Ecotoxicological applications of Dynamic Energy

Budget theory *

S.A.L.M. Kooijman†, J. Baas, D. Bontje, M. Broerse, C. A. M. van Gestel, and T. Jager

Faculty Earth & Life Sciences

Vrije Universiteit, de Boelelaan 1085, 1081 HV Amsterdam, NL

*subm J. Devillers (ed) Ecotoxicological Modelling, Springer
†corresponding author: bas@bio.vu.nl. File-name: kinetics.tex
Abstract

The Dynamic Energy Budget (DEB) theory for metabolic organisation specifies quantitatively the processes of uptake of substrate by organisms and its use for the purpose of maintenance, growth, maturation and reproduction. It applies to all organisms. Animals are special because they typically feed on other organisms. This couples the uptake of the different required substrates, and their energetics can, therefore, be captured realistically with a single reserve and a single structure compartment in biomass. Effects of chemical compounds (e.g. toxicants) are included by linking parameter values to internal concentrations. This involves a toxico-kinetic module that is linked to the DEB, in terms of uptake, elimination and (metabolic) transformation of the compounds. The core of the kinetic module is the simple one-compartment model, but extensions and modifications are required to link it to DEBs. We discuss how these extensions relate to each other and how they can be organised in a coherent framework that deals with effects of compounds with varying concentrations and with mixtures of chemicals. For the one-compartment model and its extensions, as well as for the standard DEB model for individual organisms, theory is available for the co-variation of parameter values among different applications, which facilitates model applications and extrapolations.

**Keywords:** Dynamic Energy Budgets, effects on processes, kinetics, metabolism, transformation
1 Introduction

The societal interest in ecotoxicology is in the effects of chemical compounds on organisms, especially at the population and ecosystem level. Sometimes these effects are intentional, but more typically they concern adverse side-effects of other (industrial) activities. The scientific interest in effects of chemical compounds is in perturbing the metabolic system, which can reveal its organisation. This approach supplements ideas originating from molecular biology, but now applied at the individual level; the sheer complexity of biochemical organisation hampers reliable predictions of the performance of individuals. Understanding the metabolic organisation from basic physical and chemical principles is the target of Dynamic Energy Budget (DEB) theory [1, 2]. In reverse, this theory can be used to quantify effects of chemical compounds, i.e. changes of the metabolic performance of individuals. This chapter describes how DEB theory quantifies toxicity as a process.

The effects can usually be linked to the concentration of compounds inside the organism or inside certain tissues or organs of an organism. This makes that toxicokinetics is basic to effect studies. The physiological state of an organism, such as its size, its lipid content, and the importance of the various uptake and elimination routes (feeding, reproduction, excretion) interacts actively with toxicokinetics, so a more elaborate analysis of toxicoki-
netics should be linked to the metabolic organisation of the organism [3]. In the section on toxicokinetics, we start with familiar classic models that hardly include the physiology of the organism, and stepwise include modules that do make this link in terms of logical extensions of the classic models.

Three ranges of concentrations of any compound in an organism can be delineated: too-little, enough and too-much. The definition of the enough-range is that variations of concentrations within this range do not translate into variations of the physiological performance of the individual. Some of the ranges can have size zero; such as the too-little-range for cadmium. Effects are quantified in the context of DEB theory as changes of (metabolic) parameters as linked to changes in internal concentrations. These parameters can be the hazard rate (for lethal effects), the specific food uptake rate, the specific maintenance costs, etc. Changes in a single parameter can have many physiological consequences for the individual. DEB theory is used not only to specify the possible modes of action of a chemical compound, but also how the various physiological processes interact. An increase in the maintenance costs by some compound, for instance, reduces growth, and since food uptake is linked to body size, it indirectly reduces food uptake, and so affects reproduction (and development). The existence of the ranges too-little, enough and too-much of concentrations of any compound directly follows from a consistency argument, where no
classification of compounds is accepted (e.g. toxic and non-toxic compounds), and many compounds (namely those that make up reserve) exist that do change in concentration in the organism, without affecting parameter values. An important consequence is the existence of an internal No Effect Concentration (NEC), as will be discussed below.

Effects at the population level are evaluated from those at the individual level, by considering populations as a set of interacting individuals [4-8]. Although DEB-based population dynamics can be complex, particular aspects of population performance, such as the population growth rate at constant environmental conditions, are not very complex. Moreover, particular simplifying approximations are possible. The focus of toxicology is typically at time scales that are short relative to the life span of the individual. That of ecotoxicology, however, are much longer, involving the whole life history of organisms; effects on feeding, growth, reproduction and survival are essential and typically outside the scope of toxicology. This has strong implications for the best design of models. While pharmacokinetics models frequently have many variables and parameters, such complex models are of little use for applications at the population level, where a strong need is felt for relatively simple models, but then applied to many species and in complex situations. DEB theory is especially designed for this task.
Chemical transformations are basic to metabolism, and transformations of toxicants are no exception. Compounds that dissociate in water should be considered as a mixture of ionic and molecular forms, and the pH affects that mixture. This makes that the effect of a single pure compound is of rather academic interest; we have to think in terms of the dynamic mixtures of compounds. Because of its strong links with chemical and physical principles, DEB theory has straightforward ways to deal with effects of mixtures. Some of this theory rests on the covariation of parameter values across species of organism and across chemical compounds. Theory on this covariation is implied by DEB theory, and is one of its most powerful aspects.

We first introduce some notions of DEB theory, and then discuss toxicokinetics and effects in the context of DEB theory.

2 The standard DEB model in a nutshell

The standard DEB model concerns an isomorph, i.e. an organism that does not change in shape during growth, that feeds on a single food source (of constant chemical composition), and has a single reserve a single structure and three life stages: embryo (which does not feed), juvenile (which does not allocate to reproduction) and adult (which allocates
to reproduction, but not to maturation). This is in some respects the simplest model in
the context of DEB theory, which is thought to be appropriate for most animals. Food
is converted to reserve, and reserve to structure. Reserve does not require maintenance,
but structure does, mainly to fuel its turnover, see Figure 1. Reserve can have active
metabolic functions and serves the role of representing metabolic memory. Reserve and
structure do not change in chemical composition (strong homeostasis). At constant food
availability, reserve and structure increase in harmony, i.e. the ratio of their amounts, the
reserve density, remains constant (weak homeostasis).

The shape (and so the change of shape) is important because food uptake is proportional
to surface area, and maintenance mostly to (structural) volume. The handling time of
food (including digestion and metabolic processing) is proportional to the mass of food
“particles”, during which food acquisition is ceased. The mobilisation rate of reserve to
fuel the metabolic needs follows from the weak and strong homeostasis assumptions [9]; a
mechanism is presented by Kooijman and Troost [10]. Allocation to growth and somatic
maintenance (so to the soma) comprises a fixed fraction of mobilised reserve, the remaining
fraction is allocated to maturation (or reproduction) and maturity maintenance.

The transition from the embryo to the juvenile stage (i.e. birth) occurs by initiating
assimilation when the maturity exceeds a threshold value, and from the juvenile to the
adult stage (i.e. puberty) by initiating allocation to reproduction and ceasing of alloca-
tion to maturation at another threshold value for maturity. Reserve that is allocated to
reproduction is first collected in a buffer, that is subjected to buffer handling rules (such
as spawning once per season, or convert the buffer content into an egg as soon as there is
enough).

Biomass consists of reserve and structure, and can, therefore, change in chemical com-
position (e.g. lipid content) in response to the nutritional condition; maturity has the status
of information, not that of mass or energy. Apart from some minor details, the presented
set of simple rules fully specify the dynamics of the individual, including all mass and
energy fluxes, such as the uptake of dioxygen, the production of carbon dioxide, nitrogen
waste and heat. It takes some time to see exactly how; Sousa et al. [9] gives a nice evalu-
ation. Aging is considered to result from a side effect of Reactive Oxygen Species (ROS),
and is so linked to the uptake of dioxygen [11]. A high food uptake results in a large amount
of reserve, so a high use of reserve, a high uptake of dioxygen, an acceleration of aging
and a reduction of life span. The induction of tumours is also linked to the occurrence of
ROS, and other reactive molecules, such as mutagenic compounds. This gives a natural
link between aging and tumour induction.
The standard DEB model has been extended into many directions for the various purposes. The allocation rule to the soma, for instance, can be refined to allocation to various body parts (e.g. organs), where growth of each body part is proportional to the allocated reserve flux minus what is required for maintenance of that part. Rather than using fixed fractions of the mobilised reserve, the fractions can be linked to the relative workload of the body part. This allows a dynamic adaptation of the body parts in interaction with their use. Tumours can be considered as body parts, and the “workload” of the tumour is the consumption of maintenance. This formulation produced realistic predictions of the effects of caloric restriction on tumour growth, and of the growth of tumours in young versus old hosts [12]. This approach can be extended to various types of tumours, where tumour growth is not linked to that of the whole body, but that of a particular body part.

Many tumours result from destruction of local cell-to-cell communication, rather than from genotoxic effects, but these different routes have similar dynamics.

Other types of extensions of the standard DEB model concern the inclusion of variations in chemical composition of food (with consequences for the transformation of food into reserve) and size-dependent selection of different food items. For example many herbivores are carnivores when young. Animals are special because they feed on other organisms. Most other organisms take the food-compounds (energy source, carbon source, nitrate,
phosphate) that they need independently from the environment, which necessitates the inclusion of more than one reserve; see Kooijman and Troost [10] for the evolutionary perspectives. Most micro-organisms grow and divide, and don’t have the three life stages delineated by the standard model. This makes that their change in shape hardly matters and that surface areas can be taken proportional to volumes, which simplifies matters considerably. The partial differential equations that are required to described the physiologically structured population dynamics of isomorphs then collapse to a small set of ordinary differential equations. Plants, on the other hand, require at least two types of structure (roots and shoots) and have a complex adaptive morphology (i.e. surface area-volume relationships); their budgets are most complex to quantify.

3 Family of toxicokinetic models

Originally (before the 1950’s) the focus of toxicology, i.e. the field that gave rise to ecotoxicology, was on medical applications of compounds in a pharmacological context. Subjects where given a particular dose, and the interest is in the redistribution inside the body, and in transformation and elimination. The aim is to reach the target organ and to achieve a particular effect that restores the health or well-being of the subject. A closely related interest that developed simultaneously was health protection (disinfection and food protection
products), with the purpose of killing certain species of pathogenic organism (especially microorganisms), or to reduce their impact.

After the 1950’s ecotoxicology began to flourish and gradually became more independent of toxicology, where the initial focus was in positive and (later) negative effects of biocides. This came with extensions of the interest in the various uptake and elimination routes that are of ecological relevance, and the environmental physical-chemistry of transport and transformation. The aim is to kill particular species of pest organism locally (insects, weeds), and to avoid effects on other, non-target, species (crop and beneficial species).

After the 1970’s the interest further generalised to an environmental concern of avoiding effects of pollutants on organisms, with an increasing attention for (bio)degradation of compounds that are released into the environment, coupled to human activity [13].

This historic development and branching of the interest in toxico-kinetics came with a narrowing of the focus on a particular aspect of toxico-kinetics in the various applications that, we feel, is counter-productive from a scientific point of view. The purpose of this section is an attempt to restore the coherence in the field, by emphasising the general eco-physiological context and the relationship of the various models with the core model for toxico-kinetics: the one-compartment model. The more subtle models account for the interaction with the metabolism of the organism, which involves its metabolic organisation.
We here focus on the logical coherence of toxico-kinetics, bio-availability and metabolism, including effects (= changes in metabolism). It is not meant to be a review. For recent reviews on toxico-kinetic models, see o.a. Barber [14] and Mackay and Fraser [15]. See Table 1 for a list of frequently used symbols.

![Table 1 about here.](image)

### 3.1 One-compartment model

The core model in toxico-kinetics is the one-compartment model, see Fig. 2. It states that the uptake rate is proportional to the environmental concentration $c$, and the elimination rate is proportional to the internal concentration $Q$:

\[
\frac{d}{dt} Q = k_e (P_{bd} c(t) - Q) \quad (1)
\]

\[
Q(t) = Q(0) \exp(-tk_e) + k_e P_{bd} \int_0^t c(s) \exp((s - t)k_e) \, ds \quad (2)
\]

\[
= Q(0) \exp(-tk_e) + P_{bd} c \left(1 - \exp(-tk_e)\right) \quad \text{for } c \text{ is constant} \quad (3)
\]

where $P_{bd} = Q(\infty)/c$ is the BioConcentration Factor (BCF) and $k_e$ the elimination rate.

The product $k_e P_{bd}$ is known as the uptake rate, and is frequently indicated with $k_u$, which
is misleading because its units are $d^{-1} \text{m}^3 \text{C-mol}^{-1}$. Even if we would work with kg rather than C-mol, and the specific density of the organism equals 1 kg dm$^{-3}$, the BCF is not dimensionless [1]. It is typically more convenient to work with molalities in soils (mol kg$^{-1}$), and with molarities (mol l$^{-1}$) in water. Molalities give the uptake rate the units $d^{-1} \text{g C-mol}^{-1}$. Many workers use gram rather than mole to quantify the compound, but this choice is less practical to compare the toxicity of different compounds. The elimination rate $k_e$ has dimension ‘per time’ and is independent of how the compound is quantified; contrary to the uptake rate, the elimination rate can be extracted from effect data and determines how fast effects build up in time, relative to the long-term effect level.

The concentrations $c$ and $Q$ must obviously exist, meaning that the environment and the organism are taken to be homogeneous. This condition can be relaxed without making the model more complex by allowing a spatial structure (such as organs), and an exchange between the parts that is fast relative to the exchange between the organism and the environment. Other implicit assumptions of the one-compartment model are that the organism does not change in size or in chemical composition, so changes in food availability must be negligible. The (bio)availability of the compound remains constant. So transformations can be excluded, and the environment is large relative to the organism and well-mixed. Sometimes, e.g. in the case of a large fish in a small aquarium, this is not true and the
dynamics of the concentration in the organism and the environment should be considered simultaneously: the 1-1 compartment model [16]. These restrictions will be removed below. Since rates generally depend on temperature, and temperature typically changes in time, the elimination rate $k_e$ can change in time as well [17]. In the sequel we will discuss rates of metabolism, and like all rates, these also depend on temperature, frequently according to the Arrhenius relationship [1].

3.2 Multi-compartment models

If transport inside the organism is not fast, relative to the exchange with the environment, multiple-compartment models should be considered [18, 19]. If exchange with the environment is only via compartment number 0, the change in the concentrations in the nested compartments number 0 and 1 is

$$\frac{d}{dt} Q_0 = k_e (P_{0d} c - Q_0) + k_{10} Q_1 - k_{01} Q_0; \quad \frac{d}{dt} Q_1 = k_{01} Q_0 - k_{10} Q_1$$ (4)

where $k_{01}$ and $k_{10}$ are the exchange rates between the compartments, see Fig. 2. The partition coefficient between the compartments equals $P_{10} \equiv Q_1(\infty)/Q_0(\infty) = k_{01}/k_{10}$, while $P_{0d} \equiv Q_0(\infty)/c$ remains unchanged. In many cases whole body measurements are used. If $M_0$ and $M_1$ denote the masses of compartment 0 and 1, the whole body partition coefficient with the environment amounts to $P_{7-d} = (M_0 + M_1 P_{10}) P_{0d}/M_+ = \ldots$
$M_0 + M_1$ is the whole body mass. This rather complex behaviour of the whole body partition coefficient can be a source of problems in fitting models to data. In many practical cases it is not possible to identify the compartments and to measure the concentrations in these compartments directly. The use of multi-compartment models cannot be recommended in such cases.

This extension still classifies as a transport model, so in a clean environment ($c = 0$), the organism will lose all its load ($Q_0(\infty) = Q_1(\infty) = 0$). Quite a few data sets on the kinetics of (“heavy”) metals in organisms show that once loaded, an organism never fully looses its load [20-22]. Such behaviour cannot be captured by multi-compartment models because this involves an extension to transformation of compounds (technically speaking, sequestered compounds belong to a different chemical species).

If $k_{01}, k_{10} \gg k_c$, we can assume that $Q_1(t) \approx P_{10} Q_0(t)$, and the kinetics Eq 4 reduces to Eq 1, with $Q$ replaced by $Q_0$. This situation is called time-scale separation.

The interpretation of the compartments can be a special tissue or organ, or, more abstract, reserve (compartment 1) and structure (compartment 0). The latter makes sense in the context of DEB theory, where both compartments are assumed to have a constant, but different, chemical composition, while reserve is relatively rich in lipids in many animal taxa. While Eq 4 assumes that the size of all compartments does not change, we will relax
on this below, when we allow more interactions with metabolism and energetics.

We can obviously include more compartments, and more complex interactions with the environment, but the number of parameters rapidly increases this way. In practice multi-compartments are used if the one-compartment model fits data badly. The introduction of more parameters generally improves the fit, but not necessarily for the right reasons. As a rule of thumb it is only advisable to use more compartment models if data on the concentrations inside the compartments are available. If lack of fit of the one-compartment model is the only motivation, alternatives should be considered that are discussed below.

3.3 Film models

Film models are conceptually related to multi-compartment models because both are extensions of the one-compartment model that include more detail in transport (so in physical factors), though in different but complementary ways. Film models are especially popular in environmental chemistry for following the transport of compounds from one environmental compartment (such as water) to another (such as air). Both compartments are assumed to be well-mixed, except for a narrow film at the interface of both compartments, where transport is by diffusion.
To follow the dynamics for the densities of the compound $n$ (mol/m), we need to define a spatial axis perpendicular to the interface and choose the origin at the boundary between the bulk and the film (on each side of the interface). Let $L_i$ be the depth of the film, $d_i$ the diffusivity of the compound in the film, and $v_{ij}$ the exchange velocity of the compound between the two media. As discussed in Kooijman et al. [16], the dynamics of the densities is given by partial differential equations (pde’s) for medium $i = 1 - j$ and $j = 0$ or 1

$$0 = \frac{\partial}{\partial t} n_i(L, t) - d_i \frac{\partial^2}{\partial L^2} n_i(L, t) \quad \text{for } L \in (0, L_i)$$

with boundary conditions at $L = 0$ (i.e. the boundary between the film and the well-mixed medium) for $v_i = d_i/L_i$

$$0 = \frac{\partial}{\partial t} n_i(0, t) - v_i \frac{\partial}{\partial L} n_i(0, t)$$

and boundary conditions at $L = L_i$ (i.e. the interface between the media where the two films meet)

$$0 = v_{ji} n_j(L_j, t) - v_{ij} n_i(L_i, t) + d_i \frac{\partial}{\partial L} n_i(L_i, t)$$

The boundary condition at $L = 0$, and the diffusion process in the film is rather standard, but we believe that the boundary condition at $L = L_i$ is presented for the first time in Kooijman et al. [16]. Users of the popular film models typically skip the formulation of the pde and directly focus at steady state situations; they typically use the concentration jump
across the interface that belongs to the situation when there is no net transport across the interface. As long as there is transport, however, the concentration jump differs from this equilibrium value.

The depth of the films is typically assumed to be small and the transport in the films in steady state, which makes that the density profiles in the films are linear. This leads to the 1-1-compartment kinetics for the bulk densities

$$\frac{d}{dt} n_i(0) = k_{ij}(P_{ij} n_j(0) - n_i(0)); \quad k_{ij} = \frac{v_i/L_i}{1 + P_{ij} v_j/v_i - v_j/v_{ij}}$$

(8)

where $L_i$ is the depth of the medium. This approximation only applies if $v_i v_j < v_{ij} v_i + v_{ji} v_i$ and the transport in the film is rate limiting. The 1-1-compartment kinetics also results, however, if the film depths reduce to zero and if the diffusivities are high. The rate from $i$ to $j$ then reduces to $k_{ij} = v_i L_i^{-1} (1 - v_i/v_{ij})^{-1}$. In these two situations, transport in the film is no longer rate limiting.

The applicability of film models to toxico-kinetics in organisms is still an open question. It can be argued that a stagnant water film sticks to aquatic or soil organisms (and air to a terrestrial organism), and that the skin (or cuticula) is not well served by the internal redistribution system (blood) of the organism. If toxico-kinetics is fully limited by transport in the film, and if it is not limited by the film, one-compartment kinetics results; only in the intermediary situation we can expect some deviations. Yet the discussion is not completely
academic, since these details matter for how the elimination rate depends on the partition coefficient [16, 23].

3.4 Uptake and elimination routes

We now consider extensions of the one-compartment model due to biological factors by accounting for various uptake and elimination routes. These routes depend on the type of organism, its habitat and properties of the compound. Animals that live in (wet) soil are in intense contact with the water film around soil particles, and their situation has similarities with that of aquatic animals. Direct transport through the skin can be important, which involves the surface area of an organism. Some parts of the skin are more permeable, especially that used by the respiratory system for dioxygen uptake and carbon dioxide excretion. The uptake rate might be linked to the respiratory rate, which depends on the energetics of the organism. Generally, the respiration rate scales with a weighted sum of surface area and volume, but the proportionality constants depend on the nutritional conditions of the organism [1]. For terrestrial animals, uptake via the lungs from air and via skin contact with the soil must be considered. Sometimes uptake is via drinking; the DEB theory quantifies drinking via the water balance for the individual and involves a.o. metabolism and transpiration. The details can be found in Kooijman [1].
A second important uptake route is via food and the gut epithelium. The feeding rate depends on food availability, food quality, and the surface area of the organism [1].

The elimination can follow the same routes as uptake, but there are several additional routes to consider, namely via products of organisms. The first possibility is the route that excreted nitrogen waste follows (urination). Reproductive products (mostly eggs and sperm) can also be an important elimination route. Moulting (e.g. ecdysozoans, including the rejection of gut epithelium, e.g. collembolans) or the production of mucus (e.g. lophotrochozoans) or milk (e.g. female mammals) are other possible excretion routes.

The DEB theory quantifies reproductive and other products as functions of the amounts of reserve and structure of the individual. In the standard DEB model they work out to be cubic polynomials in body length, but the coefficients depend on the nutritional conditions (amount of reserves per structure) [1].

3.5 Changes in body size and composition

The body size of an organism matters in the context of toxico-kinetics for several reasons [24]. As exchange is via surface area, and is proportional to concentration, surface area-volume interactions are involved. This problem also applies to compartment and film models, but gets a new dimension if we consider changes in body size, which are linked to
the nutritional condition of organisms (lipid content), and so to (changes in) body composition. Small changes in size can have a substantial effect on the shape of accumulation curves.

If an organism does not change in shape during growth (so it remains isomorphic), surface area is proportional to volume$^{2/3}$, or to squared length. Moreover, dilution by growth should be taken into account, even at low growth rates. This modifies Eq 1 to

$$\frac{d}{dt} Q = (P_{bd}c(t) - Q) \frac{v_e}{L} - Q r$$

with

$$r = \frac{d}{dt} \ln L^3$$

where $L$ is the length, and $v_e = L_mk_e$ is the elimination velocity for maximum length $L_m$.

The last term, $Qr$, represents the dilution by growth. If it equals zero, we can replace $v_e/L$ by the constant elimination rate $k_e'$, but its meaning still matters if we compare the kinetics in two organisms of different size. DEB theory specifies how the change in (cubed) length depends on the amount of reserve and structure of the organism, and how the change in reserve depends on these state variables and food availability. Food intake and maintenance play an important role in growth and together they control the maximum size an organism can reach, since food intake is proportional to a surface area and maintenance to structural volume. Wallace had this insight in 1865 already [25].

The DEB theory allows for particular changes in body composition, because reserve and structure can change in relative amounts and both have a constant composition. Food
(substrate) is first transformed into reserve, and reserve is used for metabolic purposes, such as somatic and maturity maintenance, growth, maturation and reproduction. The change in reserve density for metabolic use is proportional to the reserve density per length, which makes that high growth rates come with high reserve densities, i.e. the ratio of the amounts of reserve and structure.

Reserves are in many animal taxa relatively enriched in lipids, which might have a strong influence on the kinetics of hydrophobic compounds. The body burden of eel in a ditch that is polluted with mercury or PCB might greatly exceed that of other fish partly because eel is relative rich in lipids. This illustrates the importance of lipid dynamics. Freshly laid eggs consist almost exclusively of reserve, which makes egg production a potentially important elimination route for lipophyllic compounds. The reserve allocation to reproduction is via a buffer that comes with species-specific buffer handling rules. Many aquatic species spawn once a year only (e.g. most bivalves and fish), which implies that the buffer size gradually increases between two spawning events and makes a sharp jump down at spawning. The body burden can also make a jump at spawning (up or down, depending on the properties of the compound).

The difference in lipid content between reserve and structure invites for the application of a nested two-compartment model, where the exchange with the environment is via structure.
This links up nicely with food uptake, because reserve does not play a role in it, and food uptake is also proportional to squared structural length. An important difference with the nested two-compartment model is, however, that the size of the compartments typically changes in time, especially the reserve. When redistribution of the compound between the compartments reserve and structure is relatively fast, and the nested two-compartment model for the compound reduces to a one-compartment one, reserve dynamics still affects toxico-kinetics, because the lipid content is changing in time. The resulting dynamics for active uptake from food amounts to

$$\frac{d}{dt} Q = \left( P_{vd} c_d + P_{vx} f c_X \right) \frac{v_e}{L} - Q \left( P_{vw} \frac{v_e}{L} + r \right)$$

with

$$P_{vw} = 1 + P_{ev} (m_E + m_{ER})$$

(10)

where $c_d$ and $c_X$ are the concentrations of the compound in the environment and in food, $f$ is the scaled functional response, $P_{vd}$ and $P_{vx}$ are the partition coefficients of the compound in structure and environment or food. The reserve density $m_E$ and the reproduction buffer density $m_{ER}$ now modify the partition coefficient between structure and biomass (i.e. reserve plus structure), via the partition coefficient between reserve and structure $P_{ev}$. DEB theory specifies how structural length $L$, the reserve density $m_E$ and the reproduction buffer density $m_{ER}$ change in time.

The reproduction buffer is not of importance in all species, and not always in males. If food density is constant, the reserve density $m_E$ becomes constant. In those situations
the structure-biomass partition coefficient $P_{VW}$ is constant as well. If also the dilution by growth can be neglected, i.e. $r = 0$ and $L$ is constant, Eq 10 still reduces to the one-compartment model Eq 1.

Many accumulation-elimination experiments are done under starvation conditions; e.g. it is hardly feasible to feed mussels adequately in the laboratory. The reserve density decreases during the experiment, so the chemical composition is changing, which can affect the toxico-kinetics [26].

Some situations require more advanced modelling of the uptake and eliminations route, where e.g. gut contents exchanges with the body in more complex ways, and defecation might be an elimination route.

3.6 Metabolism and transformation

Both uptake and elimination can depend on the metabolic activity [27]. Respiration is frequently used as a quantifier for metabolic activity. This explains the popularity of body size scaling relationships for respiration [28], and the many attempts to relate many other quantities to respiration. In the context of DEB theory, however, and that of indirect calorimetry, respiration is a rather ambiguous term, because it can stand for the use of dioxygen, or the production of carbon dioxide or heat. These are not all proportional
to each other, however. Moreover all these three fluxes have contributions from various
processes, such as assimilation, maintenance, growth etc. Since the use of reserves fuels
all non-assimilatory activities, this is an obvious quantifier to link to the rate at which
compounds are transformed or taken up. For uptake of compounds via the respiratory
system, the use of dioxygen might be a better quantity to link to uptake under aerobic
conditions.

Respiration rates turn out to be cubic polynomials in structural length in DEB theory,
which resemble the popular allometric functions numerically in great detail. The coeffi-
cients depend on the nutritional conditions in particular ways. Since elimination rates are
inversely proportional to length because of surface area-volume interactions, as has been
discussed, and the specific metabolic rate is very close to this relationship, it is by no means
easy to evaluate the role of metabolism in direct uptake and elimination in undisturbed
subjects.

The role of metabolism is easier to access for elimination via products and if the elimination
rate is not proportional to the internal concentration, but has a maximum capacity. The
classic example is the elimination of alcohol in human blood [29]. This type of kinetics can
be described as

$$\frac{d}{dt} Q = k_e P_{\text{off}} c - k_e Q / (K_Q + Q)$$  \hspace{1cm} (11)
where $K$ is a half saturation constant for the elimination process. It reduces to the one-compartment model Eq 1 for small internal concentrations, relative to the half saturation constant, $K_Q \gg Q$. The elimination rate can now be linked to metabolic activity, and so to body size. If particular organs are involved, such as the liver in the case of alcohol, the DEB theory can be used to study adaptation processes to particular metabolic functions.

In the case of alcohol, the uptake term should obviously be replaced by a more appropriate one that applies to the particular subject.

Many toxicants are metabolically modified. This especially applies to lipophyllic compounds, which are typically transformed into more hydrophyllic ones, which are more easily excreted but also metabolically more active. The rate of transformation can be linked to the metabolic rate, and so depends on body size and nutritional conditions. These metabolic products can be more toxic than the original lipophyllic compound.

### 4 Bio-availability

Compounds are not only transformed in the organism, but also in the environment which affects their availability. Many have an ionic and a molecular form, which are taken up at different rates; the ionic species requiring counter ions, which complicates their uptake. Speciation depends on the concentration of compound and environmental properties, such
as the pH. It can vary in time and also occurs inside the organism, but the internal pH usually varies within a narrow band only. Models for mixtures of chemicals can be used in this case (see section on effects). Internal concentration gradients could develop if transport inside the organism is slow; film models should be used in this case. A nice example of a case where concentration gradients result from transformation in combination with transport is the fluke *Fasciola* which has an aerobic metabolism near its surface with the micro-aerobic environment inside its host, but an anaerobic metabolism in the core of its body [30].

Another problem, which occurs especially in soils, is that the transport through the medium can be slow enough for concentration gradients to develop around the organism. Film models should then be used again.

A major problem in the translation of laboratory toxicity tests to field situations is the formation of ligands with (mainly) organic compound that are typically abundant in the field, but not in the bioassay. Ligands reduce the availability substantially, and typically has a rather complex dynamics. Moreover compounds can be transformed by (photo)chemical transformation and by actions of (micro)organisms. This implies that the concentration of available compounds changes in time, and our methodology to assess effects of chemical compounds should be able to take this into account.
These processes of transformation require compound-specific modelling and this short section demonstrates that bio-availability issues interact with toxico-kinetics and effects of chemical compounds in dynamic ways, which calls for a dynamic approach to effects of chemicals [3, 31].

5 Effects at the individual level

Compound affect individuals via changes of parameter values as functions of the internal concentration [32, 33]. The parameter values are independent of the internal concentration in the “enough”-range of the compound. This implies the existence of an internal No Effect Concentrations (NECs) at either end of the “enough”-range; the upper end is typically of interest for ecotoxicological applications. Outside the “enough”-range the value of the target parameter is approximately a linear function of the internal concentration as long as the changes in parameter value are small; the inverse slope is called the tolerance concentration (a large value means that the compound is not very toxic). Small changes in parameter values do not necessarily translate into small changes of some end-point, such as the cumulative number of offspring [34] or the body size [35] at the end of some standardised exposure period. DEB theory specifies how exactly changes in parameter values translate into the performance of the individual. Typical target parameters are the specific
maintenance costs, or the yield of structure on reserve, or the maximum specific assimila-
tion rate or the yield of reserve on food or the yield of offspring on reserve. For effects on
survival, the hazard rate serves the role of target parameter, and the inverse “tolerance con-
centration” for the hazard rate is called the killing rate. Mutagenic compounds can induce
tumours [36], but also accelerate ageing by enhancing the effects of ROS. The partitioning
fraction for mobilised reserve can be the target parameter for endocrine disruptors.

Using sound theory for how effects depend on internal concentrations, DEB-based theory
can handle varying concentrations of toxicant [37-40], even pulse exposures [41]. DEB
theory applies to all organisms, including bacteria that decompose organic pollutants.

A proper description of this process should account for adaptation [42], co-metabolism
[43] and the fact most bacteria occur in flocculated form in nature [44], which affect the
availability of the compound.

The model of linear effects of internal concentrations on parameter values has been ex-
tended into several directions, such as adaptation to the compound, inclusion of the recent
exposure history via receptor dynamics [45] and attempts to include particular molecular
mechanisms [46].
5.1 Mixtures and NECs

Mixtures of compounds affect parameters values via addition of the effects of single compounds, plus an interaction term which is proportional to the products of the internal concentrations of the compound [47]. This interaction term can be positive or negative; a construct that is the core of the analysis of variance (ANOVA) model and rests on a simple Taylor series approximation of a general non-linear multivariate function, which only applies for small changes of parameter values; the non-linearities of the effects should be taken into account for larger changes. These non-linearities might well be specific for the compound and the species and, therefore, lack generality. Notice that linear effects on parameter values translate into non-linear effects of the performance of the individual because the DEB models are non-linear. Also notice that each DEB parameter has a NEC value for any compound; the lowest value among all parameters might be considered as the NEC of a compound for the organism, but its estimation requires to study effects on all 13 parameters of the standard DEB model, in principle. Since this study can be demanding, it is in practice essential to talk about the NEC of a compound for an organism for a particular DEB parameter.

The NEC reflects the ability of the individual to avoid changes of performance. From a statistical point of view, this robust parameter has very nice properties [48-50]. The NEC
is not meant to imply that some molecules of a compound don’t have an effect, while other molecules do. The removal of a kidney in a healthy person can illustrate the NEC concept: the removal implies an effect at the sub-organismic level, but this effect generally does not translate into an effect at the individual level. The NEC, therefore, depends on the level of observation. We can delineate three cases of how compounds in the mixture combine for the NEC

- the presence of other compounds is of no relevance to the NEC of any particular compound
- the various compounds add, like they do for effects, and at the moment effects show up, the amounts of the compounds that show no effects remain constant
- the various compounds add, like they do for effects, and the amounts of the compounds that show no effects continue changing with the internal concentration of the compounds; if a compound continues accumulation more than other compounds, its NEC increases while that of the other compounds in the mixture decreases

The third case is possibly most realistic, but also computationally the most complex. In many practical situations the results are very similar to the second case, which can be used as an approximation. If compounds in a mixture are equally toxic, and so all have
the same NEC, the second case is formally identical to this special case [51]. This way of described effects of mixtures turns out to fit well with experimental data [47] and each pair of compounds have a single interaction parameter, which does not change in time. If there are \( k \) compounds in a mixture, there are \( k(k-1)/2 \) interaction parameters, just like in ANOVA.

A further reflection on the NEC might clarify the concept. Any compound affects (in principle) all DEB parameters (including the hazard rate), but the NEC for the various parameters differ. This makes that, if the internal concentration increases, the parameter with the lowest NEC first starts to change, but other parameters follow later. In a mixture of compounds, this can readily lead to a rather complex situation where in a narrow range of (internal) concentrations of compounds in a mixture several parameters start changing [51]. Even in absence of the above-mentioned chemical interactions of compounds on a single parameter, interactions via the energy budget occur, which are hard to distinguish from the chemical interactions on a single parameter. Chemical interactions are typically rare, but interactions via the budget always occur.
5.2 Hormesis

Hormesis, the phenomenon that low concentrations of a toxicant seem to have a stimulating rather than an inhibiting effect on some endpoint, can result from interactions of the compound with a secondary stress, such as resulting from very high levels of food availability. If a compound decreases the yield of structure on reserve, it reduces growth and delays birth (if an embryo is exposed) and puberty (in the case of juveniles), but also reduces the size at birth. A reduction of growth indirectly reduces reproduction, because food uptake is linked to size. Since it also reduces size at birth, the overall effect can be a hormesis effect on reproduction (in terms of number of offspring per time) [52]. Indirect effects on reproduction differ from direct effects by not only reducing, but also delaying reproduction. This has important population dynamical consequences.

5.3 Co-variation of parameter values

A very powerful property of the standard DEB model and the one-compartment model is that they imply rules for how parameter values co-vary among species and compounds [53, 23, 9]; this variation directly translates into how expected effects vary. These expectations can be used to fill gaps in knowledge about parameter values, but cannot replace the need for this knowledge. Evolutionary adaptations and differences in mode of ac-
tion of compounds can cause deviation from expected parameter values for species, and
compounds, respectively.

The reasoning behind the scaling relationships for the standard model rests on the ass-
sumption that parameters that relate to the local biochemical environment in an organism
are independent of the maximum body size of a species, but parameters that relate to
the physical design of an organism depends on the maximum size. Strange enough, this
simple assumption fully species the covariation of parameter values. The application is
best illustrated with the maximum length \( L_m \) an endotherm can reach in the standard
DEB model. This length is a simple function of three parameters, \( L_m = \kappa \{ p_{Am} \}/[p_M] \),
where \( \kappa \) is the fraction of mobilised reserve that is allocated to the soma, \( \{ p_{Am} \} \) is the
surface-area specific maximum assimilation energy flux and \([ p_M ]\) is the volume-specific so-
matic maintenance cost. Since \( \kappa \) and \([ p_M ]\) depend on the local biochemical environment,
they are independent of maximum length, which implies that \( \{ p_{Am} \} \) must be proportional
to maximum length. All other parameters can be converted in simple ways to quantities
that depend on the local biochemical environment; these transformations then defined how
they depend on maximum length. When we divide the maturity at birth and puberty by
the cubed maximum length, we arrive at a maturity-density, which reflects the local bio-
chemical environment and should not depend on maximum length. So the maturity at
birth and puberty are proportional to the cubed maximum length. Many quantities, such as the use of dioxygen by an individual, can be written as functions of parameter values and amounts of reserve and structure. So the maximum respiration rate of a species is a function of parameter values, while we know of each parameter how it depends on maximum length. It can be show that maximum respiration rate scales between a squared and a cubed maximum length, and the weight-specific respiration with weight to the power $-1/4$, a well-known result since Kleiber [54].

The reasoning behind the scaling relationships for the one-compartment model rests on the assumption that transport to and from the compartment is skewly symmetric [16]. The ratio of the concentrations in the compartment and the environment at equilibrium is a ratio of uptake and the elimination rates, just like the maximum length of the individual in the standard DEB model. This implies, see [16], that the uptake rate is proportional to the square root of the partition coefficient, and the elimination rate is inversely proportional to the square root of the partition coefficient. Film models are extensions of the one-compartment model, that behave at the interface between the environments basically in the same way as an one-compartment model; only around this interface they differ because film models account for concentration gradients. This deviation can be taken into account with the result that elimination rates are (almost) independent of the partition coefficient.
for low values of the partition coefficient and inversely proportional to it at high values.

Effects parameters can be included into the scaling reasoning is similar ways, with the result that the NEC is inversely proportional to the partition coefficient, and the tolerance concentration or the killing rate is proportional to the partition coefficient.

The octanol-water partition coefficient is frequently taken as a substitute for the body-water partition coefficient, with the advantage that reliable computational methods exist to evaluate this partition coefficient from the chemical structure of the molecule. In practice, however, octanol is not an ideal chemical model for organisms which change the chemical composition of their bodies. This makes that the co-variation of NECs, elimination and killing rates show less scatter and can be expected on the basis of the variation between each of these three parameters and the octanol-water partition coefficient [23].

We are unaware of any descriptive model for toxico-kinetics and/or metabolic organisation for which theory on the co-variation of parameter values is available, and we doubt that it even possible to derive such theory for descriptive models. Co-variation theory is not available for the so-called net-production models [55], for instance, where maintenance needs are first subtracted from assimilation before allocation to storage, growth or reproduction. The fact that the predicted relationships of now over thirty eco-physiological quantities, such as length of the embryonic and juvenile periods, maximum reproduction rate, maxi-
mum growth rate, maximum population growth rate, vary with the maximum body size of
species in ways that match empirical patterns provides strong support of the DEB theory.

The one-compartment and standard DEB models share the property that the independent
variable (the partition coefficient in the case of toxicokinetics and the maximum length in
the case of budgets) can be written as a ratio of an incoming flux (of toxicant and food,
respectively) and an outgoing flux (excretion and maintenance, respectively). This shared
property seems to be crucial for the core theory.

One of the many possible applications of the scaling relationships is in the effects of mix-
tures of compounds with similar modes of action, such as the poly-chlorinated hydro-
carbons. Suppose we know the concentrations and the partition coefficients of the com-
pounds in the mixture. We then link the elimination rates, the NECs and the tolerance
concentrations to the partition coefficients in the way described, and estimate the three
proportionality constants for the results of a bioassays with the mixture.

The sound theoretical basis for effects of toxicants in combination with rules for the co-
variation of parameter values offers the possibility for extrapolation, from one individual
to another, from one species of organism to another, and, sometimes, from one type of
compound to another [23]. These crosslinks partly reduce the need for a huge experimental
effort that should be invested in more advanced forms of environmental risk assessment,
such as discussed in Brack et al. [13]. Moreover, the theory simplifies to parameter poor
models under particular conditions. It has been demonstrated that many popular empirical
models turn out to be special cases of the general theory [1]. This might help in particular
applications.

6 Effects at the population and ecosystem level

At high food levels, organisms grow and reproduce fast and the maintenance costs com-
prise only a tiny fraction of the budget of the individuals. If a compound increases the
maintenance cost for individuals, say by a factor two, these effects are hardly felt by a
fast-growing population. Fast growth never lasts long in nature, due to the depletion of
food resources. At carrying capacity, where the generation of food resources just matches
the maintenance needs of a population (this is the maintenance needs of the collection of
individuals plus a low reproductive output that cancels the mortality), maintenance costs
comprise the dominant factor of the budget of individuals. If a toxic compounds now in-
creases the maintenance cost by a factor two, it in fact reduces the carrying capacity by
a factor two. This simple argument shows that the effects of toxicants on populations is
dynamic, even if the concentration of the compound would be constant [56]. It also shows
that no single quantifier for toxicity can exist at the population level.
If a toxic compound increases the cost of growth or reproduction, the effects hardly depend on the growth rate of the population, so on the food level, which shows that the mode of action is important for how effects on individuals translate to those on populations. It might be difficult to tell the various modes of action apart on the basis of the results from a standardised toxicity bioassay. The reason why the mode of action is still important is in the biological significance of the observed effect, which must be found at the population level. Details in the reproduction strategy of populations turn out to be important for how effects on reproduction translate to the population level [57].

Although bioassays with meso-cosms have the charm of being close to the actual interest of effects of toxicants to be avoided, the experimental control is extremely weak which results in a huge scatter of trajectories of experimental meso-cosms. The result is that the effects have to be huge to recognise them as effects [58]. Moreover the expected long term behaviour of chemically perturbated ecosystems is very complex, as shown by bifurcation analysis [59].

The specific population growth rate integrates the various performances of individuals naturally, and can rather easily be evaluated [60]. A delay of the onset of reproduction can be at least as important as a reduction of the reproduction for the fate of the population.
7 Concluding remarks

We argued that models for effects of chemical compounds should have three modules:

- dynamic energy budgets for how organisms generally deal with resource uptake and allocation
- toxico-kinetics for how organisms acquire the compounds
- chances of budget parameters as function of the internal concentrations

We discussed the basics for each of these modules: the standard DEB model, the one-compartment model and the linear change in target parameters. We also indicated where and how these models can be extended, from simple to more complex, to include particular phenomena. We discussed how budgets affect both the kinetics and the effects and, therefore, have a central role in effects models. Practice teaches that the restriction of realistic modelling is not in the model formulation as such, but in the useful application of these models to data. More complex models have more parameters and many of these parameters are by no means easy to extract from available experimental data. They require knowledge of physiological and ecological processes that are typically outside the scope of typical (eco)toxicological research. Kooijman et al. [61] discusses why any particular application of DEB theory requires only a limited set of parameter values, and how these
values can be obtained from simple observation on growth and reproduction at several levels of food availability.

The practical need to fill in gaps in knowledge about parameter values is the reason why due attention has been given to theory for the co-variation of parameter values; this theory naturally follows from the logical structure of the one-compartment model and the standard DEB model. Extensions of both models can modify the co-variation of parameters, as has been discussed.

Contrary to descriptive models, models with strong links with underlying processes can be used for a variety of extrapolation purposes, from acute to chronic exposure, from one species to another, from one compound to another, from individuals to populations, from laboratory to field situations [31]. Such extrapolations are typically required in environmental risk assessment, where NECs should play a key role [62]. The use of models to predict exposure in the environment is frequent, but to predict effects is still rare. The complexity of the response of organisms to changes in their chemical environment doubtlessly contributed to this. Yet we think that thirty years of applications of DEB theory to quantify effects of compounds on organisms have demonstrated that the theory is both effective and realistic. Many of the computations behind the models in this chapter can be done with the freely downloadable software package DEBtool: http://www.bio.vu.nl/thb/deb/deblab/
8 Acknowledgements

This research has been supported financially by the European Union (European Commission, FP6 Contract No. 003956 and No 511237-GOCE).

References


Kooijman SALM, Troost TA (2007) Quantitative steps in the evolution of metabolic organisation as specified by the dynamic energy budget theory. Biol Rev 82:1–30


<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The standard DEB model with fluxes (moles per time) and pools (moles). Assimilation is zero during the embryo stage and becomes positive at the transition to the juvenile stage (birth) if food is available. Age is zero at the start of the embryo stage. Reproduction is zero during the juvenile stage and becomes positive at the transition to the adult stage (puberty), when further investment into maturation is ceased.</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>The scheme of the one- and two-compartment models. The factor $P_{bd}$ converts an external concentration into an internal one; all rates labelled $k$ have dimension ‘per time’. In the two-compartment model $P_{bd}$ does not have the interpretation of the bioconcentration factor.</td>
<td>51</td>
</tr>
</tbody>
</table>
Fluxes of food and reserve

1. feeding
2. assimilation
3. mobilisation
4. somatic maintenance
5. maturity maintenance
6. reproduction

Figure 1:
Figure 2:
<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>List of frequently used symbols, with units and interpretation. The unit C-mol represents the number of C-atoms in an organism as multiple of the number of Avogadro.</td>
<td>53</td>
</tr>
<tr>
<td>symbol</td>
<td>units</td>
<td>interpretation</td>
</tr>
<tr>
<td>--------</td>
<td>-------</td>
<td>----------------</td>
</tr>
<tr>
<td>$t$</td>
<td>d</td>
<td>time</td>
</tr>
<tr>
<td>$c$</td>
<td>M</td>
<td>concentration of compound in the environment</td>
</tr>
<tr>
<td>$n_i$</td>
<td>mol m$^{-1}$</td>
<td>density of compound</td>
</tr>
<tr>
<td>$Q$</td>
<td>mol C-mol$^{-1}$</td>
<td>concentration of compound in an organism</td>
</tr>
<tr>
<td>$r$</td>
<td>d$^{-1}$</td>
<td>specific growth rate of structure</td>
</tr>
<tr>
<td>$k_e$</td>
<td>d$^{-1}$</td>
<td>elimination rate</td>
</tr>
<tr>
<td>$k_{01}, k_{10}$</td>
<td>d$^{-1}$</td>
<td>exchange rates between compartments</td>
</tr>
<tr>
<td>$P_{0d}$</td>
<td>mol C-mol$^{-1}$ M$^{-1}$</td>
<td>BioConcentration Factor (BCF)</td>
</tr>
<tr>
<td>$v_{ij}, v_e$</td>
<td>m d$^{-1}$</td>
<td>velocity, elimination</td>
</tr>
<tr>
<td>$d_i$</td>
<td>m$^2$ d$^{-1}$</td>
<td>diffusivity</td>
</tr>
<tr>
<td>$m_E, m_{ER}$</td>
<td>C-mol C-mol$^{-1}$</td>
<td>reserve density, reproduction buffer density</td>
</tr>
</tbody>
</table>