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"Effects of feeding conditions on toxicity for the purpose of extrapolation"

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These are preliminary lecture notes, intended only for distribution to participants.

Effects of feeding conditions on toxicity for the purpose of extrapolation

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ABSTRACT

1 Mathematical models can be most helpful in attempts to understand the way effects of toxic substances show up under various conditions. They are essential for extrapolation and prediction, especially when effects have to be quantified.
2 In some cases, there is a tight relation between effects and toxicokinetics, i.e. uptake / elimination behaviour of compounds.
3 It can be shown that, at least for some compounds, the toxicokinetics depends on feeding conditions. This alone makes it necessary to account for energetics in the use of laboratory observations for predictions and interpretation concerning field data.
4 It can also be shown that, given certain effects on individuals, the consequences for populations depend sensitively on energetics. This leads to an even tighter link between ecotoxicology and energetics.
5 A basic problem is that realistic models involve a relatively large number of parameters, even under the most simple assumptions about kinetics and effects. This constraints possibilities for extrapolation and prediction.
6 All in all, a close link between experimental and modelling programs is necessary.

INTRODUCTION

The aim of this paper is to present some general ideas underlying a joint technical research program of our Theoretical Biology group, and experimentally oriented groups at the Free University and MT-TNO. For technical details, one should consult articles in the reference list, which provide ample discussion of the relevant literature. In this paper, I will focus on animals although the concepts used are applicable to other organisms as well.

Although ecotoxicology is basically a quantitative science which calls for a modelling approach, the investment in model based research is small in comparison with that in purely experimental research. For this reason, it might be helpful to make a few remarks on the usefulness of models in an ecotoxicological context.

Mathematics as a language is very useful in the formulation of quantitative relationships and in the development of concepts. One should real-

ize that syntactically (mathematically) correct formulations need not make sense. The indiscriminate use of P and r^2 values to underpin quantitative statements in many papers on ecology reveals a worrying lack of interest in the statistical backgrounds. Such a costume confuses rather than contributes to the reasoning.

The first useful application of mathematics is directly connected with experiments. Within a proper modelling framework, it is much easier to
- set priorities in experimental programs (Kooijman *et al.*, 1987a; Kooijman, 1988)
- design experiments like the choice of the exposure period in relation with the properties of the chemical and the size of the test animals, the choice of concentrations and number of test animals to be applied etc. (Kooijman, 1981, 1983; Kooijman *et al.*, 1987a)
- interprete data like the relation between no-effect levels (NEL) and LC50 values (Kooijman, 1981)
- quantify like the estimation of the NEL as affected by the experimental set-up.

The second type of application relates to the comparison of data obtained under different circumstances (Kooijman and Van Haren, 1990). Usually a lot of factors contribute to uptake and elimination rates, concentration factors etc. Without models it is hard to evaluate the data involved and to obtain useful conclusions. The interpretation of data from biomonitoring programs frequently suffers from this problem.

The third field of applications is in relating different levels of biological organization (Kooijman and Metz, 1983; Kooijman, 1985; Kooijman *et al.*, 1987b; Hallam *et al.*, 1989). For example, effects on individuals have consequences for populations (see Van der Hoeven, 1991) and ecosystems. It is seldom easy to evaluate these consequences. Without models it is impossible.

A basic problem in ecotoxicology is that ecosystem responses to stress from pollution are perhaps the most relevant (Kooijman *et al.*, 1987a; Kooijman, 1988; see the Discussion), but also the most difficult to quantify and interpret. It is easy to understand that people involved in legislation are pressing for research programs on this topic. It is not always easy to convince them that lack of knowledge on fundamental issues in physiology and ecology strongly limits the feasability of the short-term programs. Since mathematical models need to implement such knowledge, they can not be used to generate it without adequate experimental back-up. When one is

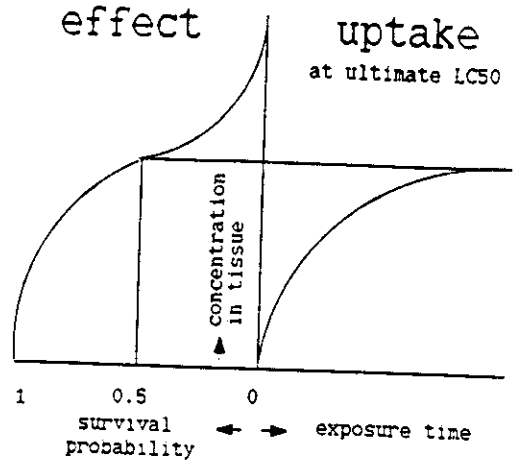

Figure 1: The relation between uptake and survival.

serious in the aim to predict ecosystem responses to pollution, one has to accept that it is a long term aim which only comes into perspective when one is willing to support long-term research programs in this field. The practical problems of today and tomorrow have to be solved in another way (Kooijman, 1987), which can only be unsatisfying. In the end, when we have finally generated sufficient insight into ecosystem dynamics we still might arrive at the obvious message: don't pollute.

UPTAKE *vs* EFFECTS

An important link between toxicology and ecotoxicology concerns the relation between toxicokinetics and effects. Toxicology, which has its roots in human health problems and pharmacokinetics, has a rather strong focus on metabolic transformations and suborganismal compartmentation. However, a compound is of interest to ecotoxicology only through its effects on the ecological behaviour of organisms.

As a first approximation effects seem to show up as soon as the concentration in the organism exceeds some threshold value, which might scatter among individuals (see e.g. Tas and Opperhuizen, 1991; McCarty, 1990; and Fig. 1). When all individuals in a cohort follow the same uptake kinetics of a toxicant from the environment, the fraction of individuals showing no effects at a certain exposure period corresponds to the fraction of individuals whose threshold concentration in the tissue is below the acquired concentration. The log logistic and log normal distribution are popular choices to describe the scatter of threshold values among individuals (Kooijman, 1981). Since their motivation is purely empirical, it is a weakness in theory based on this description. It is perhaps surprising that when we assume a simple first order linear kinetics for the uptake and elimination, the observed survival

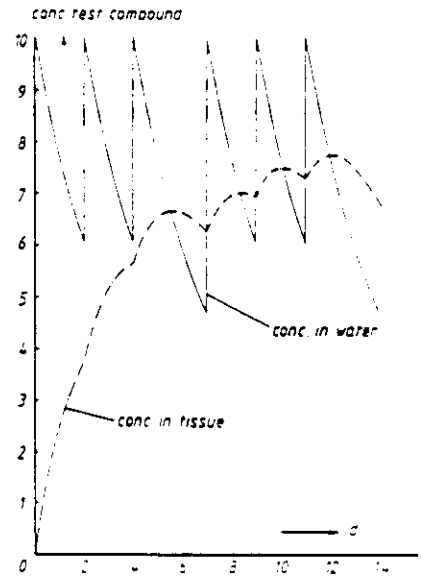

Figure 2: The expected concentration of xenobiotic in tissue, when the concentration in the water decreases exponentially and the uptake-elimination behaviour follows a first order kinetics.

pattern for simple compounds can be described very well (see Kooijman, 1983; McCarty, 1990).

This even works in the slightly more complex situation where the concentration of the compound is not constant (Kooijman, 1981). In a chronic toxicity test with daphnids, where the media are refreshed every 2, 2 and 3 days, compounds like cadmium absorb to the algae, which settle down. Cadmium is thus growing less available after refreshment of the medium. This process approximately follows a linear first order kinetics. So, we expect a concentration in the water and in the tissue as shown in Fig. 2. The survival pattern of the daphnids is well described, see Fig. 3. The point is now that the disappearance rate of cadmium can be estimated from the survival data. It corresponds very well with the one measured in the media, see Fig. 4. This provides a strong support for the assumption that toxicokinetics and effects are coupled.

Besides effects on survival, those on reproduction are of relevance to ecotoxicology. It can be affected directly, or indirectly via e.g. a change in growth, maintenance or feeding behaviour. Animal energetics provides a useful framework to evaluate this indirect effect to reproduction quantitatively (see e.g. Kooijman *et al.*, 1987a; Widdows and Donkin, 1991). The size of the effect can again be related to the concentration in the tissue.

UPTAKE *vs* PHYSIOLOGY

Under controlled conditions, many compounds seem to follow a simple first order kinetics in their uptake and elimination behaviour. The rates frequently

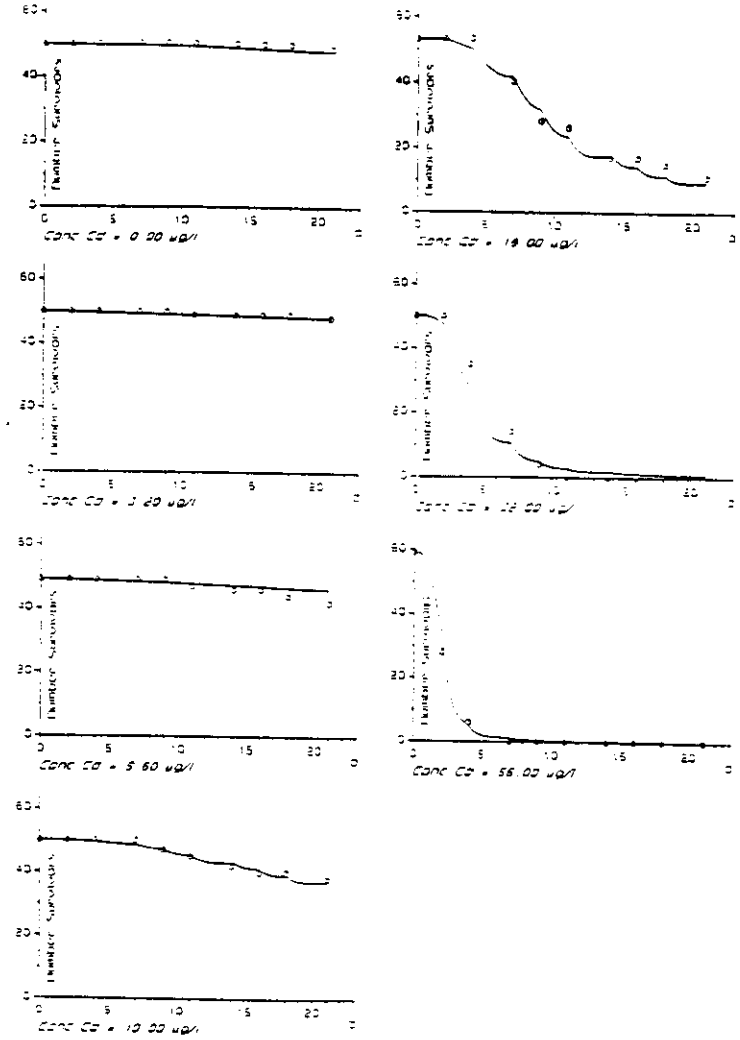

Figure 3: The observed and expected number of surviving *Daphnia* exposed to different exponentially decreasing concentrations cadmium chloride, as described by a first order kinetics for the uptake-elimination behaviour.

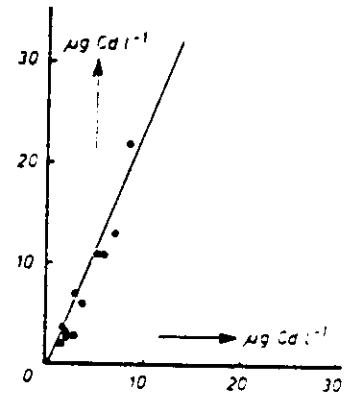

Figure 4: The measured concentration of cadmium in the water after refreshment of test media plotted against that just before refreshment. The line is not based on the points shown, but on the survival data presented in Fig.3.

depend on body size (see e.g. Hickie *et al.*, 1990) and on environmental conditions such as food availability. To explain connections between these factors, I will discuss the process in more detail (Kooijman, 1981). The assumption that food availability is a major factor modulating effects is based on three arguments. Firstly, uptake via food can be an important uptake root (see e.g. Schrap, 1991). Secondly, it is tightly linked to lipid content in the animal (see e.g. Van den Heuvel *et al.*, 1990; Van de Guchte *et al.*, 1990 for its importance). There exists a voluminous literature on the relation between bio-accumulation factors and K_{ow} values of compound (see e.g. Gobas, 1991). Thirdly, the consequences of effects on individuals for the behaviour of populations can sensitively depend on food availability (Kooijman *et al.*, 1983; Kooijman, 1985; Hallam *et al.*, 1989).

The general idea is presented in Fig. 5. Assume that an animal can be decomposed into structural biomass (i.e. a certain combination of carbohydrates, proteins and lipids) and in reserve materials (i.e. another combination of carbohydrates, proteins and lipids), which primarily function as energy reserves. Suppose that it has control over its chemical composition, such that structural biomass and reserves do not change in composition, a property known as homeostasis. We assume further that the partitioning of the xenobiotic over the watery fraction, carbohydrates, proteins and lipids, is fast compared to the exchange of the xenobiotic in the watery fraction and the environment. The uptake via food and the exchange with the environment is taken to be a linear first order process, with a rate proportional to the surface area. Since the feeding rate is also taken proportional to surface area and the animal is assumed to be isomorphic (i.e. it does not change shape during development), this set of assumptions leads to a rather simple kinetics for the xenobiotic.

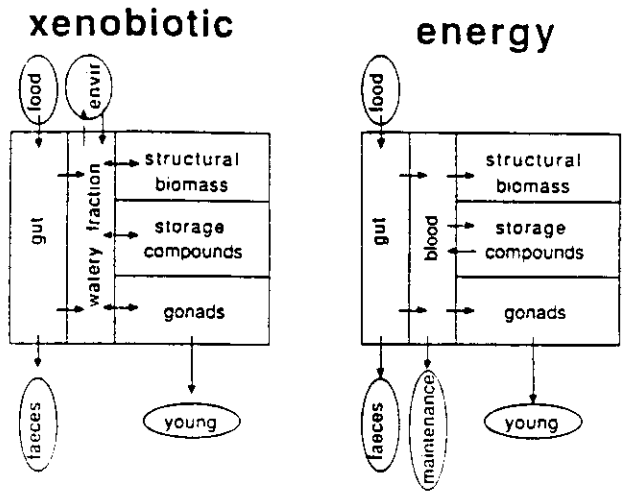

Figure 5: The toxicokinetics and energetics of an individual. The two-sided arrows refer to rapid exchanges.

These assumptions have to be supplemented with additional ones concerning energetics. The following assumptions seem to apply at least approximately for a wide variety of species: there are three size-defined life stages, embryos which do not feed, juveniles which do not reproduce, and adults; food uptake depends hyperbolically on food density; the reserve density, i.e. the ratio of the amount of reserves and body volume, follows a linear first order process with a relaxation time proportional to body length; a fixed fraction of the utilized energy is spent on growth plus maintenance, the rest on reproduction plus development; the maintenance costs are proportional to body volume; the initial size of an embryo is negligibly small and the reserve density at hatching equals that of the mother at egg production.

The kinetics can be classified as a linear first order kinetics with variable coefficients. The variation in the coefficients depends on changes in size, reserves, feeding rate and reproduction. When the exposure period is short enough, we are back at our familiar first order kinetics with constant coefficients. So the model represents an extension of simple first order kinetics, which is an alternative for the frequently applied more-compartment models (see e.g. Timmermans *et al.*, 1990). Since these models have more parameters than the one-compartment model, they usually give a better fit, but it is hard to identify the different compartments physically. Therefore their value for understanding the process is limited.

The combination of isomorphism, homeostasis and instantaneous partitioning is a strong one, which makes modelling workable. An important consequence is that when the concentration in one organ exceeds some threshold value specific for that organ, the concentration in another organ will exceed some other threshold value. So we do not have to bother about details of the cause of the effects. We even might relate effects to the concentration in a non-target organ or to that in the whole body.

The derivation of the model is a bit complicated due to the fact that exchange rates depend on concentration differences, i.e. on intensive quantities, while the mass preservation law involves absolute amounts, i.e. on extensive quantities. When volumes are changing, this leads to minor complications, especially with respect to the process of accumulation of reserves and the way they contribute to measurements of quantities such as wet weight and dry weight.

The application of the model is illustrated in Fig. 6. The fit is satisfying, but for the moment it is not possible to use this as a critical test for the model because too little is known about the energetics during the experiments. This gives too much freedom in the choice of parameter values. This is a major problem with existing data in the literature: we were not able to locate sources giving adequate information on both toxicokinetics and energetics.

DISCUSSION

A model like the one presented has to be supplemented with models for the transport and the fate of compounds in the environment and with population dynamics and foodweb considerations, to make it useful in a wider ecotoxicological perspective (Kooijman *et al.* 1987a, 1987b). It can perhaps help to understand why concentration-effect curves based on animals from laboratory cultures tend to be much steeper compared with curves based animals collected from the field. The significance of this phenomenon lies in the interpretation of no-effect levels based on laboratory animals.

The variety of compound-specific kinetics and effects, and of species-specific energetics, that have been described is huge. This calls for simplifying outlines of the main processes that are a common denominator. For many purposes it is essential to keep the picture as simple as possible. The risk of loosing the finer details in particular applications will have to be taken for

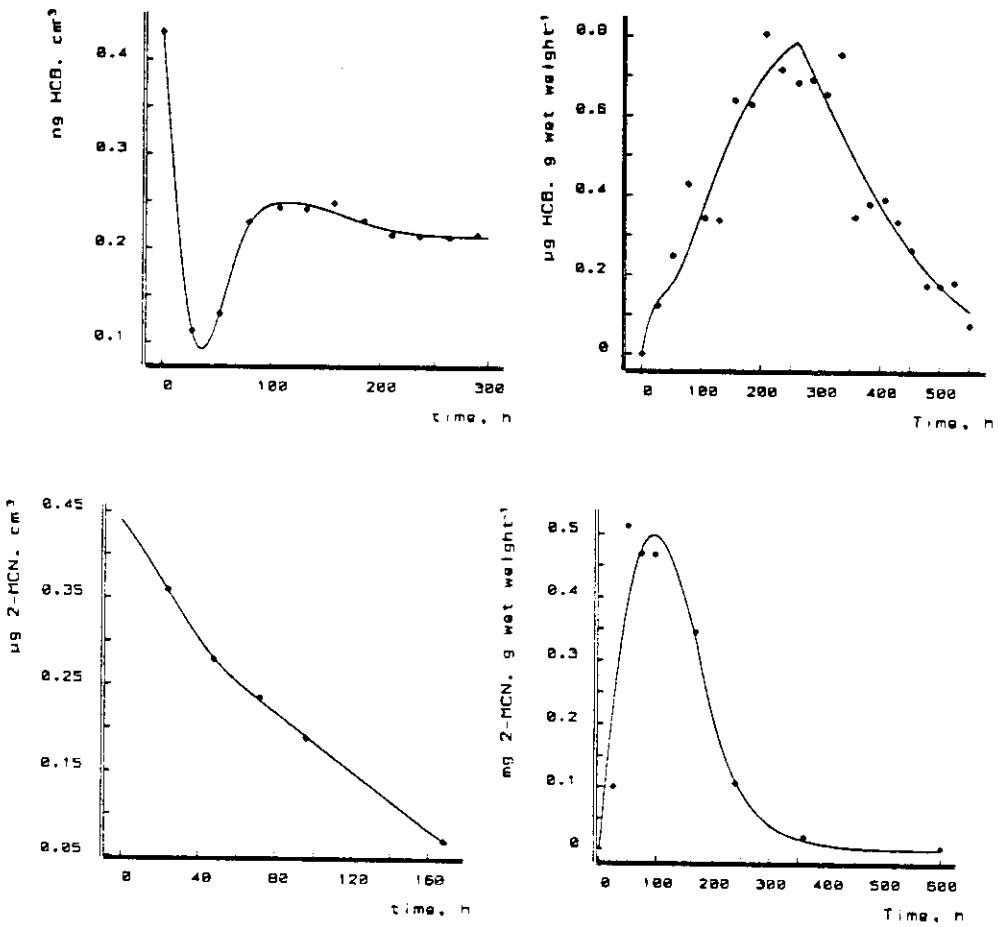

Figure 6: The uptake-elimination of hexachlorobenzene (log K_{ow}=5.45) in *Elliptio* (above) and of 2- monochloronaphtalene (log K_{ow}=3.9) in *Poecilia* (below). Left: concentration in the water with cubic spline functions used for interpolation. Right: concentration in the tissue with model based descriptions. Data from Russel and Gobas (1989) and Opperhuizen (1986), respectively.

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granted.

Some processes are too poorly understood at the moment to allow a quantitative description which is not highly species and compound specific. The process of metabolic transformation is an example (see Sijm *et al.*, 1991; Keizer *et al.* 1990), who point out that the transformation is highly species-specific). At the moment, it is only implicitly incorporated into the present model-formulation. When it follows a first order kinetics, it only affects the value of the elimination rate. When it follows a different kinetics, it has to be incorporated in an explicit way. Another example is that of physiological adaptation, which may involve a wide variety of mechanisms (see Calow, 1991). Although is not too difficult to do some wild guesses for model formulations, the result is inevitably that the number of parameters is increased, which make it more difficult to test it critically.

When a response to a compound is species-specific and when one wants to evaluate its implications for integrated systems like communities, one has to realize that models with many parameters have not contributed to our understanding of the behaviour of such systems so far. I believe that it is possible to formulate useful and parameter sparse models for integrated systems on the basis of input/output behaviour. Such models can only be used to study global problems. The fate of a particular species is not one of them unless it has a major impact on the dynamics of the integrated system. The classification of questions into specific ones or global ones is obviously simplistic. A continuum would be more realistic. It is not yet clear to what extent ecosystem models can in the end be used to study less global questions.

When an integrated system breaks down due to pollution, one can reasonably infer that most species cannot survive. On the other hand, when an integrated system is hardly affected in its global performance by a given stress, it is surely possible that many species will disappear altogether. Depending on the scale in time and space, this is highly undesirable. Problems of ecotoxicological concern are at a variety of levels of integration (see De Kruijf, 1991). I hope that problems raised in ecotoxicology stimulate physiological and ecological research to provide a useful framework to interconnect these levels. Although some progress has been made, much remains to be done.

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