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Simulating Toxicity of Carbaryl to *Gammarus pulex* after Sequential Pulsed Exposure

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Aquatic nontarget organisms are typically exposed to sequential pulses of contaminants with fluctuating concentrations. We use the semimechanistic threshold damage model (TDM) to simulate survival of the aquatic invertebrate *Gammarus pulex* after sequential pulsed exposure to carbaryl and compare it to a simpler model based on time-weighted averages (TWA). The TDM is a process-based model and we demonstrate how to parametrize it with data from an uptake and elimination experiment together with data from a survival experiment with sequential pulses. The performance of the two models is compared by the fit to the first survival experiment and the simulation of another, independent survival experiment with different exposure patterns. Measured internal concentrations in the first survival experiment are used to evaluate the toxicokinetic submodel of the TDM. The TDM outperforms the TWA model, facilitates understanding of the underlying ecotoxicological processes, permits calculation of recovery times (3, 15, and 25 days for pentachlorophenol, carbaryl and chlorpyrifos respectively) and enables us to predict the effects of long-term exposure patterns with sequential pulses or fluctuating concentrations. We compare the parameters of the TDM for carbaryl, pentachlorophenol and chlorpyrifos and discuss implications for ecotoxicology and risk assessment.

Introduction

Pesticides are broadly applied within the open environment and may reach water bodies via various pathways. Thus they have a relatively large potential for effects on nontarget organisms. Aquatic nontarget organisms are typically exposed to sequential pulses with fluctuating concentrations (*1*, *2*), but current risk assessment generally relies on standard toxicity tests performed at constant concentrations and over fixed durations. Any extrapolation to more realistic patterns of exposure must rely on modeling, and we need a theoretically sound framework for this fundamental problem in environmental risk assessment as well as appropriate mathematical models to relate fluctuating field exposures to laboratory effects data (*3*, *4*).

The advantage of modeling is that we can extrapolate to a wide range of exposure scenarios, but when we evaluated and compared the theoretical base of available models, we found that there were no generally applicable and validated methods to link sequential or fluctuating exposure to effects (*5*). Subsequently we were able to combine two approaches, one originating from the damage assessment model (*6*) and the other from the DEBtox concept (*7*) to form the threshold damage model (TDM) (*8*). The TDM is a new, process-based model and we used it to simulate survival of the aquatic invertebrate *Gammarus pulex* after fluctuating and sequential pulsed exposure to pentachlorophenol and chlorpyrifos (*8*).

Toxicokinetics are important for the assessment of effects because the toxicant has to enter the organism and reach the site of action to exert an effect (*9*, *10*). The TDM combines the toxicokinetics (uptake and elimination) with the toxicodynamics (damage accrual and recovery and exceedence of a damage threshold) in one consistent ecotoxicological model. We can simulate the processes leading from exposure to effect in a more realistic manner enabling us to assess fluctuating or sequential exposure. The differences in the toxicokinetic and toxicodynamic parameters of the models could facilitate a better understanding of the differences in sensitivity of different organisms and sensitivity to different compounds.

In this study we demonstrate the application of the TDM to simulate survival of *Gammarus pulex* after sequential pulsed exposure to the carbamate pesticide carbaryl. In addition to evaluating the TDM for a mode of action that differs from previous studies (*8*) we also test the toxicokinetic submodel with measured internal concentrations in a survival experiment with repeated pulsed exposure. The toxicokinetic parameters are derived from a short uptake and elimination experiment (A). The resulting simulation of internal concentrations is evaluated with measured internal concentrations in a survival experiment (B), which is also used to estimate the toxicodynamic parameters. Then we extrapolate and simulate the effects resulting from different exposure patterns in a third experiment (C).

We compare the performance of the TDM with a much simpler model based on time-weighted averages (TWA). The TWA pulses model (*8*) is also calibrated using the survival data of experiment B and then simulates survival in experiment C.

Materials and Methods

Organisms, Exposure Water, and Handling. *Gammarus pulex* (mixture of males and females) were collected from a small stream, Bishop Wilton Beck, ca. 20 km northeast of York, UK. The mean weight of the *Gammarus pulex* was 28.51 mg ($n = 90$, standard deviation (SD) = 11.22 mg) in the uptake and elimination experiment (experiment A), 24.80 mg ($n = 107$, SD = 8.54 mg) in experiment B and 28.82 mg ($n = 6$, SD = 16.62 mg) in experiment C. Assuming equal proportions of male and female *Gammarus pulex* of the same average age, we can estimate the age of the organisms from their wet weight (*11*). The average age was estimated to be 187 days in the uptake and elimination experiment A, 176 days in experiment B, and 188 days in experiment C. Prior to experiments, organisms were kept for 3−4 days in aerated streamwater under the same conditions as in the experiments and were fed in excess with rehydrated horse chestnut leaves.

Streamwater was also collected from Bishop Wilton Beck and stored at 5 °C. Bishop Wilton streamwater (pH 9) was used in between pulses, but during the exposure pulses we used buffered streamwater from Keys Beck (North Yorkshire Moors, upstream catchment completely in drinking water protection area). Keys Beck water was buffered at pH 6.75 with 750 mg/L MOPS (3-N morpholino propane sulfonic acid, (*12*)) and NaOH. Buffering is necessary because carbaryl is

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not stable under alkaline conditions. The resulting changes in pH are likely to cause an additional stress on the organisms, but we assume that the effect is small compared to the toxic stress caused by carbaryl. The control group is subject to the same pH regime, hence the background mortality rate (as fitted to control mortalities) accommodates for any effects of pH on mortality.

Carbaryl. ^{14}C-labeled carbaryl [1-naphthyl methylcarbamate] (ring-labeled, 100% purity, 503 MBq/mmol, batch no. XI/39) was purchased from Institute of Isotopes, Budapest, Hungary. Unlabeled carbaryl was purchased from Sigma-Aldrich Ltd. (Gillingham, UK, 99.8% purity). Dosing solutions were made in methanol by mixing labeled with unlabeled carbaryl.

Design of Uptake and Elimination Experiment (A). The uptake and elimination experiment followed the method of Ashauer et al. (*13*) with slight modifications. The uptake phase, during which the organisms were exposed to carbaryl lasted 1.85 days, then the organisms were transferred to fresh streamwater and kept there for three more days (Figure 1). Seven replicate beakers were dosed with a nominal concentration of 6 μg/L of total carbaryl and three control beakers were used. Each beaker contained 20 *Gammarus pulex* at the start of the experiment. During the whole experiment we sampled one *Gammarus* from each replicate beaker at twelve times (after 0.13, 0.33, 0.79, 1.04, 1.31, 1.85, 2.05, 2.22, 2.41, 2.89, 3.89, and 4.85 days). Each *Gammarus* was removed from the beaker, blotted dry, weighed on a precision balance (XS205, Mettler-Toledo Inc.) and frozen until analysis.

Design of Survival Experiments (B and C). The *Gammarus pulex* were treated with carbaryl using various exposure patterns and we observed survival over time. Experiment B (Figure 2) lasted for 22 days and consisted of two treatments (1 and 2) and controls. In each treatment there were six replicate beakers with 10 *Gammarus* and three replicate beakers with 25 organisms at the start of the experiment. Daily counts of surviving organisms were made in the six beakers with 10 *Gammarus* at the start. We sampled organisms for the measurement of internal concentrations from the three beakers with 25 *Gammarus* at the start. The two control beakers contained 10 organisms at the start.

Experiment C lasted for 15 days and contained three treatments and a control group. Each group consisted of five replicate beakers with ten *Gammarus* at the start of the experiment (Figure 3).

Sampling and Analysis. Test solutions were sampled (1 mL) immediately after spiking and frequently thereafter. Radioactivity present in water was quantified with liquid scintillation counting (Beckman LS6000 TA liquid scintillation counter, Beckman Instruments Inc., Fullerton, U.S.) after adding 10 mL of Ecoscint A scintillation cocktail (National Diagnostics, Hessle, UK). Samples were counted three times for 5 min. Sample counts were corrected for background activity by using blank controls. Counting efficiency and color quenching were corrected using the external standard ratio method. Determination of internal concentrations of carbaryl in *Gammarus* involved extraction with Soluene-350 tissue solubilizer (Packard BioScience B.V., Groningen, The Netherlands) and analysis of radioactivity by liquid scintillation counting following the method of Ashauer et al. (*13*).

Data Analysis and Statistics. Parameter estimation was carried out using ModelMaker version 4 (AP Benson, Wallingford, UK). The parameter values for chlorpyrifos from the robust fit in Ashauer et al. (*8*) were used as starting values in the parameter estimation procedure for carbaryl. Simulations of internal concentration, damage, and survival as well as statistical calculations were undertaken with Mathcad 2001i Professional (MathSoft Engineering & Education Inc., Cambridge, U.S.).

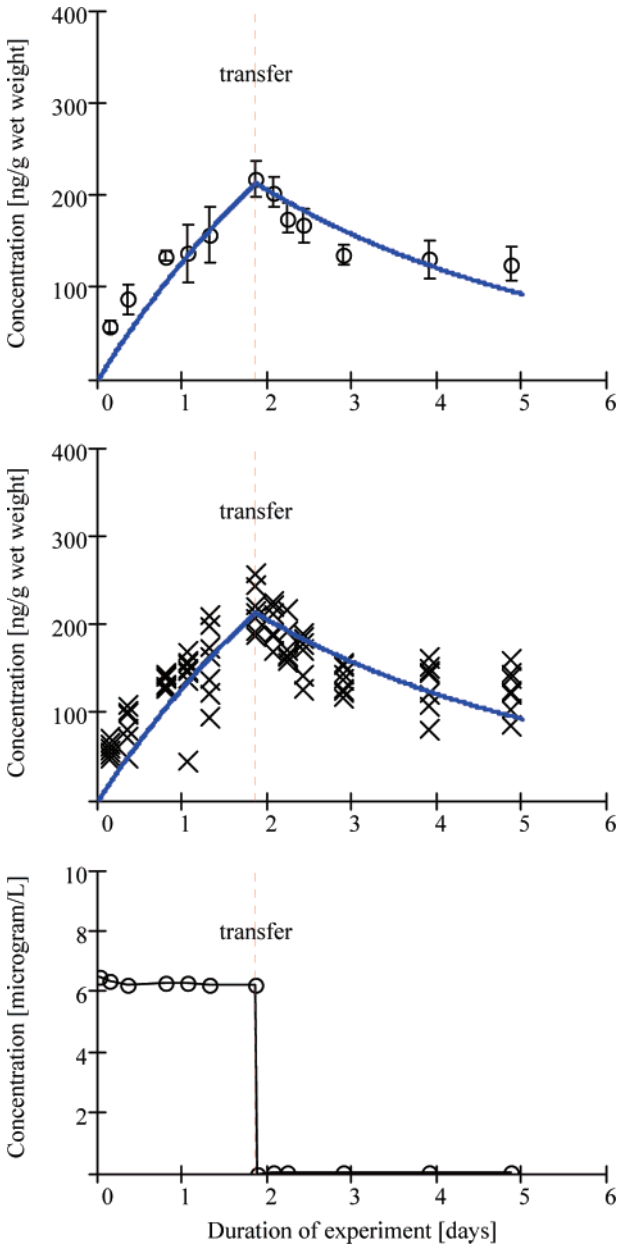

FIGURE 1. The measurement of uptake and elimination of carbaryl in *Gammarus pulex* (experiment A). Mean aqueous carbaryl concentrations (○, $n = 7$, bottom graph), measured internal concentrations (×, middle graph) and mean internal concentrations (○, $n = 7$, top graph, error bars show 95% confidence intervals). The solid line (middle and top graph) shows the fitted toxicokinetic model (eq 1) with $k_{in} = 23.4$ L kg^{-1} day^{-1} and $k_{out} = 0.27$ day^{-1}.

Modeling

The Threshold Damage Model (TDM). Equations 1−4 constitute the TDM. Equation 1 is the one-compartment first-order kinetics model, which simulates the toxicokinetics, i.e., the time course of the internal concentration in relation to the concentration in the water phase surrounding the organism. Internal concentration acts as a surrogate for the concentration at the target site.

$$\frac{dC_{int}(t)}{dt} = k_{in} \times C(t) - k_{out} \times C_{int}(t) \qquad (1)$$

where C_{int} is the internal concentration [amount × mass^{-1}], C the concentration in the water [amount × volume^{-1}] and

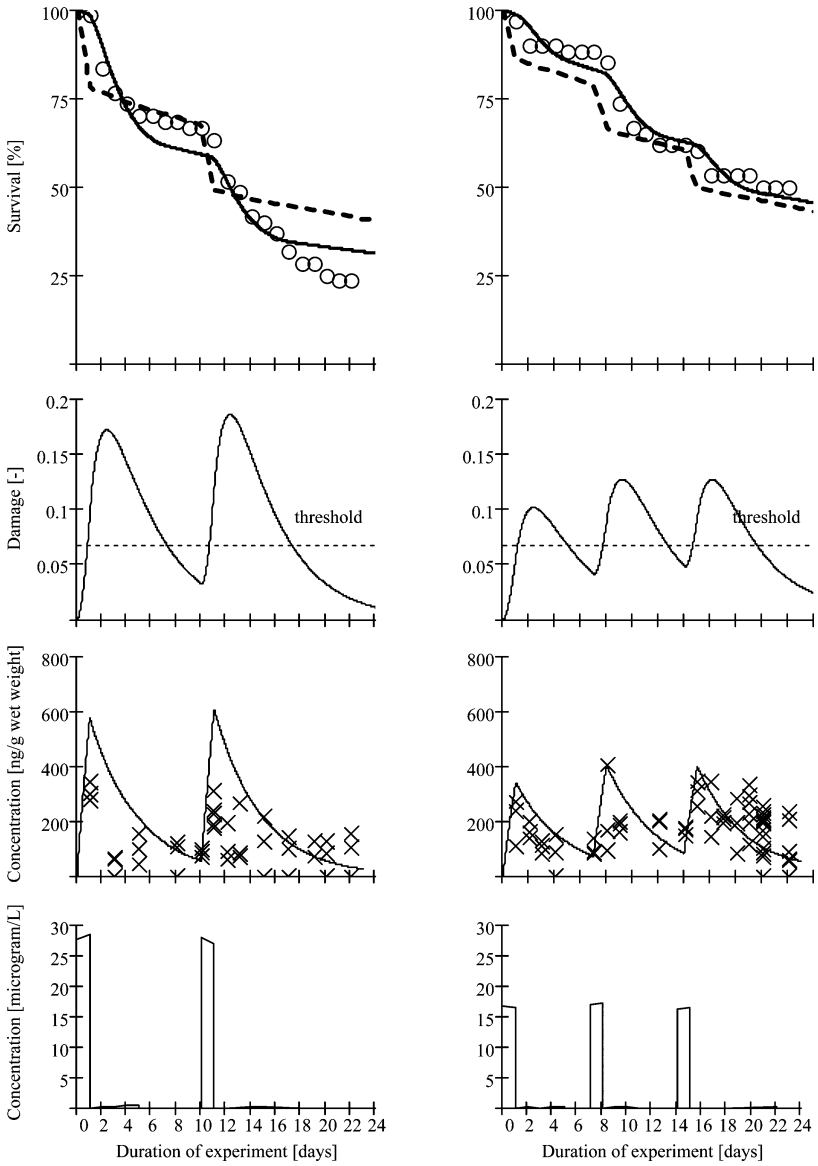

FIGURE 2. Treatment 1 (left) and treatment 2 (right) of experiment B. The graphs show aqueous concentrations of carbaryl (bottom) as well as measured (×) and predicted (solid line) concentrations of carbaryl in *Gammarus pulex* (second from bottom). Above that is the simulated time course of damage in the TDM (third graph from bottom) and the observed (○) survival with the fitted threshold damage model (solid line) as well as the fitted TWA pulses model (dashed line) in the top graph.

k_{in} and k_{out} the uptake rate constant [volume × mass^{-1} × time^{-1}] and the elimination rate constant [time^{-1}], respectively.

Equation 2 simulates the first part of the toxicodynamics as an accrual and, in the second term of eq 2, the recovery or repair of damage:

$$\frac{dD(t)}{dt} = k_k \times C_{int}(t) - k_r \times D(t) \quad (2)$$

where k_k is a killing rate constant [mass × amount^{-1} × time^{-1}], k_r is the rate constant for damage recovery or repair [time^{-1}] and $D(t)$ is damage [−]. The differential of $H(t)$, as used in eq 3 is the hazard rate, which is the probability of the organisms dying at a given time. In eq 3 the hazard rate rises above zero when a threshold for damage is exceeded:

$$\frac{dH(t)}{dt} = \max [D(t) - \text{threshold}, 0] \quad (3)$$

where threshold is a dimensionless threshold parameter [−].

The killing rate constant is a combined parameter, describing both damage accrual and the proportionality factor to the hazard rate and reflects the toxic potency of the compound. The recovery rate parameter lumps all processes leading to recovery, such as repair mechanisms on a cellular scale (e.g., reactivation of blocked enzymes or synthesis of new enzyme) or adaptation of the physiology and other compensating processes. The toxicodynamic parameters in eqs 2 and 3, and thus the speed of recovery, depend on the mode of action of the compound.

In eq 4 we use the standard approach of linking hazard to survival:

$$S(t) = e^{-H(t)} \times S_{background}(t) \quad (4)$$

where $S(t)$ is the survival probability [−] (probability of an organism surviving until time t) and $S_{background}(t)$ is the survival probability resulting purely from the background (or control) mortality [−].

The TWA Pulses Model. The time-weighted average (TWA) method is inherently based on Haber's law and can be

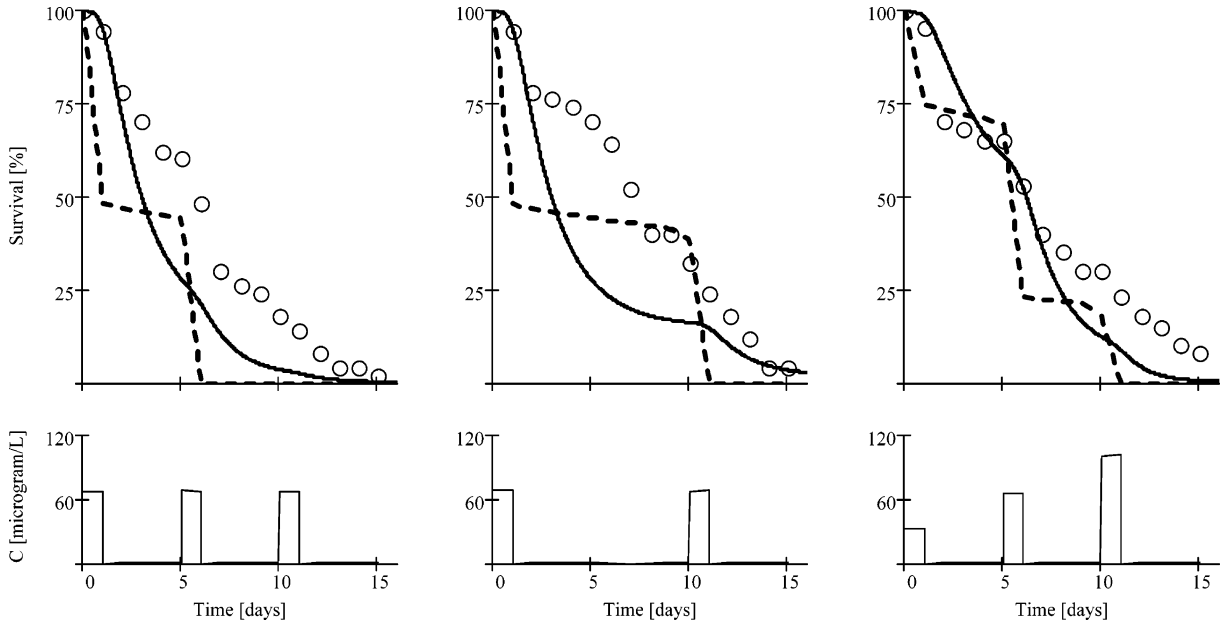

FIGURE 3. Treatments 1 (left), 2 (middle), and 3 (right) of experiment C with aqueous concentrations of carbaryl (bottom) and observed (○) survival with the predictive simulations of the threshold damage model (solid line) and the TWA pulses model (dashed line) in the top graph.

modified to simulate the survival over time if we assume a linear relationship between dose and effect (*5*). The survival probability is then calculated as follows:

$$S(t) = 1 - f\_\text{TWA} \times \int_0^t C(t)dt \qquad (5)$$

where f_TWA is a scaling factor [volume × mass^{-1} × time^{-1}], and C is the concentration in the exposure solution [mass × volume^{-1}]. Fitting eq 5 to the survival data in experiment B yields the scaling factor f_TWA. When simulating experiment B or C the survival probability in the TWA model is corrected for background mortality as in the TDM (eq 4) by multiplication with the survival probability resulting from background mortality.

Estimation of Toxicokinetic and Toxicodynamic Parameters. The toxicokinetic model (eq 1) was fitted to all individual measured internal concentrations of experiment A simultaneously (Figure 1, middle) to estimate uptake and elimination rate constants. The best fit was found using the Levenberg−Marquardt algorithm to minimize the ordinary least-squares (no weighting of data points).

Once the toxicokinetic parameters were known, the remaining toxicodynamic parameters of the TDM k_k, k_r, and threshold were estimated by fitting the TDM to the survival data of experiment B (Figure 2, top). The uptake and elimination rates were kept fixed; only k_k, k_r, and threshold were adjusted automatically until the best fit was found. The toxicodynamic parameters of chlorpyrifos (*8*) served as starting values.

The long interval between the pulses in experiment B (9 days in treatment 1 and 6 days in treatment 2) allows the mortality to return to background levels before the next pulse. This is important for a successful estimation of the TDM parameters, as is the requirement that the measured data are consistent between the treatments.

After calibration, the survival of *Gammarus pulex* in experiment C was simulated with both models (Figure 3). These simulations were driven only by the measured concentrations in the exposure solution and adjusted for background mortality.

Results and Discussion

Structure of the TDM. Traditional aquatic risk assessment views the organism under scrutiny as a black box where concentrations of contaminants in the water phase are directly related to effects on the organism. The threshold damage model (TDM) follows a different, semimechanistic approach. It is a compromise between a detailed description of processes (bottom-up) and top-down modeling. The level of complexity is dictated by the purpose of the model, here the simulation of survival over time after fluctuating or sequential pulsed exposure to a toxicant. Hence the TDM includes uptake and elimination as two lumped processes (eq 1) and damage accrual and repair/recovery as two processes (eq 2). More detailed processes such as enzyme regeneration and de novo synthesis of enzyme for repair/recovery are again lumped into one process. The hazard rate (probability of dying) increases when the damage level rises above a threshold (eq 3), i.e. the whole organism only responds with increased mortality if a certain level of internal damage is exceeded. That accommodates for compensating mechanisms as well as for the change in scales: eq 2 describes processes on the scale of cells or sites of action, whereas the survival probability in eq 4 only has meaning at the scale of the whole organism.

Hence the TDM is a generic model for aquatic organisms that emulates ecotoxicologically relevant processes. Different parameter sets represent different combinations of organisms and toxicants and can be interpreted with respect to the characteristics of the organism and compound. Once the parameter sets are available, the TDM allows simulation of effects from any kind of exposure pattern for that combination of compound and organism.

Parameters. All parameters could be estimated successfully (Table 1). Simulated annealing confirmed that the toxicodynamic parameters represent a minimum of the goal function within the realistic parameter space. The toxicokinetic parameters represent a global minimum. They are k_{in} = 23.4 L kg^{-1} day^{-1} and k_{out} = 0.27 day^{-1}. The bioconcentration factor at steady state, calculated as k_{in}/k_{out}, is 87 L kg^{-1}. The elimination rate constant (k_{out} = 0.27 day^{-1}) is smaller than that of chlorpyrifos (k_{out} = 0.45 day^{-1} (*13*)), which

TABLE 1. Model Parameters[a]

parameter	symbol	chlorpyrifos[d]	carbaryl	pentachlorophenol[d]	units[e]
uptake rate constant[b]	k_{in}	747 ± 61	23.4 ± 0.9	89 ± 7	L × kg^{-1} × day^{-1}
elimination rate constant[b]	k_{out}	0.45 ± 0.05	0.27 ± 0.04	1.76 ± 0.14	day^{-1}
killing rate constant[c,f]	k_k	0.134 ± 0.022	0.42 ± 0.1	0.061 ± 0.003	g $_{wet.w.}$ × day^{-1} × $\mu g_{a.i.}{}^{-1}$
		{47.0 ± 7.7}	{84.5 ± 20.1}	{16.2 ± 0.8}	{g $_{wet.w.}$ × day^{-1} × $\mu mol_{a.i.}{}^{-1}$}
recovery rate constant[c]	k_r	0.169 ± 0.04	0.97 ± 0.24	66 ± 3	day^{-1}
threshold[c]	threshold	0.022 ± 0.0045	0.067 ± 0.01	0.037 ± 0.006	
scaling factor pulses [c]	f_TWA pulses	321 ± 9	7.6 ± 0.4	0.0206 ± 0.0004	L × mg^{-1} × day^{-1}

[a] Parameter value ± standard error from fit. [b] Carbaryl parameters from uptake and elimination experiment (experiment A). [c] Carbaryl parameter set from fit to experiment B. [d] Chlorpyrifos and pentachlorophenol parameter set from fit to all data in (*8*). [e] g $_{wet.w.}$ is g wet weight and $\mu g_{a.i.}$ is μg of active ingredient. [f] Killing rate constants are also given on a per mole basis {in brackets}.

is surprising because chlorpyrifos has a larger log K_{ow} than carbaryl (chlorpyrifos: log K_{ow} = 4.7, carbaryl: log K_{ow} = 1.85; (*14*)). The slow elimination of carbaryl (see Figure 1 middle and top) leads to a build up of carbaryl in the organism in experiment B (Figure 2, middle). The time to depurate 95% of the carbaryl after transfer to clean water is 11 days.

The elimination rate constants measured by Landrum et al. (*15*) for *Pontoporeia hoyi* and *Mysis relicta* are even smaller than ours for *Gammarus pulex*. They measured carbaryl uptake and elimination rate constants of 90 ± 15 L kg^{-1} day^{-1} (value ± SE) and 0.0048 ± 0.0038 day^{-1}, respectively, in *Pontoporeia hoyi* (BCF = 18750 L kg^{-1}) and 3.2 L kg^{-1} day^{-1} and 0.0216 ± 0.0144 day^{-1} (value ± SE) respectively in *Mysis relicta* (BCF = 148 L kg^{-1}).

Since we measured radioactivity, we have also detected any metabolites present in the organism. Only metabolites that are eliminated much more slowly than the parent compound would yield a large error in our measured internal concentrations of the parent compound. Landrum et al. (*15*) suggested that the slow elimination of carbaryl might be due to ionization and subsequently slower transfer across membranes (ion trapping) of the major metabolite, 1-napthol (estimated log K_{ow}: 2.7 (*16*)). Further research is necessary to investigate whether there is significant metabolism of carbaryl in *Gammarus pulex* and whether any metabolites are more hydrophobic (hence depurated slower) than the parent compound or whether indeed ion trapping occurs.

The recovery rate constant k_r = 0.97 day^{-1} results in a time to 50% damage recovery of 17 h when assessing damage recovery separately from toxicokinetics. The time to 95% recovery of damage is 3 days. Acetylcholinesterase (AChE) inhibited by carbamates such as carbaryl shows faster reactivation than that after inhibition by organophosphates such as chlorpyrifos (*17*). Hence the faster damage recovery compared to chlorpyrifos (k_r = 0.169 day^{-1}, from Ashauer et al. (*8*)) is plausible. The recovery rate constant of carbaryl is smaller than that of pentachlorophenol, an uncoupler of oxidative phosphorylation, which is also plausible.

Further indication of the plausibility of our estimated recovery dynamics comes from studies by Kallander et al. (*18*) who showed significant recovery in the number of affected *Chironomus riparius* between two 1 hour pulses of carbaryl when there were at least 6 h for recovery between the pulses. Nevertheless comparisons and extrapolations between species have to be made with great care, because the possibility of recovery from carbaryl exposure varies between species (*19*). Parsons et al. (*20*) did not find significant differences in toxicity of two 1 hour pulses of carbaryl to *Aedes aegypti* larvae when recovery time between the pulses was increased from 0 to 24 h.

The toxicodynamic parameters of carbaryl can be compared (Table 1, Figure 4) with those of chlorpyrifos and pentachlorophenol (*8*). The killing rate of carbaryl (84.5 g $_{wet.w.}$ × day^{-1} × $\mu mol_{a.i.}{}^{-1}$) is five times larger than that of pentachlorophenol (16.2 g $_{wet.w.}$ × day^{-1} × $\mu mol_{a.i.}{}^{-1}$) and

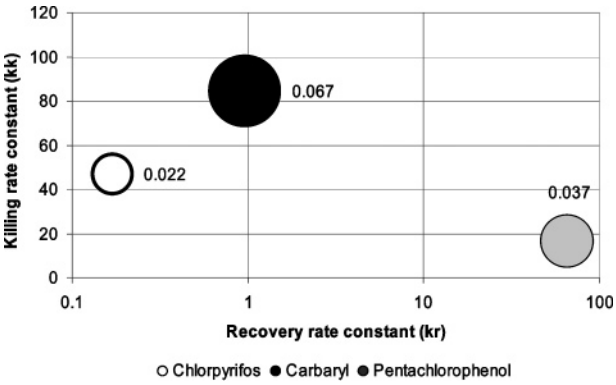

FIGURE 4. Comparison of toxicodynamic parameters of chlorpyrifos, carbaryl and pentachlorophenol. The recovery rate constant k_r [day^{-1}, logarithmic scale] and the killing rate constant k_k [g $_{wet.w.}$ × day^{-1} × $\mu mol_{a.i.}{}^{-1}$] define the axes. The bubble size of each compound is proportional to the value of threshold [−] (value shown in graph). Recovery from damage caused by carbaryl (carbamate AChE inhibitor) is faster than for chlorpyrifos (organothiophosphate AChE inhibitor) but slower than for pentachlorophenol (uncoupling of oxidative phosphorylation).

double that of chlorpyrifos (47.0 g $_{wet.w.}$ × day^{-1} × $\mu mol_{a.i.}{}^{-1}$). This comparison with chlorpyrifos and pentachlorophenol suggests that carbaryl has a larger inherent toxic potency, which is counteracted by the relatively fast recovery and the larger threshold.

Interpretation of these parameter values and application of the model assumes that (i) the TDM is an appropriate model and (ii) no metabolites have significantly obscured our measured uptake and elimination rate constants. We are currently not able to quantify the uncertainty in the parameter values due to variability between experiments and different *Gammarus pulex* populations and parameter estimation remains a critical step due to parameter correlation; hence, the parameter values should be interpreted with great care.

If we try to estimate the toxicodynamic parameters from experiment C, the estimation fails or results in nonsensical parameter values (e.g., negative k_r). The time between pulses in experiment C is too short and the observed survival data are inconsistent. For example mortality after 9 days in treatment 2 is similar to that in treatment 1 even though treatment 1 had received double the dose of treatment 2 at that point in time (Figure 3).

We can use the TDM to calculate LC50 values (concentration at which 50% of the population dies in a test with constant exposure): 24 h LC50 = 618 μg/L, 48 h LC50 = 108 μg/L, 72 h LC50 = 44 μg/L. Only the 72 h LC50 compares favorably with the measured values (24 h LC50 = 35 μg/L, 48 h LC50 = 29 μg/L, 72 h LC50 = 25 μg/L) by Bluzat et al. (*21*). The discrepancy can be explained by the more sensitive organisms in the experiments of Bluzat et al. (*21*). For

TABLE 2. Indicators of Model Performance

	mean and maximum errors [%][a]		r^2	
	TDM	TWA pulse	TDM	TWA pulse
experiment B: model fit	4 (9)	7 (21)	0.95	0.86
experiment C: predictive simulation	14 (42)	17 (48)	0.81	0.76

[a] In % of initial population, maximum error in parentheses.

comparison, the first pulses in experiment B (1 day pulses) with concentrations of 28 μg/L and 17 μg/L in treatment 1 and 2, respectively, cause 2 and 3% mortality after 1 day and 23 and 10%, respectively on day three (in clean water after day one). Hence the discrepancy is not a modeling problem but rather one of extrapolation between experiments.

Model Performance. Both, the TDM and the TWA pulses model were used to simulate survival following exposure to repeated pulses. Indicators for the goodness-of-fit (experiment B) and the performance in the independent simulations (experiment C) are shown in Table 2. All statistical indicators show a better performance of the TDM in all treatments.

The small mean errors (4% for TDM and 7% for the TWA pulse model) indicate a very good fit of both models to survival data from experiment B. Use of the toxicokinetic submodel to predict internal concentrations of carbaryl in experiment B (Figure 2, second graph from bottom) overestimates peak concentrations in treatment 1, but is more accurate for treatment 2. Toward the end of the experiment both treatments show a build up of internal concentrations and thus a better agreement with the model simulation. The overall performance of this submodel of the TDM is encouraging given the possible variability between the uptake and elimination experiment A and experiment B.

Overall doses in experiment C were much larger than in experiment B, but the TDM simulation is in good agreement with the observed survival, especially toward the end of experiment C (Figure 3). This indicates that even predictions of scenarios that are very different to the calibration experiment are possible, but the model performance in experiment C (mean errors 14 and 17% and maximum errors 42 and 48% for the TDM and the TWA pulses model, respectively) is not as good as in the calibration experiment B. The performance in experiment C may be distorted because the survival data are partly inconsistent. If we do not consider the inconsistent data of treatment 2 in experiment C, the TDM performs better (mean errors 14 and 9% for treatments 1 and 3, $r^2 = 0.91$ and 0.97 respectively). The TWA performance in treatments 1 and 3 of experiment C is also better (mean errors 21 and 13% for treatments 1 and 3, $r^2 = 0.81$ and 0.92 respectively), but still worse than that of the TDM.

The TWA pulses model simulates that all organisms die in all treatments of experiment C (as early as on day 6 in treatment 1), illustrating one of the main shortcomings of this model: for sufficiently long exposure or large doses it will always predict 100% mortality. This is inherent in the empirical model structure and highlights the fact that the TWA pulses model cannot extrapolate to scenarios that are very different to its calibration.

Ecotoxicological Implications. Both models could be used to simulate survival of aquatic organisms after exposure to sequential pulses or fluctuating concentrations of contaminants, but in this study the TDM performs consistently better than the TWA pulses model. The TWA pulses model is simple and thus easily understood and it requires less data than the TDM because it does not need uptake and elimination rate constants. Omission of process knowledge such as uptake and elimination or toxicodynamics limits our confidence in the TWA pulses model when we extrapolate to different scenarios.

The disadvantage of the TDM, that it requires uptake and elimination rates and, hence, more experiments, is outweighed by the fact that knowledge of the time course of the contaminant concentration in the organism is of great benefit for the risk assessment in itself, especially when dealing with repeated exposure events. The TDM links exposure with effects through a chain of cause−effect relationships that are representations of the underlying ecotoxicological processes. Hence we have more confidence in the TDM predictions when we are extrapolating to different scenarios. Nevertheless lab to field extrapolations are subject to uncertainties because other relevant aspects are not addressed in the TDM (indirect effects, competition, environmental factors, migration, reproduction, etc.).

The TDM parameter values reflect the chemical mode of action and can be used to calculate the times that organisms require to recover. In the simulation with the TDM, as in reality, elimination and recovery occur simultaneously so the actual simulated times to recovery differ from those calculated by adding up the individual times for 95% elimination and 95% recovery when they are calculated separately. In the TDM simulation it takes 15 days for *Gammarus pulex* to completely recover (95% of maximum damage is recovered) from a 1-day pulse that kills 50% of the population. This compares with 25 days for chlorpyrifos and 3 days for pentachlorophenol. For carbaryl, the time is mainly needed for the slow elimination process (toxicokinetics, eq 1), whereas in the case of chlorpyrifos it is the actual recovery of damage that is slow (toxicodynamics, eq 2). For pentachlorophenol, both the toxicokinetics and the toxicodynamics, are characterized by fast rates. This information is very useful for risk assessment and it highlights the consequences of the different modes of action of the three compounds.

The picture is completed when we take the value of the threshold into consideration. The rate of mortality of the organism simulated in the TDM will return to background levels as soon as the damage level falls below the threshold. Even though there is still internal damage that might contribute to effects of a subsequent pulse, it does not result in any effects on the organism scale because levels are below the threshold. The time required for the damage level to fall below the threshold (same pulse as above) is 9 days for carbaryl, 17 days for chlorpyrifos, and 3 days for pentachlorophenol.

The TDM explicitly models toxicokinetics and toxicodynamics as separate groups of processes and combines them in one model, hence, fulfilling the criteria and satisfying the need for predictive models in aquatic ecotoxicology (*10*) as well as facilitating assessment of risk from realistic exposure scenarios. We hypothesize that compounds with the same mode of action cluster together in the toxicodynamic parameter space (Figure 4). Once a sufficient database is established, the parameters of new or less well-researched compounds could be estimated based on their mode of action and properties. Furthermore, we believe that parametrizing the TDM for different species could facilitate a better understanding of the causes for the distribution of species sensitivities toward toxicants, hence, leading to new approaches for interspecies extrapolation of toxicity.

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Supporting Information Available

Additional experimental details are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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