Chapter 1

General Introduction

Understanding effects of toxicants in our environment is of course of great importance. And although great improvements can be seen over the years there is still a lot to be done. One of the aspects is effects of mixtures of compounds. Almost our whole legislative system is based on effects of single compounds (with a few exceptions) but in the environment ‘there is no such thing as a single chemical exposure’.1

Over the past years effects of mixtures got more attention, resulting in two major European funded projects MIXTOX (1997–2004) and its successor Novel Methods for Integrated Risk Assessment of Cumulative stressors in Europe (NoMiracle), which started in 2004 and ends in 2010. The aim of NoMiracle is to help increase knowledge on the transfer of pollutants between different environmental compartments, and on the impact of cumulative stressors, including chemical mixtures. This will facilitate human and ecosystem health monitoring by providing the link with information concerning the condition of air, water, soil and the built environment. By developing and using improved assessment tools and novel models, the project will quantify and aim at reducing uncertainty in current risk assessment and screening methodologies, for example by improving the scientific basis for setting safety factors. The new methods will take into account geographical, ecological, social and cultural differences across Europe.

The research that is described in this thesis was carried out between 2005 and 2009 within the framework of the NoMiracle project.

Basically there are two possible ways to assess effects of mixtures: in the first, empirical approach, the effect of the mixture is regarded as a whole, without knowing the constituents of the mixture. Organisms are exposed to a sample and then the effect is monitored. The result only applies to that specific mixture and the approach has to be repeated for every sample taken.

It is also possible to use model based approaches to assess the effect of a mixture by building up the effect of the mixture from its known constituents, the so called
bottom up approach. The most used models for the bottom up approach are: Concentration Addition (CA) and Effect Addition (EA), see e.g. Jonker et al.2. CA is expected to occur if a chemical acts like a dilution of another, meaning that the effect can be obtained by replacing one chemical totally or in part by the equieffective amount of another chemical. Concentration addition is expressed mathematically as:

\[
\sum_{i=1}^{n} \frac{c_i}{EC_{X_i}} = 1
\]

Where \(n\) is the number of mixture components, \(EC_{X_i}\) is the concentration of the \(i\)th mixture component that provokes \(x\%\) effect when applied singly and \(c_i\) is the concentration of the respective component in the mixture. Each fraction \((c_i/EC_{X_i})\) represents the concentration of a mixture component scaled for its relative toxicity and is generally termed the toxic unit of that component. If, at a total concentration of the mixture provoking \(x\%\) effect, the sum of the toxic units equals one, concentration addition holds. Otherwise, more or less than (concentration) additive effects are assessed.

The concept of EA (also known as independent action) is based on the assumption that the compounds of a given mixture act independently. Mathematically EA is expressed as:

\[
E(c_{mix}) = E(c_1 + \ldots + c_n) = 1 - \prod_{i=1}^{n}[1 - E(c_i)]
\]

Where \(E(c_{mix})\) denotes the predicted effect (scaled to 0–1) of an \(n\)-compound mixture, \(c_{mix}\) is the total concentration in the mixture, \(c_i\) is again the concentration of the \(i\)th compound, and \(E(c_i)\) is the effect of that concentration if the compound is applied singly.

Both these approaches to assess effects of mixtures start from effects and ignore the fact that toxic effects depend on time. Investigations addressing this fact explicitly are still scarce and the focus is on single timepoint endpoints instead. Even process based parameters like growth are often based one point estimates (see chapter two of this thesis). The CA/EA approaches are mainly used to find statistical interactions for binary mixtures (expressed as deviations from the standard models CA or EA) or to predict effects of a more complex mixture at some fixed point in time. Effects on growth, reproduction or survival for one single organism are interpreted separately because CA and EA do not offer a theoretical framework to incorporate these endpoints.

Already 1997 it was shown that interactions do strongly depend on time\(^4\), which was confirmed by this research (see chapter five). In addition it was shown\(^4\) that interactions at some point in time for binary mixtures are hardly reproducible experimentally. Furthermore we have shown (see chapter five) that small random varia-
tions in the experimental results can greatly influence the type of interaction that can be found in a mixture. But most important statistical interactions do not reveal the underlying mechanisms and allow extrapolation to different compounds, different points in time or even to different organisms. Shortcomings of the standard approaches were explicitly shown in chapter eight of this thesis where the CA/EA approaches were not able to make reliable predictions on the effects of mixtures as they can be found in the environment.

So if standard procedures that are based on single time point measurements are not reproducible, do strongly depend on time, can not incorporate effects on different endpoints within one consistent framework and do not allow extrapolations this calls for a different approach to the interpretation of effects of mixtures. Research on effects of mixtures is only meaningful if the underlying mechanisms can be understood and described. There are simply far too many mixtures available to experimentally assess only a tiny fraction of possible mixtures. In this research we present a mechanistic framework for the interpretation of effects of mixtures, based on dynamic energy budget (DEB) theory\textsuperscript{5,6}. This framework starts from the organism and interprets effects of toxicants in general as having an effect on the energy budgets of an organism. Organisms need energy to maintain themselves, to grow, or to reproduce. Toxicants affect the energy pathways dedicated to the different general processes in the organism, resulting in specific effects on growth and or reproduction depending on their toxic mode of action. In chapter three the general conceptual framework for the interpretation of effects of mixtures is presented. This includes effects on growth, reproduction or survival.

The approach is based on the effects of individual compounds from which the effect of the mixture is built up. This calls for a description of how individual compounds affect the survival of an organism when it is exposed to a toxicant. The focus of our research was directed on effects on survival, building up from effects of binary mixtures to effects of mixtures as they can be found in the environment (chapters four to eight). Effects of mixtures on survival are easiest to interpret and therefore also easier to extrapolate than effects on sub lethal endpoints. The basic assumption in our approach is that compounds first have to be taken up before they can exert an effect. Uptake and elimination are described by the one-compartment model, in which an organism takes up the toxicant from the environment by an uptake rate and excretes the toxicant to the environment with an elimination rate. Then we assume that death is a chance process, which can be described by the hazard model, resulting in a probability to die. The hazard rate can be written as:\textsuperscript{7}

\[
    h(t;c) = k_f(c(1 - e^{-kt}) - c_0)
\]
Where $h(t,c)$ is the hazard rate, $k_i$ is the killing rate, $k_e$ is the elimination rate, $c$ is the external concentration and $c_0$ is the No Effect Concentration (NEC). The hazard rate is integrated over time to obtain the survival probability. The NEC is the environmental concentration below which no effects occur, even after a prolonged exposure period. When applied to mixtures for each compound the hazard rate is calculated, these are combined to obtain the survival probability for the mixture (see chapter 5 and 6). In this approach if the NEC is zero the CA and EA models become the same.

The behavior of the NEC for mixtures was essential in this research. Implicitly it is assumed that each organism in a cohort has the same NEC and that it can be estimated from experimental data on the build up of toxic effects as can be found in e.g. a standard toxicity test for *Daphnia magna*. In real life organisms will not be exactly the same but are expected to each have their own NEC, so we first conceptually investigated how this influences the estimates of the NEC from a toxicity test (see chapter four). It proved that the estimate of a NEC is very robust, also if the variation is high. This allowed to proceed with effects on mixtures. We first tested our approach on the effects of binary mixtures (see chapter five). For binary mixtures it is difficult to obtain the behavior of the NEC from experimental data. This behavior becomes much more outspoken if more compounds are present in the mixture (see chapter 6 and 7). In chapter 6 we also showed that it is possible to use physical parameters of the compounds in a mixture to predict the effect of a mixture, assuming there are no interactions. We show that for narcotic compounds (over 60% off all toxic compounds have a narcotic mode of action) one would only need data on toxic effects of one component in a mixture to be able to predict the whole time course of the effect of the mixture. In chapter seven we show that the major assumptions of chapter six could be underpinned by experimental data. An experiment was designed and carried out which was specifically designed to research the behavior of the NEC for a mixture of supposedly similar acting components. In this chapter we show that using the best possible prior knowledge allows to reduce the experimental effort in assessing effects of mixtures and still allows to predict the whole time course of the effect of a mixture of four PAHs. The experimental effort for this mixture of four PAHs was comparable to that of a mixture of two compounds.

Being able to make reliable predictions for effects of mixtures under laboratory conditions allowed us to go one step further and to assess effects of complex mixtures as can be found in real life. We showed that it is possible to make reliable predictions on effects of mixtures containing pesticides, metals, PAHs, PCBs, nutrients and salts on the survival of in situ exposed *Daphnia magna*, see chapter eight. Here we show that the concept of a NEC is a very powerful means of assessing such a complex mixture, we also show in this chapter that effects of mixtures are of importance in real
life; mixtures can show effects in concentration ranges where individual compounds do not show effects (we predicted this in chapter six). This is something that is not specifically addressed in legislation, so in chapter nine we make a comparison of measured concentrations, No Effect Concentrations for the survival of daphnids with environmental standards in the form of maximum permissible concentrations.

REFERENCES


