Dynamic links between lipid storage, toxicokinetics and mortality in a marine copepod exposed to dimethylnaphthalene

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ABSTRACT

Efficiently assessing and managing the risks of pollution in the marine environment requires mechanistic models for toxic effects. The General Unified Threshold model for Survival (GUTS) provides a framework for deriving toxicokinetic-toxicodynamic (TKTD) models for the endpoint survival. Two recurring questions in the application of GUTS concern the most appropriate death mechanism, and whether the total body residue is a proper dose metric for toxic effects. We address these questions with a case study for dimethylnaphthalene in the marine copepod *Calanus finmarchicus*. A detailed analysis revealed that body residues were best explained by representing copepods with two toxicokinetic compartments: separating structural biomass and lipid storage. Toxicity is most likely related to the concentration in structure, which led to identification of 'stochastic death' as the most appropriate death mechanism. Interestingly, the parameterised model predicts that lipid content will have only minor influence on short-term toxicity. However, the toxicants stored in lipids may have more substantial impacts in situations not included in our experiments (e.g., during diapause and gonad maturation), and for contaminant transfer to eggs and copepod predators.

INTRODUCTION

Human activities at sea, such as oil and gas production, can pose a threat to marine ecosystems. To allow for efficient risk management and mitigation measures, we need methods to predict the consequences of pollution in the marine environment. Most information available on the toxicity of chemicals comes from (standardised) laboratory assays with constant exposure concentrations and controlled environmental conditions. In the field, the environmental conditions are not constant, and organisms are exposed to complex mixtures, with time-varying concentrations and composition. To efficiently translate effects from laboratory assays to relevant field conditions, mechanistic models are essential. In contrast to the classic dose-response analysis, mechanistic models include a (strongly) simplified representation of the underlying processes and explicitly include the factor 'time'.[1-3]

For effects on individual organisms, mechanistic models belong to the class of toxicokinetic-toxicodynamic (TKTD) models,[3] and for the endpoint survival, almost all published TKTD models can now be viewed as special cases of the General Unified Threshold model for Survival, GUTS.[4, 5] GUTS is thus not a single model, but a framework from which specific models can be derived by fixing one or more model parameters. An important achievement of GUTS is that it unifies two distinctly different death mechanisms that have classically been used: individual tolerance (IT) and stochastic death (SD). IT assumes that immediate death occurs when the internal concentration in an individual exceeds a threshold. Not all individuals die at the same time in the same exposure treatment because individuals differ in the value of the threshold (the threshold follows a frequency distribution in the population). SD assumes that all individuals are identical, but that death itself is a probabilistic process. Once the internal concentration has exceeded the threshold, an individual has an increased probability to die. Not all individuals die at the same time as some are simply luckier than others. The IT concept has been most popular in ecotoxicology so far, and is linked to the well-known concept of critical body residues (CBR).[6, 7] This concept was also used as the basis of an effects module for assessing impacts of oil pollution.[8] However, IT has a range of problems associated with it,[9, 10] making it unlikely that this concept can cover the prediction of survival patterns all by itself. Unfortunately, it turns out to be very difficult to prove which of the two mechanisms is most appropriate, and it is likely that the truth is best represented by a combination of the two concepts.[4, 5]

All TKTD models apply the assumption that it is not the external concentration that causes the toxic effect, but that the chemical first needs to be taken up into the body. The first module in a GUTS model will therefore be a toxicokinetics (TK) model. However, which internal concentration is the relevant one? Can we use the total concentration in the organism, or do we need to distinguish between specific fractions in the body? This question is particularly pertinent for marine zooplankton at higher latitudes, as these species generally build up large lipid stores to survive adverse conditions and/or to fuel their maturation and reproduction before the phytoplankton blooms.[11] The storage and use of lipids will have a large effect on the toxicokinetics of hydrophobic chemicals, such as components of oil pollution, and thereby, potentially, on their toxicity.

If we want to use TKTD models such as GUTS to predict effects of oil (and other) pollution in the field from laboratory assays, we need to address several questions related to the issues explained above. For any TKTD model, we need to establish how lipid storage affects toxicokinetics, and which part of the body residue is actually linked to the toxic effects. Specifically for GUTS, we need to establish if one of the two death mechanisms dominates, or whether we are forced to use a combination of SD and IT. Here, we address these questions with a case study for dimethylnaphthalene in the marine copepod *Calanus finmarchicus*, a dominant zooplankton species in the northern Atlantic and sub-arctic waters, in which both survival and body residues have been determined over time. This compound is a methylated polycyclic aromatic hydrocarbon (PAH). Naphthalenes, in general, form a quantitatively very important component of the water-soluble fraction of crude oils.[12] As persistence to volatilisation increases with alkylation, dimethylnaphthalene tends to become more dominant over other naphthalenes during weathering.

METHODS

Experimental tests. Test animals (*Calanus finmarchicus*) were obtained from the continuous laboratory culture at the SINTEF/NTNU SeaLab. Details regarding the culturing have been described elsewhere.[13] Late copepodites (CV) and early adults (all females) were used for all tests, the test temperature was 9±1 °C, and animals were not fed during the experiments. The experimental system utilises previously published methodology where dispersions of oil were continuously produced, filtered inline to remove particulate oil droplets, and feeding exposure vessels (borosilicate bottles, 0.5 L) with dissolved oil components.[14] Briefly, a stock solution of approximately 4 mg/L of 1,3-dimethylnaphthalene (Sigma-Aldrich) in filtered (0.22 μm) seawater was produced by dispersing liquid dimethylnaphthalene into seawater using a syringe pump (Aladdin), a water pump (Fluid Metering, Inc), and a dispersion generator.[14] Thereafter, the solution was filtered through glass wool (10 g) and GFC and GFF filters to remove any undissolved dimethylnaphthalene, as described previously for crude oil.[14] This filtered stock solution was continuously generated and fed through three-way solenoid valves (Cole-Parmer, USA), programmed (using Mini Bee, Bee Step 14 v. 3.2) for delivering predefined concentrations of dimethylnaphthalene by diluting the stock solution with filtered seawater. The desired exposure concentrations were introduced continuously into a series of exposure vessels where 7 (acute toxicity test) or 21 (toxicokinetics test) copepods had been introduced.

For the acute test, a series of six concentrations ranging 0.04-4.0 mg/L (nominal) was used (n=4 for all exposures, n=8 for the control treatment), and mortality was determined every 24 hours up to 144 hours. For the toxicokinetics experiment, one constant exposure concentration (nominal 0.1 mg/L) was used in a total of 56 exposure vessels. Exposure over a period of 96 hours was followed by a 96-hour recovery period. For both experiments, water samples were taken twice from each exposure vessel. Samples were injected directly, through an Agilent DBX C18 reverse phase column, into a high-performance liquid chromatography with diode-array detection (HPLC-DAD). Dimethylnaphthalene was detected using UV and quantified using a standard curve with a linear range up to 30 mg/L. For the acute toxicity test, actual measured concentrations were used for the modelling, and the first two exposure treatments were combined as the actual concentrations turned out to be very similar (within 3%). The measured water concentration in the toxicokinetics experiment was 0.18 mg/L (s.d.=0.02, n=36), and this concentration was used to analyse the body-residue data. For the toxicokinetic experiment, copepods were sampled after 6, 12, 18, 24, 48, 72 and 96 h of exposure and after 6, 12, 18, 24, 48, 72 and 96 h of recovery for body-residue analyses (4 vessels

were used per time point, pooling all 21 copepods in a single vessel for analysis). The method for copepod extraction and PAH body-residue analyses using gas chromatography-mass spectrometry (GC-MS) has been given elsewhere.[15] Body residues are reported on a wet-weight basis.

Raw data from the experiments is provided in SI Section 2.1.

Model and optimisation. The GUTS framework has been described in detail elsewhere,[4] but the model equations used in this study are also provided in SI Section 1.2. Here, we use both the reduced and the full GUTS cases (Fig. 1A and 1C) for the two death mechanisms in isolation (SD, IT). We ignore the damage stage of GUTS as we assume that dimethylnaphthalene is a narcotic chemical, and we expect that the kinetics of the internal concentration (in the relevant body compartment) is a good proxy for the kinetics at the target site. We started by fitting the reduced model to the survival data alone, linking the scaled-internal concentration to the death mechanism (Fig. 1A). The use of the scaled internal concentrations allows a TKTD model to be fitted to data in the absence of information on body residues; the elimination rate (k_e) is estimated from the survival pattern over time.[16]

Body-residue data were fitted with the standard one-compartment model, and also with a two-compartment model[17] (Fig. 1B; model equations in SI Section 1.1). The two-compartment model delineates a structural compartment and a lipid storage, which exchanges chemicals with the structural compartment only. This is a reasonable setup as the lipid storage in *C. finmarchicus* is present in the form of a discrete sac within the body. The two-compartment model specifies the size of all compartments on volume basis. For comparison to the measured data on body residues, model predictions were translated to weight basis by assuming a density of the total body of 1 kg/L.[18] We take the volume of the lipid sac, relative to the structural component, as 0.2 L/L. This is approximately the median value observed for the CV copepodites in our culture.[17] This factor cannot be independently estimated from the body-residue data in our study (see SI Section 1.1 and 3.3).

In the last analysis, the full GUTS model was used, in combination with the two-compartment TK model (Fig. 1C), fitting survival and body-residue data together. The death mechanism was linked to the concentration in structure, as the chemical associated with the storage lipids is unlikely to be a direct cause of toxicity. This model is very similar to the model presented by Gergs and co-workers,[19] who also included the influence of body size on TK.

Optimisation was performed by likelihood maximisation, assuming a multinomial distribution for survival data, and a normal distribution after square-root transformation for the residuals of the body-residue data.[4, 20] All parameters are constrained to avoid the optimisation routine trying negative values, or values were we know (from model structure and the observation times in the data set) that the objective function will always be flat (SI Section 2.2). The only parameter that was affected by these constraints was the elimination rate for the lipid compartment in the two-compartment TK model (k_{eL}). Therefore, its uncertainty is presented as a half-open confidence interval in Table 1. Raw data used for modelling, more information on the statistical treatment of the data, and a link to the code used can be found in SI Section 2. All calculations were performed in Matlab 2017a, using the BYOM modelling platform (www.debtox.info/byom.html).

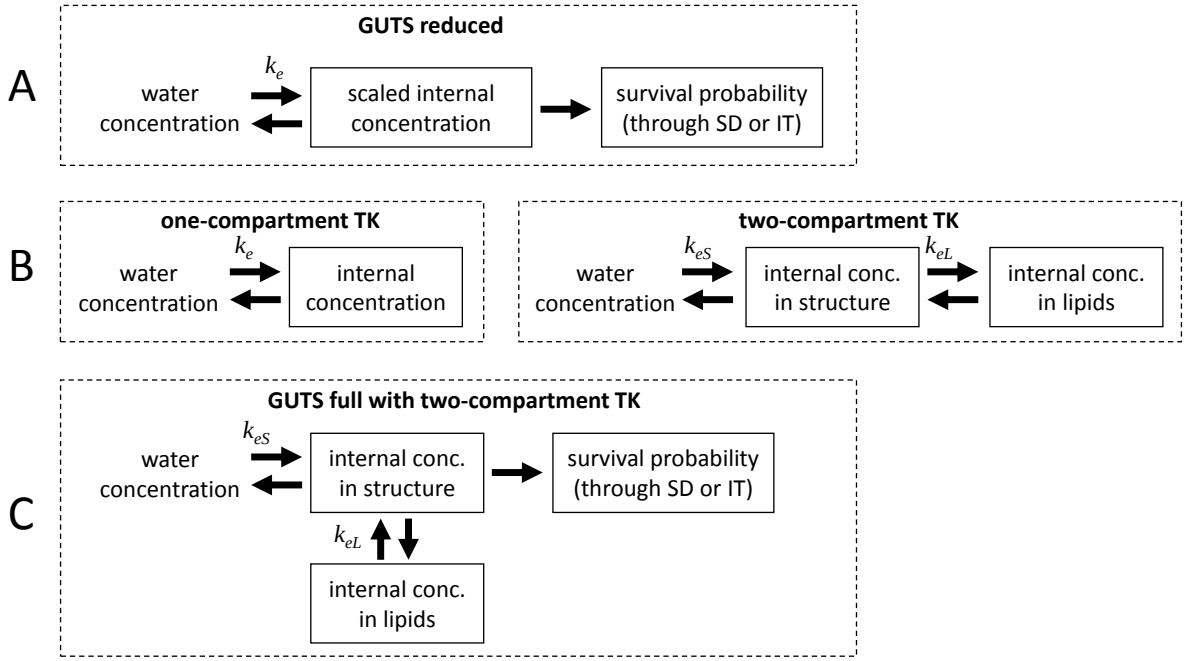

Figure 1. Schematic representation of the models used in this study: A) reduced GUTS models to analyse survival data in isolation, B) one- and two-compartment toxicokinetics models to analyse body-residue data in isolation, and C) the GUTS model with two-compartment toxicokinetics to fit both types of data simultaneously. The various elimination rates are shown, as plotted for cases A and B in Figure 3.

RESULTS AND DISCUSSION

Fits on survival data only. We start by fitting the two reduced GUTS models (Fig. 1A) to the survival data. These cases use either individual tolerance (IT) or stochastic death (SD) as the death mechanism, and apply a scaled TK model. The use of a scaled TK model implies that the elimination rate constant is estimated solely from the pattern of survival over time. In principle, this rate constant combines the elimination from the body with the toxicodynamic 'damage recovery'.[4] However, as dimethylnapthalene is a baseline toxicant, there should be no build-up of damage; narcotic effects relate to the number of molecules in the cell membrane, and the effect is completely reversible. Hence, we can expect the rate constant estimated from the survival data to represent the elimination rate. Jager & Kooijman[16] showed that the rate constants derived from survival data for narcotic compounds in fathead minnows were indeed consistent with the expected elimination rates from the body.

The fits of IT and SD to the survival data are visually very good and very similar (Fig. 2); the difference in Akaike Information Criterion (AIC) is 3.2 (see SI Section 3.1). The survival pattern thus cannot be used by itself to select the most realistic death mechanism. However, these good fits are established using a very different estimate for the elimination rate constant (Fig. 3): a good fit of IT requires a much lower rate constant than a good fit for SD, and the confidence intervals do not overlap. This is a general pattern observed in application of GUTS models.[4, 5, 21, 22]

Fits on body-residue data only. The body-residue data are reasonably-well represented by the standard one-compartment TK model (Fig. 2). Interestingly, the resulting elimination rate constant is consistent with the estimates based on the survival data using IT (Fig. 3), thus providing support for this death mechanism. However, a typical misfit is observed: the last two observation times on body residues show that elimination does not continue as expected from the one-compartment model. The two-compartment model (Fig. 1B) provides a significantly better fit to the data (likelihood-ratio test, α=0.05, df=2), by assuming that the measured body residues are the combined result of the concentrations in a relatively fast structural compartment and a relatively slow lipid storage.

Looking at the elimination rate constants of the two-compartment model (Fig. 3), the best estimate for the elimination rate of the lipid compartment (k_{eL}) is very low and hitting the lower boundary set for the optimisation (see Methods section). This does not hamper the analysis, but it implies that the parameters for the lipid storage cannot be properly identified from these data. The elimination rate constant for structure is consistent with the elimination rate constant required for the SD mechanism (Fig. 3). The target sites for toxicants (in this case the cell membranes) are associated with the structural part of the body and not with the wax esters stored in the lipid sac. Therefore, this comparison of elimination rate constants strongly supports SD as the most appropriate death mechanism.

Further support for SD can be obtained from Ashauer and co-workers,[21] working on the amphipod *Gammarus pulex* and various chemicals from different chemical classes, and combining measured body residues and survival over time. These authors demonstrated that almost all tested chemicals require a much smaller rate constant for damage recovery when assuming IT instead of SD as the relevant death mechanism. For the narcotic chemicals tested, damage recovery under SD is so high, that damage effectively does not play a role, and the probability to die is thus directly related to body residues.

Clearly, the IT mechanism can also provide a good fit to the survival data, but not with the elimination rate that is derived from body-residue data using the two-compartment model. This raises questions about the validity of using a QSAR for elimination rates (derived from body-residue data) combined with an IT-interpretation of toxicity data, as done by French-McCay.[8] The combination of such QSARs combined with the SD mechanism (as done by Baas *et al.*[23]) is more promising, given our results, at least for narcotic chemicals.

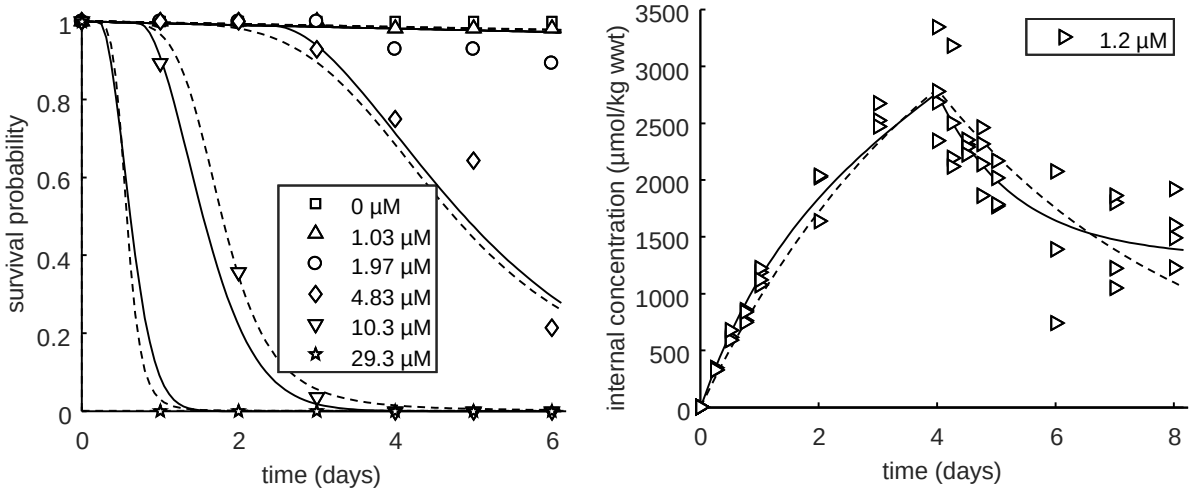

Figure 2. Left panel: fit of the reduced GUTS models for IT (broken line) and SD (solid line) to the survival data only. Right panel: fit of the one-compartment (broken line) and two-compartment (solid line) model to the body residue data.

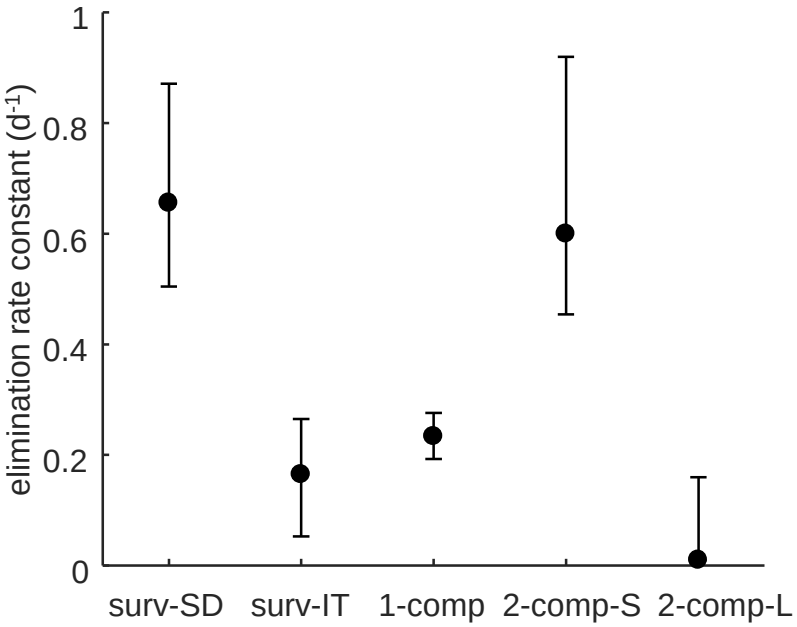

Figure 3. Comparison of the elimination rate constants from the two fits on survival data, and the elimination rates from two fits on the body-residue data. For the two-compartment model, values are shown for the structural (S) and lipids (L) compartment. Error bars represent the 95% confidence interval.

Simultaneous fit on survival and body-residue data. As a final step, we combined GUTS with the two-compartment TK model (Fig. 1C), and simultaneously fitted the body-residue data and the survival patterns. The internal concentration in structure is linked to the toxic effect. The fits to the data are shown in SI Section 3.3, as they are very similar to the fits in Figure 2; the solid lines for the SD fit, and the broken lines for the IT fits. Not surprisingly, given the results in Figure 3, SD provides a much better fit to the combined data than IT. The difference in AIC is more than 20, which basically implies that there is no support for the IT interpretation. We also fitted a combined SD-IT GUTS model to the data, but this model fit degenerates to the pure SD case; the best fit is obtained by assuming that there are no differences in sensitivity between the individuals (see SI Section 3.3). The parameter estimates for the SD fit are shown in Table 1. It should be noted that the lipid content (V_L/V_S) was fixed; its uncertainty was therefore not propagated in the confidence intervals. The uncertainty in the lipid content mainly affects k_{uL} (see SI Section 3.3), and the absolute value of this parameter, and its confidence interval, should thus be treated with care.

To check whether the two compartments can indeed represent structure and lipid storage of the copepods, we calculate the partition coefficients structure-water and storage-water from the four TK rate constants (see SI Section 1.1). These partition coefficients can then be compared to the octanol-water partition coefficient of the compound, log K_{ow} = 4.3-4.4 (EPI Suite 4.11, estimated and experimental value). This calculation serves as rough indication as the rate constants (especially for the lipid storage) cannot be accurately determined from these data. The ^{10}log partition coefficient structure-water is 3.4. The affinity of the chemical for structure is thus roughly a factor of 10 lower than for octanol. This makes sense as structure has a substantial amount of water and less hydrophobic biomass such as proteins. The ^{10}log partition coefficient storage-water is 5.3; one log unit higher than the K_{ow}. As storage in *C. finmarchicus* mainly consists of highly hydrophobic wax esters, such a high partition coefficient is not unreasonable. The TK rate constants are thus consistent with the view that the two compartments represent structure and lipid storage.

Table 1. Parameters for the fit of the GUTS-SD model with the two-compartment TK model. N.e. is not estimated. Asterisk marks where the optimisation hits the lower constraint applied in this study. Subscripts in units refer to water (W), structure (S) or lipids (L).

Symbol	Explanation	Best fit (95% CI)	Unit
k_{eS}	Elimination rate constant structural biomass	0.553 (0.451-0.700)	d^{-1}
k_{uS}	Uptake rate constant for structural biomass	1440 (1290-1620)	$L_W L_S^{-1} d^{-1}$
k_{eL}	Elimination rate constant lipid storage	0.01* (<0.197)	d^{-1}
k_{uL}	Uptake rate constant for lipid storage	0.825 (0.609-1.76)	$L_S L_L^{-1} d^{-1}$
m_S	Median no-effect concentration, referenced to structure	7.94 (6.40-8.99)	mmol L_S^{-1}
h_b	Background hazard rate	4.50 (1.41-10.5) 10^{-3}	d^{-1}
b_S	Killing rate constant, referenced to structure	0.260 (0.182-0.361)	L_S mmol^{-1} d^{-1}
V_L/V_S	Ratio of lipid volume to structural volume	0.2 (n.e.)	$L_L L_S^{-1}$

Link between GUTS and the CBR concept. The best estimate for the internal toxicity threshold in structure (m_S) in the final fit is 7.9 mmol/L (Table 1), which is just within the range of the reported critical body residues (CBRs) for narcotic effects of 2-8 mmol/kg,[7] assuming a density of structure of 1 kg/L. But can we directly compare the threshold m_S to these CBRs? The CBR for lethality is usually interpreted as the internal concentration associated with 50% mortality in a test cohort. This is generally taken to be constant over time, which (implicitly) assumes the IT mechanism for mortality. In the IT model (without a damage module), there is no toxicodynamics; there is a static link between the internal concentration and the survival probability (all individuals with a threshold value below the internal concentration die immediately). In our SD model, the threshold m_S is a model parameter specifying the internal concentration above which a chemical starts to affect the *probability* to die. In SD models, there is a dynamic link between the internal concentration and the survival probability; the probability is calculated by integrating the hazard rate over time.

The use of CBRs thus rests on the assumption that TD does not play a role in mortality, and that a certain internal concentration is directly linked to a certain percentage of survival in the test population. It should be clear that this represents an exceptional situation rather than a general rule. For survival, this situation only occurs when IT is the true death mechanism, damage does not play a role (i.e., very fast damage recovery), and we are able to determine the internal concentration in the relevant compartment (in our case: structural biomass). For sub-lethal effects, this situation is even rarer as the link between the internal concentration and the observed effect is more indirect and changes over the life cycle of the organism.[20] As a result, the ECx values for body size and reproduction do not need to decrease over time, but can also increase.[2] In summary, the CBR concept can be used as a rule-of-thumb, but it is not a useful concept in a dynamic modelling framework; in general, we need to consider both TK and TD.

Consequences of lipid content. The LC50, as predicted from the model parameters in Table 1, decreases in time (Fig. 4), ultimately approaching an asymptotic minimum, known as the incipient LC50. Using the parameterised model, we can predict the LC50-versus-time pattern for animals with a higher lipid content, or no lipid storage whatsoever. Increasing lipids leads to somewhat higher values of the LC50s (Fig. 4). Thus, lipid storage is expected to protect organisms against the toxic effects of dimethylnaphthalene, although the level of protection is rather small.

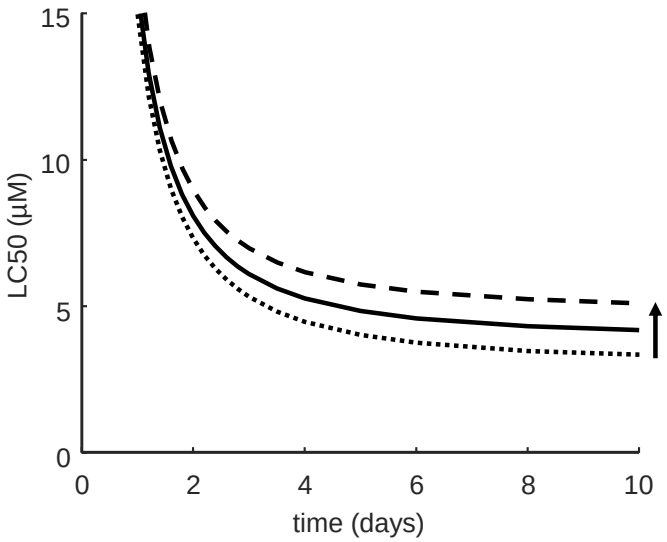

Figure 4. Predicted LC50 versus time for different lipid contents (dotted line V_L/V_S=0, solid line V_L/V_S=0.2, broken line V_L/V_S=0.4). The arrow points in the direction of increasing lipid content. LC50s are predicted from the model parameters in Table 1.

The reason for the protective effect of lipid storage lies in the concentration pattern for the structural compartment. With the current parameterisation, the concentration in the structural compartment follows a pattern that is very close to that of a one-compartment model. This is illustrated in Figure 5 using simulations for a scenario of 10-day constant exposure followed by 10-day depuration in clean water. More lipids leads to a lower pseudo steady-state concentration that is more rapidly achieved. The reason is that the flux from structure to the lipid storage acts as another elimination flux from the perspective of structure (worked out mathematically in SI Section 1.1). In this way, a substantial lipid storage will protect to some extent against toxic effects. However, this effect is limited (see Fig. 4) and temporary; eventually, the structural compartment will be in steady state with the lipid storage, and at that point, there will be no difference in the structural concentration between animals with and without lipid storage.

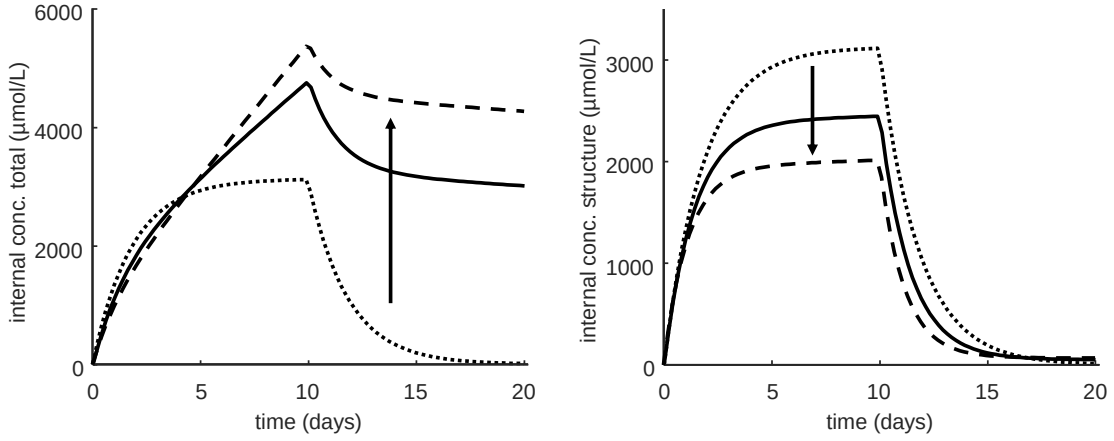

Figure 5. Simulated concentrations in total body (left) and structure only (right) at different lipid contents (dotted line V_L/V_S=0, solid line V_L/V_S=0.2, broken line V_L/V_S=0.4). The arrow points in the direction of increasing lipid content. Exposure scenario is 10-day constant exposure to 1.2 □M, followed by 10 days depuration in clean water.

In earlier work with *C. finmarchicus*, we observed a clear difference in oil toxicity between two experimental tests: one test showing no effects at exposure levels where the animals in the other test suffered some 75% mortality.[17] The only obvious difference between both tests was that the animals in the test without mortality had a markedly higher average lipid content. In that study, we attempted

to explain the observed sensitivity differences using the same two-compartment TK model (Fig. 1), but using a simplifying assumption about toxicokinetics: we assumed that exchange between structure and lipids would be fast, relative to exchange with water. Using this assumption, the two-compartment model reduces to a one-compartment model where the kinetics slow down with increasing lipid content. Slow kinetics postpones the onset of toxic effects, giving rise to 'survival of the fattest': individuals with the highest lipid content are expected to survive the longest during constant exposure.[24] However, the differences in lipid content were insufficient to explain the observed differences in survival. Furthermore, measured lipid sac volume at the end of the tests did not reveal preferential survival of lipid-rich individuals, nor lipid depletion as a result of toxic stress.[17] The current data set suggests that exchange between lipids and structure is not instantaneous, but a slow process. Lipids are still expected to protect against toxic impacts, but the effect is very limited (Fig. 5). This prediction is thus consistent with the lack of preferential survival of lipid-rich individuals in the test where mortality was observed, but also fails to explain the observed differences in sensitivity between the two tests in this earlier study.

In another recent study, we compared the acute toxicity of oil between different life stages of *C. finmarchicus*.[25] There were no differences between the sensitivity of the lipid-rich CV and females (somewhat less lipid-rich) or nauplii (lacking lipid storage completely). That result is therefore consistent with the very small effect of lipid content on toxicity as predicted in the current study. However, early copepodites and males were markedly more sensitive than the other stages.

Even though our conclusions on dimethylnaphthalene may not be directly transferrable to oil, a complex mixture of hydrocarbons, these two earlier studies strongly indicate that sensitivity for toxicant stress in copepods is more complex than the effect of the lipid sac on toxicokinetics alone.

Outlook on extrapolation to field situations. Both SD and IT can provide a good explanation of the survival data (Fig. 2), but they do so with different assumptions about the underlying TK (Fig. 3). A detailed analysis of the body-residue data suggests that the animal needs to be divided into (at least) two compartments: a structural compartment and a lipid storage (Fig. 1). We expected the concentration in structure to relate to toxicity, which clearly identifies SD as the most likely mechanism of action. So much so, that inclusion of an additional IT component does not improve the fit at all. Measuring body residues over time, next to toxicity assays, can thus provide a means to identify the contribution of each death mechanism. Even though TK follows a two-compartment model, the concentration pattern in the structural compartment is influenced only very little by the lipid content (Fig. 5). Using GUTS-SD with a scaled one-compartment TK model will thus provide a good approximation of mortality, both in analysing survival data from laboratory experiments (Fig. 2, left panel, solid line) and also in short-term extrapolation to other exposure scenarios. However, it remains to be tested whether this conclusion also holds for other hydrocarbons.

Even though lipid storage does not have a substantial impact on mortality under the test conditions, care must be taken when linking measured total body residues to toxicity. Furthermore, the toxicological consequences of the lipid storage will be more complex in the field. A substantial lipid sac means that total body residues in these copepods will remain high, even long after exposure has ceased. This might have consequences for diapause and gonad maturation (which is largely paid from lipid stores, thus remobilising the stored chemicals), but also for maternal transfer of contaminants to offspring, and for exposure of animals feeding on copepods.

In extrapolating toxicity over longer exposure durations, we need to assume that the animals remain the same. In reality, animals will feed, grow and develop, which will affect TK and possibly TD as well. In some studies, differences in sensitivity between life-stages could be related to size-dependent TK.[19, 26] However, our earlier work with *C. finmarchicus* shows that this cannot be the whole story; some differences in TK and/or TD, not logically related to size or lipid content, occur between tests, life stages and sexes.[17, 25] Furthermore, for extrapolation to chronic exposure, we need to assume that no additional mechanisms of action emerge. Figure 4 shows small effects in the lowest two exposure treatments that seem to be dose-related, and are not captured by the model. If this is a true effect of the chemical, and not some random variation in background mortality (or a consequence of the lack of feeding during the test), it could lead to underprediction of toxic effects in situations with long-term low-level exposure. Multiple mechanisms of action were also suggested in other studies with GUTS models.[25, 27]

Dimethylnaphthalene is a representative compound for oil pollution, but oil is a complex mixture of a wide range of chemicals. Progress has been made in the GUTS context regarding mixture toxicity[23, 28] and patterns in parameter values for across chemicals.[16, 21] However, more structural testing efforts will be needed to turn these proofs-of-concept into a reliable tool for predicting the consequences of oil pollution under field conditions.

ASSOCIATED CONTENT

Supporting information

The Supporting Information provides an extensive model description (including equations), the raw data, link to download the code used, more detailed information on the statistical procedures, and details of the various model fits. This information is available free of charge on the ACS Publications website at …

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Notes

The authors declare no competing financial interest.

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