Dynamic effects of compounds on animal energetics and their population consequences[*]

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Abstract

Effects of toxic compounds on populations can, under certain simplifying assumptions, be deduced from effects on individuals, and specifically from effects on the processes of substrate uptake and use by individuals. These effects can be understood in three steps: toxicokinetics (how external concentrations relate to internal ones), effects on target parameters (how internal concentrations change the value of on a target parameter), effects on endpoints (how a change in a target parameter relates to the endpoint of interest, such as reproduction rate or cumulative number of offspring in a standardised exposure period). Effects of toxicants occur at various levels of organisation. At the molecular level, all molecules have (potential) effects; at the level of the individual, No Effect Concentrations exist, and at the population level, effects can be varying, even if the concentrations in the organisms are constant.

1 Introduction

The actual behaviour of populations depends on many factors, and is frequently difficult to predict. This is a handicap for recognising effects of toxicants on population dynamics, while the general feeling is that such effects should be avoided. Spatial and temporal inhomogeneities, the presence of a rich biodiversity with complex trophic interactions all contribute to this lack of predictability. In the context of environmental risk assessment for chemicals it is only practical to refrain from concrete predictions, for the time being, and to consider potential effects of chemicals on populations in idealised simple environments. A practical choice for such an environment is a spatially homogeneous one, without temporal variation of environmental variables including food availability. It is needless to mention that this environment is highly artificial indeed. Another, and perhaps more relevant choice for an artificial environment is that of an idealised fed-batch culture, where a population lives in a spatially homogeneous environment, while it receives a constant flux of food, and is harvested at a constant rate.

Despite the simplicity of these artificial simple environments, the behaviour of populations is still difficult to understand. This makes that effects of toxicants on populations can only be understood within a modelling context where population behaviour is described in terms of properties of individuals. The reason for the latter restriction is that toxicants affect individuals and not populations directly. This calls for

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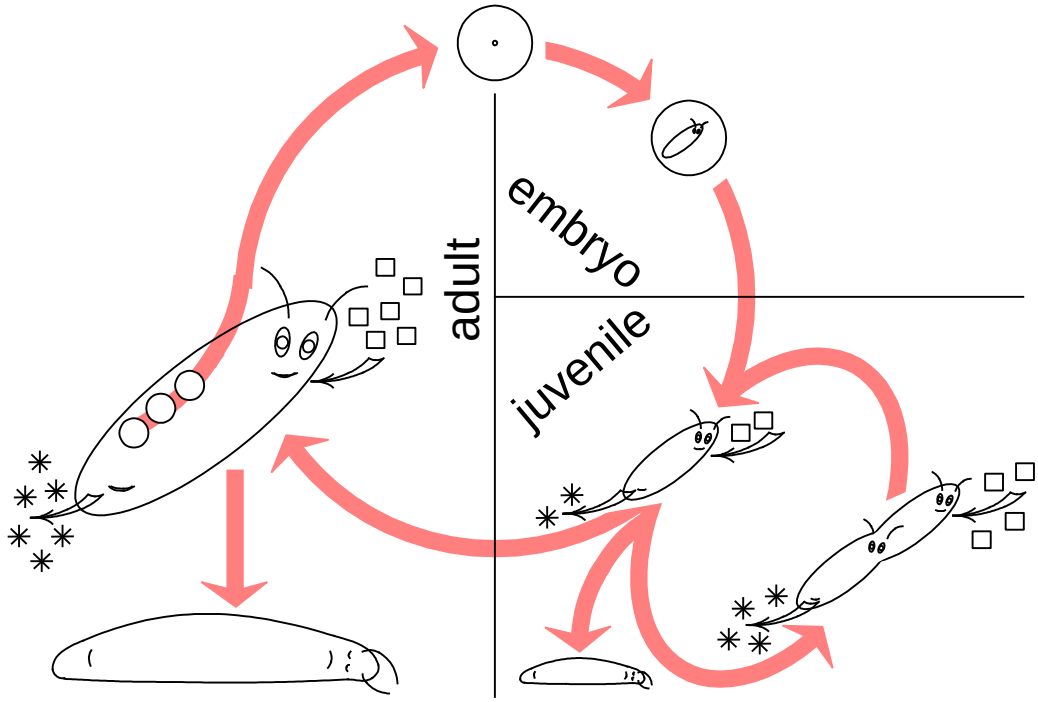

theory for physiologically structured population dynamics, which explicitly deals with the physiology of individuals (Hallam et al., 1989).

Figure 1. Dynamic Energy Budget theory aims to quantify the energetics of individuals as it changes during life history. The key processes are feeding, digestion, storage, maintenance, growth, development, reproduction and ageing. The theory amounts to a set of simple rules, summarised in Table 1, and a wealth of consequences for physiological organisation and population dynamics. Although some of the far-reaching consequences turn out to be rather complex, the theory is simple, with only one parameter per key process. Intra- and inter-specific body-size scaling relationships form the core of the theory and include dividing organisms, such as microbes, by conceiving them as juveniles.

2 Dynamic Energy Budgets

Dynamic Energy Budgets (DEBs) specify how the processes of substrate uptake and use change during the full life cycle, see Figure 1. Since ageing has tight links with respiration, via free radicals, and respiration with the use of reserves, ageing, and so life span, is specified by DEBs as well. Since population dynamics directly relate to the processes of feeding, reproduction and survival by individuals, knowledge about DEBs is basic to population dynamics and so to ecosystem dynamics.

2.1 Individuals

The assumptions on which the DEB model is based are listed in Table 1. A flow diagram for energy and mass is given in Figure 2. Food is ingested by an animal, transformed into faeces and egested. Energy derived from food is taken up via the blood, which has a low capacity for energy but a high transportation rate. Blood exchanges energy with the storage, and delivers energy to somatic and reproductive tissues. A fixed part, κ, of the

utilisation rate, i.e. the energy delivered by the blood, is used for (somatic) maintenance plus growth, the rest for development and/or reproduction. The decision rule for this fork is called the κ-rule. Maintenance has priority over growth, so growth ceases if all energy available for maintenance plus growth is used for maintenance. Energy used for development in embryos and juveniles is similarly partitioned into energy for maintenance of a certain degree of maturation and energy for an increase in the degree of maturity. The energy spent on increasing the degree of maturity in juveniles is allocated to reproduction in adults.

Table 1 The assumptions that lead to the DEB model as formulated for multicellular animals and modified for unicellulars

General

1 Structural body mass and reserves are the state variables of the individual and they do not change in composition (strong homeostasis).

2 Food is converted into faeces and assimilates derived from food are added to reserves, which fuel all other metabolic processes. These processes can be classified in three categories: synthesis of structural body mass, and of reserves (including the embryonic reserves via reproduction), and processes that are not associated with synthesis of biomass. Products that leave the organism may be formed in direct association with these three categories of processes, and with the assimilation process.

3 If the individual propagates via reproduction (rather than via division), it starts in the embryonic stage that initially has a negligibly small structural body mass (but a substantial amount of reserves)

Specific

3a The reserve density of the hatchling equals that of the mother at egg formation.
Foetuses develop in the same way as embryos in eggs, but at a rate unrestricted by energy reserves.

4 The transition from embryo to juvenile initiates feeding, and from juvenile to adult initiates reproduction, which is coupled to ceasing maturation. The transitions occur when the cumulated energy invested in maturation exceeds a threshold value.
Unicellulars divide a fixed time after initiation of DNA duplication, which occurs when the cumulated energy invested in maturation exceeds a threshold value.

5 Somatic and maturity maintenance are proportional to body volume, but maturity maintenance does not increase after a given cumulated investment in maturation. Heating costs for endotherms are proportional to surface area.

6 The feeding rate is proportional to surface area and depends hyperbolically on food density.

7 The reserves must be partitionable, such that the dynamics is not affected; the use of reserves does not depend on food density; the reserve density at steady state does not depend on structural body mass (weak homeostasis).

8 A fixed fraction of energy, utilised from the reserves, is spent on somatic maintenance plus growth, the rest on maturity maintenance plus maturation or reproduction (the κ-rule).

9 Under starvation conditions, individuals always give priority to somatic maintenance and follow one of two possible strategies:
they do not change the reserve dynamics (so continue to reproduce), or
they cease energy investment in reproduction and maturity maintenance (thus changing the reserves dynamics).

10 Ageing related damage accumulates in proportion to the concentration of damage inducing compounds, which accumulate in proportion to the volume-specific metabolic rate. For unicellulars damage is lethal, therefore, it does not accumulate. Apart from `accidents', the

hazard rate is proportional to accumulated damage, but death occurs if somatic maintenance costs can no longer be paid.

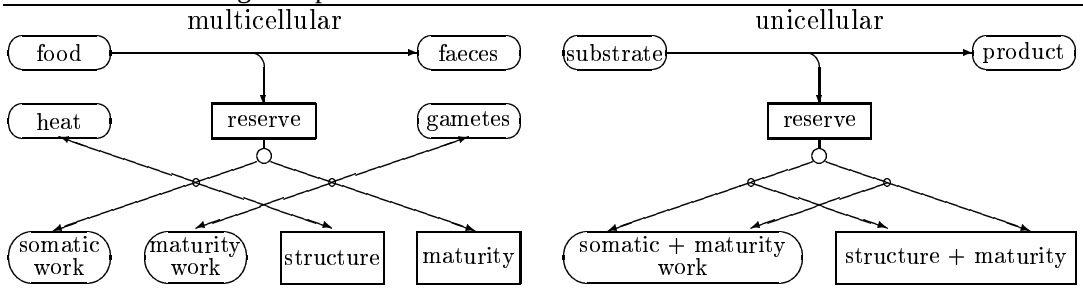

Figure 2 Energy fluxes through a heterotroph. The rounded boxes indicate sources or sinks. All fluxes contribute a bit to heating, but this is not indicated in order to simplify the scheme.

Substrate is taken up and processed by unicellulars (including prokaryotes) in a way conceptually comparable to food by animals, although defecation and utilisation share partly the same machinery to mobilise energy. The coupling between mass and energy fluxes, particularly relevant to micro-organisms, follows directly from the model assumptions. Detailed description of the model may be found in Kooijman (1993).

The primary parameters of the DEB model are listed in Table 2. For unicellulars, the list is shorter, since they do not reproduce (so they pay no overhead costs for reproduction). They only have one life stage (so maturation investment for birth and puberty should be replaced for that in division) and they do not heat. For unicellulars ageing acceleration should be replaced by ageing rate (which means that a particular cell is or is not hit by the ageing process). If maturity maintenance costs has a particular value relative to the somatic maintenance costs, the cell divides at a fixed size, and the role of the partition coefficient for catabolic power disappears from the energy budget.

For many applications it is not necessary to know all parameter values. Knowledge about energy parameters is only essential if energies have to be described or predicted. As long as the interest is in feeding, survival and reproduction, only some ratios of energy parameters are needed such that the dimension energy drops out. This usually involves a small number of compound parameters only.

Table 2 The primary parameters of the DEB model for animals

saturation constant	max specific ingestion rate
max specific assimilation rate	energy conductance
somatic maintenance costs	maturity maintenance costs
costs for growth	partition coefficient for catabolic power
maturation investment for birth	maturation investment for puberty
overhead fraction for reproduction	ageing acceleration
specific heating costs (endotherms only)	

Although the list of assumptions in Table 1 hardly mentions masses, it does specify all mass fluxes, including respiration (oxygen use or carbon dioxide production), nitrogen waste production, and even the water flux (Kooijman, 1995; Kooijman et al., 1999; Kooijman, 2000).

One of the more remarkable implications of the DEB model is that body size scaling relationships for parameter values follow naturally, without any empirical argument or extra assumptions. Each of the primary parameters is either intensive (so does not relate

to maximum body size), or extensive, such that ratios of extensive parameters are intensive again. The classification is purely on the basis of physical principles. In other words: the parameters tend to covary across the different species. A first guess for particular parameter for a species with maximum body size V_{m1} can be obtained from that of a species with maximum body size V_{m2} by correcting for the differences in body size. This is obviously of great help to obtain approximative parameter values. The crux of the argument is in the fact that the maximum body length of an individual is a simple function of three parameters: the partition coefficient for catabolic power times the ratio of the maximum specific assimilation rate and the specific somatic maintenance costs. The maximum specific assimilation rate is the only extensive parameter, which makes that it must be proportional to maximum body length.

This reasoning can be extended to any life history parameter, such as life span, length of the juvenile period, maximum reproduction or growth rate, etc. They can all be written as functions of primary parameters, and, therefore, as functions of maximum body size. This also holds for mass fluxes, such as weight-specific respiration, which turns out to decrease with maximum body size in a way that is fully consistent with experimental data.

2.2 Populations

Suppose that individuals only interact via competition, and that they live in a homogeneous environment. At constant food density the population will eventually grow exponentially at a rate that depends on the survival probability and the reproduction rate, as given by the characteristic equation, see Box 1.

Since the target of risk assessment is the ecosystem, rather than the population, relationships between populations are of interest. Studies of the behaviour of food chains revealed complex dynamics as soon as the chains have more than 2 trophic levels (Kooi and Kooijman, 1994; Kooi and Kooijman, 1995; Kooi et al., 1997a; Boer et al., 1998; Kooi et al., 1998b; Kooi et al., 1999; Boer et al. 1999b; Boer et al., 1999a; Hanegraaf et al., 1999). Multiple attractors appear and some of them are of the chaotic type. Methods have been developed to analyse bifurcation patterns systematically (Boer et al., 1999a) and to reduce the complexity (Kooi et al. 1998a), but we still have to invest a considerable effort along this line. Realistic models for food chain dynamics are found to be inconsistent with some popular formulations in the literature, which turn out to violate the conservation law for mass (Kooi et al., 1997b), and frequently leads to new insights. We mention the result that an increase in complexity of the food web, or in biodiversity if you wish, can increase stability (Kooi and Kooijman, 1999), while the reverse is generally stated by mathematical biologists, on the basis of the work of May in the early 70's, who used linear functional responses to describe trophic relationships among (unstructured) populations. A more subtle treatment of variations in the nutritional values of prey for predators turned out to have a substantial stabilising impact on food chain dynamics (Hanegraaf et al., 1999). The study of reactions of food webs to perturbations has only be initiated (Berg, 1998).

With the extension of the DEB theory to include mass fluxes, it became evident that predator-prey relationships comprise only one aspect of interactions among populations. For a proper understanding of the dynamics of ecosystems it is essential to consider all types of mass exchange between populations, including the release of nutrients, the growth of producers on these nutrients and the indirect effects on consumers (Kooijman

and Nisbet, 1999). The environment in which the system is living and the nutrient exchange across its borders has a major impact on the long-term behaviour of the system, with intriguing implications for evolutionary theory. Most of the food chain analyses have been done in chemostat environments, where the dynamics of the chain breaks down at throughput zero, because these analyses did not include recycling of nutrients. With an extended DEB theory that covers both heterotrophic and autotrophic systems, we now feel ready to include these trophic interactions via nutrients in simple ecosystems. A start has been made with the formulation of a Canonical Community, which has three basic components, producers, consumers and decomposers, and is fully closed in terms of mass, and fully open in terms of energy. A preliminary study of its dynamical properties indicates that an attempt to include simple food webs is feasible and instructive.

Box 1

The characteristic equation and stable age distribution

t	time	a	age
$\dot{R}$	reproduction rate	$a_\dagger$	age at death

Let the food density be constant, and let $n(a,t)\,da$ denote the number of females at time t with age $\in \{a, a+da\}$; the total number of females is given by $N(t) = \int_0^\infty n(a,t)\,da$

The recursive relationship for survival is $n(a,t) = n(0,t-a)\Pr\{\underline{a}_\dagger > a\}$

The relationship between birth and reproduction is $n(0,t) = \int_0^\infty n(a,t)\dot{R}(a)\,da$

Substitution gives the integral equation

$$n(0,t) = \int_0^\infty n(0,t-a)\Pr\{\underline{a}_\dagger > a\}\dot{R}(a)\,da$$

Let the founder population be given by $n(a,0) = n_0(a)$, which leads to

$$n(0,t-a) = n_0(a-t)/\Pr\{\underline{a}_\dagger > a-t\} \quad \text{for } a > t$$

Substitution into the integral equation gives the renewal equation

$$n(0,t) = \int_0^t n(0,t-a)\Pr\{\underline{a}_\dagger > a\}\dot{R}(a)\,da + \int_t^\infty \frac{\Pr\{\underline{a}_\dagger > a\}}{\Pr\{\underline{a}_\dagger > a-t\}} n_0(a-t)\dot{R}(a)\,da$$

The second term relates to the founder population.

If it is small for $t > t_1$, the characteristic equation results

$$\begin{aligned} n(0,0)\exp\{\dot{r}t\} &= \int_0^{t_1} n(0,0)\exp\{\dot{r}(t-a)\Pr\{\underline{a}_\dagger > a\}\dot{R}(a)\,da \quad \text{or} \\ 1 &= \int_0^{t_1} \exp\{-\dot{r}a\}\Pr\{\underline{a}_\dagger > a\}\dot{R}(a)\,da \end{aligned}$$

The stable age distribution is given by

$$\phi_{\underline{a}}(a)\,da = \lim_{t\to\infty} \frac{n(a,t)\,da}{N(t)} = \frac{\exp\{-\dot{r}a\}\Pr\{\underline{a}_\dagger > a\}}{\int_0^\infty \exp\{-\dot{r}a_1\}\Pr\{\underline{a}_\dagger > a_1\}\,da_1}$$

as $n(a,t) = n(0,t-a)\Pr\{\underline{a}_\dagger > a\} = n(0,0)\exp\{\dot{r}(t-a)\}\Pr\{\underline{a}_\dagger > a\}$.

3 Effects of chemical compounds on biota

Toxicants can affect the physiology of organisms, which can lead to a change in the reproduction and/or survival of individuals. This again has consequences on the population and ecosystem level. Dynamic Energy Budgets fully specify the reproductive output of individuals. As a consequence all effects of toxicants on reproduction can be translated into effects on Dynamic Energy Budgets, even in the case that no direct relationship with energy is involved. Energy budgets function to quantify fluxes in organisms, in a way that is independent of any particular compound. It represents just one aspect. The DEB theory has been extended to include nutrient limited growth by autotrophs, for instance, which shows that nutrient limitation has energy aspects as well.

It is important to recognise that several levels of organisation are involved in judging effects of chemical compounds. At the molecular level, no-effect concentrations are always zero, because each molecule does something. A small effect on the liver, for instance, does not necessarily effect the individual in terms of survival or reproduction, as long as the liver function is not exploited at full capacity. Individuals usually have several options to cope with physiological handicaps, such as adapting preferences in diet to relief liver duties. Cat-owners know that by experience. Old cats usually suffer from a reduction of the kidney-function, which leads the cat to drink more, so reducing labour by the kidney. This means that no-effect concentrations exist at the level of the individual, but these levels might depend on environmental conditions.

Similar problems apply to the translation of effects of individuals to populations. If a toxicant affects the maintenance requirement of an individual, which can be measured directly, this hardly affects fast growing populations, so populations living at abundant food. The reason is that only a small fraction of the available energy budget is used for maintenance, and a doubling of the costs has hardly any consequences. This obviously changes dramatically when food density, and so population growth rate, is dropping. Since food levels naturally fluctuate, this means that the effect of toxicants on populations fluctuates, even if individuals would have a constant amount of toxicant.

3.1 Effects on individuals

The effects on individuals can be decomposed into three steps

- toxicokinetics, which translates external to internal levels of toxicants
- effects on target parameters, which translate internal levels of toxicants to changes in one or more target parameters, as identified by the DEB model
- effects on specific end points, which translate changes in target parameter to the end point of interest (e.g. survival, reproduction, respiration etc.).

3.1.1 Toxicokinetics

A natural first choice for a model of toxicokinetics at low levels of exposure is the first order process, where uptake is proportional to the external concentration and elimination is proportional to the internal concentration. This model is also known as the one-compartment model. It is simple and popular; the motivation rests on first-term Taylor approximations for both the uptake and the elimination processes. The implications of first order kinetics are far reaching, such as how the parameters of the process depend on

the octanol-water partition coefficient (Kooijman, 2000). This can be evaluated on the basis of the fugacity principle, which takes transportation rate of compound between 2 media proportional to the ratio of the binding forces between compound and media, see Box 2.

The goodness of fit of first-order kinetics with experimental data is not always striking, and a series of natural extensions frequently repairs this problem. We mention four types of extensions in decreasing priority.

Box 2

The fugacity principle

explains how the rates of first order kinetics depend on the octanol-water partition coefficient (P_{ow}), the ionization constant pK and the acidity pH.

- uptake rate $\propto \sqrt{P_{ow}}$
- elimination rate $\propto 1/\sqrt{P_{ow}}$
- if ionization is much faster than transport:

$$P_{ow}(\mathrm{pH}) = \frac{P_{ow}(-\infty) + P_{ow}(\infty)10^{\mathrm{pH}-\mathrm{pK}}}{1 + 10^{\mathrm{pH}-\mathrm{pK}}}$$

$$\dot{k}_e(\mathrm{pH}) = \sqrt{\frac{\dot{k}_e^2(-\infty) + \dot{k}_e^2(\infty)10^{\mathrm{pH}-\mathrm{pK}}}{1 + 10^{\mathrm{pH}-\mathrm{pK}}}}$$

where pH= $-\infty$, ∞: all in molecular, in ionized form
$\dot{k}_e$: elimination rate of molecules plus ions

The first extension accounts for the process of dilution by growth. If the animal grows during exposure, even a little bit, this affects the shapes of concentration versus time curves dramatically. Since transport in three dimensions occurs across two dimensions, it is natural to take exchange rates proportional to surface areas of the animal. This does not imply that all parts of the surface are equally important, but for 3D-isomorphs (i.e. animals that do not change in shape during growth) the surface areas of gills, and of guts, is proportional to the total surface area. If elimination is proportional to the surface area, and to the internal concentration (i.e. volume^{-1}), the elimination rate is inversely proportional to a length measure (i.e. volume$^{-1/3}$). The growth rate is specified by the DEB model, which fully specifies this extension of first order kinetics.

The next extension accounts for the delineation of the various uptake and elimination routes in more detail. Is the compound taken up directly from the environment, and/or via food? Does the animal eliminate via reproduction? For animals such as daphnids, this elimination route might be really important, because a female produces offspring at a rate of 0.25 times her own weight each day. Does the fat content change during exposure? This would affect the kinetics of lipophyllic compounds dramatically. These types of changes are fully specified by the DEB model, again.

The third type of extensions involves metabolic transformations. Lipophyllic compounds are frequently transformed into less lipophyllic ones, which can be eliminated

more rapidly. The rate of metabolic transformation is usually directly linked with the general metabolic activity of the organism, and fully specified by the DEB model again.

The forth type of extension involves a slow internal partitioning of the compound across the various body parts (organs). This leads to more compartment models. Since these types of models are very popular in pharmacology, a substantial literature exists on multi-compartment models, but these models do not account for the earlier mentioned extensions of first order kinetics. A basic problem with this type of extensions is that many parameters are involved.

Models that are more complex than first order kinetics require measurement of internal concentrations to be applicable. More-compartment models should only be used if the compartments are identified and their internal concentrations measured. The improvement of goodness of fit usually does not balance the number of extra parameters.

3.1.2 Effects on target parameters

The simplest, and probably most effective, description of effects on target parameters is to let the change in the value be proportional to the internal concentration that exceeds internal NEC (no-effect concentration). The value of the target parameter, par_C, at internal concentration $c(t)$ relates to the values in the blank as

$$\begin{aligned} parc(t) &= par0 \times (1 + (int.conc(t) - int.\ nec)/int.tolerance\ conc.) \quad \text{or} \\ &= par0 \times (\ 1 + (conc(t) - nec)/tolerance\ conc.) \end{aligned}$$

where conc(t) is defined to be the internal concentration(t)/BCF, while BCF stands for the Bio Concentration Factor (i.e. the ratio of the ultimate internal and the external concentration). The tolerance concentration quantifies the change in the parameter value per unit of concentration that exceeds the internal NEC. The name refers to the fact that high values relate to compounds that are not toxic.

The target parameter can be any of the list mentioned in Table 2, depending of the mode of action of the compound of interest. We never encountered effects on size at birth and at puberty, which means that effects on maturation investment for birth and puberty, and the maturity maintenance costs are perhaps less likely target parameters. This also holds for specific heating costs. Due to lack of data, we do not know about effects on feeding (saturation constant, and maximum ingestion rate), and assume that all observed effects on assimilation should be interpreted as effects on both maximum assimilation and ingestion, such that the ratio (i.e. the digestion efficiency) is not affected. Endo-parasites, and possibly endocrine disrupters, are found to increase the partition coefficient for catabolic power, and to intercept allocation to reproduction. This leads to an increase in body size. Genotoxic compounds, such as nitrite are found to affect the ageing acceleration, which does not come as a surprise because their mode of action resembles that of free radicals.

Effects on reproduction can be classified as direct and indirect effects. Direct effects on reproduction do not affect the physiology of the mother in terms of feeding, growth, maintenance and development. They reduce the survival probability of the embryo, or increase the energy costs per offspring, which obviously leads to a reduced offspring production. Indirect effects on reproduction reduce the assimilation, or increase the maintenance or growth costs. These modes of action increase the juvenile period, and decrease the reproduction, due to the coupling of traits as specified by the DEB model.

As long as the concentrations are small, the idea is that only the most sensitive process is affected, and only a single parameter is involved. Things rapidly become more complex when at concentration increase several parameters are affected simultaneously.

Consistent with the ageing module in the DEB model, effects on survival are modelled as

$$\text{hazard}_C(t) = \text{hazard}_0 + \text{killing rate} \times (\text{int.conc}(t) - \text{int.NEC})/\text{BCF} \quad \text{or}$$
$$= \text{hazard}_0 + \text{killing rate} \times (\text{conc}(t) - \text{NEC})$$

where the killing rate quantifies the effect of the compound on the hazard rate. If death during exposure that is not related to the compound is just accidental, the blank hazard rate is a constant. If some ageing mechanism is involved, e.g. as specified by the DEB model, it is a function of time as well. Other sources of death can easily be introduced as long as they are independent of each other, and of death due to the compound; such contributions to the hazard rate just add.

Notice that the value of the target parameter(s) change in time as long as the internal concentration is changing, but the parameters that describe the effect (the NEC and the tolerance concentration for sublethal effects, and the NEC and the killing rate for lethal effects) do not change in time.

The motivation for this linear model is that effective molecules (i.e. the molecule that exceed the NEC) operate independently. It can also be considered as a first-term Taylor approximation approximation for small effects.

The linear effect module for target parameters is very useful for evaluating the effects of mixtures of compounds, since the effects of the various compounds can just be added, as a first approximation. If necessary, interaction terms can be introduced to describe synergistic effects, similar to what is standard in the analysis of variance.

Although the change of the parameter values is taken to be linear in the internal concentration, this does not imply that concentration-response relationships are supposed to be linear. This is because the change in the parameter value first has to be translated into the response.

3.1.3 Effects on endpoints

The relationship between the hazard rate and the survival probability does not require any modelling, since it is defined to be hazard(t) = - d(ln survival probability)/dt. This results in the familiar sigmoid relationships between the survival probability and the logarithm of the external concentration for a given exposure time. The (maximum) slopes of these sigmoidal relationships increase during exposure.

If toxicokinetics is fast, relative to the exposure period, and the internal concentration is constant during most of the exposure, the hazard rate is constant as well and linear in the concentration. For small NECs this means that the logarithm of the survival probability is proportional to the product of the concentration and the exposure time. This well-known empirical result is fully consistent with the present quantitative framework for the understanding of effects of toxicants.

The DEB model can be used to translate changes in target parameters to changes in endpoints, such as reproduction rates, cumulative reproductive outputs etc. This results in the familiar sigmoid relationships between the cumulated number of *Daphnia* offspring during a fixed exposure period and the logarithm of the external concentration.

3.2 Parameter estimation

The bioassays for the measurement of toxic effects, as standardised by the OECD are suitable to obtain estimates for the NEC and the effect parameters (tolerance concentrations or killing rates) (Bedaux and Kooijman, 1994). The software package DEBtox (Kooijman and Bedaux, 1996a; Kooijman and Bedaux, 1996b; Kooijman and Bedaux, 1996c; Kooijman and Bedaux, 1996d; Kooijman et al., 1996b) does all the required computations. That package can also be used to obtain the more familiar measures, such as LCx and ECx values (including confidence intervals), which depend on the exposure time. This option is only included to assist comparisons, since these measures are frequently reported in the literature.

The package DEBtox also extracts the elimination rate from effect data. This parameter occurs in effect data because it quantifies how rapidly the effects build up during exposure to their ultimate levels. The parameter, therefore, quantifies the dynamic component of the response, which is independent of the toxicity of the compound. The value of the elimination rate can (and must) even be extracted if effects are only measured at a single exposure time. The value affects details in the shape of the concentration response curve. Needless to mention that the uncertainty of the value is substantial in such a case, which translates into the value of the confidence interval for other parameters, such as the NEC. The confidence intervals reduce substantially if the data actually show how fast effects built up during exposure. We have to think, in the context, about effects in terms of response *surfaces* rather than response *curves*, so of effects as functions of concentrations *and* exposure times.

Another approach to deal with the dynamic aspect of toxic effects is to use knowledge about the value of the elimination rate directly, and avoid the extraction of these values from observed effects. Toxicokinetic data can be used for this purpose, and/or physical chemical information. Elimination rates of related compounds can be used, corrected for differences in P_{OW} values, pH, temperature, and body size of the organism.

3.3 Effects on populations

Effect on populations can be deduced from that on individuals after adding assumptions about processes at the supra-individual level, as has been discussed in the section on populations.

Figure 3 gives the population growth rate as a function of the concentration in the environment, for five modes of action (between brackets: abbreviation as used in Figure):

1. increase in the overhead fraction for reproduction (repro)
2. direct effect on egg survival (egg surv)
3. increase in the costs for growth (growth)
4. decrease in the maximum specific assimilation rate (assim)
5. increase in the somatic maintenance costs (maint)

Debtox will be extended in the near future to produce these types of plots, and to obtain ECx values with confidence intervals for the population growth rates, based on the bioassay for reproduction.

The population growth rates of unicellulars (and duckweed) can already be analysed with DEBtox, for three modes of action: effects on the inoculum (i.e. an initial killing, with no further adverse affects), on survival, and on the growth rate (Kooijman et al., 1996b; Kooijman and Bedaux, 1996a). This can be extended to include other modes of action.

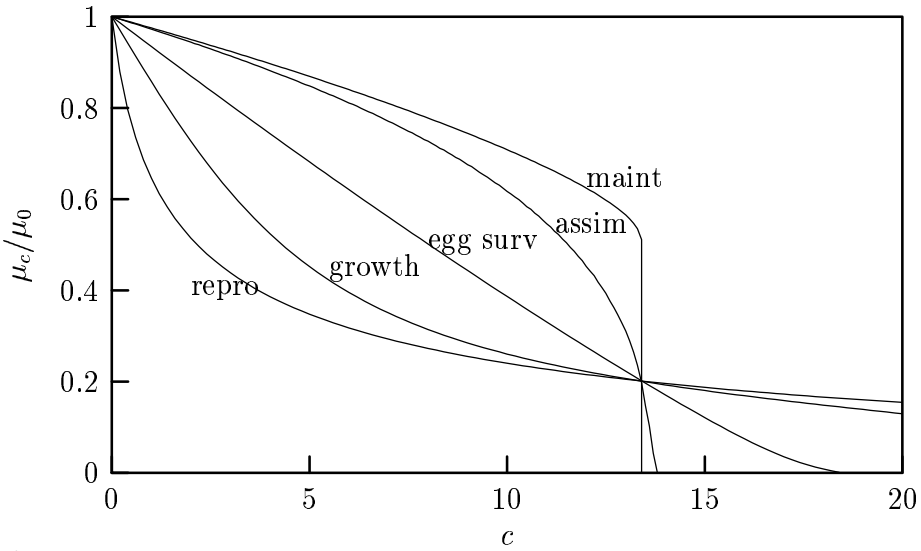

Figure 3 Scaled population growth rate as a function of concentration, for the five modes of action. All curves are calculated with NEC = 0, and the tolerance concentrations are chosen in such a way that there is a common EC_{80} value. The abbreviations near the curves denote the mode of action. See text for explanation

4 Discussion

Models based on different assumptions about the mode of action of the compounds usually turned out to fit data on cumulative reproduction approximately equally well. This means that such data are of little use to tell the various modes of action apart, and other type of information must be available. One could think of additional data on feeding, respiration, body size, etc. This points to a need to develop experimental protocols that allow an optimal extraction of information. The extensions could be modest; the measurement of body lengths at the end of the experiment does not take much extra time, and hardly increases the financial costs of the experiment, while it would contribute substantially to the information content. Knowledge about the mode of action is essential to translate effects on individuals to that on populations.

We also observed that the NEC was not very sensitive for the choice of mode of action of the compound. So if the only aim of the experiment is to estimate the NEC, knowledge about the correct mode of action might be less essential. This observation gives hope in the sense that we do not have to generate a lot of knowledge about the effects of compounds, as long as our aim is to avoid effects in the environment. This is quite a relief given the fact that about 2000 new chemical compounds appear yearly on the European market.

We also came to the conclusion that it makes little sense to standardise toxicity experiments in terms of exposure time. The relevant length of exposure, and the required observation times to monitor the build up of effects should depend on physical chemical properties of the compound, in combination with the body size of the test animals.

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