Chapter 10

Discussion
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1 INTRODUCTION

This thesis is about mechanistically understanding effects of mixtures. This is of importance as it is impossible to experimentally assess effects of mixtures on organisms. Even not discussing the moral obligation to minimize experiments carried out with living organisms. Because experimentally assessing effects of mixtures is time consuming, experiments usually focus on effects of binary mixtures. Apart from the sheer endless combination of mixtures found in the environment this even further stresses the need to be able to have a framework in which measurements can be interpreted and which allows for extrapolation. This thesis is about such a framework and the main focus in this thesis is on effects on survival. We have shown that mixtures can exert effects in concentration ranges where individual compounds do not show effects. Focus of our research was to understand the mechanisms behind this effect and to interpret the results in terms of what this means for our legislative system.

In this discussion we will first go into some of the details of our approach to assess effects of mixtures. Then we will make a comparison with the traditional methods and other approaches. We will end with an example of the assessment of a complex mixture as can be found in Dutch surface waters with our process based method to show the advantages of this approach and we will give directions for future possibilities to assess effects of mixtures and further research.

2 PROCESS BASED MODELING OF EFFECTS OF MIXTURES

We developed a process-based description of the effects of mixtures of toxicants on the population relevant endpoints mortality, growth and reproduction within a single consistent theoretical framework, the theory on Dynamic Energy Budgets\textsuperscript{1,2}. The starting point in this approach is how an organism uses the available energy. Effects of toxicants on an organism are interpreted as effects on the energy household of the organism. Toxicological endpoints in general must not be viewed separately (as is the case in standard approaches); they reflect toxic effects in the same organism. Within the framework of DEB theory, modes of action are based on physiological allocation processes, and are therefore not directly related to 'mode of action' approaches, describing molecular mechanisms (e.g. AChE inhibition) or overall toxic syndromes (e.g. narcosis).

The starting point in this approach is that toxicants must be taken up by the organism before they can exert an effect. The internal concentration determines the effect and, once it is built up above a certain threshold level, effects start to show. Once the toxicant is inside the organism the internal concentration affects one or
more parameters in the model. Affected parameters can be the feeding rate, the maintenance costs, the costs for an egg, the probability to die, etc.. A schematic view of the modeling framework is shown in Figure 1. This approach can be applied to a large variety of different species without modifications. This in contrast to the physiology based pharmacokinetic pharmacodynamic (PBPK-PD) approaches, which are very species specific.

![Figure 1](image)

**Figure 1** Schematic view of the modeling system. The first step is a toxicokinetic module, followed by a description of how subsystems are affected by a toxicant with a feedback on the kinetics, resulting in an observed effect.

For survival, the focus of this thesis, a less elaborate modeling framework can be used than for growth and reproduction. For survival we can focus solely on the stochastic aspects of hazard modeling, neglecting the much more complicated modeling framework associated with effects on growth and reproduction.

### 2.1 Hazard modeling for mixtures with survival as an endpoint

We describe survival by a hazard model with a threshold concentration, the No Effect Concentration (NEC). The toxicant, once inside the organism, may increase the probability of death. If the organism changes size during the exposure the kinetics change and corrections have to be made. The corrections however, do not change the conceptual framework. With this approach three parameters per compound are needed to describe effects:

- The no effect concentration (expressed as an environmental concentration)
- The killing rate (1/(concentration.time))
- The elimination rate (1/time)
One extra parameter is needed to correct for the mortality in the controls, this parameter does not depend on the effects of the mixture. The no effect concentration (NEC) is the time independent concentration to which an organism can be exposed during for a prolonged period of time without an effect on survival. Note that the NEC might be zero, in that case any exposure, however small will ultimately have an effect. The 'strength' of the effect is described by the killing rate, the more toxic the compound, the higher the killing rate. The last parameter is the elimination rate. This parameter describes how fast the equilibrium between internal and external concentrations is set.

If an organism is exposed to a mixture of components the number of parameters increases rapidly if all possible interactions are taken into account. For a mixture of $n$ components we need $1 + 3n + n(n-1)/2$ parameters to describe the effect and all interaction parameters (see also section 2.3).

### 2.2 The behavior of the No effect concentration in mixtures

We derived three approaches for the behavior of the NEC in mixtures. In the first approach all compounds have their own NEC irrespective of one another. In the second and the third approach the components in the mixture share the NEC. This can be done in two different ways; the concentrations of the compounds sharing the NEC can be fixed at the moment the NEC is exceeded. This can be viewed as a irreversible binding model. And in the second approach the concentrations are not fixed, but can change after the NEC is exceeded. However, it appears that the differences between these two approaches are too subtle to be experimentally verifiable. So basically we are left with two behaviors of the NEC; independent or shared NEC. The sharing of the NEC was experimentally verified for compounds with the same mode of toxic action. The implication of this is that in mixtures effects can be expected in concentration ranges where the individual compounds making up the mixture do not show effects.

This approach proved to be very successful in predicting effects of complex mixtures on the survival of daphnids (see section 5 of this discussion). The observed survival or mortality could reliably be predicted without any further assumptions. Traditional methods, without a threshold, failed for the same data.

### 2.3 Interactions

Strong interactions in mixtures appear to be an exception, especially in mixtures with increasing numbers of components. But there are some examples where they exist. For binary mixtures estimating interaction parameters is feasible, but for mixtures containing more compounds this rapidly becomes impossible.
Interestingly in our measurements on the survival of springtails exposed to binary metal mixtures we showed that over the whole time period (21 days) there were no interactions in this mixture. But when the data were analyzed for each timepoint individually, interactions differed substantially for the different points in time. In addition Cedergreen et al.\textsuperscript{9} showed that interactions measured for individual points in time have very poor reproducibility. This leads to the conclusion that finding interactions for some mixture at some point in time does not give reliable results and should not be the aim of ones research.

2.4 Experimental effort

2.4.1 General considerations
We are frequently asked how much extra effort it takes to conduct an experiment that suits a DEB interpretation of the results. This is especially important as the experimental effort in assessing effects of mixtures is considerable in itself. This question becomes even more important as it is impossible to experimentally assess only a tiny fraction of all possible mixtures. Given these considerations it is of major importance that experiments carried out with mixtures allow for extrapolation. Extrapolation to compounds not taken up in the measurements, extrapolation to other organisms, extrapolation to other points in time etc. So finding some significant statistical interaction, for some organism exposed to some (binary) mixture at some point in time, without further interpretation should never be the aim of ones research.

The most important consideration in conducting an experiment (not only in mixtures) is to have a really clear view on the questions that have to be answered by an experiment and from there guide the experimental setup. Establishing a framework that allows to extrapolate ones results should be the starting point. So, if one has a soil dwelling organism and one is interested in the mechanisms behind effects of mixtures this means that effects in the medium should be separated from effects in the organism. Interactions in the medium can easily dominate the processes in the organism. Keeping the organism on filter paper or on compacted soil can then be of great help in interpreting the experimental results. For springtails we showed that keeping the organisms on top of the soil allowed for a daily count of the number of surviving organisms. Still the resulting $LC_{50}$ values were similar to experiments where the springtails were allowed to dwell in the soil, so the ecological relevance of the test was not compromised. Keeping the organisms on top of the soil allows daily monitoring of growth, survival and reproduction and allows for an easier interpretation of the experimental results. The ecological relevance of an experiment might be subordinate to the wish of unraveling toxicological mechanisms. The experimental effort that is needed in achieving ones goal is of course important, but there is no
point in performing an experiment that fits the available time but will not answer ones questions.

The additional experimental effort that is needed for a DEB interpretation compared to a standard test depends on the organism. The most eye catching difference is that for the interpretation of toxic effects with a process based approach measurements at different points in time are needed. Further one has to be aware that an interpretation based on DEB theory requires that the relevant endpoints in terms of the energy balance of an organism have to be followed: growth and reproduction. For a full scale mechanistic interpretation of measured data the most important features that have to be caught by the measurements are:

- maximum length, compared to the control,
- growth curve compared to the control,
- time of first reproduction compared to the control,
- number of juveniles compared to the control,
- survival in time.

The effect surface should be covered by the experimental design. Whether a full factorial design or a ray design is chosen is not important for the interpretation of the experimental data.

If more data become available patterns might occur and the predictability of effects of mixtures therefore might increase. This may lead to a considerable reduction in the experimental effort in assessing effects of mixtures. As we have already shown for the interpretation of a four component PAH mixture experiment was carried out with only 27 treatments, including the controls and single compounds. In this case the experimental effort was reduced considerably by making use of the best available prior knowledge and we showed that this works (see chapter 7). In this experiment only one concentration level for each single compound was used (see chapter 7 of this thesis). Especially if a mixture contains more compounds, the experimental effort may be considerably reduced by applying the best possible prior knowledge available (or estimates) of the effects of the compounds making up the mixture. Also in general the number of replicates can be reduced leaving space for more treatments. The extra noise from having less replicates is usually compensated by having extra treatments.

Note that it is commonly accepted that a PBPK approach is experimentally demanding, but can also lead to very interesting insights in underlying mechanisms. The same applies to DEB. The discussion should not be about what does it cost, but what does it bring us. The major advantage of Process-based modeling is that data are interpreted in one single consistent framework, which allows extrapolation. Within
the framework of DEB theory extrapolation is possible to (see also section 6 Outlook of this discussion):

- other species,
- other compounds,
- other points in time,
- pulsed exposure,
- changing food conditions.

For the additional experimental effort that is needed for a DEB based analysis of experimental results of a study on mixtures we have to make a distinction between soil dwelling organism and aquatic organisms.

2.4.2 Aquatic organisms
A number of standard test protocols for aquatic organisms demand that effects on survival and reproduction are monitored at intermediate points in time, this is prescribed both for acute tests as well as for chronic tests for fish and daphnids. These intermediate time point data are later discarded, which is completely inconceivable, as these measurements do contain valuable information. But growth does not have to be followed, so the extra effort is in following also growth. Compared to the standard test protocol this would lead to some extra effort, but not a major effort, in a well equipped laboratory with experienced personnel something like an hour per day (looking at growth every second day is enough to get a growth curve). This means an extra effort of less than 10% of the normal experimental effort appears to be a reasonable estimate, irrespective of the number of components in the mixture.

2.4.3 Soil-dwelling organisms
For soil dwelling organisms things are much more complicated. For *Folsomia candida* for instance a standard test takes 28 days without any intermediate points in time. If intermediate points in time are needed extra jars can be used that can be sacrificed at intermediate points in time. In this case the additional work can be quite considerable, the number of treatments was close to doubled. However the preparation of the soil, concentration measurements etc. takes much more time than the actual experiment (as it runs). So preparing some extra soil and putting it in extra jars is only part of the work. An approach like this will add approximately 30% of the time that one needs for a normal experiment, also here irrespective of the number of components in the mixture.
3 CONCENTRATION ADDITION AND INDEPENDENT ACTION MODELING OF MIXTURES

The tools that are most often used to assess effects of mixtures are the classical Concentration Addition (CA) and Independent Action (IA) models. These models are conceptually different, but both are descriptive models that use dose-response curves of single compounds to predict effects of mixtures or to find (synergistic) interactions for binary mixtures at a single point in time. Jonker et al. specifically designed a framework dedicated to find interactions in binary mixtures. Interactions are defined as statistically significant deviations from the standard models. CA is generally used for mixtures of compounds with the same assumed mode of action and IA is generally used for mixtures of compounds with an assumed different mode of action.

The CA/IA approaches for assessing effects of mixtures have some major drawbacks. The most important drawbacks are that every endpoint is considered independently of other endpoints and that these models can not integrate different points in time. As the CA/IA models can not integrate different points in time for a single endpoint and can not integrate different endpoints, extrapolation efforts become very difficult if not impossible. If one looks at effects on one organism it is more natural to interpret effects on growth, reproduction and survival in one framework, using the same parameters, than regarding the different endpoints as unrelated and deriving (statistical) interaction parameters for each specific endpoint at different points in time. In addition EC₅₀ values are often chosen to find interactions on different sublethal endpoints. We show in chapter 2 of this thesis that EC₅₀ values should be treated with great care as an EC₅₀ for the growth rate has a different pattern in time than an EC₅₀ based on absolute growth for example. Interactions should not depend on whether growth is expressed as a growth rate or as an absolute growth.

3.1 Application of CA/IA to survival

We analyzed a dataset where survival of Folsomia candida exposed to a binary mixture of metals was monitored for 21 days (see chapter 5). This analysis showed that statistical interactions can vary substantially over time, see table 4.

The IA model suggests a synergistic effect over the whole time period for the mixture of copper and cadmium. When time progressed the synergistic effect became dose-level dependent, but the model still gave a synergistic effect. When the CA model is used as a base model, the variation in interactions is much larger. During the first few days there is no interaction, then there is a synergistic effect, then again no interaction and later in the experiment a dose-level interaction
with synergism at low doses and antagonism at high doses. The switching point from antagonism to synergism is about twice the LC50 value (parameter b, see Jonker et al13, is close to 0.5). But care must be taken in interpreting the data: the spreadsheet looks for deviations from the standard model and attributes the deviations to interactions. This means that (small) random variations in the survival data may change the best fitting model. The more elaborate the exposure experiment (in terms of concentration ranges and exposed organisms) the less problems random variations give. For this experiment, containing 6 x 6 concentrations levels and 30 exposed organisms, random variations can be problematic. The best fitting model can be switched from a dose-level dependent interaction to no interaction if survival is changed by only a few organisms for two or three data points. For example we measured 25, 26, 18, 19, 13, 8 surviving organisms at one of the 36 available mixtures, this lead to a statistically significant interaction, whereas a measurement of 26, 25, 20, 17, 13, 8 survivors would not. Moreover Cedergreen et al.9 showed that specific interactions are hardly reproducible. This all appears to indicate that the identification of interactions with the classical models may be an artifact, following from either random errors at a particular time point (as was shown by Baas et al14), or differences in toxicokinetics between the compounds. Then the choice of the standard model (CA or IA) must be made on the basis of assumptions about the mode of action of the compounds in the mixture. When an incorrect working mechanism is selected, observed effects may be wrongly attributed to an interaction.

Note also that for use of the CA or IA models, at least 5 parameters per time point are needed to describe the effects of the mixture when no interaction takes place. When there is an interaction, this can increase to 7 parameters per time point. In

Table 4 Results for the interactions obtained when applying the spreadsheet model to the data for the mixture of copper and cadmium on the survival of Folsomia candida in Lufa 2.2 standard soil. (CA: Concentration addition model, IA independent action model, A: antagonism S: Synergism)

<table>
<thead>
<tr>
<th>Time (d)</th>
<th>Interactions CA as base model</th>
<th>Interactions IA as base model</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 – 3</td>
<td>No interaction, CA</td>
<td>Synergism</td>
</tr>
<tr>
<td>4 – 5</td>
<td>Synergism</td>
<td>Synergism</td>
</tr>
<tr>
<td>6</td>
<td>Synergism</td>
<td>Dose level dependent Synergism</td>
</tr>
<tr>
<td>7 – 9</td>
<td>Dose level dependent Synergism</td>
<td>Dose level dependent Synergism</td>
</tr>
<tr>
<td>10 – 15</td>
<td>No interaction, CA</td>
<td>Dose level dependent Synergism</td>
</tr>
<tr>
<td>16</td>
<td>Dose Ratio dependent interaction</td>
<td>Dose level dependent Synergism</td>
</tr>
<tr>
<td>17</td>
<td>No interaction, CA</td>
<td>Dose Ratio dependent interaction</td>
</tr>
<tr>
<td>18 – 21</td>
<td>low doses S, high doses A*</td>
<td>Dose level dependent Synergism</td>
</tr>
</tbody>
</table>

* Change from antagonism to synergism at about 2 x LC50.
the experiment described here, 119 parameters would be required to describe the results with the CA model and 133 parameters with the IA model. This number of parameters may be reduced by describing the time dependence of the $LC_{50}$ and slope values in the mixture (adding new parameters). The process-based model needs only 8 (!) parameters to model the complete time series though measurements in time are essential.

A very good example of the limitations of CA/IA approaches in assessing effects of mixtures is shown in chapter 8 of this thesis. We analyzed survival data for in situ exposed *Daphnia magna*. In this case an effect of the mixture was observed at one specific point in time (168 hrs of exposure). In most cases there were no direct toxicity data available for the individual compounds for this point in time. So an effort to link the observed survival to the chemical contamination in the surface waters using CA or IA approaches immediately fails because of lack of data. In addition a more or less random choice has to be made for how many toxic units in a mixture are needed to kill the organisms. So here CA/IA do not allow an absolute approach. Furthermore if a ranking is made, based on the measured concentrations in comparison with their 48hr $LC_{50}$ values, the metals would be the most important group of compounds responsible for an effect on survival. We have shown with a DEB based approach (which directly led to reliable predictions on the occurrence of mortality without extrapolation problems and random choices), that the pesticides have to be the cause of effects on the survival of the daphnids and not the metals.

### 3.2 Application of CA/IA to sublethal effects

For sub-lethal endpoints, there are very few studies that follow endpoints in time. An example is the study by Jager et al.\textsuperscript{16} and by Van Gestel and Heusbergen\textsuperscript{17}. In Van Gestel and Heusbergen\textsuperscript{17} each time step was analyzed as a new data set. And also here it was shown that the apparent mixture interactions changed over time. Recently the DEB-approach was extended to sublethal effects and applied to effects of binary mixtures of PAH and nickel and chlorpyriphos to daphnids (unpublished results). The whole time series of toxic effects on growth, reproduction and survival could be interpreted within one consistent framework with the same parameters for the different endpoints.

For sublethal effects the DEB approach implies interactions can occur through toxicokinetics, as toxicokinetics is influenced by body size and growth rate. Therefore, it is to be expected that a model analysis based on energy budgets shows deviations from classical CA and IA even without imposing statistical interactions between the compounds.
3.3 Conclusions

The concepts of CA and IA are principally not suitable for integrating effects over different points in time and it was shown that:

- interactions differ at different points in time
- small random variations may result in large deviations in the interactions found
- the reproducibility of interactions is poor at best

Therefore the statistical interactions found with these approaches may offer artifacts of these models. In addition the statistical interactions do not explain why these interactions occur so there is no (at best very limited) possibility to make predictions for compounds that were not measured. So the main drawback of these concepts is the lack of a mechanistic basis, which does not allow extrapolation to other compounds, other organisms, other points in time or fluctuating concentrations is very difficult if not impossible. Furthermore with these approaches the interpretation of effects in the same organism on growth or survival within one consistent framework is impossible.

4 OTHER PROCESS-BASED APPROACHES

For survival there are other approaches to assess effects of mixtures. For a combination of survival and sublethal endpoints so far the DEB approach is the only approach where effects of mixtures are interpreted in one single consistent theoretical framework.

For survival the best known approaches apart from DEB are those by Lee et al.\textsuperscript{21} and Ashauer et al.\textsuperscript{22}. The different approaches and underlying assumptions were recently compared by Ashauer et al.\textsuperscript{22}. The most striking difference is that the damage assessment model by Lee and coworkers mortality is deterministic, whereas the model by Ashauer and coworkers and the DEB model for survival are based on the hazard model, implying that mortality is a chance process. Instead of instant death when exceeding a threshold, it is assumed that death is an inherently stochastic process. Dedicated fish studies showed that the stochastic component dominated for mortality\textsuperscript{24}.

Both the method by Ashauer et al. and Lee et al. use one extra kinetic step to assess damage inflicted by a chemical in the recent past, and therefore require an extra parameter (per component in a mixture) compared to the DEB approach for survival. This extra parameter is estimated from measured or estimated internal concentrations. Jager and Kooijman\textsuperscript{25} applied a method that is very similar to that of Ashauer et al.\textsuperscript{22}, also involving an extra kinetic step, to raw survival data for exposure to
single components but the extra parameter hardly improved the fits of the model to the survival data. It did give different values for the elimination rate however. But the data available did not include internal concentrations, so the elimination rates could not compared with experimental data. Especially for complex mixtures data on internal concentrations are usually not available and require an elaborate experimental and modeling effort.

5 APPLICATION OF THE DEB BASED APPROACH TO COMPLEX MIXTURES

A very nice example of the DEB-based approach for mixtures is in the assessment of effects of mixtures of narcotic compounds (PAH, Chlorinated benzenes, PCB, etc etc.) on survival. The hazard model predicts that the parameters that describe the toxic effects are related to their $K_{ow}$ values, which were shown to have a strong support from experimental work\textsuperscript{16}. This means that for a mixture of e.g. PAHs one only needs to do experimental work on one of the PAH and then the whole mixture effect for any point in time can be calculated, using the $K_{ow}$ values to obtain the other toxicological parameters\textsuperscript{5,16}. Such an extrapolation potential allows for a vast reduction in experimental effort in assessing effects of mixtures and is unknown to the CA/IA approaches.

We have shown that our approach is capable of reliably predicting effects of complex mixtures, based on readily available literature data\textsuperscript{29} (see chapter 8). Also here it proved extremely valuable to be able to extrapolate the effect of single compounds to other points in time than the actual measurements. We were able to predict the effect of a mixture containing over 80 different compounds (pesticides, metals, PAHs, PCBs, nutrients etc) to the survival of in situ exposed \textit{Daphnia magna} to Dutch surface waters. But most importantly we were able to directly link the chemical constituents to the ecological status of water bodies.

The most important assumptions made were first the existence of a NEC and secondly that toxicants can be divided in classes of compounds with the same mode of action, sharing a NEC and that different groups of compounds act independent of the other groups of compounds (a similar approach was followed in\textsuperscript{17}).

The general picture, that emerges from this study is that high concentrations of relatively few compounds can be linked to observed effects in field situations. This has been reported earlier\textsuperscript{18,19,20}. But we also expect that if partial or sub lethal effects would be evaluated, that effects are mainly caused by mixtures of compounds and not by individual components in the mixture.
6 OUTLOOK

Looking into the future, one of the most challenging areas in chemical mixture research is answering the question on how to deal with the infinite number of combinations of chemicals and other stressors? Tools that can mechanistically interpret experimental data and allow extrapolations are a necessity to try to address this problem. DEB theory describes simple rules for how organisms acquire and use resources for growth, development and reproduction. Toxic effects in combination with (some) natural stressors can then be viewed as a disruption of allocation processes. This means that we have to treat growth and reproduction for what they are: tightly interlinked processes in time, and make assumptions on how toxicants interfere with these processes.

To fully understand the effects of (chemical) stress on life-history responses, such as growth, reproduction and longevity, there is a need to link the effects of chemicals at the detailed mechanistic level with higher organization changes in resource allocation and trait performance. Process-based models, such as DEB, can provide a framework to understand the physiological basis of life history in terms of energy allocation. Mode of action prediction derived from DEB can provide a useful indication of the physiological basis of the toxic effect. However, these modes of action still represent changes in very broad metabolic processes. Studies of detailed molecular mechanisms on the other hand may provide detailed understanding of the molecular target site of a chemical, but not always how interactions at this site may result in different patterns of change for the whole organism. Combined, however, the two can meet the objective of understanding both the detailed basis of chemical effect and how these translate to biologically meaningful effects on key traits. The first tentative steps in this direction are set and data are being gathered that are suitable for this kind of interpretation. If more data become available patterns might occur and the predictability of effects of mixtures therefore might increase. This may lead to a considerable reduction in the experimental effort in assessing effects of mixtures. As we have already shown for the effect of mixtures of narcotic compounds.

The nature of the deviations from model expectations will provide more information on the underlying mechanisms than descriptive statistical interaction terms, and thus delivers directions for further mechanistic research. Dealing with sub-lethal effects, it is essential to have a model capable of showing the relations between feeding, maintenance, growth, development and reproduction. This approach allows understanding why toxicological effects change under changing food conditions\textsuperscript{26}. As organisms in the environment mostly live under periodical food stress and laboratory testing is mostly done under ad libitum food conditions it is important to have a framework to interpret these effects\textsuperscript{7}. As mixture models develop we expect a more tight interaction between the modelers and the experimentalists, to arrive
at natural quantifiers for effects and solve more specific questions in unraveling the mechanisms behind effects of mixtures and further rationalize experimental design for mixtures.

Process-based modeling has clear advantages in understanding effects of mixtures. The major advantage is that data are interpreted in one single consistent framework, which allows extrapolation. Within the framework of DEB theory extrapolation is possible to:

- other species,
- other compounds,
- other points in time,
- pulsed exposure,
- changing food conditions.

Extrapolation between species (e.g. from laboratory species to related field species of interest) is possible, because many metabolic parameters vary with body size in a predictable way\(^2\). Analogously, test data for different species may be combined to yield a coherent set of information on a chemical. Process-based methods also aid in extrapolating between chemicals, because the model parameters often have relationships with chemical properties such as hydrophobicity (e.g., \(^{16, 27, 28}\)). This predictability of effects, as long as the mechanism of action remains the same, is especially valuable in that it allows predictions for untested compounds\(^5\). Furthermore, process-based modeling facilitates an educated extrapolation from single-species test results to population consequences, which is impossible using the standard summary statistics.

Because processes are modeled explicitly in time, the results of short-term test can be extrapolated to chronic timescales, and vice versa. This claim rests on two preconditions: 1) that the model parameters must be accurately fixed by the data, and 2) that no other modes of action of the chemical appear after prolonged exposure. It is also possible to make predictions of effects resulting from time-varying concentrations, even when the test is performed under constant exposure.
REFERENCES


