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TIME-VARYING EFFECTS OF AROMATIC OIL CONSTITUENTS ON THE SURVIVAL OF AQUATIC SPECIES: DEVIATIONS BETWEEN MODEL ESTIMATES AND OBSERVATIONS

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Abstract: There is a need to study the time course of toxic chemical effects on organisms because there might be a time lag between the onset of chemical exposure and the corresponding adverse effects. For aquatic organisms, crude oil and oil constituents originating from either natural seeps or human activities can be relevant case studies. In the present study the authors tested a generic toxicokinetic model to quantify the time-varying effects of various oil constituents on the survival of aquatic organisms. The model is based on key parameters applicable to an array of species and compounds with baseline toxicity reflected by a generic, internal toxicity threshold or critical body burden (CBB). They compared model estimates with experimental data on the effects of 8 aromatic oil constituents on the survival of aquatic species including crustaceans and fish. The average model uncertainty, expressed as the root mean square error, was 0.25 (minimum–maximum, 0.04–0.67) on a scale between 0 and 1. The estimated survival was generally lower than the measured survival right after the onset of oil constituent exposure. In contrast, the model underestimated the maximum mortality for crustaceans and fish observed in the laboratory. Thus, the model based on the CBB concept failed to adequately predict the lethal effects of the oil constituents on crustaceans and fish. Possible explanations for the deviations between model estimates and observations may include incorrect assumptions regarding a constant lethal body burden, the absence of biotransformation products, and the steady state of aromatic hydrocarbon concentrations in organisms. Clearly, a more complex model approach than the generic model used in the present study is needed to predict toxicity dynamics of narcotic chemicals. *Environ Toxicol Chem* 2017;36:128–136. © 2016 SETAC

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INTRODUCTION

Crude oil can be introduced into the aquatic environment via natural seeps and human activities like oil extraction, transportation, and consumption [1]. Oil drilling activities lead to discharge of water contaminated with oil constituents and added process chemicals. Furthermore, accidents during shipping and drilling can cause the release of large amounts of crude oil to the environment, resulting in mass mortality of aquatic organisms from physical contamination and oil toxicity [2]. This has been demonstrated by the immediate mortality of crustaceans, fish, and mammals after oil spills, for example, from the supertanker *Amoco Cadiz* and the Deepwater Horizon oil rig [3,4].

Oil has the tendency to accumulate in biota [5]. Microcosm and laboratory studies allow for the examination of oil effects on aquatic species. Although the number of experiments has increased over the last decade [2,6–12], effect data of oil constituents are still lacking for a large number of marine and freshwater species. Lethal effects on individuals, measured in single-species toxicity experiments for a selection of species and chemicals, can be used in mechanistic models to estimate effects on survival for oil substances and species that have remained untested. Various models simulate the time course of toxic effects on organisms by translating external concentrations to internal concentrations and subsequently linking these internal concentrations to effects on organisms [8]. In particular, the critical body residue (CBR) model and the damage assessment model have been used to estimate the time course of toxic effects (residue at 50% mortality) of a few polycyclic aromatic hydrocarbons (PAHs) in 2 amphipods and a midge [13,14]. The CBR or critical body burden (CBB) concept assumes an immediate adverse effect of a chemical on an organism if an internal concentration threshold is exceeded. Because the toxicity threshold for a given species is assumed invariant, variability in response is attributed to toxicokinetics [15]. A toxicokinetic–toxicodynamic model that simulates energy budgets in organisms and uses a time-dependent damage variable, DEBtox, has been used to estimate effects of the oil constituents fluoranthene and pyrene on the survival and reproduction of the water flea *Daphnia magna* [7]. To relate a metabolic parameter to the body burden in an organism DEBtox uses an internal no-effect concentration and a tolerance concentration [7].

In toxicokinetic–toxicodynamic modeling, there is a trade-off between the level of detail and the number of parameters that need to be estimated from experimental data [16]. Application of species-specific and substance-specific models may generate accurate predictions yet require more input data, which may give rise to difficulties in the parameterization when being used for untested species and chemicals. By contrast, the OMEGA model represents a modeling approach based on relatively few and easily retrievable chemical properties and biological traits,

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such as the chemical's octanol–water partition coefficient (K_{OW}) and the species' body weight [17,18]. The model has been successfully applied to estimate the time-varying population development of copepod (*Eurytemora affinis*) and white-tailed eagle (*Haliaeetus albicilla*) populations exposed to metals and organic pollutants (polychlorinated biphenyls and dichlorodiphenyldichloroethylene), respectively [19,20]. However, these applications were based on substance-specific toxicity threshold values (50% effect concentration and 50% lethal concentration). It has not yet been evaluated whether the OMEGA model can be profitably used to assess toxic effects of oil constituents based on a generic, internal toxicity threshold or CBB.

The main goal of the present study was to parameterize and test the CBB–based OMEGA model to quantify the time-varying effects of oil constituents on the survival of aquatic organisms. First, we estimated the body burden of oil constituents in aquatic organisms over time [17,21]. Next, we assumed survival to be a log-logistic function of the body burden to estimate the toxic impact of oil constituents on aquatic organisms [22,23]. For parameterization of the model equations, we used generic values where applicable and chemical-specific or species-specific data where needed. Finally, the model results were compared with measured effects of 8 selected oil constituents (monocyclic, dicyclic, and PAHs) on the survival of crustaceans and fish. While the equations should be applicable for different exposure scenarios, we tested the model for constant exposure only because 1) its validity for simple cases should be known before proceeding to complex situations, and 2) experiments with variable oil concentrations have not been carried out yet.

MATERIALS AND METHODS

Model equations

Bioaccumulation. The OMEGA bioaccumulation model [17] estimates the body burden in an organism (i.e., internal chemical concentration) based on the uptake and elimination rate constants of the chemical. These rate constants are quantified as a function of the K_{OW} of the chemical and the organism's wet weight, lipid content, and trophic level [17]. In the present study we estimated the absorption of an oil constituent via the water phase ($k_{0,in}$; liters per kilogram wet wt daily). Uptake via food or oil droplets was assumed negligible [24]. Elimination from the organism was assumed to occur via water ($k_{0,out}$), feces ($k_{1,out}$), dilution by biomass as a consequence of growth or reproduction ($k_{2,out}$), and biotransformation of the chemical ($k_{3,out}$). The total elimination rate constant was the sum of these 4 elimination rate constants ($\Sigma k_{j,out}$; kilogram wet wt/kilogram wet wt daily). The model did not include the possible body burdens of products formed by biotransformation. Assuming first-order kinetics, the time-varying concentration of a chemical c in an organism of species level s (micrograms per kilogram wet wt) was calculated as [17]

$$\frac{d\mathrm{BB}_{s,c}}{dt} = k_{0,\mathrm{in}} \times C_{w,c} - \sum_{j=0}^{j=3} k_{j,\mathrm{out}} \times \mathrm{BB}_{s,c} \qquad (1)$$

which represents the absorption from water with exposure concentration $C_{w,c}$ (micrograms per liter) and the elimination from the organism with a chemical residue $\mathrm{BB}_{s,c}$ (micrograms per kilogram wet wt). A conceptual diagram of the OMEGA model can be found in De Hoop et al. [21], and the model equations used to determine $k_{0,in}$ and $\Sigma k_{j,out}$ are available in Table 1.

Effects on survival. The effects of oil constituents on the survival of aquatic organisms were calculated relative to the survival representative of a control situation (no unit; Equation 2). We assumed the effects to be a logistic function of the estimated body burden [23,25],

$$\text{Fraction survival}_t = \frac{1}{1 + \left(\frac{\max\ \mathrm{BB}_{s,c,t}}{\mathrm{LBB}}\right)^{\text{slope}}} \qquad (2)$$

where $\max\mathrm{BB}_{s,c,t}$ is the highest body burden that occurred until time t (millimoles per kilogram lipid), the lethal body burden translates to LBB (millimoles per kilogram lipid; i.e., the CBB), and "slope" is the interindividual variation in LBB as represented by the corresponding concentration–response curve [26]. The model assumed an individual tolerance distribution, meaning that individuals die at different body burdens because they are assumed to have different sensitivities to chemicals [8]. Furthermore, consistent with the CBB concept, death occurs immediately if the LBB is exceeded and the model assumes no effect of a chemical on the metabolic processes of the organisms. The estimated body burden ($\mathrm{BB}_{s,c,t}$) was converted from micrograms per kilogram wet weight to millimoles per kilogram lipid weight with the molar mass (grams per mole) of the oil constituent and the lipid fraction of the organism.

Model input and parameters

Bioaccumulation. We parameterized the model with generic data where applicable (e.g., the allometric regression exponent) and chemical-specific or species-specific data where needed (e.g., K_{OW}, species' body wt; Table 1). To facilitate comparison of the model outcomes with experimental data from survival experiments (see section *Comparison with experimental data*), we used the oil constituent concentrations in water ($C_{w,c}$) as well as the wet weight and lipid content of the species from the survival experiments themselves. In most experiments a nominal $C_{w,c}$ was reported, except for *Pimephales promelas* and *Hyalella azteca* exposed to pyrene and fluorene [8,9]. In 5 out of the 6 survival experiments the test solutions were changed daily or every other day to achieve the initial concentration specified [7–9,14,27]. If weight or lipid content was not reported, we used a value obtained from other experimental studies on the same species of a similar developmental stage (Supplemental Data, Table S1). Lipid fractions reported on a dry weight basis were converted with a default dry-to-wet weight ratio for the species' taxonomic group [28]. If no measured lipid fraction could be obtained, we used default values specific to the species' trophic level (Table 1). The molecular weight and K_{OW} of the oil constituents were obtained from the CONCAWE database as compiled in the PETROTOX model (Table 2 [29]). Data needed to calculate the absorption ($k_{0,in}$) and elimination ($k_{0,out}$, $k_{1,out}$, $k_{2,out}$) rate constants were obtained from the literature [17]. Biotransformation rate data ($k_{3,out}$) were not available for most invertebrate species and oil constituents, except for *H. azteca* and *Pandalus platyceros* exposed to fluoranthene and benzo[*a*]pyrene, respectively [21,30,31]. We therefore did not include biotransformation rate constants for crustaceans. For fish, whole-body primary biotransformation rate constants for oil constituents were estimated using quantitative structure–activity relationships (QSARs) based on the K_{OW}, biological half-life, and molecular weight of a

Table 1. Generic parameter values and variables used for estimating the effect of oil constituents on the survival of aquatic species

Symbol	Description	Unit[a]	Typical value/calculated from	Reference
Kinetics (Equation 1)				
i	Trophic level[b]		1 = algae and plants, 2 = herbivores, 3 = carnivores	
j	Medium		0 = water, 1 = food, 2 = biomass	[16]
$k_{0,in}$	Absorption rate constant	L/kg d^{-1}	$\frac{w^{-\kappa}}{\rho_{H_2O,0}+\frac{\rho_{CH2,i}}{K_{ow}}+\frac{1}{\gamma_0}}$	[6]
$k_{0,out}$	Excretion rate constant	d^{-1}	$\frac{1}{p_{CH2,i}\times(K_{ow}-1)+1}\times\frac{w^{-\kappa}}{\rho_{H2O,0}+\frac{\rho_{CH2,i}}{K_{ow}}+\frac{1}{\gamma_0}}$	[6]
$k_{1,out}$	Egestion rate constant	d^{-1}	$\frac{1}{p_{CH2,i}\times(K_{ow}-1)+1}\times\frac{w^{-\kappa}}{\rho_{H2O,1}+\frac{\rho_{CH2,i}}{q_T\times K_{ow}}+\frac{1}{p_{CH2,i-1}\times K_{ow}\times(1-p_1)\times q_T\times\gamma_1}}$	[6]
$k_{2,out}$	Dilution rate constant	d^{-1}	$q_T\times\gamma_2\times w^{-\kappa}$.	[6]
$K_{3,out}$	Biotransformation rate	d^{-1}	QSAR for fish	[26,27]
$C_{w,c}$	Concentration in water	μg/L	Variable	[c]
$BB_{s,c}$	Concentration in organism	μg/kg	Variable	[16]
K_{OW}	Octanol–water partitioning coefficient	—	Variable	[27,28]
w	Species body weight	kg	Variable	[d]
$p_{CH2,i}$	Lipid fraction of species	kg kg^{-1}	Default: 0.01 ($i=1$), 0.03 ($i=2$), or 0.05 ($i=5$)	[17,29]
$p_{CH2,i\text{-}1}$	Lipid fraction of food	kg kg^{-1}	Trophic level: 1 = 0, 2 = 0.01, 3 = 0.03	[29]
κ	Rate exponent		0.25	[16]
$\rho_{H2O,j}$	Water layer diffusion resistance	d $kg^{-\kappa}$	2.8×10^{-3} ($j=0$), 1.1×10^{-5} ($j=1$)	[16]
$\rho_{CH2,i}$	Lipid layer permeation resistance	d $kg^{-\kappa}$	4.6×10^{3} ($i=1$), 6.8×10^{1} ($i\geq2$)	[16]
$p_{1,i}$	Fraction ingested food assimilated	kg kg^{-1}	0 ($i=1$), 0.4 ($i=2$), 0.8 ($i=3$)	[16]
q_T	Temperature correction factor	kg kg^{-1}	1 (cold-blooded organisms)	[16]
γ_0	Water absorption–excretion coefficient	kg^{κ} d^{-1}	200 (water-breathing organisms)	[16]
$\gamma_{1,i}$	Food ingestion coefficient	kg^{κ} d^{-1}	0 ($i=1$), 5.0×10^{-3} ($i\geq2$)	[16]
γ_2	Biomass (re)production coefficient	kg^{κ} d^{-1}	6.0×10^{-4} (all organisms)	[16]
Dynamics (Equation 2)				
LBB	Lethal body burden	mmol/kg lipid wt	65.6 (min–max: 12.3–280.0, $n=95$)	[e]
Slope	Slope of concentration–response curve	—	3.0 (min–max: 0.9–24.9, $n=16$)	[e]

[a] Kilograms are in wet weight.
[b] Crustaceans are considered herbivores; fish are considered carnivores.
[c] See Supplemental Data, Table S4.
[d] See Supplemental Data, Table S1.
[e] See Supplemental Data, Table S3.

chemical [21,32,33]. Table 2 shows an overview of the estimated absorption and elimination rate constants per oil constituent.

Effects on survival. For the parameterization of Equation 2, we collected toxicity data from the literature pertaining to chemicals with a narcotic toxic mode of action and aquatic species. A narcotic toxic mode of action is believed to be the result of nonspecific disturbance of membrane integrity and functioning because of partitioning of toxicants into biological membranes [34,35]. The majority of oil constituents are expected to exhibit this so-called baseline toxicity based on their chemical structure consisting mainly of carbon and hydrogen [36]. In a previous study, measured mean lethal effect concentrations (50% hazard concentration) for aquatic species corresponded well with estimated lethal effect concentrations (50% lethal concentration) expected from a narcotic toxic mode of action for the oil components naphthalene and 2-methyl-naphthalene [37]. In the present study, we therefore parameterized the model with a generic LBB and slope based on internal concentration–response curves pertaining to multiple narcotic chemicals, including oil constituents, and aquatic species.

We determined a geometric mean LBB of 66 mmol/kg lipid (minimum–maximum, 12–280 mmol/kg lipid wt) based on 11 aquatic species exposed to chemicals with an expected narcotic toxic mode of action, such as PAHs, fluorobenzenes,

Table 2. Estimated absorption rates ($k_{0,in}$) and elimination rates via water ($k_{0,out}$), feces ($k_{1,out}$), dilution by biomass ($k_{2,out}$), and biotransformation ($k_{3,out}$) for several oil constituents in crustaceans and fish

Species	Chemical	K_{OW}	Molar mass (g/mol)	$k_{0,in}$	$k_{0,out}$	$k_{1,out}$	$k_{2,out}$	$k_{3,out}$
Crustacea								
Chironomus tentans	Fluoranthene	$10^{5.25}$	202.3	2353.3	1.04	0.05	0.01	
Daphnia magna	Pyrene	$10^{5.18}$	202.3	4283.8	0.95	0.04	0.02	
Daphnia magna	Fluoranthene	$10^{5.25}$	202.3	4320.1	0.81	0.04	0.02	
Diporeia spp.	Fluoranthene	$10^{5.25}$	202.3	2787.2	0.26	0.01	0.01	
Hyalella azteca	Fluoranthene	$10^{5.25}$	202.3	2671.2	0.61	0.03	0.01	
Hyalella azteca	Fluorene	$10^{4.05}$	166.2	1583.7	5.67	0.03	0.01	
Hyalella azteca	Pyrene	$10^{5.18}$	202.3	2648.7	0.72	0.03	0.01	
Fish								
Clupea pallasii	Benzene	$10^{2.00}$	78.1	78.7	13.14	0.03	0.03	7.64
Oncorhynchus mykiss	Phenanthrene	$10^{4.65}$	178.2	441.5	0.20	0.00	0.00	0.35
Oncorhynchus mykiss	Retene	$10^{6.24}$	234.3	524.4	0.01	0.00	0.00	0.28
Pimephales promelas	Trimethylbenzene	$10^{3.42}$	120.2	182.7	1.38	0.00	0.00	0.87
Pimephales promelas	Naphthalene	$10^{3.35}$	128.2	160.5	1.43	0.00	0.00	0.30

chlorobenzenes, and bromobenzenes (Table 1; Supplemental Data, Table S2). Most scientific publications do not report the slopes of concentration–response curves [8]. We therefore calculated slopes ourselves by fitting concentration–response functions to the reported raw internal concentration–response data (in millimoles per kilogram lipid wt and percentage survival). An arithmetic mean slope of 3.0 was determined based on narcotic chemicals, such as PAHs, bromobenzenes, chloroethanes, and chlorobiphenyls, affecting the survival of a midge, amphipods, and fish (Table 1; Supplemental Data, Table S2). An overview of the LBBs slopes of concentration–response curves, and the corresponding chemicals and species is shown in Supplemental Data, Tables S2 and S3.

Comparison with experimental data

We compared our model estimates on survival with experimental data on the survival of 4 arthropod species (Branchiopoda and Malacostraca) and 3 fish species (Actinopterygii) exposed to various oil constituents: pyrene, fluoranthene, fluorene, phenanthrene, retene (i.e., PAHs), naphthalene, and 2 benzenes (Table 2; Supplemental Data, Table S4) [7–9,14,27,38]. The experimental survival data were relative to the survival representative of the control situation. One of these studies reported the measured body burdens in addition to the measured effect on the survival of an aquatic species [14]. This enabled us to compare estimated and measured body burdens to separately evaluate the performance of the kinetic part of the model. The experimental data used for comparison were reported averages of the body burdens and effects on survival measured in multiple replicates per experimental treatment. None of the experimental studies reported the variability in measurements between the replicas.

Model performance statistics

We calculated the root mean square error (RMSE) to evaluate the overall goodness of fit of the model [39]. The RMSE is a relative measure for the performance of the model. First, we calculated the RMSE per species, chemical, and exposure concentration:

$$\mathrm{RMSE}_{s,c,Cw} = \sqrt{\frac{\sum \left(O_{s,c,Cw,t} - P_{s,c,Cw,t}\right)^2}{n}} \quad (3)$$

where $O_{s,c,Cw,t}$ and $P_{s,c,Cw,t}$ are the measured and estimated fraction survival (between 0 and 1) for species s, chemical c, exposure concentration C_w, and time t, respectively, and n is the number of times the fraction survival was measured during the experiment. Second, the typical RMSE was determined by simply averaging the RMSE_{Cw} values,

$$\mathrm{RMSE} = \frac{\sum \mathrm{RMSE}Cw}{m} \quad (4)$$

where m denotes the number of experiments. The RMSE summarizes both random error and systematic bias [40].

RESULTS

Overall, the estimated time-varying survival deviated from the measured survival dynamics for crustaceans and fish exposed to 8 oil constituents. In general, the maximum effect of the oil constituents on the survival of several crustaceans and fish estimated with the model was reached within 4 d (Figures 1 and 2). Right after the onset of exposure, the model overestimated the lethal effect of pyrene and fluorene on *H. azteca* and pyrene and fluoranthene on *D. magna* (Figure 1A,B, D,E). The model also overestimated the lethal effect of fluoranthene on *H. azteca*, *Chironomus tentans*, and *Diporeia* spp. during the first days of exposure (Figure 1C,F,G). Furthermore, we found that the estimated body burdens of fluoranthene reached a steady state earlier than the measured body burdens for *H. azteca* and *C. tentans* (Supplemental Data, Figure S1). For *Diporeia* spp. the body burdens were overestimated during the first days of exposure days and underestimated at the last day of exposure (day 28).

The model underestimated the maximum mortality for most crustaceans except for *D. magna* exposed to fluoranthene (Figure 1E) and *Diporeia* spp. exposed to 250 μg/L fluoranthene (Figure 1G). Figure 1B,D shows minor differences between estimated and measured survival for *H. azteca* and *D. magna* exposed to 698 μg/L fluorene and 70 μg/L pyrene, respectively. For fish, the model underestimated the mortality except for *P. promelas* exposed to trimethylbenzene (Figure 2A) and to 6050 μg/L naphthalene (Figure 2B). The average uncertainty in the modeled effects on survival, expressed as the RMSE, was 0.25 with a minimum and maximum RMSE_{Cw} of 0.04 and 0.67, respectively (Table 3). More specifically, the RMSE_{Cw} ranged from 0.04 to 0.67 for crustaceans and from 0.07 to 0.55 for fish.

DISCUSSION

In general, the present study showed that the generic and dynamic OMEGA model, based on the CBBs concept, overestimated the mortality right after the onset of exposure and underestimated the maximum mortality for crustaceans and fish exposed to oil constituents.

The CBB approach thus failed to predict the dynamic effects of chemicals with a baseline toxicity (narcosis) on the survival of organisms. In the next section, *Model deviations*, we discuss potential reasons for the deviations found.

Model deviations

The geometric mean of measured LBBs (66 mmol/kg lipid) was in the range of the LBBs estimated using QSARs for fish exposed to 124 narcotic chemicals (i.e., 40–160 mmol/kg lipid) [41–43]. In addition, the geometric mean LBBs determined for oil constituents (64 mmol/kg lipid) and narcotic chemicals excluding oil constituents (75 mmol/kg lipid) were significantly similar ($p > 0.05$; Supplemental Data, Table S3). The performance of the model improved slightly from an RMSE of 0.25 (RMSE_{Cw} 0.04–0.67) to 0.23 (RMSE_{Cw} 0.02–0.56) when optimizing the mean LBB from 66 mmol/kg lipid to 89 mmol/kg lipid because the reduced differences between measured and estimated mortality right after the onset of exposure outweigh the increased deviations at maximum mortality.

In addition, a sensitivity analysis was performed to evaluate the influence of the LBB on the model fit. Overall, a factor 2 lower LBB did not improve the average model performance (RMSE 0.34 and RMSE_{Cw} 0.02–0.84). A factor 2 higher LBB resulted in a similar average RMSE of 0.25 compared to no change in LBB, but the RMSE_{Cw} range improved slightly to 0.01 to 0.48. In particular, the difference between survival estimates and measurements reduced by 46% to 78% for *D. magna* exposed to fluoranthene and 67% for *P. promelas* exposed to trimethylbenzene (Supplemental Data, Table S5 and

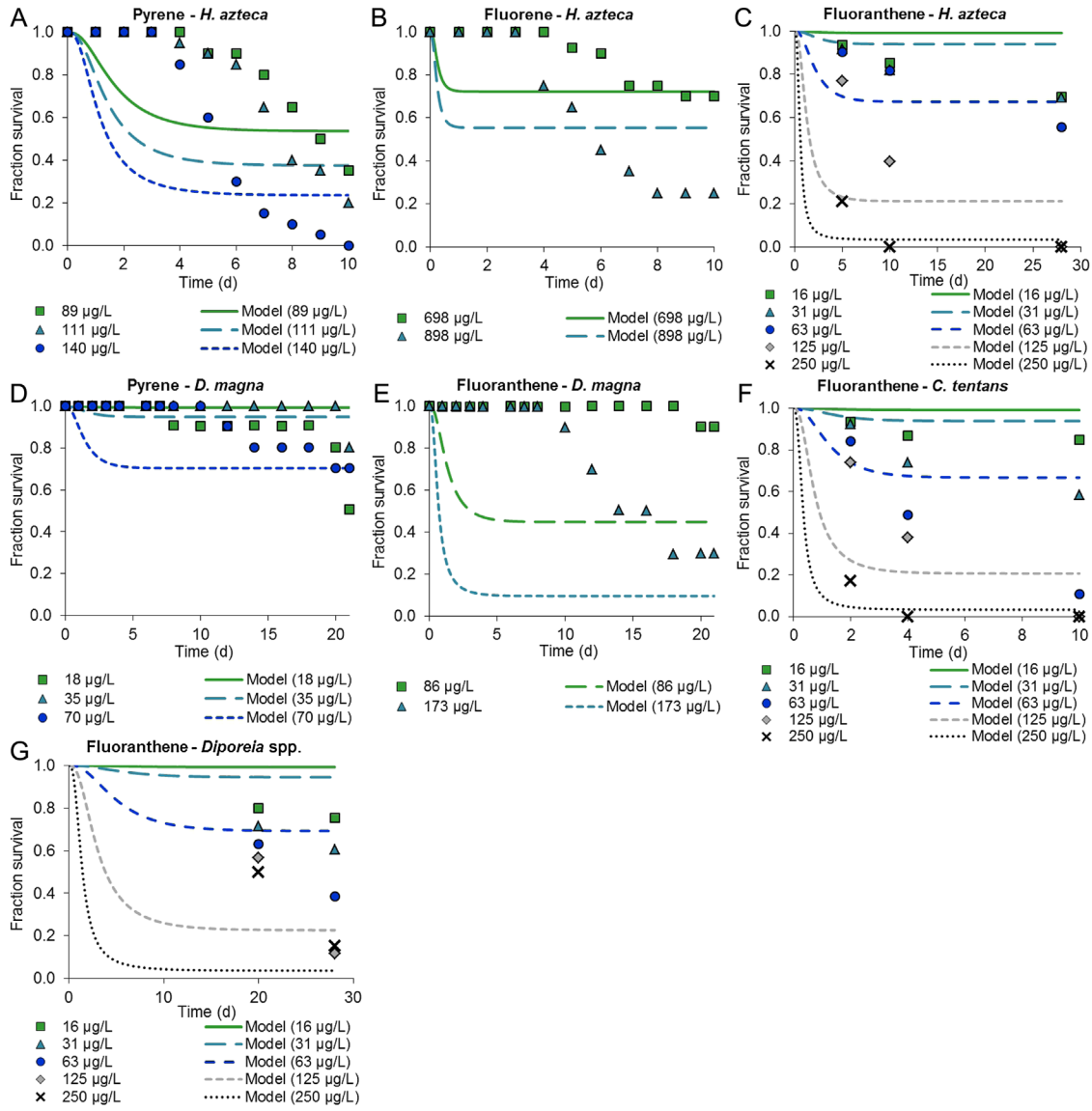

Figure 1. Fraction survival measured experimentally (dots) and estimated with Equation 1 and Equation 2 (lines) for the crustaceans *Hyalella azteca* (**A–C**), *Daphnia magna* (**D**,**E**), *Chironomus tentans* (**F**), and *Diporeia* (**G**) exposed to different concentrations of oil constituents.

Figures S2 and S3). Nevertheless, the model still overestimated the survival fraction in the first days of chemical exposure. In addition, species-specific and chemical-specific measured LBBs were reported for *H. azteca*, *C. tentans*, and *Diporeia* spp. exposed to fluoranthene: 71 mmol/kg lipid, 19 mmol/kg lipid, and 85 mmol/kg lipid, respectively [14]. The relatively low LBB for *C. tentans* indicated higher species sensitivity to fluoranthene. Yet, when estimating the survival using the species-specific LBB instead of the narcotic LBB, the $RMSE_{Cw}$ for *C. tentans* exposed to different fluoranthene concentrations increased from a range of 0.07 to 0.30 to a range of 0.08 to 0.45. Concluding, the LBB influences the model performance for few species exposed to specific aromatic hydrocarbons, but the sensitivity analyses indicated no general pattern for all exposure concentrations. For example, the model fit right after the onset of exposure remained erratic.

The average slope (i.e., 1/β) of 3.0 for internal concentrations was similar to a previously reported slope of 3.1 (minimum–maximum, 0.6–4.8) of the external concentration–response curves of crustaceans exposed to chemicals with a narcotic toxic mode of action [44]. The average slope of 4.2 for 4 oil constituents was higher than the slope of 2.7 for narcotics excluding oil constituents (Supplemental Data, Table S3). The best possible model fit, that is, an RMSE of 0.22 ($RMSE_{Cw}$ 0.03–0.50) instead of 0.25, was obtained by reducing the slope from 3.0 to 1.1, thereby suggesting a very high interindividual variation in LBBs. A sensitivity analysis showed a change in average RMSE from 0.25 to 0.27 ($RMSE_{Cw}$ 0.00–0.75) and 0.22 ($RMSE_{Cw}$ 0.04–0.54) using a factor 2 lower and higher slope, respectively (Supplemental Data, Table S5 and Figure S2). Overall, the factor 2 higher slope slightly reduced the difference between estimates and measurements, in particular for *Diporeia* spp. exposed to fluoranthene (11–46% reduction). In line with the LBB, the slope influences the model performance for few species but indicated no general pattern for all exposure concentrations.

In 4 survival experiments a nominal exposure concentration, $C_{w,c}$, was reported [7,14,27,38]. Although test solutions were changed daily or every other day to achieve the initial concentration specified, sorption and volatilization could have contributed to a reduced water concentration. We evaluated if exposure concentration and time could be explanatory variables

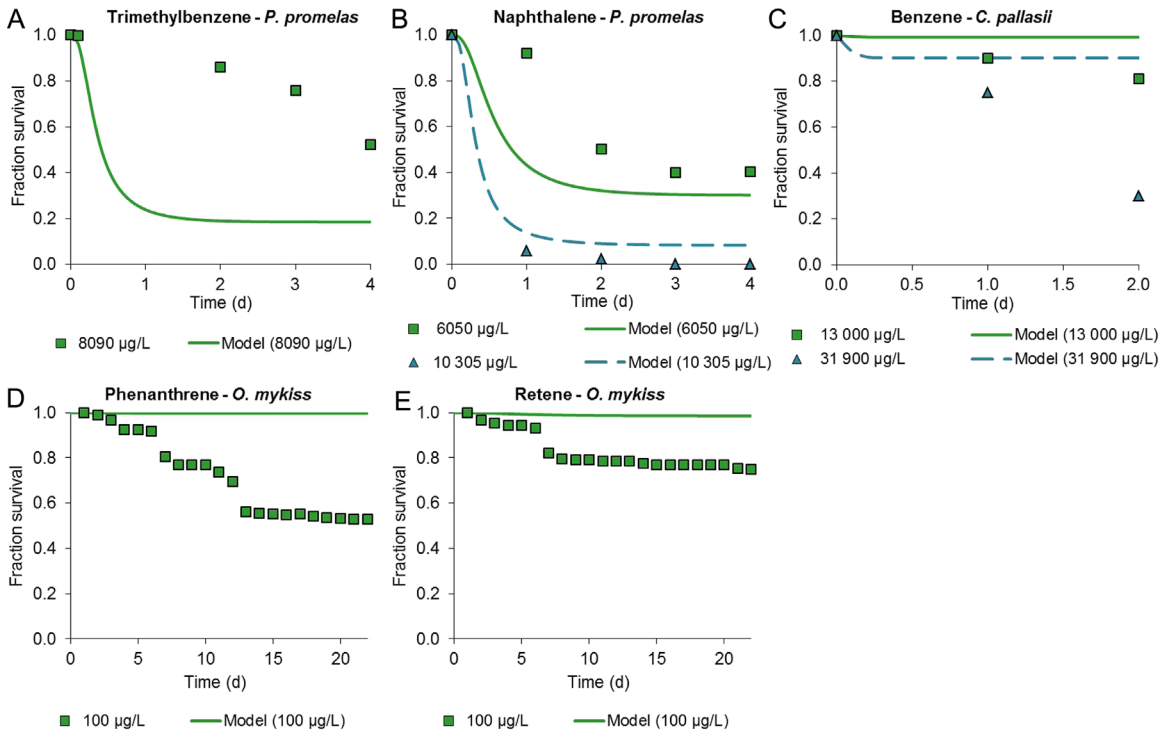

Figure 2. Fraction survival measured experimentally (dots) and estimated with Equation 1 and Equation 2 (lines) for the fish *Pimephales promelas* (**A**,**B**), *Clupea pallasii* (**C**), and *Oncorhynchus mykiss* (**D**,**E**) exposed to different concentrations of oil constituents.

for the degree of deviation between the estimated and measured survival. A factor underestimation or overestimation per data point, calculated using $P_{s,c,Cw,t}/O_{s,c,Cw,t}$, was related to the corresponding time t or exposure concentration $C_{w,c}$ using linear regression. Over all species and oil constituents, the relative deviation showed a significant positive trend in relation to $C_{w,c}$ and time ($p = 0.04$ and <0.01, respectively). Yet, these trends for $C_{w,c}$ and time explained only 1.7% and 3.1%, respectively, of the variation in the estimated/measured ratios (Supplemental Data, Figure S3).

The estimated fraction survival reached a steady-state situation earlier than observed in the experiments. This could partly be explained by overestimated body burdens in the first exposure days, as shown for *H. azteca*, *C. tentans*, and *Diporeia* spp. exposed to fluoranthene (Supplemental Data, Figure S1). We evaluated the performance of the kinetic part of the model by calculating the RMSE using log-transformed measured and estimated body burdens in Equations 3 and 4. The model overestimated the body burdens of fluoranthene in *H. azteca* by a factor of 1.4 to 1.7 (factor $= 10^{\mathrm{RMSE}}$) and in *C. tentans* by a factor of 1.3 to 2.0 (Supplemental Data, Table S6). For *Diporeia* spp. the body burdens were overestimated for the first exposure days and underestimated at the last day, resulting in an overall overestimation by a factor of 1.3 to 2.5.

Overestimation of the body burdens, and thus mortality, right after the onset of exposure may be partly explained by a possible underestimation of the weight or lipid fraction of the organisms. Except for the lipid weight of *H. azteca*, *C. tentans*, and *Diporeia* spp. exposed to fluoranthene, we used values obtained from other experimental studies. An underestimated weight would lead to overestimated absorption and elimination rates, causing the maximum estimated mortality to be reached more quickly compared with the measured mortality. We performed a sensitivity analysis to evaluate the influence of the weight and lipid fraction on the model fit. We set both variables on no change and an order of magnitude decrease and increase (Supplemental Data, Table S7). Overall, a factor 10 decrease and increase in wet weight had a small impact on the relative error (RMSE_{Cw} 0.05–0.69 and 0.04–0.63, respectively) compared to no change in wet weight (RMSE_{Cw} 0.04–0.67). Except for *H. azteca* exposed to pyrene, *P. promelas* to naphthalene (6050 µg/L), and *C. tentans* to fluoranthene (125 µg/L), the RMSE reduced by 22% to 40% using a factor 10 increase in wet weight (Supplemental Data, Table S7). An order of magnitude change in lipid fraction resulted on average in lower model performance as the RMSE increased from 0.25 to 0.28 (RMSE_{Cw} 0.03–0.76) and 0.26 (RMSE_{Cw} 0.01–0.73) using a factor 10 lower and higher lipid fraction, respectively. The model fit improved with 41% to 68% using a factor 10 higher lipid weight for some individual cases: *D. magna* exposed to 173 µg/L fluoranthene, *Diporeia* spp. to 250 µg/L fluoranthene, *H. azteca* to fluorine, and *P. promelas* to trimethylbenzene (Supplemental Data, Table S7). A factor 10 deviation in wet weight is, however, expected to be more likely than a similar high deviation in lipid weight. Eventually, 1 order of magnitude change in the input variables wet weight and lipid fraction did not produce a general improvement of the model performance (Supplemental Data, Figures S3A,B and S4).

The exclusion of biotransformation rates ($k_{3,\mathrm{out}}$) of oil constituents in crustaceans may also contribute to the overestimation of mortality. The present model included biotransformation as an additional elimination route for the parent compound and excluded the possible body burdens of products formed by biotransformation. An underestimated elimination rate as a result of exclusion of biotransformation would therefore lead to overestimated body burdens and mortality. Only metabolic transformation rates of 1.15 ± 0.1 d^{-1} and 0.06 pmol min^{-1} g^{-1} have previously been reported for *H. azteca* and *P. platyceros* exposure to fluoranthene and benzo[*a*]pyrene, respectively [30,31]. However, after including

Table 3. The number of data points (n) and root mean square errors of the fraction survival of different aquatic organisms exposed to different oil constituents

Chemical	C_w (μg/L)	Species Latin name	Species common name	n	$RMSE_{cw}$	Reference
Fluoranthene	16	*Chironomus tentans*	Midge	4	0.10	[14]
Fluoranthene	31	*Chironomus tentans*	Midge	4	0.21	[14]
Fluoranthene	63	*Chironomus tentans*	Midge	4	0.30	[14]
Fluoranthene	125	*Chironomus tentans*	Midge	4	0.27	[14]
Fluoranthene	250	*Chironomus tentans*	Midge	4	0.07	[14]
Pyrene	18	*Daphnia magna*	Water flea	15	0.15	[7]
Pyrene	35	*Daphnia magna*	Water flea	15	0.04	[7]
Pyrene	70	*Daphnia magna*	Water flea	15	0.20	[7]
Fluoranthene	86	*Daphnia magna*	Water flea	15	0.49	[7]
Fluoranthene	173	*Daphnia magna*	Water flea	15	0.67	[7]
Fluoranthene	16	*Diporeia* spp.	Amphipod	3	0.18	[14]
Fluoranthene	31	*Diporeia* spp.	Amphipod	3	0.24	[14]
Fluoranthene	63	*Diporeia* spp.	Amphipod	3	0.18	[14]
Fluoranthene	125	*Diporeia* spp.	Amphipod	3	0.21	[14]
Fluoranthene	250	*Diporeia* spp.	Amphipod	3	0.28	[14]
Fluoranthene	16	*Hyalella azteca*	Amphipod	4	0.17	[14]
Fluoranthene	31	*Hyalella azteca*	Amphipod	4	0.14	[14]
Fluoranthene	63	*Hyalella azteca*	Amphipod	4	0.14	[14]
Fluoranthene	125	*Hyalella azteca*	Amphipod	4	0.30	[14]
Fluoranthene	250	*Hyalella azteca*	Amphipod	4	0.09	[14]
Fluorene	698[a]	*Hyalella azteca*	Amphipod	11	0.18	[9]
Fluorene	898[a]	*Hyalella azteca*	Amphipod	11	0.30	[9]
Pyrene	89[a]	*Hyalella azteca*	Amphipod	11	0.27	[9]
Pyrene	111[a]	*Hyalella azteca*	Amphipod	11	0.36	[9]
Pyrene	140[a]	*Hyalella azteca*	Amphipod	11	0.38	[9]
Benzene	13000	*Clupea pallasii*	Pacific herring	3	0.12	[38]
Benzene	31900	*Clupea pallasii*	Pacific herring	3	0.36	[38]
Phenanthrene	100	*Oncorhynchus mykiss*	Rainbow trout	15	0.40	[37]
Retene	100	*Oncorhynchus mykiss*	Rainbow trout	15	0.22	[37]
Naphthalene	6050[a]	*Pimephales promelas*	Fathead minnow	5	0.24	[8]
Naphthalene	10305[a]	*Pimephales promelas*	Fathead minnow	5	0.07	[8]
Trimethylbenzene	8090[a]	*Pimephales promelas*	Fathead minnow	5	0.55	[8]
$RMSE_{model}$					0.25	

[a] The measured exposure concentration.
RMSE = root mean square error.

a biotransformation rate of 1.15 d^{-1} in the model, the differences between the estimated and measured time-varying survival decreased for *D. magna*, yet increased for *H. azteca* (see Supplemental Data, Figure S5). Furthermore, this particular biotransformation rate was not included in the model estimations because in the survival experiment with *H. azteca* the body burdens were expressed as total fluoranthene equivalent residues, that is, the total internal concentration of parent and metabolite compounds [14].

Narcosis was the suggested toxic mode of action of the parent and metabolite compounds for fluoranthene, justifying body burden addition [14]. Metabolites could also exhibit a more specific toxicity than narcosis; for instance, some metabolites of phenanthrene can cause toxic effects by a nonnarcotic and nonphototoxic mode of action in juvenile fish [45]. Some parent PAHs are also known to cause specific (chronic) effects, such as cardiotoxicity [46] and dioxin-like aryl hydrocarbon receptor–mediated effects [47]. For fish, the QSARs used to predict biotransformation rates do not provide predictions for the formation of metabolites, some of which may be at least as toxic as the parent compound [32]. Nevertheless, in the present study differences between the modeled and measured survival for retene (dioxin-like toxic mode of action) are comparable with the differences of the other oil constituents with an expected narcotic toxic mode of action.

In a toxicity study with a light and a heavy oil type it was suggested that the toxicity of heavy oil is higher because of a toxic mode of action other than narcosis: physical soiling. Very heavy oil constituents may contribute to physical soiling of the organisms depending on the amount of oil present in the sediment [48]. In the present study, the molecular mass of the oil constituents ranged between 78 g/mol for benzene and 234 g/mol for retene. Although the performance of our model was similar for the light and heavier chemicals, it should be taken into account that physical effects might also contribute to a reduced survival of organisms.

Model assumptions

Body burden was immediately linked to survival in our model because we assumed a steady state to occur rapidly for chemicals with baseline toxicity [8]. However, especially for *H. azteca* and *D. magna* exposed to pyrene, fluoranthene, and fluorene, no effect was observed in the first 4 d to 8 d of the experiment, respectively, resulting in a large deviation between the measured and estimated mortality rates. If the time-varying body burdens cannot explain the time course of survival, alternative approaches could be used. For example, it could be assumed that the body burden leads to damage, which in turn leads to mortality [8,14]. Damage would then be used as a dose metric to simulate delayed effects in the toxicodynamic part of the model [48].

In accordance with previous studies, the LBB of chemicals with a narcotic toxic mode of action was assumed to be independent of exposure-related parameters such as time and concentration [43,49]. In various studies, this concept of a constant LBB (e.g., in the CBR model) has been tested by measuring LBBs and the exposure duration until mortality (time to death) of aquatic species exposed to organic chemicals.

Depending on the method used, the LBB varied or remained constant over time. For example, within 1 experimental treatment (e.g., 1 exposure aquarium) the variation in organism sensitivity led to an increase in LBB with increasing exposure duration for *P. promelas* exposed to naphthalene and 1,2,4-trichlorobenzene [50] and *H. azteca* exposed to 3 PAHs [9,13]. In contrast, comparing a mean LBB and exposure duration over different treatments resulted in a decreased or a constant LBB with time for 2 fish, a crab, and an amphipod species exposed to biocides, chlorobenzenes, and PAHs [13,50]. Despite these contrasting outcomes, these findings indicate that temporal variation in the effects of oil constituents on the survival of aquatic species may be the result not only of time-varying body burdens but also of changes in LBB with increasing exposure duration [13].

In the present study the model was based on the individual tolerance hypothesis. An alternative hypothesis is stochastic death, which assumes that all individuals have an equal chance of dying and the probability of dying increases when exceeding the LBB [25]. The individual sensitivities of crustaceans and fish in the experiments were unknown because they were not measured; therefore, both model hypotheses could have been applicable. To evaluate the performance of the model when assuming stochastic death, the fraction survival was estimated by calculating the probability that an individual survives until the next day given a certain chemical concentration. The fraction survival on day n was subsequently calculated by multiplying the survival probabilities of all preceding days (see Supplemental Data for equations). A comparison of the measured and estimated effects for crustaceans and fish mainly showed an overestimated mortality when using a model with stochastic death assumptions (Supplemental Data, Figure S6) that underlined that neither of the model hypotheses was most valid for toxicodynamic modeling. This is in accordance with experimental and modeling studies that estimated the survival of *Gammarus pulex* in propiconazole exposure [25] and the time to stupefaction in zebra fish (*Brachydanio rerio*) exposed to benzocaine and lethality in mosquitofish (*Gambusia holbrooki*) exposed to sodium chloride [51].

Implications and recommendations

A visual comparison of our results to the results of the DEBtox model [7,8], a toxicokinetic–toxicodynamic model, showed that the DEBtox model fitted better to the measured survival data than the OMEGA model for *D. magna* exposed to pyrene and fluoranthene and *P. promelas* exposed to trimethylbenzene. For *P. promelas* exposed to naphthalene, performance was comparable between the 2 models. Compared with OMEGA, the DEBtox model includes more information on energy fluxes in organisms, such as the volume-specific costs for structure and fraction of reserve flux to maturation [52]. Yet, experimental observations needed as input for DEBtox can be missing for species and chemicals as most toxicity experiments are not designed with a DEB-based analysis in mind [53].

We assumed the exposure concentration to be constant over time, which is in accordance with the survival experiments in which the test solutions were changed daily or every other day [7–9,14]. Contrastingly, in field situations concentrations of oil can decrease rapidly as a result of processes such as physical dilution [54]. Exposure conditions after open ocean spills are therefore expected to be of short duration (e.g., hours), which is in the range where our model overestimated the mortality. In theory, the model can be used for fluctuating exposure concentrations; yet constant exposure concentrations already yielded deviations that require additional research.

In conclusion, the estimated time-varying survival generally deviated from the measured survival dynamics for crustaceans and fish exposed to 8 oil constituents. The average uncertainty in the generic OMEGA model, expressed as the RMSE, was 0.25 (minimum–maximum, 0.04–0.67) on a scale between 0 and 1. Thus, the model based on the CBB approach failed to adequately predict the lethal effects of chemicals with a baseline toxicity (narcosis). Possible explanations for the deviations between model estimates and observations may include uncertainties in model parameters as well as incorrect assumptions regarding the absence of biotransformation products, the constant LBB, and the steady state of aromatic hydrocarbon concentrations in organisms. Model performance might be improved by including a delay between accumulation and effect, for example, by addition of a damage factor as is done in the damage assessment model [48], a time-varying LBB instead of a constant LBB, or toxic effects induced by biotransformation products. In short, a more complex model approach than the generic approach used in the present study is needed to predict toxicity dynamics of narcotic chemicals.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3508.

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Data availability—Data are available on request from the corresponding author (L.deHoop@science.ru.nl).

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