Announcement ECB-Workshop on Biology-Based Modelling *

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1 Introduction

Chemical risk assessment aims to ensure that the manufacture and use of chemicals will not endanger human health and the environment. In the European Union, the guidelines for chemical risk assessment are laid down in Technical Guidance Documents (TGD, [6]). For the environment, the assessment boils down to a comparison of exposure levels (PECs) to predicted safe concentrations for various populations and ecosystems (PNECs). For exposure assessment, the available dataset contains physico-chemical properties of the chemical in question, such as hydrophobicity, water solubility, and results from (bio-)degradation tests. To calculate the PEC, process-based fate models are commonly used. In such models, the available data are used to estimate environmental process parameters such as volatilization rates, degradation rates and partition coefficients. Using these models, it is possible to simulate the chemical's fate and transport in the environment for different release scenarios without actually measuring concentrations. For effects assessment, the available data in the initial tiers typically include the results from standard toxicity tests, usually reported as NOECs or ECx[1] (after a specified exposure time). NOECs are derived using a statistical hypothesis test (comparison of effects to the control response), whereas the ECx is an interpolated value, yielding x% effect, based on a descriptive regression model.

Unlike exposure assessment, the method to derive the PNEC does not use process-based models. Instead, the approach relies on the use of assessment factors (generally multiples of 10) to derive a predicted no-effect concentration (PNEC) as "a concentration below which an unacceptable effect will most likely not occur" [6]. The assessment factors reflect the translation from laboratory tests (short term, high exposure, one species, and controlled environment) to the field (long term, low exposure, multiple species, variable environment). Although ecotoxicological experience and common sense forms the basis of these factors, the scientific rigor of the extrapolation from test result contrasts the process-based models used for the exposure assessment.

It may be questioned whether we have enough knowledge about ecotoxicological processes to be able to accurately extrapolate laboratory test results to field populations. However, we will argue that valuable process information can already be extracted from the same standard ecotoxicological laboratory tests. The current methods for analyzing toxicity test results are largely descriptive and do not incorporate all of the data from the toxicity test. Furthermore, these statistics do not allow for educated extrapolation to populations under field conditions (which is why we have to resort to the use of assessment factors). For these reasons, alternatives to the statistical methods currently used for the analysis of ecotoxicity tests should be considered.

A promising alternative are so-called biology-based models, which make explicit assumptions regarding the processes behind the toxic effect. At this moment, biology-based methods are not used within the regulatory context. However, these methods are discussed (next to the more classical approaches of hypothesis testing and regression analysis) in recent ISO/OECD guidance documents on the statistical analysis of ecotoxicity data [21, 9]. In 2007, the European Chemicals Bureau (ECB) will organise a workshop to introduce biology-based methods to the regulatory

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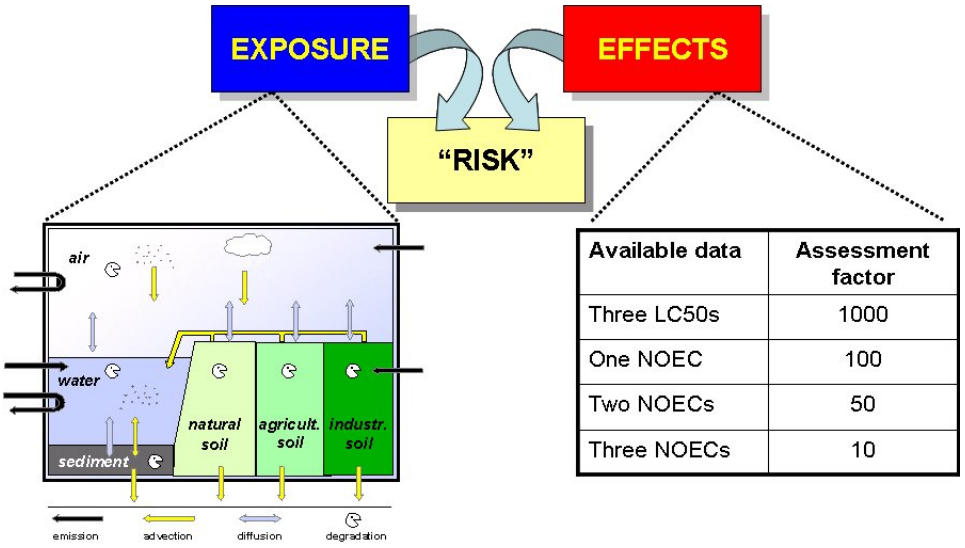
[1]Here, we will use the term ECx for all endpoints, including survival.



Available data	Assessment factor
Three LC50s	1000
One NOEC	100
Two NOECs	50
Three NOECs	10

Figure 1: Illustrating the conceptual difference in the approaches for exposure assessment and effects assessment in the EU-TGD.

community, and explore the possibilities for future implementation into risk assessment methodology. Technical contents for this workshop will be provided by the department of Theoretical Biology from the Vrije Universiteit, Amsterdam.

2 Limitations of current methods for dose-response analysis

The current approaches for dose-response analysis can be classified in two categories: hypothesis testing and regression analysis. The methods for hypothesis testing try to select the highest test concentration that does not have a significant effect, in comparison to the control. This concentration is generally designated as a NOEC (no-observed effect concentration), although other terms are also used. Over the past decades, the NOEC and similar statistics have received serious critique (e.g. [5, 8, 4, 19, 25]). The main objection raised against the NOEC is that it is based on the absence of *statistically significant* effects. However, the ability to detect significant effects depend on the experimental design, variation within the treatments, and the power of the statistical test. In general, poorly conducted tests (few animals, large variation in the response) lead to high (unprotective) NOECs. Even in standardised tests, the observed effect at the NOEC may be considerable: in one review of literature data, the mean effect was generally 10-34%, but in some cases even higher [4]. The following conclusion was reached at an OECD workshop in 1996 in Braunschweig, Germany [20]: "It was concluded that the NOEC, as the main summary parameter of aquatic ecotoxicity tests, is inappropriate for a number of reasons ... and should therefore be phased out."

The use of ECx values has several advantages over the NOEC. Regression analysis is used to fit a curve (the log-logistic is a popular choice) to all of the data at a single exposure time, and the concentration yielding x% effect is interpolated (x can be any number, but generally 5, 10 or 50% are used). A result of this procedure is that the summary statistic is, in comparison to the NOEC, less dependent on details of the experimental design and can be estimated with a confidence interval. The main concerns raised against the NOEC are thus addressed. However, limitations remain, as discussed in detail in [12, 11]:

- As clearly stated in the ISO/OECD reports [21, 9]: "A statistical regression model itself does not have any biological meaning, and the choice of the model (expression) is to some extent arbitrary". The EC50 is relatively unsensitive to the choice of model, but for small values of x the ECx becomes increasingly sensitive to model details. Because the model has no biological meaning, educated extrapolations are impossible (e.g., acute to chronic, individual to population, constant to time-variable exposure, high-food to low-food conditions).

- Inefficient use of the experimental data is made in the derivation of ECx. Many standard test protocols require that measurements are taken at several points in time. In practice, only the results at the last time point are used [2]. Regression analysis cannot make use of data in time (at best only as a covariate in the regression).
- The value of the ECx itself depends on the exposure time (just like the NOEC). For lethality, ECx tends to decrease in time until it reaches a so-called incipient level [24] (see e.g., Figure 5). For sub-lethal endpoints, ECx may show a much less predictable relationship with time [1].

Figure 2 serves to illustrate the last point. In this example, it is clearly impossible to select any EC10 as *the* EC10 for these compounds. Depending on the selected test duration and the selected endpoint, a different EC10 is obtained. Taking the EC10 at the end of the test does not necessarily produce the worst-case value (as most clearly shown for body length in the pentachlorobenzene case), and there is no simple relationship between the endpoints. Generally, the EC10 for reproduction is lower than for body length, but their ratio changes with exposure time. The example in Figure 2 is for a nematode species, as detailed life-cycle experiments have been performed. However, there is no reason to believe that the situation is different for the standard test species. The ECx-time relationship will depend on the selected endpoint, properties of the compound (e.g., mechanism of action and hydrophobicity), and properties of the organism (e.g., body size) [1]. The fact that ECx changes with time is a source of bias in the comparison of toxicity between chemicals and the comparison of sensitivity between species.

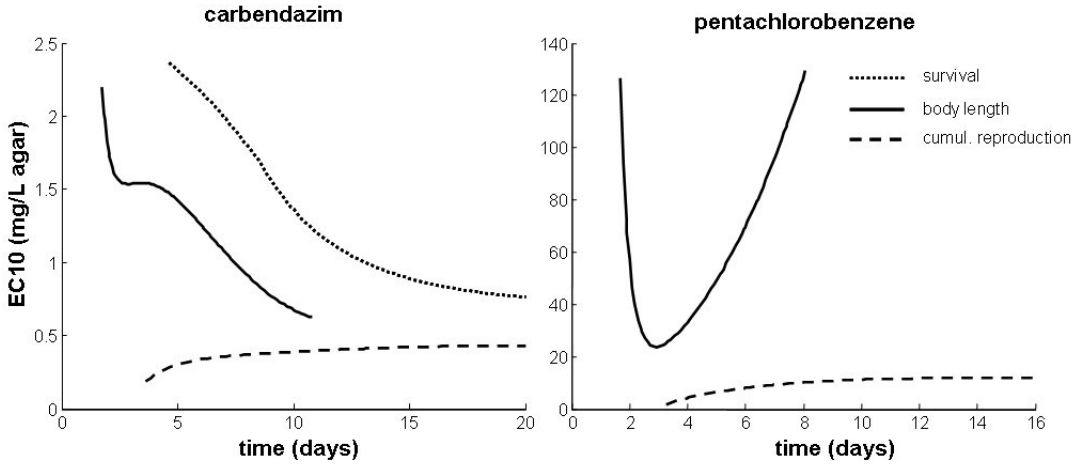


Figure 2: Example of the time-dependence of the ECx. This example is for two compounds in the nematode *Caenorhabditis elegans*, exposed in agar [1]. For pentachlorobenzene, no effects on survival were observed at the tested concentrations.

3 Biology-based modelling

The limitations of current practice were recognized at the 1996 OECD workshop in Braunschweig, Germany [20] on the statistical analysis of aquatic ecotoxicity data. At this workshop, the following conclusion was reached: "The time course of effects should be incorporated in the analytical procedures ... If different models are equivalent and give adequate fits to data, and if assumptions are valid, mechanistic models are preferred over empirical models." With biology-based modelling, we attempt to explain the toxic effects on all endpoints from a set of consistent assumptions about the underlying mechanisms (biological and toxicological). One of the most basic features of such a model is that it explicitly incorporates these processes as a function of time.

There are several approaches available that can be called "biology-based", but the DEBtox method is the only available approach that deals with both lethal and sub-lethal effects, and is

[2]For the NOEC, even less information from the data is used: in general, only the observations with "low" effects are used, and when a non-parametric test is used, the absolute values are discarded.

able to deal with ecotoxicity data as currently produced using standard OECD test protocols. The DEBtox method is a spin-off of the theory of Dynamic Energy Budgets (DEB [13, 14]). Dealing with sub-lethal effects, it is essential to have a model capable of showing the relations between feeding, maintenance, growth, development and reproduction. This approach allows questions to be rephrased in a more meaningful framework. For example, if a chemical decreases reproduction, the question could be, not how to derive an ECx, but rather, *why* is reproduction decreasing. The "why question" is relevant because this will help to us to better understand what the toxicant is doing to the organisms and thus helps to predict and prevent unacceptable toxic effects in the environment. An observed decrease in reproduction can be explained from the perspective of energy balance; offspring are produced from food, so one possibility is that ingestion has declined as a result of toxic stress. Alternatively, feeding may be unaltered, but there may be an additional energy drain for metabolic repair, leaving less energy for the production of eggs. Other hypotheses as to the observed reproductive effects can be put forward, obviously. The DEB theory provides a framework for addressing this question by describing how individuals acquire and use energy, based on a set of simple rules for metabolic organization. Within this theory, organisms are treated as dynamic systems with explicit mass and energy balances.

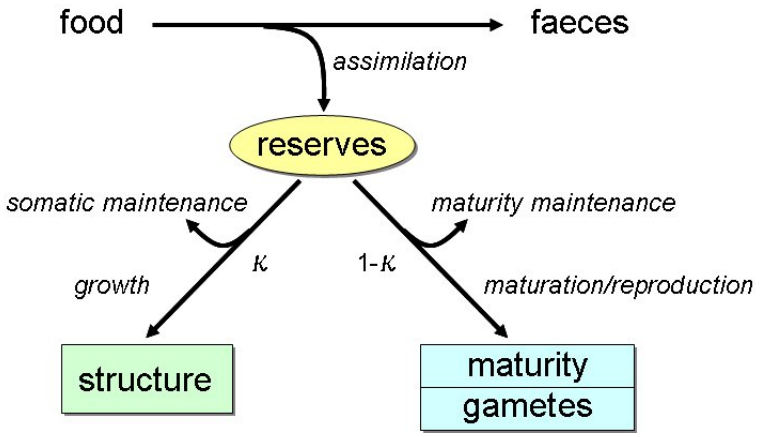


Figure 3: Schematic representation of resource allocation in the DEB theory.

When we expose an organism to a toxicant, the compound is first taken up and distributed within the organisms' body (toxicokinetics). Next, the internal concentration affects resource allocation. In principle, all parameters in the DEB model may be subject to change under influence of the toxicant. Potential target parameters are a.o., maintenance costs, assimilation efficiency, and costs for reproduction. A change in any of these target parameters will have specific consequences for the growth and reproduction as a function of exposure time and concentration (which have indeed been observed in experiments). These modes of action are based on resource allocation, which is not directly comparable to the more familiar use of the term (e.g., as in narcosis and uncoupling). Knowing the resource-based mode of action is of particular importance for the extrapolation to populations.

The first version of DEBtox was developed in 1996, and has been described in a book [15], and a series of papers [3, 16, 17, 18]. A free version of the Windows-based software protocol can be downloaded from our website (http://www.bio.vu.nl/thb/deb/deblab/). This software can be used to analyse data from acute survival studies, fish growth, *Daphnia* reproduction, and algal population growth. The observations (in time) from the experimental test are entered, and the software estimates the parameter values that provide the best fit of the model to the data. One of these parameters in a no-effect concentration: the concentration that produces no effect whatsoever, even after prolonged exposure. Advantages of the DEBtox software include:

- Use of all of the data in time in a single analysis. This implies that more information is used to fit the model, thus resulting in more precise parameter estimates. On the other hand, more precision means that we can still obtain good results when using less test animals. As such, DEBtox can help to decrease the number of experimental animals used.

- A time-independent no-effect concentration (NEC) can be derived with a confidence interval. Unlike ECx and NOEC, this NEC does not change in time. The NEC is a parameter in the model, and determines effects on all sub-lethal endpoints (and thus has the same value for effects on growth and reproduction). These properties make the NEC a more robust candidate for risk assessment purposes.

- The resulting parameters have a physiological interpretation. The advantage of this is that these parameter estimates can than be used to make educated extrapolations, e.g., to populations under food limitation (e.g., [10]).

The current version of the software is specifically intended for the analysis of data resulting from standard OECD test protocols. However, the DEBtox method itself has far more possibilities, which have not been included into the current version of the software, such as: simultaneous analysis of multiple endpoints [10], dealing with time-varying exposure [22, 23], toxicity of mixtures [2], prediction of population effects [10], etcetera. The large flexibility of this approach has great benefits for the analysis of non-standard data (e.g., tests with non-constant exposure, different test durations, tests without a control, or even tests at a single exposure concentration).

Of course, a DEB model is a simplification of the resource allocation in an organism, just like the multimedia fate models are a simplification of the fate processes environment. Nevertheless, as long as we capture the dominant processes, such simplifications can be of great help for risk assessment, for example by simulating different scenarios (e.g., discontinuous releases into the environment, and different environmental temperatures). Just as exposure assessment has moved in the past from simple scoring systems to process-based fate models, in the future we need to take a similar step for the effects assessment.

4 Examples of DEBtox applications

To illustrate the philosophy behind biology-based methods, we show here two DEBtox fits on real data sets. The first example is a 4-day acute fish (fathead minnow) study for hexachlorobutadiene (data from [7]). The standard regression analysis is shown in Figure 4, only using the data at the end of the test.

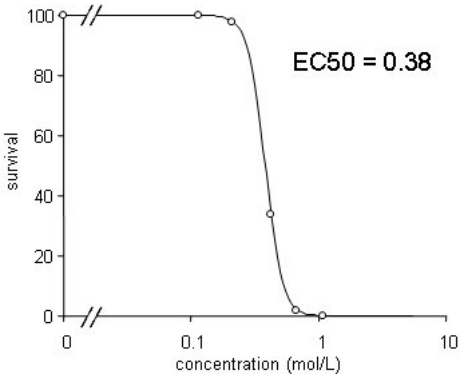


Figure 4: Standard regression analysis for survival of fathead minnow (*Pimephales promelas*) exposed to hexachlorobutadiene.

The test protocol prescribes daily observations on mortality, and the DEBtox model uses all data in one integrated analysis (Figure 5). The model requires four parameters to be estimated from the data. In contrast, standard regression analysis would require two parameters per time step, making a total of eight for the entire data set. The resulting NEC is 0.13 (0.091-0.16) mol·L^{-1}. This is nearly a factor of three lower than the 4-day EC50 resulting from the regression analysis. The DEBtox analysis shows that the iso-effect lines converge at the NEC for long exposure times. This implies that the dose-response curve gets steeper in time, until it is nearly vertical. In this case, the kinetics of the compound are not fast enough to reach the NEC (which equals the "incipient EC50") by the end of the test. In other words, in this case the EC50 would

have decreased further had the test been continued for longer than the standard four days. It must be noted that the decrease in EC50 for survival in time is governed by properties of the compound (e.g., hydrophobicity) and of the test organism (e.g., body size). For compounds with fast toxicokinetics, it is likely that the 4-day EC50 would be close to the NEC.

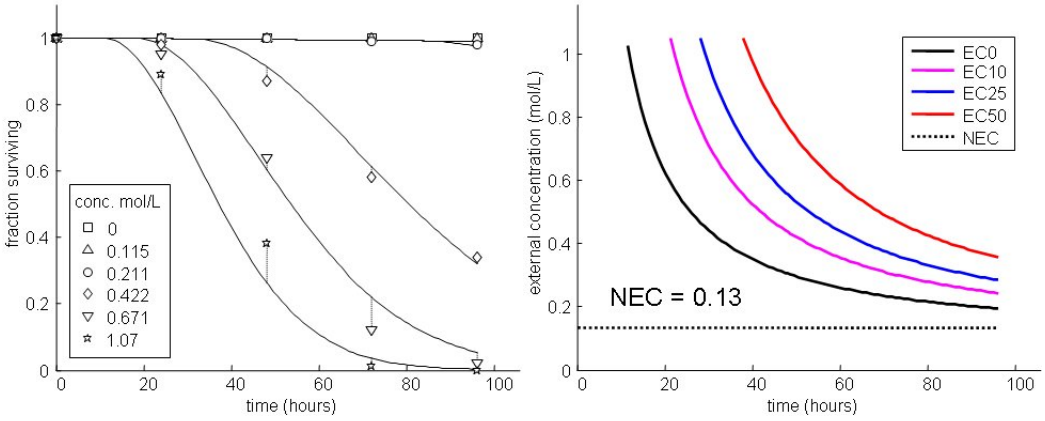


Figure 5: DEBtox fit for survival of fathead minnow (*Pimephales promelas*) exposed to hexachlorobutadiene. The right panel shows the iso-effect lines for several effect levels.

The second example is a standard 21-day reproduction test with *Daphnia magna* exposed to cadmium (data from an OECD ring test). Figure 6 shows a standard regression analysis on the total offspring per female at the end of the test. The regression analysis requires three parameters to fit the data at one time point.

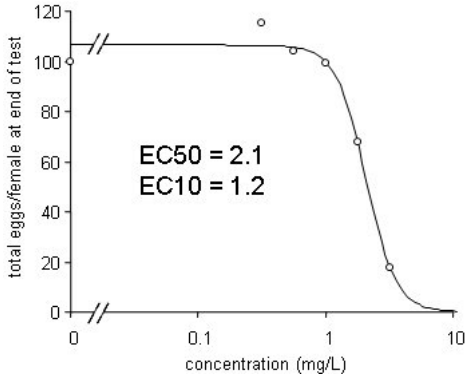


Figure 6: Standard regression analysis for reproduction in *Daphnia magna* exposed to phenol.

Again, the test protocol prescribes regular observations in time. All these observations are used by DEBtox model to fit four model parameters, including a NEC (0.82 mg/L, with confidence interval 0.52-0.91). The best fit was obtained by choosing one particular allocation-based mode of action (assuming that phenol increases the energetic costs for growth). The DEBtox model requires several additional physiological parameters to fit the data, more specifically, data on growth, which is usually not determined in these toxicity tests. For these parameters, default values are used, which is a valid option for such well-known species and standardised test conditions. In this case, the iso-effect lines do not converge in time, but rather diverge in time. This illustrates that there is no such thing as an "incipient ECx" for sub-lethal endpoints. In this example, a longer test duration leads to a higher (less protective) ECx value, raising the question of the representativeness of the 21-day ECx. This behaviour of the iso-effect lines is related to the specific properties of this endpoint (a cumulative continuous response), and the particular properties of the compound (mode of action and toxicokinetics).

These examples illustrate the basic DEBtox philosophy of fitting the effects data as processes in time. Further, these examples show that DEBtox can already work with data as produced

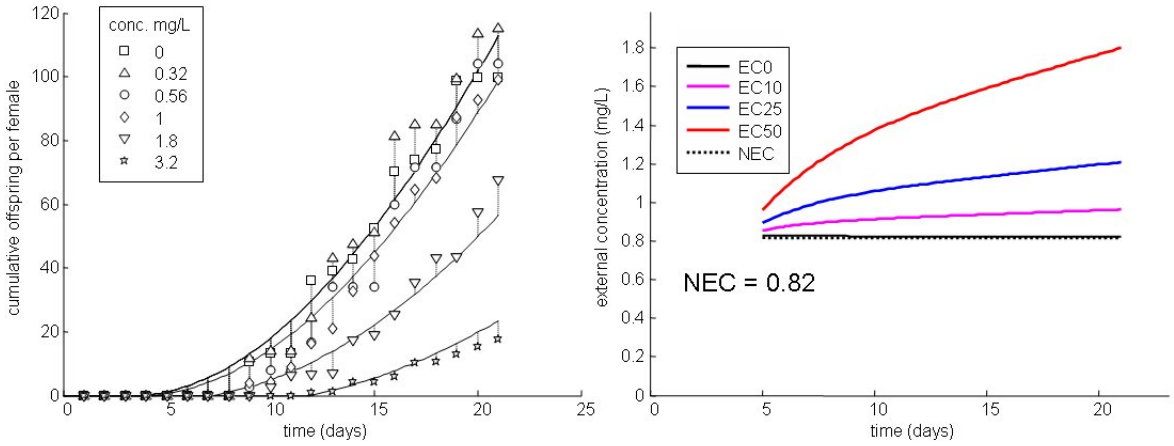


Figure 7: DEBtox fit for reproduction in *Daphnia magna* exposed to phenol. The right panel shows the iso-effect lines for several effect levels (note that the NEC overlaps with EC0).

according to standard OECD test protocols. The next question is how to use these results in a risk assessment. The NEC may replace NOEC and ECx as summary statistic. On the other hand, because this method is based on processes, it offers far more possibilities. For example, the parameter estimates resulting from the fit of the model to the data may be used to make predictions for effects under other scenarios (such as intermittent exposure).

5 Contents and aim of the workshop

In our opinion, the exposure assessment is well ahead of the effects assessment in terms of flexibility, scientific rigor, and possibilities to accommodate various scenarios. The effects assessment could benefit from adopting a more process-based approach, not only by improving the efficiency of extracting information from toxicity tests, but also by allowing for a reduction the number of animals per test (and perhaps even in the number of tests, as better use can be made of available non-standard data). The aim of the two-day workshop, to be organised by the ECB (aiming for the week of 4-8 June 2007), will be to introduce biology-based methods to the regulatory community, and explore the possibilities for future implementation in risk assessment methodology. At least one month before the start of the workshop, a background document on biology-based methods will be distributed among the participants. In this document, the DEBtox method will be explained in further detail, along with a set of worked out examples, to demonstrate how this methods deals with standard test data. Furthermore, the document will present and discuss several options for a possible implementation of such methods in the regulatory context (i.e., how methods such as DEBtox fit into the current effects assessment scheme). This background document will be discussed at the workshop. The discussion will focus on the feasibility of implementing such methods in risk assessment, and what the best option would be. We emphasise that it is the intention to focus a constructive and open discussion on the general concepts behind biology-based methods, and their possibilities, without stranding in a detailed technical discussion, or a discussion of statistical techniques.

The current DEBtox software will act as starting point for the discussions. We will give a demonstration, and invite participants to get hands-on experience with the software (preferably by bringing their own data sets). Examples will be discussed in the group, focusing on the usefulness for risk assessment, and the interpretation of the outcomes in a regulatory context. Using the DEBtox software as a reference point, the workshop can identify the user requirements for software to perform such calculations. More information on DEB and DEBtox (including the last version of the software) can be obtained from our website: http://www.bio.vu.nl/thb/deb/deblab. Publications from our department can be viewed from http://www.bio.vu.nl/thb/research/bib.

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