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*Hazard/Risk Assessment*

# USING A BIOLOGY-BASED MODEL (DEBTOX) TO ANALYZE BIOASSAYS IN ECOTOXICOLOGY: OPPORTUNITIES AND RECOMMENDATIONS

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**Abstract**—The conventional analysis of bioassays does not account for biological significance. However, mathematical models do exist that are realistic from a biological point of view and describe toxicokinetics and effects on test organisms of chemical compounds. Here we studied a biology-based model (DEBtox) that provides an estimate of a no-effect concentration, and we demonstrated the ability of such a model to adapt to different situations. We showed that the basic model can be extended to deal with problems usually faced during bioassays like time-varying concentrations or unsuitable choices of initial concentrations. To reach this goal, we report experimental data from *Daphnia magna* exposed to zinc. These data also showed the potential benefit of the model in understanding the influence of food on toxicity. We finally make some recommendations about the choice of initial concentrations, and we propose a test with a depuration period to check the relevance and the predictive capacity of the DEBtox model. In our experiments, the model performed well and proved its usefulness as a tool in risk assessment.

**Keywords**—No-effect concentration Time-varying concentrations Acute toxicity *Daphnia magna* Model

## INTRODUCTION

Several toxicity parameters, like no-observed-effect concentration (NOEC) or the concentration leading to 50% of effect (EC50), are derived from bioassays results [1]. They are used to calculate benchmark concentrations of chemicals in the environment. The estimation of a NOEC is usually done without a model but has been severely criticized in a number of publications on both theoretical and practical grounds. Chapman et al. [2] reviewed these critics and cautioned that the use of NOEC in legislation was inappropriate.

In contrast to the NOEC, the ECx (concentration leading to *x*% of effect) parameters are mostly model dependent, but the models usually used (logit, probit) have no biology-based assumptions that allow questions about the relevancy of such models. As an attempt to face this problem, the log-logistic model tries to account for an interaction between the xenobiotic and the receptor in the organism using the Hill equation [3]. It is a first step toward biological relevancy. Nevertheless, it can provide a concentration responsible for only a certain percentage of effects (reduction of 50% of the number of eggs, death of 50% of the organisms, and so on), and therefore it is problematic for use in risk assessment [4].

Kooijman and Bedaux [5] have proposed a model based on the dynamic energy budgets (DEB) theory [6], DEBtox, to estimate a no-effect concentration (NEC) for the effects of chemicals on survival, growth, and reproduction. This parameter is the highest concentration having no effect on the test organism for any time point during the bioassay. It is one alternative to the NOEC. In the DEBtox model, the concentration of the xenobiotic inside the organism is derived from the concentration in the solution according to a simple kinetics model of one compartment. Effects of chemicals are modeled by a change in the parameters of the dynamic energy budget of the test organisms. This change is a function of the tissue concentration of the compound [5]. Nevertheless, unlike empirical models that do not depend on toxin-specific mechanisms, problems arise for the DEBtox model when metabolic transformations of the toxicant occur or when a changing lipid content is present due, for instance, to changing feeding conditions [5].

In the DEBtox model, time is incorporated in the model only to account for the toxicokinetics of the compound. The effect of the toxicant is supposed to depend only on the body concentration (see further the presentation of DEBtox). Thus, the NEC theoretically does not depend on the duration of the bioassay, contrary to the NOEC and the ECx. This provides more relevant information for risk assessment [4]; since the NEC and the effect parameters do not depend on exposure time, it is easier to extrapolate observed effects from a particular compound to that of other compounds with a similar mode of action but a different lipophilicity [4].

Another advantage of the model is that it can take into account problems usually faced when performing bioassays. In a previous paper [7], we showed that the DEBtox model can be successfully extended to tackle the problem of time-varying concentrations of the test compound in a bioassay. Since one of the problems usually faced when feeding the test organisms is the decrease of the compound concentration due to adsorption on food surfaces, we think that the extensions we proposed to take into account time-varying concentrations also provide an opportunity to deal with the question of the influence of food on the toxicity. In this paper, we aim to test this assumption.

We also propose an optimal design of bioassays to obtain the most relevant possible analysis of data with DEBtox. First,

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as the choice of the number and values of the concentrations used in a bioassay affects the precision of the parameters' estimate, we performed Monte Carlo simulations to find optimal designs. Such simulations have already been carried out to identify the optimal number and location of concentrations for the estimation of LCx (concentration leading to *x*% of lethality) and ECx [8,9]. Second, we proposed a way to check experimentally the relevance and the predictive capacity of the DEBtox model. Some people have little confidence in the concept of NEC or in the use of a simple kinetics model, such as the one-compartment model. To address this concern, we proposed to set up a short depuration period after the exposition period to check if the observed survival is in agreement with the DEBtox model. This model predicts an exponential decrease of the concentration in the tissues leading to a progressive return to background values for death rates.

We conducted two experiments with *Daphnia magna* exposed to zinc. The first one was conducted over 8 d with fed organisms. To assess the influence of food on zinc toxicity, the second one was conducted with starving organisms. As such an experiment can hardly last for more than 2 d, this assay was conducted over 48 h. The results with zinc, which is an essential metal, would probably support the existence of a range of concentrations without any lethal effect. Our experiments could furthermore contribute to meet the ''need for better ways to determine NEC levels to be able to assess environmental hazard of long-term zinc exposure'' expressed by some authors [10]. We decided to use adults because we wanted to avoid dilution by growth. On the other hand, Calow et al. [11] showed that for strongly iteroparous species, such as *D. magna*, reduction of the adult survival has a considerably stronger impact on the population dynamics than reduction of the juvenile survival.

### *DEBtox model: From exposure to effects*

The DEBtox model was fully described in Kooijman and Bedaux [5]. We recall here the main equations of the survival model.

*Kinetics module: From exposition to concentration in the body.* The kinetics of the compound is a simple linear one-compartment treatment. The uptake of the compound is assumed to be proportional to its concentration in the solution and the elimination proportional to its concentration in the body. This leads to the equation

$$\frac{dci}{dt}(t) = ke \cdot (ce(t) - ci(t)) \tag{1}$$

where $ke$ is the elimination rate, $ce(t)$ is the concentration in the solution, and $ci(t)$ is the concentration in the tissue scaled by the bioconcentration factor (BCF). This scaled concentration is thus expressed as

$$ci(t) = \frac{Ci(t)}{\text{BCF}} \tag{2}$$

If $ce(t)$ is a constant, when equilibrium is reached, we obtain, as expected

$$Ci(t) = \text{BCF} \cdot ce \tag{3}$$

The scaled concentration $ci(t)$ is proportional to the concentration in the tissue $Ci(t)$ but has the dimension of an external concentration. It has a very useful role in practical applications because internal biotic concentration can be more relevant than exposure concentration in risk assessments [12], but it plays the role of a hidden variable because it is not measured. In this paper, we assume that the initial amount of compound can be neglected, that is, $ci(0)$ equals 0, to keep a reasonable number of parameters in the model.

*Effects module: From concentration in the body to effects.* The survivor probability at time $t$, $q(t)$ is defined as the probability to survive until time $t$ and can be expressed as the exponential of minus the cumulated hazard:

$$q(t) = \exp\left(\int_0^t h(\tau)\, d\tau\right) \tag{4}$$

where $h(\tau)$ is the hazard rate at time $\tau$. For a small interval, $d\tau$, $h(\tau)d\tau$ represents the probability of dying between $\tau$ and $\tau + d\tau$ for an organism that has survived until time $\tau$. In the DEBtox approach, we suppose that a NEC exists, that is, a concentration in the bioassay having no effect on the survival of the organisms within any duration of the bioassay. As soon as the scaled concentration in the organism, $ci(t)$, exceeds this NEC, the hazard rate is supposed to increase proportionally to the difference between $ci(t)$ and the NEC:

$$h(t) = \begin{cases} b(ci(t) - NEC) + d & \text{if } ci(t) > NEC \\ d & \text{if } ci(t) < NEC \end{cases} \tag{5}$$

where $b$ is the so-called killing rate and $d$ is the background death rate, which is assumed to be constant. The model being a hazard model, the description of the data is based on the percentages of dead organisms between two measurements. These percentages are supposed to be statistically independent.

## MATERIALS AND METHODS

We performed two assays with *D. magna*. The first one was short (48 h), and the organisms were not fed. The second one was longer (8 d for the exposure period and 5 d for the depuration period), and consequently the organisms were fed.

### *Optimal choice of initial concentrations*

We used Monte Carlo simulations to find the optimal choice, in terms of accuracy, of the estimation of the number and values of the initial concentrations for the long-term bioassay. We had a total of 300 organisms to allocate. In general, the number of organisms is the most important design feature, but this factor has already been studied by Kooijman and Bedaux [5]. To simulate the expected variations of the concentration, we supposed that the concentrations decreased exponentially with a rate of decrease of 0.3/d. We assumed the concentration to be renewed every 3 d and the survival to be measured every day. For the first series of simulations, we fixed the parameters values NEC = 6, $ke$ = 0.2/d, $b$ = 0.05/d, and $d$ = 0.01/d. These are realistic values, comparable with previous estimates of survival data using DEBtox [4]. With given number and values of initial concentrations, we generated 1,000 sets of survival data using the DEBtox model and a random number generator. For each living organism, DEBtox provided its probability to die between two measurements, which was, for instance, *x*%. If the random number was below *x*% of the upper number that could be produced by the random number generator, the organism died, and if not, it survived during this particular time period. This operation was repeated for all the measurements. For each data set, we then estimated the parameters values using the DEBtox model according to the maximum log-likelihood theory, as explained in Kooijman and Bedaux [5]. We then calculated the mean and standard

deviations of the 1,000 estimates. This showed whether the estimation was biased and gave an idea of the accuracy of the estimation.

We already had some information about *D. magna* and zinc. The 48-h LC50s range generally from 0.7 to 5.3 mg/L [13–15] except in one study, where a value of 0.05 mg/L was found [16]. For the NOEC, values of 0.1 and 0.05 mg/L are reported [13,17]. We could then use the results of our simulations together with these data to choose optimal initial concentrations.

### *Experimental design*

Concentrations, pH, and hardness were measured daily except for days 1 and 7. Concentration measurements of the acidified samples (with 1% nitric acid) were performed using atomic absorption spectrometry according to AFNOR (Agence Française de Normalisation, Saint-Denis, France) standard NF T 90112.

*Daphnia magna* had been cultured prior to the experiment according to standard methods. For both bioassays, one three-week-old organism was added at random to each of the 0.4-L beakers. Two beakers were used per each of the six concentrations, resulting in a total of 40 and 30 test organisms per concentration for the long- and the short-term bioassay, respectively. In the long-term bioassay, the organisms were fed daily with the algae *Pseudokirchneriella subcapitata* cultured in a medium without zinc. The tests were conducted at 21°C with a 16:8-h light:dark photoperiod. The medium was hard water medium ($NaHCO_3$ 192 mg/L, $CaSO_4$ 120 mg/L, $MgSO_4$ 245.6 mg/L, KCl 8 mg/L). Survival data were reported every 24 h for the long-term bioassay and every 8 h for the short-term bioassay. Newborn organisms were removed every day. After exposure, the remaining organisms were put in clean water beakers for 5 d for a postexposure observation in clean water.

### *Data analysis*

The estimation of parameters was done using software we previously made to analyze survival data with time-varying concentration of the test compound [6]. The DEBtox model can be applied only with continuous concentration data. Thus, the software uses a purely descriptive method using cubic splines to describe the concentration data. We showed [6] that it is possible to use this method to describe properly the data and get an accurate estimation with the software. The statistical analysis of the survival data is based on the maximum log-likelihood theory. The software provides a 95% confidence interval for the estimate of the parameters of DEBtox.

## RESULTS

### *Optimal choice of concentrations*

Table 1 presents the results of the Monte Carlo simulation (mean and standard error for the 1,000 NEC estimates). We observed that the use of one concentration is not optimal to get a precise estimate. However, provided that at least two concentrations are used, the key factor is the upper concentration. It should not be as low as 18, which is three times the NEC, nor as high as 200, which is about 30 times the NEC. If possible, we recommend an upper concentration of about 10 times the NEC. Simulations performed with other theoretical parameters values (see the results in Table 2) did not affect our conclusions, except when the elimination rate was high. In this case, an upper concentration five times the NEC has to be favored.

Table 1. Consequences on no-effect-concentration (NEC) estimate (mean and standard error of 1,000 simulations) of the choice of initial concentrations of a 12-d bioassay with a total of 300 organisms. Simulations were performed assuming an exponential decrease of the concentration with disappearance rate 0.3/d, a renewal every 3 d, a measure of survival every day, and theoretical values of parameters: NEC = 6, $ke$ = 0.2/d, $b$ = 0.05/d, and $d$ = 0.01/d

| No. of concentrations | Concentration values | Mean of the estimate | Standard deviation |
|---|---|---|---|
| 1 | 10 | 5.7 | 2.9 |
| | 18 | 5.7 | 2.5 |
| | 30 | 5.8 | 3.9 |
| | 50 | 5.8 | 4.5 |
| 2 | 0; 18 | 5.6 | 2.9 |
| | 18; 30 | 5.9 | 0.9 |
| | 18; 50 | 6 | 1.1 |
| 3 | 0; 5; 18 | 5.7 | 3.2 |
| | 0; 18; 30 | 6 | 1.1 |
| | 10; 18; 30 | 6 | 0.7 |
| | 18; 30; 50 | 6 | 0.8 |
| | 18; 50; 100 | 5.9 | 0.6 |
| | 50; 100; 200 | 6.2 | 2.2 |
| 6 | 1; 3; 5; 10; 18; 30 | 6.2 | 0.7 |
| | 0; 10; 18; 30; 40; 50 | 5.8 | 1 |
| | 5; 10; 18; 30; 40; 50 | 6.2 | 0.6 |

Prior to the experiments presented here, we carried out a preliminary experiment. The data indicated that the NEC for zinc and *D. magna* was likely to be higher than 0.5 mg/L. Our choice of initial concentrations was 0, 0.15, 0.3, 0.6, 1.3, and 2.5 mg/L for the 8-d-exposure bioassay. We chose the initial concentrations of 0, 0.6, 1.3, 2.5, 5, and 10 mg/L for the 48-h-exposure bioassay.

These concentrations are similar to environmental concentrations. Van Hattum et al. [18] reported concentrations from 0.26 to 0.34 mg/L in a tributary of the Meuse River located in the Netherlands. A concentration of 8.17 mg/L was observed in a stream that received metals from a small abandoned mine [19].

### *Concentration measures*

In the assay with starving organisms, the concentration remained constant. This confirmed the results of Timmermans et al. [20], who observed no zinc disappearance from the water

Table 2. Consequences on no-effect-concentration (NEC) estimate (mean and standard error of 1,000 simulations) of the choice of initial concentrations of a 12-d bioassay with a total of 300 organisms. Simulations were performed assuming an exponential decrease of the concentration with disappearance rate 0.3/d, a renewal every 3 d, and a measure of survival every day. The theoretical parameters values are those used in Table 1 except for one of them as indicated in the first column

| Change of parameter value | Concentration values | Mean of the estimate | Standard deviation |
|---|---|---|---|
| $d$ = 0.001/d | 0; 18; 30 | 6 | 1.2 |
| | 18; 50; 100 | 6 | 0.6 |
| | 10; 18; 30 | 6.2 | 0.4 |
| $ke$ = 2/d | 0; 18; 30 | 6 | 1.2 |
| | 10; 18; 30 | 6.1 | 0.3 |
| | 18; 30; 50 | 7.4 | 2.2 |
| | 18; 50; 100 | 7.5 | 2.2 |
| $b$ = 0.01/d | 0; 18; 30 | 5.6 | 2.2 |
| | 10; 18; 30 | 6.1 | 1.6 |
| | 18; 50; 100 | 5.8 | 1.3 |

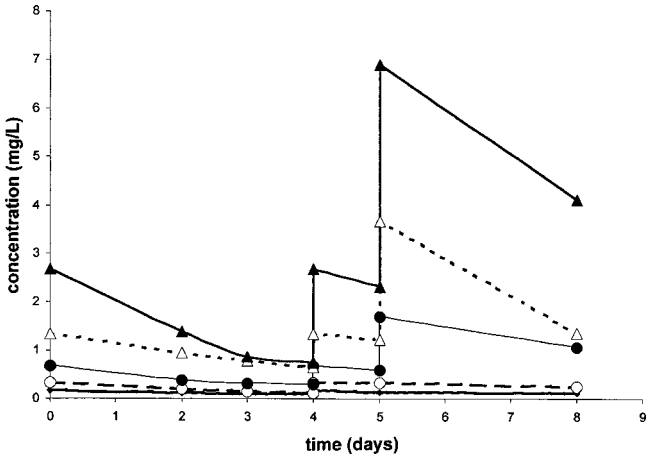


Fig. 1. Concentration variations of dissolved zinc for the bioassay with fed *Daphnia magna*. The concentration data are described with cubic splines.

column below a nominal concentration of 10 mg/L. On the contrary, a substantial disappearance was observed during the assay with fed organisms (Fig. 1). The concentration was renewed at day 4 and was tripled at day 5 because mortality was too low to lead to accurate estimates of the parameters (the survival data are available in Table 3). Tripling the actual concentration was possible because our data analysis is adapted to time-varying concentrations. Hardness (180 mg/L) and pH (7.8) were constant during the bioassays.

*Parameters estimates*

Survival data are presented in Table 3. The data analysis gave the following results: NEC = 0.75 mg/L, $ke$ = 1.8/d, $b$ = 0.06 L/mg/d, and $d$ = 0.014/d with a 95% confidence interval $0 < \text{NEC} < 1.1$ mg/L for the experiment with fed organisms. The fact that the confidence interval was large was a direct consequence of the very low mortality observed in the beginning of the experiment. Nevertheless, it would certainly have been larger without having increased the actual concentrations. As for the experiment with starving organisms, the analyses provided the following estimates: NEC = 2.2 mg/L with a 95% confidence interval $1.9 < \text{NEC} < 2.4$ mg/L, $ke$ = 2/d, $b$ = 1 L/mg/d, and $d$ = 0.037/d. First, the elimination rate estimates were quite comparable. Second, as can be expected, the starving organisms had a greater background death rate than the fed organisms. Moreover, they reacted more strongly to zinc when the NEC was exceeded (the killing rate was much greater), which could be a direct consequence of their weakness due to lack of food. In terms of toxicity, this compensated for the differences observed for the NEC estimates. Indeed, even if the NEC was higher for nonfed organisms compared to fed organisms, the hazard rate for nonfed organisms was much higher than the hazard rate for fed organisms when the external concentration was higher than 3 mg/L. The differences between the NEC estimates could be due to differences in the uptake. Indeed, fed organisms could be contaminated via food and water, whereas starving organisms were contaminated only via water.

We also tried to estimate the parameters with the data obtained after half the duration of the experiment. After 24 h of exposure, the estimate of the NEC for the assay with starving organisms was 1.2 mg/L, with a large confidence interval (as expected with only four survival measures) $0 < \text{NEC} < 2.8$ mg/L, which does not contradict the time independence of this parameter. A proper estimation after 4 d was impossible for the long-term assay because the observed hazard rate was too low for the highest exposure concentration.

Background mortality was low. Consequently, no mortality due to density effects should have occurred as predicted by the works of Burns [21], who showed that no density effect was observed in *Daphnia* species below 85 organisms/L (we used a maximum of 40 organisms/L).

*Detoxification period*

After exposure, the organisms were put in clean water for 5 d. We measured survival data each day except on day 4. The observed survival data are presented in Figure 2 for the two highest concentrations, together with predictions from the DEBtox model based on the estimates obtained with the exposure period and from a model with persistent effects that assumes that the effects during the detoxification period are the same as those observed at the end of the exposure period.

Table 3. Number of survivors as a function of time and zinc concentration for fed *Daphnia magna* (above) and starving *D. magna* (below) exposed to zinc

| Day | Control | 0.15 mg/L | 0.3 mg/L | 0.6 mg/L | 1.3 mg/L | 2.5 mg/L |
|---|---|---|---|---|---|---|
| 0 | 40 | 40 | 40 | 40 | 40 | 40 |
| 0.8 | 40 | 40 | 40 | 40 | 40 | 39 |
| 1.3 | 40 | 40 | 40 | 40 | 39 | 35 |
| 2 | 40 | 39 | 40 | 40 | 38 | 34 |
| 3 | 40 | 37 | 39 | 40 | 36 | 34 |
| 4 | 40 | 36 | 39 | 39 | 36 | 33 |
| 5 | 40 | 36 | 38 | 37 | 34 | 31 |
| 6 | 38 | 36 | 38 | 37 | 32 | 26 |
| 7 | 38 | 36 | 38 | 37 | 30 | 21 |
| 8 | 38 | 34 | 37 | 34 | 26 | 14 |
| | | 0.6 mg/L | 1.3 mg/L | 2.5 mg/L | 5 mg/L | 10 mg/L |
| 0 | 30 | 30 | 30 | 30 | 30 | 30 |
| 0.3 | 30 | 30 | 30 | 30 | 30 | 29 |
| 0.6 | 30 | 30 | 30 | 29 | 24 | 4 |
| 1 | 30 | 30 | 30 | 29 | 9 | 0 |
| 1.3 | 29 | 30 | 29 | 28 | 6 | 0 |
| 1.6 | 29 | 30 | 28 | 25 | 5 | 0 |
| 2 | 29 | 30 | 24 | 24 | 3 | 0 |

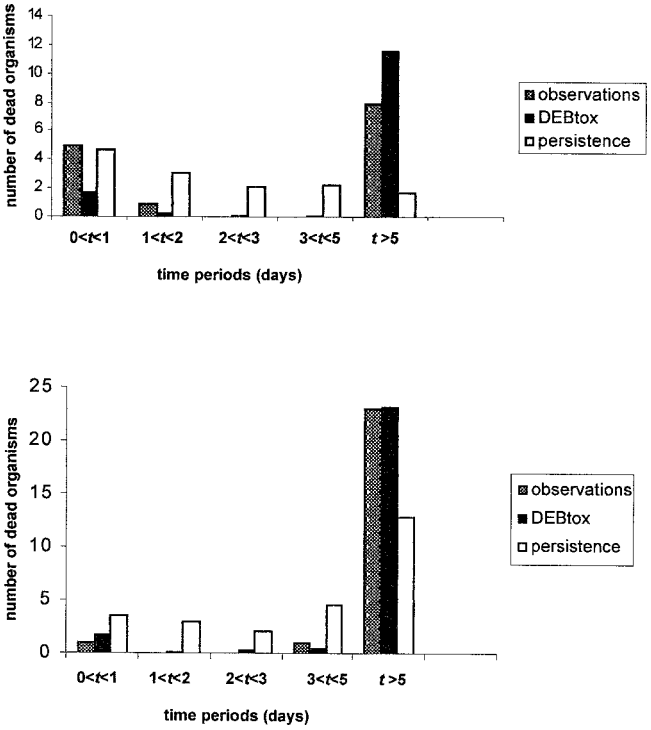


Fig. 2. Comparison between observed number of dead organisms between two measurements during the detoxification period and predictions with the DEBtox model or with a model with persistent effects (upper graph for nominal concentration 2.5 mg/L and lower graph for nominal concentration 1.2 mg/L). Only the model with persistent effects was significantly different (chi-square test, $p < 0.05$) from the observations.

With the DEBtox model, the effects disappear as soon as the internal concentration goes below the NEC. The approach of the model with persistent effects is to assume that the toxicant causes irreparable and thus persistent damage to the animal. During the detoxification period, the hazard rate is thus assumed to be equal to the hazard rate at the end of the exposure period. Here, for the two highest concentrations, mortality went back to background value after some time, and no significant difference (chi-square test, $p > 0.05$) was seen between observed data and DEBtox predictions. On the contrary, the persistent effects model failed to describe properly the observations. For the other concentrations, the predictions of both models were not significantly different from the observations.

We then incorporated the survival data of the detoxification period to get a more accurate confidence interval for the NEC. The data analysis provided the following estimates: NEC = 0.7 mg/L, $ke = 1.8$/d, $b = 0.07$ L/mg/d, and $d = 0.014$/d with a 95% confidence interval $0.15 < \text{NEC} < 1$ mg/L. This time, the NEC was significantly different from zero.

## DISCUSSION

### *Optimal choice of initial concentrations*

Our simulation results show, with little surprise, that initial concentrations should, as much as possible, be neither below nor far above the NEC. In the first case, survival data provide information only about background mortality, and in the second case, the organisms are rapidly eliminated, which gives poor survival data. Andersen et al. [22] also observed the same results when they used Monte Carlo simulations for constant concentrations to describe the influence of design characteristics on the statistical inference of the DEBtox model. In particular, they pointed out the lack of precision for the estimate of NEC if the lowest concentration is far from the NEC.

### *Time variations of zinc concentration*

The observed decline of the concentration between two renewals can be attributed to the algae. We measured the concentration at day 3, before and after removing algae from the solution by centrifugation, which indicated the partition of zinc between water and food particles. A substantial part of the compound was adsorbed on the algae, and the higher the concentration was, the more important the adsorption was (almost no adsorption for initial concentrations 0.15 and 0.3 mg/L and more than 80% of zinc adsorbed for initial concentration 2.5 mg/L). This phenomenon is probably a direct consequence of the effect of zinc on the feeding rate of *D. magna*. Allen et al. [23] showed that positively charged chemicals reduce feeding at sublethal levels. Similarly, we observed for the highest exposure levels much of the remaining food on the bottom of the beaker. Moreover, the solutions seemed to be greener than those at the low exposure levels. For high concentration levels, more algae were thus present in the solution compared to low concentration levels. Consequently, more zinc was captured. It became unavailable for the organisms when algae deposited on the bottom of the beaker. This result also explains that the actual concentrations measured after 4 d, for initial concentrations 1.3 and 2.5 mg/L, were almost the same.

No specific model exists to describe this particular way of disappearance. This confirms the usefulness of general methods such as cubic splines fitting to describe the concentration data.

As we showed with our long-term assay, models that can take into account time variations of exposure concentrations can have another use. Such models allow an increase in the concentrations during the assay. When the initial concentrations appear to be irrelevant, such a possibility can be quite useful.

### *Kinetics*

Our kinetics model can be criticized, especially for essential metals such as zinc. First, some zinc must be present initially in the tissue, but we assumed that this quantity can be overlooked when compared to the NEC. Further investigations should be conducted.

Another point concerning essential metals is that their concentration in the tissue is regulated by homeostasis. The kinetics should thus be more complicated than a simple one-compartment kinetics. Nevertheless, for concentrations above the NEC, the regulation process may be saturated because the concentration in the tissue must be higher than the background concentration to produce an effect. We suppose that when saturation occurs, the kinetics is a linear one-compartment kinetics with an upper limit value for the elimination rate. In this way, Widianarko et al. [24] showed that for the guppy *Poecilia reticulata*, the one-compartment kinetics could apply to relatively high concentrations of zinc. Moreover, the survival data we obtained during the depuration period would probably not have been predicted correctly if the elimination of the compound had been different from the one used in the model. For low concentrations, homeostatic mechanisms exist that keep the concentrations constant at a level without effect, which is thus necessarily below the NEC. Then the hazard rate is not affected by homeostatic mechanisms; it is equal to back-

ground mortality for any concentration below the NEC. In this way, for low external concentrations, that is, below the NEC, the effect is the same as described with DEBtox.

Our results suggest that the elimination rate is independent of the level of food available. However, we can expect a dependence of the uptake rate because uptake of compounds via food must be taken into account. In this paper, we implicitly assumed that the amount of toxicant in the water was proportional to the amount of toxicant adsorbed on the algae, which allowed the use of Equation 1. In this case, the consequence of uptake via food is just an increase in the bioconcentration factor and consequently a decrease in the NEC, which is in agreement with the results of our data analysis. However, other studies should be performed to confirm our assumption.

### *Effects*

The parameters of the DEBtox model do have potential benefits in risk assessment. First, the model leads to a NEC that theoretically does not depend on time. This was confirmed by our experiments when we performed a data analysis of the short-term assay after half the exposure period or when we compared the data analysis after 8 d and after 13 d for the long-term assay.

Second, it offers the opportunity to predict the consequences of different exposure conditions. Brent and Herricks [25] affirmed that conventional toxicity testing fails to predict the effects of highly variable and often brief exposure regimes of episodic pollution events. They suggested performing post-exposure observations. Nevertheless, they did not indicate how these observations could be used to assess risk. Here we propose to use them to test the ability of the model to make predictions about survival when exposure stops. When the model performs well, as it did in our experiment, risk assessment for episodic pollution events becomes possible. However, we showed here that DEBtox was able to make predictions about the lethal effects of zinc only on water fleas. More experimental work must be performed to confirm the ability of the model to deal with other organisms and other toxicants.

Third, DEBtox provides an estimate for the NEC, but together with the estimates of the three other parameters, it is possible to build an estimate for any ECx value, using Equations 1 and 5 to find the concentration that leads to a hazard rate of x/100. Here it provided 48-h LC50 estimates of 3 and 8 mg/L for nonfed and fed water fleas, respectively.

Finally, the model provides information about the influences of food. The disappearance of the compound due to the presence of food can easily be taken into account because our model can incorporate time variations of the concentration. The interpretation of the influence of food as changes in the parameters NEC and *b* seems potentially interesting even if some questions remain. Is the difference between the NEC estimates a direct consequence of a difference in terms of uptake rates, or is this uncertainty inherent to the concept of NEC? How can the killing rate be related to food quantity? Measures of the tissue concentration should help answer the first question. Other theoretical and experimental studies are needed to answer the second one.

## CONCLUSIONS AND RECOMMENDATIONS

Because they are based on biological mechanisms, biology-based models can adapt to a large range of situations, which differs from empirical models, which can be used only for the type of experiment for which they have been built.

In the present study, the DEBtox model proved able to make predictions about the lethal effects of zinc on water fleas. It also proved that it could be modified to take into account problems faced with bioassays, such as time-varying concentrations or irrelevant choices of nominal concentrations. Yet to assess properly the influence of food, more work is required.

We recommend the following for the use of DEBtox. First, if some knowledge does exist concerning the NEC, it is better to choose most of the initial concentrations slightly above this value. If the choice turns out to be irrelevant, concentrations can be changed during the bioassay. Second, performing a depuration period may be an efficient way to test the relevancy and the predictive capacity of the DEBtox model.

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