1}), b is the slope, and X is the log concentration (actual exposure concentration in μg of Cd L^{-1}).

At temperatures ranging from 10 to 26 °C, there was no mortality in the control treatment and S_0 was set at 100%. However, at the higher temperatures control mortality occurred. Therefore, S_0 was estimated by use of eq 1. Following Van Gestel and Hensbergen (*13*), the LC_{50} values obtained in the range of temperatures were tested two by two for significant differences by fitting the data simultaneously by the logistic response model, once by using a separate LC_{50} parameter (a in eq 1) for each temperature treatment and once by using the same LC_{50} parameter for both treatments. The outcomes of these fits were then compared using a likelihood ratio test (*14*). The significance level was adjusted for multiple comparisons by the Bonferroni correction (*14*). This implied that α was lowered from 0.05 to 0.002. Nonlinear regressions and statistical analyses were performed with the computer program SPSS (version 10.0.5, SPSS Inc.).

Short-Term Accumulation Experiments at 10−26 °C. After log-transforming the tissue cadmium concentrations, the differences in cadmium accumulation by daphnids exposed at 10, 20, and 26 °C were tested for significance by analysis of variance (ANOVA). When significant treatment effects were revealed ($\alpha = 0.05$), a Student−Newman−Keuls post hoc test ($\alpha = 0.05$) was used to determine which treatments differed from each other.

The cadmium concentrations in the daphnids were used to estimate uptake and elimination rate constants assuming the simple linear one-compartment model:

$$C_i = \frac{k_1}{k_2} C_e (1 - e^{-k_2 t}) + C_{i,0} e^{-k_2 t} \quad (2)$$

where C_i (mg of Cd kg^{-1} dw (dry weight)) is the tissue cadmium concentration, k_1 (L kg^{-1} dw h^{-1}) and k_2 (h^{-1}) denote the uptake and elimination rate, respectively, C_e (mg of Cd L^{-1}) is the cadmium concentration in the test medium, t (h) represents time, and $C_{i,0}$ (mg of Cd kg^{-1} dw) is the cadmium concentration in the daphnids at the start of the experiment.

The parameters were estimated by nonlinear regression. The differences between uptake and elimination rates at the three temperatures were tested for significance ($\alpha = 0.017$, Bonferroni correction for multiple comparisons (*14*)) using the method of Van Gestel and Hensbergen (*13*) described above. Nonlinear regressions as well as all statistical analyses were executed with the computer program SPSS (version 10.0.5, SPSS Inc.).

Time-Dependent Toxicity Tests. The data of the time-dependent toxicity tests were evaluated by the DEBtox model, which was used to reveal whether the influence of temperature on the response to cadmium was due to changes in the intrinsic sensitivity of the daphnids. Since the original model is not able to fit accumulation and toxicity data simultaneously, additional equations were programmed in MatLab 6.1 (The Mathworks, Inc.). A full description of the model is given in ref *7*, but the most important assumptions and equations as well as extensions of the model are summarized here (see also ref *15*). The kinetics of the compound is assumed to follow a simple linear one-compartment model as given by eq 2 but assuming that the initial tissue cadmium concentration is an inert fraction that is not eliminated. Instead of estimating k_1, DEBtox fits the ratio of k_1 and k_2, which is also known as the bioconcentration factor (BCF; L kg^{-1}), as a model parameter.

According to DEBtox, the toxicity of a chemical is given by the survival probability of individuals, which is specified via the hazard rate (h). The product $h\Delta t$ can be interpreted as the probability to die in the small time interval Δt, given that the animal has survived up to that moment. The survival probability can be expressed as:

$$q(t,C_e) = \exp(-\int_0^t h(\tau,C_e)\, d\tau) \quad (3)$$

where $q(t,C_e)$ (−) is the probability to survive until time t and $h(\tau,C_e)$ (h^{-1}) is the hazard rate at time τ, both a function of the toxicant concentration in the water (C_e).

DEBtox assumes the existence of a true no-effect concentration (NEC); a concentration causing no additional mortality of the organisms, even after long exposure. Normally, internal concentrations are not measured in toxicity experiments; therefore, DEBtox treats the internal concentration as a hidden variable: the tissue concentration (C_i) is scaled with the BCF in order to obtain a quantity that is directly proportional to the tissue concentration but has the dimension of an external concentration. In the present study, however, the elimination rate (k_2) was very small, which hampers an accurate estimation of the BCF and, as a result, the NEC. Instead, the tissue cadmium concentrations measured in the accumulation experiments were used, making it possible to estimate an internal threshold concentration (ITC), which is the internal analogue of the NEC. When the ITC is exceeded, the hazard rate is assumed to increase proportionally to the difference between $C_i(t,C_e)$ and the ITC:

$$h(t,C_e) = \begin{cases} k_\dagger(C_i(t,C_e) - \text{ITC}) + h_0(t) & \text{if } C_i(t,C_e) > \text{ITC} \\ h_0(t) & \text{if } C_i(t,C_e) \le \text{ITC} \end{cases} \quad (4)$$

where $k_\dagger$ (kg dw (mg of Cd)$^{-1}$ h^{-1}) represents the killing rate, $h_0(t)$ (h^{-1}) is the background hazard rate, and ITC (mg of Cd kg^{-1} dw) is the internal threshold concentration.

The killing rate is the proportionality factor that describes the relation between the hazard rate and the tissue concentration that exceeds the ITC. It is a measure for the toxicity of a compound and has the dimension [(tissue concentration·time)$^{-1}$]. Both the ITC and the killing rate are measures for the intrinsic sensitivity of the daphnids to cadmium.

The blank hazard rate (h_0) is assumed to be constant in the standard DEBtox model. In the current study, however, control mortality increased with temperature and time, which was probably caused by starvation. Therefore, when control mortality occurred in a temperature treatment, blank mortality was assumed to follow a Weibull function:

$$h_0(t) = \alpha t^\beta \quad (5)$$

where α and β are empirical constants.

The DEBtox model fitted the data of the toxicity and accumulation experiments simultaneously, the model parameters were estimated by maximum likelihood methods (*14*), and the 95% confidence intervals (CI) were determined using the profile likelihood (*16*).

Results

Influence of Temperature on Toxicity. In the acute toxicity tests at 10−35 °C, the average cadmium recovery in the water at the end of the experiment (as percentage of the concentration at the start of the experiment) was 103 ± 2.3%. The effect of temperature alone on survival of *D. magna* can be determined by analyzing control survival for temperatures ranging from 10 to 35 °C, which is given in Figure 1. Up to 26 °C, there was no control mortality during the exposure period, but above this temperature, survival decreased drastically to 0 at 35 °C. This decline was more severe after 48 h than after 24 h. Figure 1 also shows that the LC_{50} after

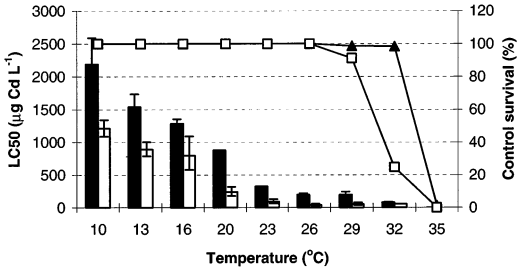

FIGURE 1. Control survival (lines) and LC_{50} (bars) for *Daphnia magna* exposed to cadmium at 10−35 °C for 24 (triangles and black bars) and 48 h (squares and white bars). Error bars represent 95% confidence intervals.

TABLE 1. Significant Differences in 24 and 48 h LC_{50} Values between Temperatures Ranging from 10 to 32 °C As Tested with a Likelihood Ratio Test ($\alpha = 0.002$)[a]

24 h								48 h							
temp (°C)	10	13	16	20	23	26	29	temp (°C)	10	13	16	20	23	26	29
13	nd							13	*						
16	*	–						16	*	–					
20	*	*	*					20	*	*	–				
23	nd	nd	nd	nd				23	*	*	–	nd			
26	*	*	*	*	nd			26	*	*	–	*	nd		
29	*	*	*	*	*	–		29	*	*	–	nd	nd	–	
32	*	*	*	nd	nd	*	*	32	nd	*	nd	nd	nd	–	–

[a] Symbols indicate the following: *, significant difference; –, no significant difference; and nd, not determined. No comparisons were made for 35 °C since there were no surviving daphnids in any of the treatments.

24 and 48 h decreased with increasing temperatures. A longer exposure time generally decreased the LC_{50} value, but the influence of temperature on the LC_{50} value showed the same trend for both 24 and 48 h of exposure. LC_{50} values were not calculated for 35 °C as no daphnids survived in any of the treatments. The results of the statistical analyses are given in Table 1. The cases where statistical differences could not be determined were caused by a poor fit of the dose−response curve or by high control mortality. Table 1 shows that all 24-h LC_{50} values at the various temperature regimes differed but that the 48-h LC_{50} values in the high-temperature range were similar. The 48-h LC_{50} obtained for the 16 °C treatment did not differ from the other treatments, which was probably due to the large confidence interval.

Influence of Temperature on Accumulation Kinetics. In the accumulation experiments at 10−26 °C, the average cadmium concentration in the water that was recovered at the end of the experiment was 107 ± 0.53% of the initial concentration. In the 26 °C treatment, a high number of animals was immobilized due to the cadmium exposure; therefore, this treatment was already ended after 24 h. Survival in control and exposed groups was 95 and 92% at 10 °C, 100% and 90% at 20 °C (45-h exposure period), and 98 and 82% at 26 °C (24-h exposure period), respectively. Control animals contained very little cadmium (4.65 ± 0.12 mg of Cd kg^{-1} dw). Figure 2 shows the cadmium concentrations in daphnids held at the three temperatures after different exposure times. Generally, elevated temperatures resulted in higher tissue concentrations. The cadmium tissue concentration could not be determined after 45 h for the highest temperature due to mortality, caused by the relatively high exposure concentration of 101 μg of Cd L^{-1}. As the results of the acute toxicity test show, this concentration is above the 48-h LC_{50} obtained at 26 °C (Figure 1). Therefore, the accumulation curve for this temperature treatment should be considered with care. From the course of the accumulated

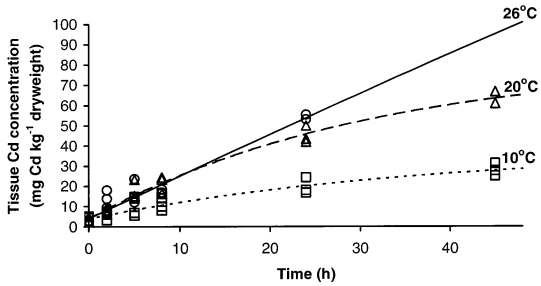

FIGURE 2. Tissue cadmium concentrations of *Daphnia magna* exposed to 101 ± 0.24 (SE) μg of Cd L^{-1} for 45 h at 10 and 20 °C and for 24 h at 26 °C. Symbols represent data points, while the lines are fitted to the data following eq 2.

TABLE 2. Uptake Rates with 95% Confidence Intervals (CI) Estimated for *Daphnia magna* Exposed to 101 ± 0.24 (SE) μg of Cd L^{-1} for 45 h at 10 and 20 °C and for 24 h at 26 °C

temp (°C)	k_1 (L kg^{-1} dw h^{-1})	95% CI
10	10.5	5.5−15
20	25.3	20−30
26	22.2	11−33

cadmium concentration over time, uptake rates were estimated (summarized in Table 2). In Figure 2 can be seen that the uptake is still in the linear range, which makes the estimation of elimination rates impossible. Statistical analysis revealed a significant difference between the uptake rates at 10 and 20 °C, but no difference between the rates at 10 and 26 °C nor between those at 20 and 26 °C (likelihood ratio test, $\alpha = 0.017$). Again, the high test concentration could have inhibited the normal functioning of the daphnids in the 26 °C treatment, hampering a further increase in the cadmium uptake rate.

Influence of Temperature on Sensitivity. The average cadmium recovery in the water at the end of the time-dependent toxicity tests was 104 ± 0.96% of the concentration that was initially present. In Figure 3, the cadmium concentration in the daphnids (upper panel) and the fraction of surviving daphnids (lower panel) are plotted against time. The simple one-compartment model used by DEBtox provides an adequate fit to the cadmium concentration in the daphnids. The accumulation data at 20 °C showed that the uptake and elimination rate constants were independent of the exposure concentration. The survival of the daphnids was described well by the estimated tissue concentrations, and the results were comparable with the toxicity tests at 10−35 °C, with toxicity increasing with rising temperature. At 20 °C, however, the best fit was obtained when mortality in the lower cadmium treatments was regarded as control mortality, resulting in an overestimation of control mortality. Otherwise, DEBtox was incapable of fitting all cadmium exposures with one ITC. The daphnids at low cadmium concentrations appeared to become more vulnerable to cadmium after a longer exposure time (resulting in a lowering of the ITC), which was presumably due to starvation, as in chronic experiments with food and the same temperature treatments no control mortality was observed (unpublished results). The daphnids at the higher concentrations were not living long enough to experience these adverse effects. For the same reason, the longer exposure period caused high control mortality at a temperature of 26 °C, in contrast with the previous toxicity experiments at 10−35 °C. Better fits for 20 and 26 °C could be obtained by assuming that the ITC decreased in time, but since it is highly speculative in which way the ITC changes in time, these model fits were not shown.

In Table 3, the estimated model parameters are given. The killing rate and the BCF are highly correlated with the

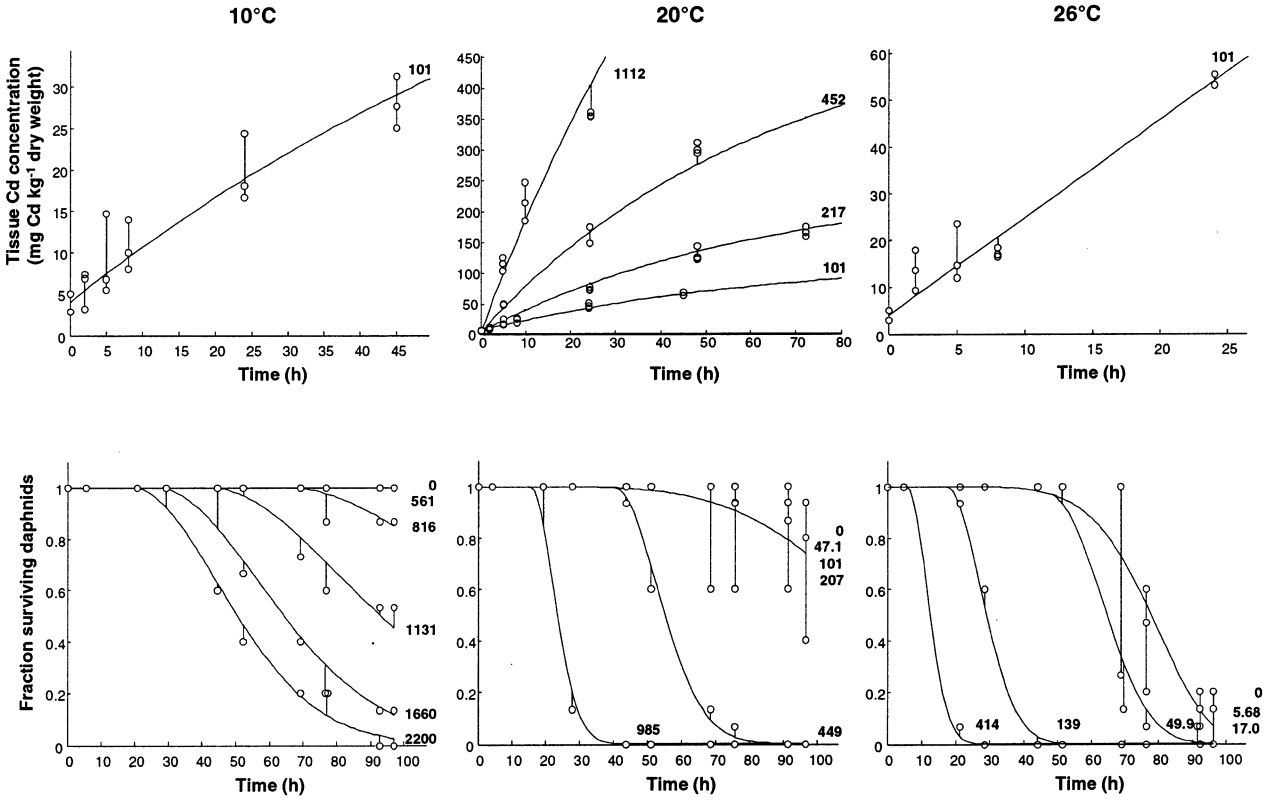

FIGURE 3. Tissue cadmium concentrations of *Daphnia magna* (upper panel) and the fraction of surviving *D. magna* at 10, 20, and 26 °C (lower panel). Test duration of accumulation experiments was 45 h (10 °C and 101 μg of Cd L^{-1} at 20 °C), 24 h (26 °C), and 0−72 h (other concentrations at 20 °C). Test duration of toxicity tests was 96 h. Symbols represent data points, while lines are fitted to the data by the DEBtox model. The numbers on the right side of each line correspond to the actual cadmium concentration in the treatment (μg of Cd L^{-1}).

TABLE 3. Parameters Estimated by DEBtox by Fitting Toxicity[a] and Accumulation[b] Data Simultaneously

symbol	description	unit	temp (°C)	parameter value	likelihood based 95% CI
α	parameter of Weibull function		10	ne[c]	
			20	1.02e-9	8.0e-15−3.0e-6
			26	3.47e-12	2.8e-15−1.4e-9
β	parameter of Weibull function		10	ne	
			20	3.60	1.7−6.3
			26	5.40	4.0−7.0
BCF	bioconcentration factor		10	6.53e2	3.6e2−6.1e4
			20	1.13e3	8.8e2−1.6e3
			26	1.00e6	2.9e3−∞
k_2	elimination rate	h^{-1}	10	1.09e-2	9.5e-5−2.4e-2
			20	1.57e-2	1.0e-2−2.2e-2
			26	2.15e-5	−∞−8.1e-3
k_T	killing rate	kg dw (mg of Cd)$^{-1}$ h^{-1}	10	8.03e-2	3.8e-2−8.6
			20	1.80	9.3e-1−3.3
			26	3.26e3	1.9e3−5.4e3
ITC	internal threshold concn for survival	mg of Cd kg^{-1} dw	10	2.70e2	2.2e2−3.3e2
			20	2.39e2	2.1e2−2.6e2
			26	5.16e1	4.2e1−6.1e1

[a] Time-dependent toxicity tests at 10−26 °C with a 96-h test duration. [b] Short-term accumulation experiments at 10−26 and 20 °C with a 24−45-h and 0−72-h test duration, respectively. [c] ne, not estimated.

elimination rate. Since the elimination rate is very small, the three parameters could not be accurately estimated, and comparisons of the parameters at the three temperatures cannot be made. However, the trend of higher killing rates at elevated temperatures suggests that cadmium became more toxic at rising temperatures. Nevertheless, the model fits resulted in reliable estimates of ITCs at the three temperatures and revealed that there is little difference between the ITC at 10 and 20 °C, but the ITC is significantly lower at 26 °C.

Discussion

Influence of Temperature on Cadmium Toxicity. The results of the acute toxicity tests showed that the effect of cadmium on the survival of *D. magna* is highly temperature-dependent and that temperature itself may become lethal as well when it exceeds the thermal tolerance limit of the daphnids. Thermal effects on survival of daphnids were also reported by Work and Gophen (*17*), who found that life span of 2−3-day-old *D. lumholtzi* decreased from 29 d at 15 °C to 17 d

at 29 °C. The toxicity data are in accordance with the findings of other authors (*18*−*20*), who observed lower cadmium resistance of *D. magna* at elevated temperatures.

Although this study as well as the available literature agrees that temperature increases toxicity, the question remains if the observed temperature-dependent toxicity is due to altered toxicant accumulation, susceptibility of the daphnids, or a combination of these factors. In the following sections, the significance of these factors is discussed.

Influence of Temperature on Cadmium Accumulation. The present study showed that cadmium uptake rates at 20 °C were significantly higher than the rate at 10 °C. Few studies considered the influence of temperature on cadmium kinetics in *Daphnia*. Stuhlbacher et al. (*19*) observed four times higher cadmium accumulation at 30 than at 10 °C in *D. magna* treated with 100 μg of Cd L^{-1} for 24 h at 10, 20, and 30 °C. Overall, the cadmium concentrations measured in the daphnids were 3−5 times higher than in the present study, probably due to differences in the test medium used.

Studies concerning thermal effects on the accumulation of cadmium by other test species showed the same trend as observed in this paper: uptake rates and the amount of cadmium accumulated during a certain period of time was higher at elevated temperatures. For instance, Bervoets et al. (*21*) found a 14 times higher cadmium uptake rate at 25 °C as compared with 5 °C in the midge *Chironomus riparius*. Higher cadmium body concentrations at elevated temperature were also reported for several other species, such as burrowing mayfly nymphs (*22*), freshwater isopods (*23*), Asiatic clams (*24*), Japanese eel (*25*), and fingerlings of perch (*26*). These observations can be explained by an increase in metabolic rate and thus oxygen demand when ectothermic organisms are exposed to a temperature rise. This causes elevated ventilation rates, which may lead to higher cadmium accumulation at higher temperatures (*1*, *24*). The increased metabolic rate may also result in higher active transport of cadmium across the membranes, which may increase accumulation rates as well (*1*).

In contrast to uptake rates, the temperature dependence of cadmium elimination appears to be less clear. The depuration rates of Asiatic clams were not altered by temperature (*27*). In contrast, a small but significant temperature effect on cadmium elimination was found in freshwater isopods since the metal was eliminated at 5 °C but not at 10 and 20 °C (*23*). Burrowing mayfly nymphs eliminated cadmium rapidly, and a small but significant increase of the elimination rate was observed when temperature was elevated (*28*).

Summarizing, in the present study as well as in others, increased cadmium uptake rates and accumulation at elevated temperatures are reported and some small but significant effects of temperature on elimination rates. Many authors suggest therefore that increased cadmium toxicity at elevated temperatures is caused by enhanced cadmium accumulation. Since it is still uncertain if changes in the sensitivity of the daphnids are involved as well, the subsequent section deals with this topic.

Temperature Effects on Sensitivity of *D. magna*. When the sensitivity of the daphnids to cadmium is not changed by temperature, the ITC and the killing rate should be equal at all temperature regimes. The temperature dependency of the ITCs estimated by DEBtox showed that this hypothesis is invalid. The ITC was smaller at 26 °C as compared with 10 and 20 °C, implying that at 26 °C less cadmium needed to be accumulated to induce lethal effects than at lower temperatures. Although the estimation of the killing rate was uncertain, the value increased strongly with rising temperature over the whole temperature range studied, indicating that cadmium effects were amplified with rising temperature. It is thus concluded that thermal effects on cadmium toxicity cannot be ascribed to accumulation kinetics alone and that altered susceptibility of the daphnids plays an important role as well.

Accumulation versus Sensitivity. The results presented in this study showed that temperature stress alone caused mortality of daphnids when the temperature reached a certain upper thermal tolerance limit. Furthermore, temperature had a major impact on cadmium toxicity, which was attributable to various mechanisms. In the lower temperature range, temperature rise accelerated uptake kinetics, causing higher cadmium toxicity. In the higher temperature range, increased uptake was less important, while the contribution of increased sensitivity of the daphnids became more significant, as shown by the lowering of the ITC. Also the temperature dependency of the killing rate indicated that the intrinsic sensitivity to cadmium, despite the uncertain estimation of this parameter, increased sharply with temperature. The present model allowed us to compare quantitatively the roles of increased uptake and susceptibility of the daphnids throughout wide temperature ranges. Determination of cadmium accumulation and survival in time proved to be vital parameters. Although accumulation kinetics as well as sensitivity of the daphnids were influenced by temperature and both processes in conjunction determined the wide ranges of cadmium toxicity observed, intrinsic temperature-dependent sensitivity to cadmium was shown to be the primary factor for toxicity in daphnids.

Many previous studies (reviewed in refs *1*, *5*, and *6*) have considered the influence of temperature on toxicity of chemicals. The generally observed temperature−toxicity relationship was thought to be related to changes in accumulation kinetics or sensitivity of the test organisms. Since these assumptions were never tested, the relative importance of the interacting processes responsible for temperature-dependent toxicity remained elusive. The present study proved that a combined approach of experiments and modeling is essential to disentangle interacting processes and to test hypotheses on the role of mechanisms of multiple stress. The present study is likely to have consequences for the wide-spread practice of extrapolating laboratory results to ecosystems that so far totally ignores temperature-modified toxicity.

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