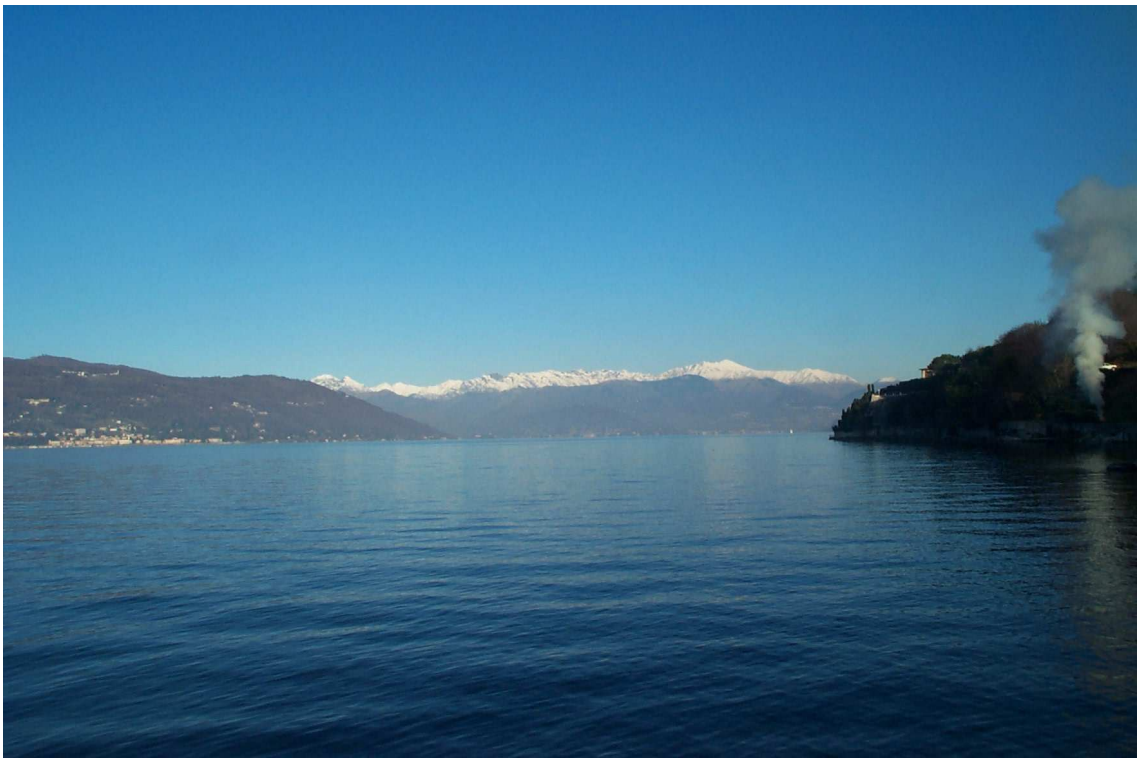
AN OVERVIEW OF CHEMICAL MIXTURES ASSESSMENT AND MODELLING IN THE AQUATIC ENVIRONMENT

Helena Maciel and José-Manuel Zaldívar

European Commission, Joint Research Centre, Institute for Environment and Sustainability, Inland and Marine Waters Unit, Ispra, Italy



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Summary

The number of chemicals produced and released to the environment is overwhelming. The impacts of a chemical depend on the released quantity, where it occurs, how chemicals are transported in the environment, how long they survive or persist, and how much toxic stress is exerted on ecosystems. Several of them - PAHs, PCBs and PBDEs, amongst others - are persistent and tend to bioaccumulate and biomagnify in the food web. For these reasons, strategies are needed to predict the effects of these compounds in the environment.

Inland and marine water pollution is, unfortunately, quite common. Whereas most of the studies and regulations focus on single compounds, waters are usually contaminated by a cocktail of chemicals. Mixtures exposure is the rule rather than the exception, indicating that exposure assessment and safety evaluation should focus on mixtures rather than on single chemicals. The fact that practically all studies and assessment have been concerned with the effect of individual chemical exposures makes the study of chemical mixtures a priority in risk assessment.

Mixtures can be of different types depending on the number and groups of chemicals present. Identifying and quantifying these chemicals is never an easy task even for simple mixtures. Furthermore, another problem arises: how will the chemicals behave in the mixture? In this case, there is a need to know the chemicals mechanism of action, which can constitute a major problem in complex mixtures where components of the mixture are not known beforehand.

In a mixture, chemicals may basically behave in two ways from a toxicological point of view: they can have a joint action or they can interact. In the first case they may act through independent action (IA), when the toxicity of the individual chemical is independent of the other compounds in the mixture, or by concentration addition (CA) when the overall toxicity equal the sum of the toxicity of the mixture. In the second case, the effects of the interaction may be antagonistic or synergistic. There is a general consensus that, in most of the chemical mixtures in aquatic environments, the toxicity acts according to concentration addition (CA) and, even for mixtures that have dissimilar modes of action (IA), at low concentrations, they still might behave according to CA toxicity approach.

To analyze and model mixtures different parameters need to be used, from biological (physiologically base toxicokinetics and toxicodynamics - PBTK and TD) to

physico-chemical properties, e.g. reactivity, octanol water partition coefficient, vapour pressure, etc. New models to assess mixtures behaviour focused on the pharmacokinetic and pharmacodynamic effects of the chemicals are needed to have a more precise prediction of potential mixture interactions and effects.

Current developments in molecular biology and chip technology will permit toxicologists to screen effects of chemical mixtures at molecular level. The use of genomic and proteomic technologies will dramatically increase the information on mechanisms of toxicity, and thus a much better understanding of combines-action it can be expected in the near future.

The objective of this review is to assess what is known on the effect of mixtures in order to develop ecological models that incorporate these effects in a coherent way, allowing for a correct description of the effects of exposure in aquatic ecosystems, and to develop thresholds for contaminant concentrations that incorporate the knowledge of the effects of chemical mixtures and are not only based on a single compound.

Contents

1. Introduction

The number, diversity and complexity of organic chemicals produced and released to the environment is overwhelming. Organic chemicals are ubiquitous and affect every possible aspect of modern life. Unfortunately, organic pollution of waters is common. The parameters that influence the partitioning of the organic chemicals in the environment include the physical and chemical characteristics of a compound, its behaviour with respect to chemical reactions or microbial degradation, and other physical conditions such as temperature, availability of water, light and oxygen (Walker *et al*., 1996). The major processes responsible for distributing synthetic organic chemicals throughout the biosphere are volatilization and atmospheric transport, transport to waters in soluble form or adsorbed to particles or movement through the food chain and environmental persistence.

Furthermore, also their physicochemical properties affect their biological activity at the subcellular level (site of action) such as cell membrane, microsomal enzymes, respiration and metabolism, which provokes the impacts on higher structure levels of a biological system. In this sense, the bioavailability is a key factor responsible for ecotoxicological effects of contaminants since only the bioavailable fraction induces ecotoxicological life effects (Fent, 2003).

Despite a large number of chemicals being produced commercially, there is only information regarding environmental fate and/or impact on human health for a small fraction (Swoboda-Colberg, 1995). However, developments in the knowledge of the way in which physicochemical properties of chemicals and physiological processes in the organism determine the compound's toxicity have greatly increased the understanding of toxicological processes and the ability to interpret experimental results (Blaauboer, 2003).

General effects of toxicants that can be observed by toxicity tests are the death of organisms, physiological change, behaviour change, growth, reproduction, genetic effects and cellular change (Manly, 2000). However, ecotoxicological effects occur at all levels of the biological organization, from the molecular to the ecosystem level.

The idea to apply an assessment of ecosystem health to environmental management emerged in the late 1980s (Jørgensen, 2005) with obvious parallels with human health. It is based on different phases: diagnosis (what is wrong?, which is the

cause of the unhealthy condition?), assessment and corrective measures. However, for the specific case of individual chemicals, quality objectives or criteria for protection of aquatic life were developed by national and international organizations (EIFAC/FAO, USEPA, EC) since the late 1960s. As early as the late 70s the problem of mixtures was recognized (Vighi *et al*., 2003). The need for quality objectives for mixtures of chemicals was stressed by research demonstrating that very low levels of chemicals may still be active, as they are additive at concentrations as low as 0.02 of the LC_{50} (Konemann, 1981a). In this sense, the Water Framework Directive (WFD, 2000/60/EC) applies the ecosystem health approach in the sense that one of its main objectives is the protection of aquatic life, with water quality status able to permit all stages in the life of aquatic organisms to be successfully completed, and not allowing conditions that alter the functioning of the ecosystem.

Aquatic organisms are rarely exposed to only one single contaminant, but typically to mixtures of numerous man-made-chemicals with varying constituents in varying concentrations and concentration ratios (Faust *et al*., 2003). However, in contrast to this environmental reality, the toxicological reality is that until recently about 95% of the resources in toxicology were devoted to studies on single chemicals (Groten, 2000). Nevertheless, toxicity data from laboratory tests with single pure chemicals provide essential input to scientific assessments of chemical risks to aquatic life. On the other hand, the behaviour of chemicals in a mixture may not correspond to that predicted from data on the pure compounds (Altenburger *et al*., 2003). But the direct testing of all the potential combinations of water contaminants is unfeasible, and thus we are confronted with the task of deriving valid predictions of multiple mixture toxicity from toxicity data on individual compounds (Faust *et al*., 2003).

Therefore, combined exposure is a reality that dictates the necessity to pay a great deal of attention to hazard identification, exposure assessment and risk characterization of mixtures at individual as well as ecosystem level.

The objective of this review is to assess what is known on the effect of mixtures in order to incorporate these effects in the ecological models we are developing to assess the effects of chemical contaminants in a coherent way, allowing for a correct description of the effects of exposure in aquatic ecosystems. Furthermore, the development of thresholds for contaminants concentrations should incorporate the knowledge of the effects of chemical mixtures and should not only based on a single compound result.

2. Chemical Mixtures

The main concerns regarding water pollution have mainly focused on surface waters in rivers and lakes (Baird, 1998). This, in principle, is justified since a large number of xenobiotic organic are loaded into these systems. These compounds are generally found at low concentrations; nevertheless, in combination they may cause severe biological effects as many of the identified compounds are highly toxic or even carcinogenic (Schrab *et al*., 1993).

Let us adopt, in this work, the US EPA definition of chemical mixtures (U.S. EPA 2000) as either:

a/ Simple mixtures: Mixtures containing more than two identifiable components which toxicity can be adequately characterized by a combination of its components toxicities and interactions

b/ Complex mixtures: Mixtures containing so many components that the above cited approach contains too much uncertainty.

Even though the aquatic environment is exposed both simultaneously and sequentially to a wide variety of compounds, regulatory limit values are generally set for single compounds (Foster *et al*., 2005). The Scientific Advisory Committee on Toxicity and Ecotoxicity (CSTE) of the European Commission defined Water Quality Objectives (WQO) for the protection of aquatic life as follows (CSTE/EEC, 1994):

- should permit all stages in the life of aquatic organisms to be successfully completed;
- should not produce conditions that cause these organisms to avoid parts of the habitat where they would normally be present;
- should not give rise to the accumulation of harmful substances;
- and should not produce conditions that alter the functioning of the ecosystem.

The general approach actually followed to define WQO consists on evaluate the NOEC (No Observable Effect Concentration) which is defined (Vighi *et al*., 2003) as the highest concentration actually tested within a specific test procedure at which the response of the exposed organism cannot be significantly distinguished from the response of untreated control organisms. However, the question that arises is if at these concentration values, below NOEC, a mixture of compounds is able to produce a significant effect. There is a need to develop water quality objectives not only for

single substances but also for mixtures of chemicals. However, the toxicity of a mixture, depends not only on the exposure concentration of each mixture constituent and its ratio but also on the means of the toxicants to act jointly (Greco *et al*., 1995). Some of the most challenging problems that toxicologists confront are: determining how biological effects of components in a complex mixture may interact; determining how these interactions affect the overall toxicity of the mixture; and determining how to incorporate this information into risk assessments of chemical mixtures.

2.1 Assessing the behaviour of mixtures

Many experimental set-ups can be used to achieve toxicological knowledge on mixtures with a restricted number of test groups. The simplest way to study effects of mixtures is to compare the effect of a mixture with the effects of all its constituents (at comparable concentrations and duration of exposure at one dose level without testing all possible combinations of chemicals). This requires a minimum number of experimental groups (n+1, the number of compounds in a mixture plus the mixture itself). However, a mixture should, preferentially, be tested both at high and at low (realistic) concentrations (Groten, 2000).

Recently regulatory guidance for conducting mixture risk assessments encourages the use of mechanistic information about individual chemicals to select models for predicting the dose response characteristics of a mixture (Borgert *et al*., 2004).

Different biological concepts, have been used to determine the extent to which chemicals exhibit similar mechanistic features: mode of action is defined as a common set of physiological and behavioural signs that characterize a type of adverse biological response, while a toxic mechanism refers to the crucial biochemical processes and/or xenobiotic-biological interaction underlying a given mode of action (Rand *et al*., 1995).

To apply a common quality objective, chemicals should be grouped based on a known similar mode of action. However for a large number of chemicals this information is not available (Vighi *et al*., 2003).

Traditional chemical analysis, frequently used to identify the chemical composition of a contaminated environment, can only provide information of the compounds present and their concentration. This provides no indication as to the effects of the chemical mixtures to the ecosystem. Chemical analysis of samples from contaminated sites will provide information about total amounts of specific pollutants,

but they provide no indication about the bioavailable fraction (exposure). Furthermore, even if toxicity data on individual compounds are available, there is still the problem of extrapolating the results obtained for high exposure concentration in laboratory to ecosystems/species being exposed to lower concentrations.

Mixtures toxicities can be assessed using single species or multispecies tests. Mixture toxicity is generally assessed with single-species tests, but in the contaminated ecosystem the mixture of chemicals interacts with a multitude of species in their ecological context. The abundance and activities of the different species as well as their individual sensitivities influence mixture toxicity. In addition, the variability of the target sites is most likely higher in a biotic community than in a population of only one species, resulting in flatter response curves (Vighi *et al*., 2003). Furthermore, toxicants in aquatic ecosystems are clearly capable of causing a variety of indirect ecological effects that can be more significant than the direct (toxic) effects of a contaminant (Fleeger *et al*., 2003).

Finally, it is important to distinguish between simple mixtures and complex mixtures (Groten, 2000). In the later case there is no assessment procedure in place.

3. Modelling mixtures toxicity

Organisms in the aquatic environment are readily exposed to chemical mixtures, and risk assessment has therefore to account for the occurrence of diverse contaminants with different toxic potentials. However, occurrence of pollutants may not signify contribution to toxic effects.

The identification of relevant contributors to observed mixture effects in site-specific assessment would offer scope for measures targeted at toxicity reduction, which is of particular interest for costly remediation efforts (Altenburger *et al*., 2004). Realistically, the testing of all chemical mixtures and possible environmental concentrations is not viable. As a consequence, different models on mixture toxicity based on the toxicity of single compounds have been developed. The objective is to reduce the amount of experiments and to be able to predict mixtures toxicity. As we will show later on, the main draw back associated with this approach is the attribution of a correct mechanism/mode of action to the involved chemicals.

3.1. Modelling toxicity of single compounds

One of the most important concepts used in toxicology to determine risk assessment and regulation is the dose-response relationship for which several models have been used. In the past, the most used approach was to consider a linear function with or without threshold, i.e. at increasing concentrations there is an increase in the response and nonlinear with saturation at 100%. Actually, dose-response curves of single chemicals are fitted to sigmoidal shape curves with values between 0-1 (0-100%). Several models have been proposed in literature (Backhaus *et al*., 2004), between them:

- Weibull:

$$f(x) = 1 - \exp[-\exp(\theta_1 + \theta_2 \log_{10} x)] \quad (1)$$

- Box-Cox transformed Weibull:

$$f(x) = 1 - \exp\left[-\exp\left(\theta_1 + \theta_2 \frac{x^{\theta_3} - 1}{\theta_3}\right)\right] \quad (2)$$

- Morgan-Mercier Flodin:

$$f(x) = 1 - \frac{1}{1 + \theta_1 \cdot x^{\theta_2}} \quad (3)$$

- Logit

$$f(x) = \frac{1}{[1 + \exp(-\theta_1 - \theta_2 \log_{10} x)]} \quad (4)$$

- Generalized Logit:

$$f(x) = \frac{1}{[1 + \exp(-\theta_1 - \theta_2 \log_{10} x)]^{\theta_3}} \quad (5)$$

where θ_1, θ_2,and θ_3 are parameters of the equations. As said before, normally the functions have a lower (L) and upper (U) asymptotes with values of 0 and 1. However, in some cases, at low concentrations chemicals shown stimulating effects (hormesis effect) having an U-type shape in the lower part of the concentration-response relationship (Calabrese and Baldwin, 2003). In this case, it is possible to move along the y-axis the function using the following expression:

$$F(x) = L + (U - L) f(x) \quad (6)$$

However, the U-type shape form cannot be reproduced with this approach (Backhaus *et al*., 2004).

Figure 1 summarizes the different functions that have been considered for dose-response, whereas in fig. 2, the individual concentration response curves for algal toxicity obtained by Faust *et al* (2003) are shown.

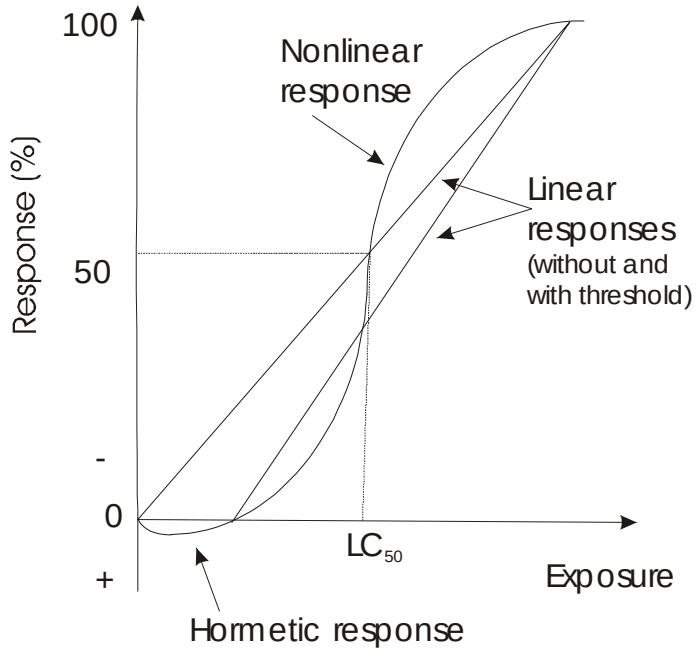

Figure 1. General dose-response functions: a/ linear with and without thresholds and nonlinear with hormesis.

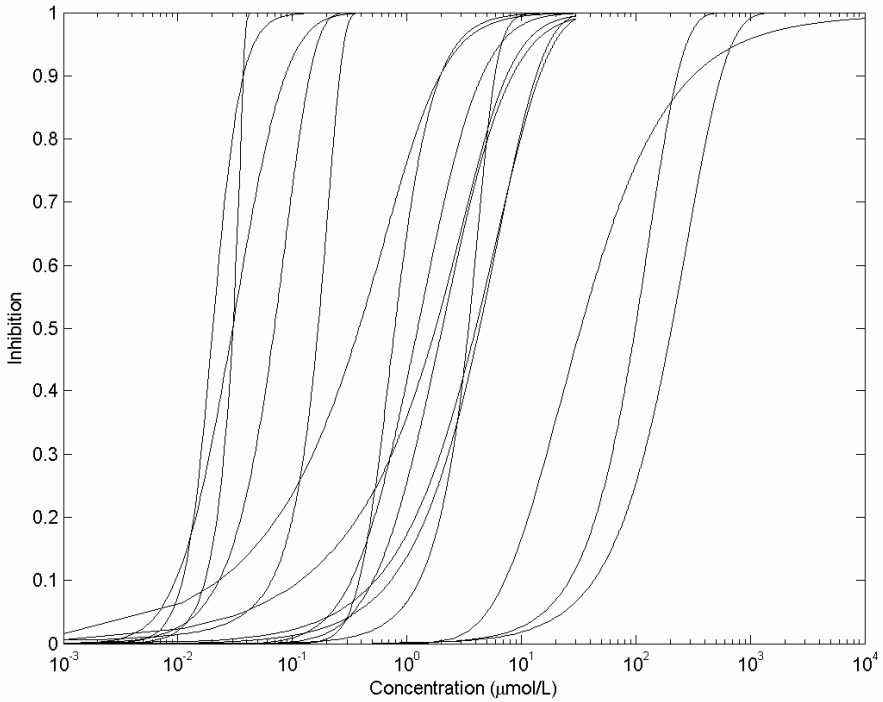

Figure 2. Individual concentration response curves for the algal toxicity of 16 dissimilarly acting chemicals (Norflurazon, Aclonifen, DTMAC, Terbuthylazine, Metazachlor, 8-Azaguanine, Paraquat dichloride, CCCP, Azaserine, Kresoxim-methyl, Triadimenol, Metsulfuron-methyl, Fenfuram, Chloramphenicol, Nalidixic acid, Metalaxyl. Fitting functions from Table 4 in Faust *et al*. (2003).

3.2. Joint Action (Non-Interactive) and Interaction models

Even though early toxicological studies were devoted to the characterization on single chemicals, Bliss defined in 1939 several categories of chemical action, which are still relevant (Dybing *et al.*, 2002). These are: Concentration Addition (CA), Independent Action (IA) and interactions.

a/ Concentration Addition (CA): Assumes that the components in the mixture have a similar action but differ only with respect to their individual potency. Introduced by Loewe and Muischnek (1926), it is also know as Loewe additivity, simple joint action or dose addition. This may be expressed in terms of toxic units (TUs) which are the ratio of the concentration *i–th* substance in the mixture to the concentration needed to provoke a certain effect (Backhaus *et al*., 2004):

$$TU_i = \frac{C_i}{ECx_i} \qquad (7)$$

whereas C_i is the concentration of toxicant i in the mixture producing x% effect (e.g. EC50). Therefore the overall toxic unit, for a mixture with n components, is equal to:

$$TU_{mix} = \sum_{i=1}^{n} TU_i = \sum_{i=1}^{n} \frac{C_i}{ECx_i} = \frac{C_{mix}}{ECx_{mix}} = 1 \qquad (8)$$

Individual concentrations can be expressed as constant proportions p_i of the total concentration C_{mix}, with $p_i=C_i/C_{mix}$. In order to calculate the ECx_{mix}, this equation can be re-written as:

$$ECx_{mix} = \frac{1}{\sum_{i=1}^{n} \frac{p_i}{ECx_i}} \qquad (9)$$

The concentration addition is the most common approach to risk assessment of mixtures and it is applicable over the whole range of exposure levels from low non-toxic levels when all chemicals in the mixture act in a similar way (Feron and Groten, 2002).

In concentration addition the components of the mixture exerting their effect via membrane perturbation as narcotic toxicants only or if the concentrations of specifically acting compounds are so low that only these baseline toxicities contribute to an overall effect (Escher and Hermens, 2002). This is the case of the studies on s-triazine mixtures on algal toxicity reported by Faust *et al*. (2001) and (2003) or for the application of toxic equivalency factors (TEF) used to describe the combined toxicity of isomers or structural analogues such as dioxins or PCBs (Dybing *et al.*, 2002) where the total potency of the combined occurrence is calculated as the sum of the concentration of each individual congener multiplied by its specific. Also toxicity of PAHs (Fent and Batscher, 2000; Ankly *et al.*, 1996; Birnbaum and DeVito, 1995; Calamari and Vighi, 1992; Konemann, 1981b), also confirmed by Swartz *et al*. (1997) and Erickson *et al*. (1999) for phototoxic PAHs. Furthermore, a number of models have been proposed to predict the toxicity of mixtures to organisms, all of which are generally based on the concept of additivity (Konemann, 1981b, Ribo and Rogers, 1990; Stratton, 1988 and Stratton, 1989).

However, it is important to considerer that the mode of action of a certain group of chemicals may only be the same for a particular species and therefore it may be not possible to generalize to other organisms.

b/ Independent Action (IA): IA, also known as Bliss independence (Bliss, 1939) and response addition (Greco *et al*., 1995), is based on a the concept of statistically independent distribution of the sensitivities of the individuals towards the toxicants. In

this case, it is assumed that the joint probability, p^s_{mix}, that an individual survives a concentration, $C_{mix} = \sum_{i=1}^{n} C_i$, is given by:

$$p^s_{mix} = 1 - \prod_{i=1}^{n} [1 - p^d(C_i)] \quad (10)$$

whereas the probability of dying p^d is the complementary of the survival probability, i.e. $p^d = 1 - p^s$. Although, originally it was formulated for mortality/survival analysis, it can be applied in dose-response analysis as:

$$ECx_{mix} = 1 - \prod_{i=1}^{n} (1 - ECx_i) \quad (11)$$

IA predicts that a mixture of chemicals will not exert an adverse effect when individual chemicals in that mixture are present below their individual No Observable Adverse Effect Level (NOAEL). According to USEPA, 2000, IA should be used for mixtures of chemicals that produce the same toxic effect in the same target organ, but which do so by dissimilar mechanisms of action (Borgert *et al*., 2004).

Both approaches have shown their validity (Faust *et al*., 2001; Faust *et al*., 2003;Vighi *et al*., 2003, a.o.), CA when used for chemical mixtures with similar action and IA when used for chemical mixtures with dissimilar action. Combination of both approaches has been also attempted (Altenburger *et al*., 2004). Although both models (CA, IA) involve summing, either the component doses or their toxic effects, differences between models may produce large differences in the risks estimated for a particular mixture. However, with a regulatory perspective, i.e. worst case, CA may be defendable as a pragmatic assumption by default since normally high mixture toxicity is predicted. Alternatively, the use of QSAR criteria was proposed by Vighi *et al*. (2003) to classify the substances as supposedly similarly or dissimilarly acting when no information is available.

c/ Interactions: In any case, both proposed approaches (CA, IA) to evaluate joint toxicity are “non-interaction” approaches, that is, they assume that chemicals are simply additive, and neither synergistic nor antagonistic, when combined in mixtures (Borgert *et al*., 2004). Several approaches have been proposed to take into account the interactions between chemicals to describe their combined effect that may result in a stronger effect (synergism, potentiation) or weaker effect (antagonism, inhibition) than expected on the basis of either CA or IA.

Antagonistic effects were explained by Escher *et al*. (1996), at the molecular level, by competition for sites in the membrane that may decrease toxicity. Synergistic effects can be explained by damage in the cell membrane. Organic solvents, in particular, will affect the membrane permeability and cause proton leak leading to uncoupling (Escher *et al*., 1999; Lewis *et al*., 1994). In order to study these effects mechanistic studies have shown (Andersen and Jennison, 2004) that interactions should be described at the level of target tissue dose and are best categorized as either pharmacokinetic (PK) or pharmacodynamic (PD). PK interactions occur when the presence of other chemical alter the relationship between the applied dose and the target tissue dose of a compound.

PD interactions occur when the presence of a second chemical alters the relationship between target tissue dose and tissue response.

Joint or interactive effects of a mixture observed at a clearly toxic-effect-levels of the individual chemicals in the mixture do not predict the joint or interactive effects of the mixture that might occur at exposure levels of the mixture similar to or lower than the highest no-toxic-effect-levels of the individual chemicals. This conclusion is highly relevant for designing further toxicity studies of mixtures as well as for low dose extrapolation of mixture toxicity data (Feron and Groten, 2002).

All three basic principles of joint action and interaction are theoretical. In reality, however, it is likely to have to deal with these concepts at the same time, especially when mixtures consist of more than two compounds and when the targets (individuals rather than cells) are more complex (Groten, 2000).

A frequent goal in mixture toxicology is primarily to determine situations where the effects of combinations of chemicals differ from the additive effects of the chemicals given individually. A great deal of effort has focused on creating various statistical methods for assessing when differences from additivity become significant and on identifying potentially important interactions that would change perceptions of the risks of mixtures of chemicals (Andersen and Dennison, 2004).

3.3. Calculating mixture's toxicity from individual components

Concentration response curves for single substances describe the intensity of a defined effect as a function of the toxicant concentration, see fig.2. Similar curves can be

obtained for mixtures when the ratio of the concentrations of the individual components is kept constant and only the total concentration is varied.

For the case the assumed action mechanism is CA and we are interested in calculating the total effect caused by a mixture there is an iterative procedure where the function:

$$error = \left(1 - \sum_{i=1}^{n} \frac{C_i}{f_i^{-1}(E(C_{mix}))}\right)^2 \tag{12}$$

has to be minimised. The procedure consists on defining an effect (E) and a mixture concentration C_{mix}, then calculate the individual concentrations that will produce this effect using the inverse of Eqs. (1-5). For example for the Box-Cox-Weibull (BCW), we will have:

$$f_i^{-1}(E(C_{mix})) = \left[1 + \frac{\theta_3}{\theta_2}(\ln[-\ln(1-E)] - \theta_1)\right]^{1/\theta_3} \tag{13}$$

Then the Eq. (12) is calculated and the procedure repeated by changing the mixture concentration until the error is minimized. Figures 3-4 show two examples for two mixtures of dissimilarly toxicants selected by Faust *et al.* (2003).

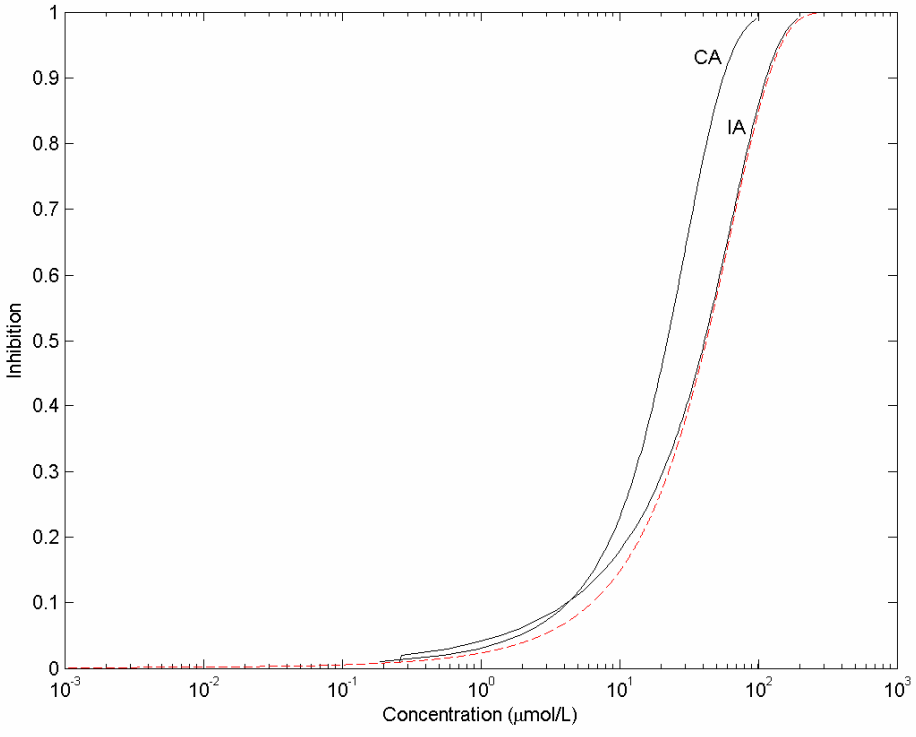

Figure 3. Observed and predicted (CA and IA) algal toxicity of the mixture of 16 dissimilarly acting substances with components mixed in the ratio of their EC50 values (Faust *et al.*, 2003; Table 5). Discontinuous red line: fitted experimental values (Faust *et al.*, 2003; Table 6).

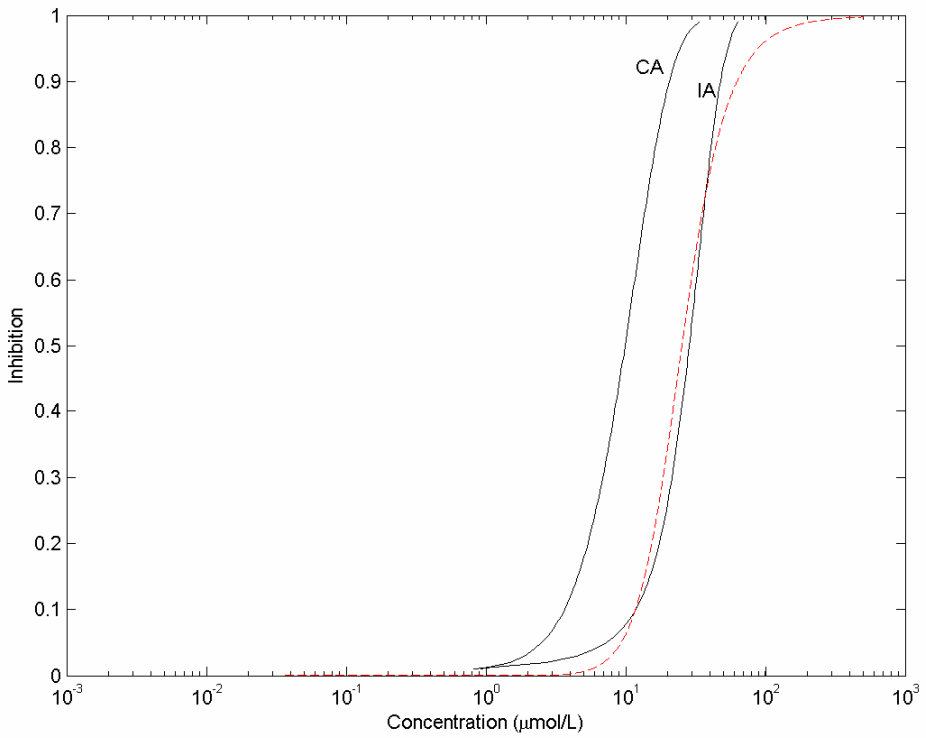

Figure 4. Observed and predicted (CA and IA) algal toxicity of the mixture of 16 dissimilarly acting substances with components mixed in the ratio of their EC1 values (Faust *et al.*, 2003; Table 5). Discontinuous red line: fitted experimental values (Faust *et al.*, 2003; Table 6).

The procedure in the case of IA also requires iteration. In this case the error to minimize is:

$$error = \left[x\% - 1 + \prod_{i=1}^{n} (1 - f_i(p_i(ECx_{mix}))) \right]^2 \qquad (14)$$

whereas the total effect is x%. In this case one defines a total effect and a mixture concentration, then calculates the individual effects of each component in the mixture at their specific concentration and evaluates Eq. (14). The procedure is repeated until the appropriate mixture concentration is obtained. Figures 3-4 show two examples for mixtures of dissimilarly acting compounds. In these two cases IA gives better results, since the chemical mixture was specifically chosen from dissimilarly acting substances.

Ii is generally accepted that for dissimilarly acting toxicants, IA will produce a better fit of the mixture toxicity (Backhaus *et al*; 2000; Faust *et al.*, 2003; a.o.), whereas in the case of similarly acting chemicals CA will adjust more accurately the experimental results (Konemann, 1981b; Calamari and Vighi,1992; Altenburger *et al*.

2000; Faust *et al*., 2001; a.o.). However, with a regulatory perspective, i.e. worst case, CA by predicting higher toxicity (see figs. 3-4) seems a more pragmatic option (Vighi *et al*., 2003). In any case, no-interactions have been assumed to occur in these two approaches so interactive aquatic toxicity is not taken into account (Gunatilleka and Poole, 1999). Thus although the additivity models are mathematically simple, they require assumptions about the mechanisms of action (only similar or dissimilar) and the high to low dose extrapolation. Therefore theoretical considerations in risk assessment of chemical mixtures should be verified by simple case studies (Groten, 2000).

General toxicity refers to narcosis that acts by non-specific disruption of the proper functioning of the cell membrane (generally thought of as the site of action). Compounds exhibiting narcotic toxicity are not reactive and do not interact with specific receptors in an organism (Verhaar *et al*., 1992). Specific toxicity refers to reactive toxicity that is realized through disruption of the function of a defined receptor site in the cell (Gunatilleka and Poole, 1999).

Effects of mixtures usually exceed those of the most active constituents alone. As a consequence, risk assessment procedures for contaminants in aquatic systems may no longer be restricted to single pure contaminants, but have to be considered combined effect resulting from multiple chemical exposures.

Typically aquatic environmental concentrations are lower than the concentrations that cause statistically significant effects in laboratory toxicity tests, they are below NOEC. Whether such low concentrations are relevant for a predictive mixture toxicity assessment is a controversial issue (Faust *et al*., 2003). Hence the relevance of low concentrations for the predictive assessment of mixture toxicity is a critical point (Konemann and Pieters, 1996). Under the assumption of concentration addition any concentration of any mixture component is expected to contribute to the overall toxicity of a mixture; there would be no threshold concentration other than zero. Under the Independent action the situation is different. Only those concentrations of individual toxicants that cause individual effects greater than zero are expected to contribute the overall toxicity.

3.4. QSARs in mixture toxicity

In this context, the purpose of QSAR (Quantitative Structure-Activity Relationships) techniques was to predict the toxicity of individual chemicals from their

physicochemical properties. Hence relationships between toxicity and these properties have been developed (Veith and Konasewich, 1975).

For QSARs, chemicals can be classified in four categories (Verhaar *et al*., 1992); the classification into one of these classes relies on the presence or absence of certain structural or substructural features:

- Class I: Non-polar narcotics
- Class II: Polar narcotics
- Class III: Reactive compounds
- Class IV: Specifically acting compounds (e.g. pesticides)

Class I: Narcosis & Baseline Toxicity

Narcosis in aquatic organisms is defined as non-specific reversible disturbance of the functioning of the membrane, caused by accumulation of pollutants in hydrophobic phases within the organisms (Albert, 1965; Crisp *et al.*, 1967 and Veith *et al.*, 1983). Narcosis is directly linked to the hydrophibicity of a compound (van Wezel and Opperhuizen, 1995). The cellular membrane (lipid phase) in the aquatic organisms is the most likely target (van Wezel and Opperhuizen, 1995). The disturbance of membrane function results in decreased activity and a diminished ability to react to stimuli, ultimately leading to death.

Narcosis is considered to correspond to the minimal level of toxicity that will be exerted by a chemical. Accordingly narcosis is also referred to as “baseline toxicity” (van Wezel and Opperhuizen, 1995). Nonpolar narcotic compounds are chemical unreactive in biological systems. A variety of chemical substances are classified as nonpolar narcotics and are enumerated in Konemann (1981a) and Veith *et al*. (1983).

Class II: Polar narcosis

There are many apparent narcotic chemicals that are more toxic than baseline narcosis predicts-these are referred to as the polar narcotics. Often the presence of a strong hydrogen bonding group on the molecule is the causal agent of greater toxicity (van Wezel and Opperhuizen, 1995).

Class III: Reactive compounds

In class III, compounds have toxicity values considerably greater than those predicted for either non-polar or polar narcosis. Reactivity can be related to several processes including competing electron-and nucleophilic, redox, and free radical processes (Cronin and Dearden, 1995).

Class IV: Specific mode of action

In class IV, compounds act by specific mechanisms of toxic action, i.e. molecules that exert their toxic action at a known and specific site, for example binding irreversibly with a receptor or disrupting enzyme systems, hence they give much higher toxicity than predicted baseline toxicity (Cronin and Dearden, 1995)

The fundamental descriptor, of physico-chemical properties, in aquatic acute toxicity is the logarithm of the *n*-octanol-water partition coefficient (log K_{ow}). However if specific types of interactions occur, and then can be electronic or steric, logK_{ow} will not be sufficient. However, only a profound understanding of the underlying mechanism and appropriate assignment of chemicals to a mode of action, or even to a mechanism makes it possible to choose the right descriptors for QSARs and to define the chemical domain appropriately (Escher *et al*., 2002).

The QSARs have been used to predict concentrations of components in mixtures from joint effects and defined mixtures ratios and have been developed to predict narcotic-type mixture toxicity from molecular descriptors that are calculated as composite properties according to the fractional concentrations of the mixtures components (Altenburger *et al*., 2003). Furthermore, QSARs were suggested to be used, when the knowledge of toxic action is not available and/or the mixture contains chemical acting with similar and dissimilar modes. The general hypothesis is that chemicals with similar structure patterns and responding to the same QSAR model would have a similar mode of action (Vighi *et al*., 2003).

3.5. PBTK and TD models

In the past 15 years physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modelling has been applied to the toxicological interactions of chemical mixtures, frequently with the name Physiologically based toxicokinetic (PB-TK) and toxicodynamic (TD) models. These models simulate the distribution of contaminants throughout an organism as estimate the rate of accumulation in target organs using processes such as gill exchange, bivalve filtration rates or temperature-dependent metabolic rate (Newman, 1995).

Mechanistic studies have shown that interactions should be described at the level of target tissue. These interactions may be pharmacokinetic (PK) or pharmacodynamic (PD). In PK interactions the presence of another toxicant alters the relationship between the applied dose and the target tissue dose of a toxic compound

whereas PD interactions occur when the presence of other toxicants alter the relationship between target tissue dose and tissue responses (Andersen and Dennison, 2004).

PB-TK modelling is used for studying possible interactions in the toxicokinectic phase. The models are based on the anatomy, physiology, and biochemistry of the exposed organism and are used to generate chemical concentration time-course predictions for specific tissues and organs (Dixit *et al*., 2003 and Nichols *et al*., 1994). This information can in turn be related to research on a compound's mechanism of action to improve understanding of relationships between applied dose and observed effect. These models are able to quantify exposure in terms of an absorbed dose (Lien *at al*., 2001). If combined with dynamic aspects via physiologically based toxicodynamic (PB-TD) modelling, a more or less complete picture is obtained (Oberemm *et al*., 2005). Escher *et al*. (2002) defend that the use of PB-TK and TD modelling will give insights into rate limiting steps in, and theoretically based mathematically model of, the whole chain of events from external dose of observable effect.

These models have become important tools in the study of mixtures for predicting conditions under which interaction are likely to alter the assumption of additivity and have permitted calculation of interaction thresholds with more confidence (Andersen and Dennison, 2004). Furthermore, they are being used in risk assessment to allow extrapolation from high dose to low dose, from one route to another route and from one species to another species, including man (Oberemm *et al*., 2005).

Recently, reaction network modelling has been added to describe metabolic pathways of complex mixtures in biologic systems, Liao *et al*. (2001).

4. Ecological models in ecological risk assessment of mixtures

In a recent survey carried out by Fleeger *et al*. (2003) the effects of contaminants in aquatic ecosystems were divided in direct and indirect effects. Direct effects from the release of a pollutant into aquatic habitats vary with intensity and duration of exposure and are frequently studied as a part of the estimation of risk of a certain chemical and the establishment of a threshold value for its permissible concentrations. These studies are normally based on laboratory toxicity tests using model species responses to a single contaminant exposure. Direct effects typically reduce organisms' abundance, e.g. increase of mortality, reduction of growth rate or fecundity, etc. However, pollutants may have other effects even on tolerant species by other ecological mechanisms, e.g. direct influences of contaminants on predators can lead to cascading indirect effects on resistant species in other trophic levels by altering competitive interactions and therefore modifying substantially its abundance and dynamical behaviour. Such effects are called indirect (or secondary) contaminant effects (Flegger *et al*., 2003) and sometimes can be as or more significant that the direct (toxic) effects of a contaminant.

Ecological models have become effective tools in evaluating direct and indirect effects, estimating and to managing ecological risks (Bartell, 1996; Pastorok *et al*., 2003). In addition, ecological models may be applied to forecast future potential risks or to estimate risks when field experiments cannot be performed, i.e. the release of a new chemical into the environment. They are useful tools for testing alternative hypothesis or to reconstruct past situations where evidence of toxic exposure cannot be demonstrated.

The main issue normally when introducing contaminants in ecological models is to infer likely the impact on the ecosystem and populations of the toxic effects observed at the individual level. Traditionally, mortality (survival rate) of organisms has been used as the ecological effect of toxicant stress in risk assessment (Bartell, 1996; Lopes *et al*., 2005, a.o.) but other approaches have been appeared recently. For example, Tanaka (2003) proposed the application of population vulnerability analysis (PVA) which consists on estimating the probability of extinction by evaluating the adverse effects of pollutant chemicals on the intrinsic rate of natural increase, r (Ginzburg *et al*., 1982).

Bartell (1990) has proposed an iterative process to analyse ecosystem (population) response using toxicological data. This approach is illustrated in fig. 5. In this sense, the risk is evaluated as the probability of detecting a specific change in an annual integrated biomass of a population of interest (O'Neil *et al*., 1982). In order to carry out this approach, it is necessary to translate single-species toxicity data to elements of an effects matrix which will in turn modify growth rates, mortality, etc. in the modelled processes in the phytoplankton, zooplankton, bacteria, etc. modules contained in the ecological model. This is a necessary step to translate toxicological effects on single population dynamics. Then this combined matrix effects is produced and the total effects at ecosystem level are evaluated. Monte Carlo approach may also be used at this level to assess uncertainty not only in the model equations but also in the environmental factors.

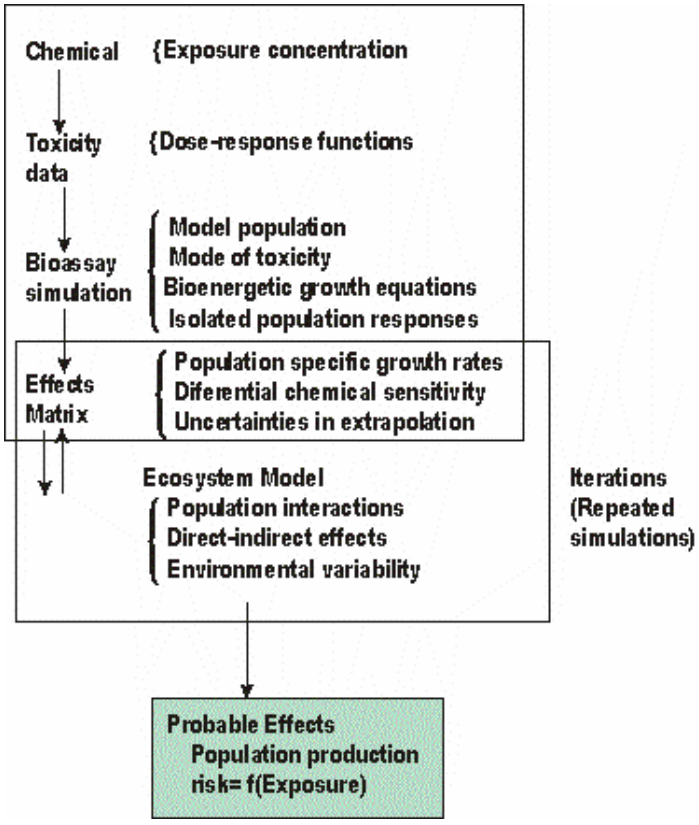

Figure 5. Estimating the ecological risk for a given chemical exposure from toxicity data. The assay simulations produce an effects matrix that modifies growth rates for populations in the food web model. The population effects from several simulations are used in calculating the risk (modified from Bartell, 1999).

5. Future directions in mixture research

To consolidate the scientific foundation of mixture toxicology, studies are in progress to re-examine the biological concepts and mathematics underlying formulas for low-dose extrapolation and risk assessment of chemical mixtures: There is a need for both a better mathematical basis for combination rules that predict effects of mixtures and a fundamental biological concept that supports quantitative formulas for risk assessment of chemical mixtures.

New developments in mixture research include the production of new computer programs applicable to mixture research, the application of functional genomics and proteomics to mixture studies, the use of nano-optical sensors for in vivo imaging of physiological processes in cells (Feron and Groten, 2002).

Much of the toxicology work performed focuses narrowly on specific endpoints or measures of responses. To the same end point, several modes of action may be connected. Due to recent advances in the application of genomics (proteomics, metabolomics, etc.), toxicology is developing new methods to measure mixtures toxicity based on chemical alterations in gene expression, i.e. toxicologists have the ability to evaluate changes in almost every gene product in the cell simultaneously. There is a requirement for the ability to see how individual compounds affect signalling and how mixtures affect a common physiological endpoint by either similar or dissimilar modes of action in the body. It is expected that, by detecting changes in gene expression, lower levels of exposure will be detected and early toxicity alterations monitored. Clearly, validation studies are necessary.

The use of gene expression technologies such as microarrays (Lettieri, 2005) seems a suitable technique to detect common or independent effects of chemical mixtures. This will allow a better understanding in the way total mixture toxicity may be calculated, i.e. CA, IA, mixed.

Future advances in mixture research will depend on progress in systems biology, a discipline that integrates information across multiple level of biological organization producing PD models of normal function and assessing under which exposures to chemicals lead to the perturbations sufficiently great to produce toxicity and disease(Andersen and Dennison, 2004).

6. Conclusions

Society is faced with the enormous task to assess numerous chemicals and complex chemical mixtures while protecting many different species and the diversity of ecosystems (Escher *et al*., 2002). The task of performing risk assessments on mixtures is overwhelming because they may contain hundreds of different and unique chemicals that cannot practically be assessed individually (Foster *et al*., 2005). Further, the physicochemical properties of these components and their relative proportions in the mixture may not be adequately known.

The concentration addition concept is the most common approach in risk assessment of mixtures. Concentration addition is easier to handle than independent action but tends to overestimate the joint toxicity of dissimilarly acting chemicals. With a regulatory perspective, however, these overestimations appear to be minor and hence concentration addition may be defendable as a pragmatic approach and a precautionary default assumption (Faust *et al.,* 2003). Nevertheless, its use is only justifiable from a scientific point of view, when all chemicals in the mixture act in the same way, by the same mechanism, and thus differ only in their toxic potencies. Hence, concentration addition models should be used to assess chemicals exhibiting similar mechanistic features and independent action models to assess chemicals exhibiting dissimilar mechanistic features (Borgert *et al*., 2004). However, it may be assumed that mixtures actually occurring in the environment are seldom composed entirely of similarly or dissimilarly acting chemicals (Vighi *et al.*, 2003).

Further conclusions can be withdraw from the literature:

- Environmental toxicologists should focus on the low-dose region of the dose effect curves - It appears that interactions are less plausible at low doses, however, dose additivity cannot be excluded (Konemann and Pieters, 1996).
- The lack of knowledge, about mechanisms of toxic action of the majority of environmental pollutants, complicates the classification of environmental pollutants as similarly or dissimilarly acting and hence may be an obstacle to the choice of the most suitable concept for predicting mixture toxicity (Faust *et al.,* 2003) - The knowledge of modes of action in ecotoxicology would be useful in setting up models and avoiding pitfalls in applied environmental risk assessment of chemicals and polluted sites (Escher *et al.,* 2002).

- The toxicity assessment of mixtures is made mainly based on the toxicity of single species, the question remains if it is valid for biologically more complex systems: Preliminary findings from mixture toxicity analyses with multi-species algal communities indicate that the applicability of the concept of concentration addition for similarly acting agents is not generally restricted to single species test (Blanck, 1999).
- Currently pharmacokinetic and pharmacodynamic models have provided a more mechanistic basis for chemical risk assessment and permitted more confidence in extrapolation to lower doses and across species (the impact of exposures to mixtures of chemicals) (Andersen and Dennison, 2004). Biokinetic modelling and toxicodynamic modelling allow the estimation of a compound's critical amount/concentration on the critical site of action, which ideally would be the basis for hazard and risk assessment (Blaauboer, 2003).
- Either QSARs and PBTK/TD models can be used for predictions in mixtures of joint action (CA or IA) or interactive.

In general, a good predictability of the toxicity of chemical mixtures can be assumed, however, one major question within the context of water quality objectives remains: what happens if the components are present in concentrations below their individual water quality objectives? Is there still a combined effect expectable and detectable (Vighi *et al.*, 2003).

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Appendix A. PAHs, PCBs and PBDEs- General

Polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are important environmental contaminants because of their persistence, bioaccumulation and widespread occurrence. They are present in a diverse number of materials and are widely used by industry. Therefore there is a concern regarding the toxicity of these compounds and its potential effects in coastal aquatic ecosystems since they have potential to alter ecosystem structure and function. Thus the selection of PCBs, PAHs and PBDEs for the study at the Thresholds Integrated Project (http://www.thresholds-eu.org) in the work packages devoted to the assessment of environmental thresholds of contaminants.

Most published data on PAHs (Altenburger *et al.*, 2004; Ankly *et al.*, 1996; Billiard *et al*., 2004; Birnbaum and DeVito, 1995; Calamari and Vighi 1992; Erickson *et al.*, 1999; Faust *et al*., 2001; Fent and Batscher, 2000; Landrum *et al*., 2003 and Scwartz *et al*., 1997), suggest that, when in mixture, and for a variety of end points, PAHs behave in an additive manner. A few exceptions are reported, e.g. Chaloupa *et al*. (1993), Hughes and Phillips (1990) and Wassenberg and de Giulio (2004).

In previous toxicity studies the toxicity and risk of PCBs mixtures was determined using the Toxic Equivalency Factor (TEF) approach (Gao *et al.*, 2000; Birnbaum and DeVito, 1995 and Safe, 1998) which assumes additivity. However, recent studies, though mostly on rats and mices, e.g. Chubb *et al*., (2004), suggests that these compounds when in a mixture behave, mainly, synergistically, however additive and antagonistic effects are also reported, this depending mainly on the concentrations tested and time of exposure (Haag-Gronlund *et al.*, 1998; Suh *et al.*, 2003 and Fadhel *et al*., 2002). The general view is that the additive behaviour is thought to be the exception rather than the rule for PCBs. Therefore the TEF approach may overestimate the risk. In addition, there are other studies, focused on the toxicity of mixtures of PCBs in relation to TCDD (Chen and Bruce, 2003 and Guosheng and Bunce, 2004) or other compounds such as PCDDs and PCDFs (Van de Berg *et al*., 1998 and Lin *et al*., 2005) or 2,-DCP and 2,4,6-TCP (Tiensing *et al*., 2002), which report mainly synergistic effects.

PBDEs are acknowledge to have similar structure and act through the same biological mechanism as PCBs (Sidiqi *et al.*, 2003 and Kodavanti *et al*., 2005). The few studies (e.g. Chen and Bunce, 2003 and Tohka and Zevenhoven, 2001) that

considered possible PBDEs interactions, were also performed mainly on rats and mices. Their effect was considered, generally, non-additive. Min *et al.* (2003) evaluated the effect of 3 different PBDEs on bacteria and concluded that the effects were mainly synergistic.

For these two groups (PCBs and PAHs), most data focus on the Ah receptor, since there is evidence that they share a common mode of action involving binding to the Ah-receptor (Larsen *et al.*, 2000; Chen and Bruce, 2003; Chen and Bruce, 2004 and Suh *et al.*, 2003).

Appendix B. PAHS, PCBS & PBDEs - Toxicity to algae

B1. PAHs

1) Grote *et al.,* 2005

Green algae *Scenedesmus vacuolatus* - inhibition of cellular reproduction

Several PAHs

Table 1 – Toxicity of several PAHs to *Scenedesmus vacuolatus.*

PAH	EC_{50} mg/L *Scenedesmus vacuolatus*
Anthracene	0.51
Benzo(a)anthrance	13.22
Benzo(a)pyrene	1.77
Benzo(b)fluoranthene	22.307
Benzo(*ghi*)fluoranthene	9.89
Benzo(*k*)fluoranthene	4.567
Fluoranthene	34.00
Indeno(1,2,3-*cd*)pyrene	1.16
Phenanthrene	595.48
2-phenylnaphthalene	57.64
Pyrene	49.73

2) Djomo *et al.*, 2004

Green algae *Scenedesmus vacuolatus* - growth inhibition

Several PAHs

Table 2 - Toxicity of several PAHs to *Scenedesmus vacuolatus.*

PAHs	EC_{50}	EC_{10}	NOEC
Benzo(a)pyrene	1.48 µg/L	0.03 µg/L	0.01 µg/L
Pyrene	18.72 µg/L	2.41 µg/L	1.44 µg/L
Anthracene	1.04 mg/L	0.01 mg/L	0.003 mg/L
Phenanthrene	50.24 mg/L	4.91 mg/L	2.74 mg/L
Naphthalene	68.21 mg/L	7.27 mg/L	4.15 mg/L

3) INERIS website

Table 3 – Acute and Chronic toxicity values for several PAHs and algae.

PAH	Organism	Value acute	Organism	Value chronic
Benzo(a)pyrene	*Scenedesmus acutus*	EC_{50} (72 h)	*Pseudokirchneriella*	EC_{10} (72 h) 0.78 µg/L
PNECaq 0.05µg/L		5 µg/L		
Anthracene	*Selenastrum capricornatum*	EC_{50} 16.1 µg/L	*Pseudokirchneriella subcapitata*	EC_{10} (72 h) 7.8 µg/L
PNECaq 0.063 µg/L		EC_{50} (22h) 37.4 µg/L	*Selenastrum capricornatum* NOEC (22h)	3 µg/L
Fluoranthene	*Pseudokirchneriella subcapitata*	NOEC (72 h)	*Pseudokirchneriella subcapitata*	NOEC (72h) 0.0086 mg/L
PNECaq 0.1 µg/L	(fresh water)	0.0086 mg/L		
Fluorene	Not available		*Pseudokirchneriella subcapitata*	EC_{10} (72h) 82 µg/L
PNECaq 0.25 µg/L				
Phenanthrene	*Selenastrum capricornutm* (fresh water)	EC_{50} (4 h) 0.94	*Pseudokirchneriella subcapitata*	NOEC (72 h) 0.0264 mg/L
PNECaq 1.34 µg/L		mg/L		NOEC 0.13 mg/L
Pyrene	Not available		*Anabaena flosaquae* *Pseudikirchneriella subcapitata*	EC_{10} (72h) 1.2 mg/L
PNECaq 0.012 µg/L				

Note: *Pseudokirchneriella subcapitata* was formerly named *Selenastrum capricornutm*.

B2. PCBs

1) Water Quality Criteria for PCBs, Aquatic life - Canada (website)

1A) Freshwater

Lethal

The lethal toxicity (2 to 30 days) of PCBs to freshwater organisms varies with PCB formulation, species, stage of development and test conditions (length of exposure, static versus flow-through tests).

Aroclors containing 40 to 54 % chlorine appear to be the most toxic formulations.

No data available for algae.

Chronic and Sublethal toxicity

The LOAE, for algae was 1.0 µg/L Aroclor 1242.

Aquatic animals' seem more sensitive to PCBs than algae and plants.

Table 4 – Chronic and sublethal toxicity of commercial PCBs to several freshwater algae.

Organism	PCBs Commercial	Conc. µg/L	Effects
	A-1232		
Green algae (*C. pyrenoidosa*)		100-1000	Transient growth reduction
	A-1242		
E. coli		10	Stimulated growth
Green algae (*C. pyrenoidosa*)		100-1000	Transient growth reduction
Diatom (*C. closteria*)		10	No notable effect
Diatom (*C. closteria*)		100	Sharply reduced growth
Green algae (*Euglena*)		10000	Depressed growth
Algae (*S. obtusiulus*)		300	Growth inhibition
Freshwater diatom (*S. acus*) & Green algae (*A. falcatus*)		1	Decreased in cell number in 9 days
Green algae (*S. quadricauda*)		5	Decreased in cell number in 9 days
Green algae (*A. falcatus*)		5	Little effect on photosynthetic activity even after 2 d

Green algae (*C. pyrenoidosa*)	A-1254	100-1000	Transient growth reduction
Green algae (C. *pyrenoidosa*)	A-1268	100-1000	Transient growth reduction
Planktonic algae	various	10-100	Decreased growth
Blue-green alga (*Phormidium*)	C-A30	50	Inhibited growth
Blue-green alga (*Phormidium*)	C-A30	60	No effect on growth

Table 5 - Toxicity of PCB congeners to freshwater algae.

Organism	PCB congener	Conc µg/L	Effects
Blue-green algae (*Phormidium*)	1, 2, 3, 4, 5, 6, 8, 9, 15, 28 & 37	100 µg/2.1 µg algae (dry weight)	Inhibited growth
Blue-green algae (*Phormidium*)	7, 18, 52, 141 & 209	100	No effect on growth

1B) Marine Environment

Lethal and sublethal toxicity

The data suggest that PCBs are as toxic to marine environments as they are to freshwater organisms.

The growth of the marine diatom (*Rhizosolenia setigera*) exposed to 0.1 µg/L Aroclor 1254 was more severely reduced at a lower temperature (10 ^{o}C) than at higher temperature(15 ^{o}C).

The marine diatom *Ditylum brightwellii*, pre-heated with sublethal concentrations of 10 to 30 µg/L over a period of 30 days, developed a resistance to PCB.

Table 6 - Sublethal and Chronic Toxicity of PCB, commercial, to marine aquatic algae.

Organisms	PCB	LC_{50} µg/l	Effects
	A-1242		
Phytoplankton communities		>1.0	Reduced carbon uptake
	A-1254		
Diatom (*T.pseudonana* 3H)		0.1	Reduced Growth rate

Diatom (*R. setigera*)	0.1	Reduced growth rate at 10 ^{o}C for first 192 h
Diatom (*T.pseudonana*)	1.0	Reduced cell division by day 3
Heptophyceae (algae) (*I. galbana*)	1.0	Reduced cell division by day 3
Chlorophyceae (algae) (*D. tertiolecta*)	1.0-50	No effect
Chlorophyceae (algae) (*D. tertiolecta*)	100	Increased cell division
Diatom (*S. costatum*)	10	Reduced cell division
Chrysophyceae (algae) (*M. lutheri*)	10	Reduced cell division
Diatom (*C. socialis*)	10	Reduced cell division
Diatom (*N. longissima*)	25	Reduced cell division by day 4
Phytoplankton communities	>1.0	Reduced carbon uptake
Phytoplankton communities	1.0-10	Reduced biomass and size
Diatom (*S. costatum*)	10	Reduced growth

2) Evandri *et al.*, 2003 –Fresh water biota

Algal - *Raphidocelis subcapitata* (= *Selenestrum capricornutum*)

Aroclor 1254

EC_{50} = 407.5 mg/L

3) Ewald *et al.*, 1976

Table 7 – Toxicity of several Aroclors to *Euglena gracilis*.

PCB Aroclor	*Organism*	Conc. tested mg/L	ID_{50} mg/L	Effect
1221	*Euglena gracilis*	2.5, 5.0, 7.5, 10	4.4	Reduction in cell growth
1232	*Euglena gracilis*	20, 35 50 and 100	55	Reduction in cell growth
1242	*Euglena gracilis*	20, 35 50 and 100	-	No inhibition in growth for concentrations up to 100 mg/L

B3. Flame Retardants (mainly PBDEs)

1) Evandri *et al.*, 2003

Algal - *Raphidocelis subcapitata* (= *Selenestrum capricornutum*)

BDE-99 not toxic to *Raphidocelis subcapitata* at up to 100 µM (56470 mg/L).

2) Europa website

Toxicity test on three marine algae indicate that growth inhibition was only observed at the highest concentration tested (1 mg/L). Therefore a precise EC_{50} cannot be calculated (EC_{50} > 1 mg/L). This concentration is at least 10000 times higher than the water solubility of the chemicals and was tested using a solvent carrier.

3) Lund *et al.* (2004)

HBCDD (hexabromocyclododecane)

Marin alga EC_{50} 72h, 11 µg/L

4) Hakk (2004)

Tetrabromobisphenol A (TBBPA)

Marine algae – *Chlorella sp*. –Not inhibited at mg/L concentrations (96 h).

Freshwater algae – *Selenastrum caricornutum* – Not inhibited at mg/L concentrations (96 h)

Nevertheless, TBBPA was toxic for 2 other marine algae strains at an EC_{50} between 90-890 µg/L.

Negative mutagenecity studies for *S. cerevisiae* and negative Ames test for 5 bacterial strains.

5) FSC America – from Birnbaum –USEPA (web publication)

TBBPA – tetrabromobisphenol A

Algae EC_{50} = 5.6 mg/L

7) Priority existing chemical assessment (2001), National Industrial Chemicals Notification and Assessment Scheme, Australia.

Commercial mixture : 33.7% TBDPE, 54.6% PeBDPE and 11.7% HBDPE

Freshwater algae, *Selenastrum capricornatum* (96 h assay) – Not conclusive the chemical absorbes into the algae.

7A) TBBPA

Marine unicellular algae:

Skeletonema costatum (72 h) EC_{50} 90-890 µg/L

Thalassiosira pseudonana (72 h) EC_{50}130-1000 µg/L

Chlorella sp. (96 h), not inhibited (at 50%) at 1500 µg/L

TBBPA maybe classified as very highly toxic to marine algae.

7B) TBBPA

Freshwater – *Selenastrum capricornutum* (96 h), conc varied between 0.64 and 5.6 mg/L. Growth not reduced

7C) HBCD

Marine unicellular algae:

Skeletonema costatum (72 h) EC_{50} 9.3-12 µg/L

Thalassiosira pseudonana (72 h) EC_{50} 0.05-0.37 µg/L

Chlorella sp. (96 h), EC_{50} >1500 µg/L

8) Stockholm convention (2005)

Deca-BDE is reported to have low general toxicity comparing to penta and octa-BDE. This is also verified by Birnbaum (USEPA, web presentation) - Ecotixicity: PeBDE>> OBDE>DBDE. The author reports as well, a NOEC for algae of 3 µg/L and a PNEC = 0.03 µg/L.

9) Milojøstyrelsen report for the Danish Toxicology Center (web publication)

Table 8 – Toxicity of several PBDEs to algae.

PBDE	Organism	EC_{50} mg/L	Comments
TBBPA	Algae	0.09 (72 h)	Very toxic to aquatic organisms
5BT			No data available for algae
2,4,6-tribromphenol			Toxic to aquatic organisms
Vinylbromide			No data available for algae
Decabromodiohenylether (DeBDE)	*Skeletonema costatum*	1 (72 h)	
	Chlorella sp.	1 (96 h)	
HBCD	*Scenedesmus Subspicatus*	>500 µg/L (96h)	
	Selenastrum capricornutum	>2.5 µg/L (4 d)	
	Skeletonema costatum	9.3-12.0 µg/L (72h)	
	Thalassiosina pseudonana	50-370 µg/L (72h)	

		Chlorella sp.	>1500 µg/L(96 h)	
DBNPG				No data available for algae
DeBB				No data available for algae
PeBDE				No data available for algae
OBDE				No data available for algae
Brominated homopolymer	styrene			No data available for algae

APPENDIX C. Environmental Concentrations of PAHs, PCBs & PBDEs

C1. PAH

C1.1 Water

Individual PAHs Concentrations

Table 9 - PAH concentration (pg/L) in the North Atlantic and Mediterranean water columns (Lipiatou *et al.* 1997).

Compound	Mediterranean Sea		North Atlantic	
	Dissolved	Particulate	Dissolved	Particulate
Phenanthrene	240	170	400	1.8
Fluoranthrene	350	40	110	1.9
Pyrene	67	28	74	1.3
Benzo(a)anthracene	10	3	6	0.5
Chrysene+triphenylene	7	7	2	1.8
Benzofluoranthenes	14	15	7.5	2.5
Benzo(e)pyrene	24	0.5	1.6	0.5
Benzo(a)pyrene	7	0.7	1.5	0.5
Benzo(ghi)perylene	0.5	1.9	0.4	0.5
Indenol(1,2,3-cd)pyrene	0.5	1.7	0.4	0.5

Table 10 - Maximum concentration (ng/L) of several PAHs in different locations (Hellou *et al.*, 2005).

Location	Phenanthrene	Fluoranthene	Pyrene	phase
Iceland/North Atlantic	0.03	0.009	0.007	Dissolved
Norway, reference site	9.9	120	33	D
Chesapeak bay	4.1	22.1	10.6	D
England and Wales	2130	313	205	D
Norway, sewage effluent	1117	515	248	D
Halifax harbor	0.643	5.717	2.876	D
Baltic sea	1.310	3.930	2.00	D+ particulate
Greece seawater	58	37	50	D+ particulate
Greece sewage effluent	1987	452	1371	D+ particulate
Greece waste water	900	100	167	D+ particulate
Montreal, influents	333	150	138	D+ particulate

Total PAH Concentrations

Table 11 - Summary of total PAH concentration, maximum, in sub-surface water from various sites in the world (Zhang *et al.* 2004 and Hellou *et al.,* 2005).

Location	ng/L
Eastern Mediterranean	0.489
Baltic sea	0.594
Chesapeake bay, USA	65.7
Halifax harbour, USA	250
Danube estuary	0.214
Seawater around England and Wales	24821
Seine river and estuary	36
Northern Greece	856
Western Xiamen sea, China	945

C1.2 Sediment

Individual PAHs Concentrations

Table 12 - Sediment concentration, maximum, (ng/g) from different water depths - Alborean sea (southwestern Mediterranean) (Dachs *et al.* 1996).

PAHs	250 m	500 m	750 m
Phenanthrene	230	225	275
Methylphenanthrene	160	175	210
Dimethylphenanthrenes	150	115	120
Anthracene	20	15	25
Dibenzothiopene	100	90	140
Methyldibenzothiophenes	145	125	145
Dimethyldibenzothiophenes	210	90	100
Fluoranthene	45	60	75
Pyrene	70	100	125
Benzo(a)anthracene	20	20	18
Chrysene	50	40	38
Total benzofluoranthene isomers	55	50	45
Benzo(e)pyrene	35	30	25
Benzo(a)pyrene	25	25	15
Perylene	15	15	10

Table 13 - PAH distribution, maximum, in surficial sediments (ng/g) of western Mediterranean sea (Lipiatou *et al.,* 1997).

	Rhone Delta	Ebro Delta	Gulf of Lions	Balearic Sea	Open Sea	Open Sea
Phenanthrene	180	6	65	25	20	10
Anthracene	25	2.5	2	8	2	2.5
Fluoranthrene	150	16	45	55	22.5	17.5

Pyrene	125	17.5	32	45	12.5	12
B(a)anthracene	75	7.5	15	25	10	7.5
Chrysene	100	12.5	35	50	25	17.5
Benzofluorene	200	22	45	130	39	16
Benzo(e)pyrene	75	10	15	45	19	7.5
Benzo(a)pyrene	100	7.5	12.5	35	9	3.5
Indenopyrene	75	6.5	-	55	10	12.5
Benzo(ghi)perylene	100	8	7.5	50	11	11

Table 14 - PAH concentration, maximum, of 16 parent PAHs for sediments of the Niger Delta, Nigeria (Olajaire *et al.*, 2005).

PAH	ng/g dw
Naphthalene	8.92
Acenaphthylene	1.76
Acenaphthene	6.71
Fluorene	7.27
Phenanthrene	16.86
Anthracene	5.98
Fluoranthene	5.00
Pyrene	3.80
Benzo(a)anthracene	1.81
Chrysene	2.66
Benzo(b)fluoranthene	2.57
Benzo(k)fluoranthene	2.32
Benzo(a)pyrene	1.31
Dibenzo(a,h)anthracene	0.4
Benzo(g,h,i)perylene	2.08
Indeno(1,2,3-cd)pyrene	1.11

Table 15 - Concentration (ng/g dw) sediment, Ariake Sea (Nakata *et al.*, 2003)

PAHs	Tidal flat	Coastal water
Anthracene	4.4	<0.02
Chrysene	16	<0.02
Benzo(a)anthracene	19	<0.2
Benzo(b)fluoranthene	39	<0.05
Benzo(k)fluoranthene	16	<0.02
Benzo(a)pyrene	20	<0.03
Indeno(1,2,3-cd)pyrene	23	<0.2

Total PAHs Concentrations

Table 16 - Summary of PAH concentration (maximum) in sediments from various sites in the world (Zhang *et al.* 2004 and Qiao *et al.,* 2005).

Location	ng/g dw
Casco bay, USA	20748
Chesapeake bay, USA	180
England and Wales	102471
Kitimat harbour, Canada	528000
Kyenoggi bay, Japan	1400
Masan bay, Korea	1100
Penobscot bay, USA	8800
San Diego bay, USA	20000
San Francisco bay, USA	27680
Todos santos bay, Mexico	813
Victoria Harbour, Hong Kong	26100
Western Xiamen sea China	33000
Jiulong river estuary, China	1177
Pearl river delta, China	10811
Bohai sea and the yellow sea, China	5534
Yangtze estuary, China	11740
Western Baltic sea	30100
Northwestern Black sea	269
Humber plume, North sea	1700
Kara sea and adjacent rivers, Russia	810
River Tonghui, Beijing, China	928
Minjiang river estuary, China	877
Guba Pechenga, Barents sea, Russia	208
Yalujiang River, China	1500
Deep bay, China	726
Minjiang River Estuary, China	887
Lingding Bay, China	1006
Bohai Sea, the yellow sea, China	5734
Zhujiang River, China	10811
Izmit Bay, Turkey	25000
Kiel Harbour	30000
Meilang Bay, Taihu Lake	4754

Table 17 – Data from Lipiatou *et al.* (1997), total PAH concentration in Mediterranean surficial sediments.

Area	ng/g	Water depth/m
Northwestern Mediterranean	620,750	2500,1700
Coastal shelf between Monaco and Rhone Delta	128-238	-
Rhone Delta	376-1878	10-80
Rhone Delta	1225-2457	23-90
Western Mediterranean central cyclonic gyre	179	2970

Rhibe Delta	1070-6364	4-95
Ebro Delta	200-6500	10-1000
Ebro Delta	50-170	30-50
Ligurian Sea off Monaco	599-723	250
Adriatic Sea	12-174	29-252
French Riviera-Marseilles	103-1582	-
French Riviera-Toulon	912-8525	-
French Riviera-Cannes	393-661	-
West Coast-Corsica	3.5-54	-
Gulf of Lions	182-763	69-2200
Gulf of Lions shelf, slope, fan	470-590	80-1500
Balearic/Catalan sea	100-500	1000-1500
Coastal area near urban centres of Barcelona and Valencia	1396-2313	10-25

In unpolluted coastal area PAHs occur at concentrations up to 1 µg/g.

C1.3 Water and Sediment

Individual PAHs Concentrations

Table 18 - Concentrations (maximum) in water and lake sediments of Lac Saint Louis (Mackay and Hickie, 2000).

PAH	Water ng/l	Sediments ng/g dw
Anthracene	0.6	5
Benzo(a)pyrene	0.48	43
Chrysene	1.2	43
Fluoranthene	4.1	46
Phenanthrene	8.1	15
Pyrene	3.1	24
Benzo(a)fluoranthene	4.2	91

Table 19 - PAH concentrations (maximum) in Ninjiang river estuary, China (Zhang *et al*., 2004).

PAH	Water µg/L	Pore water µg/l	Sediment ng/g
Naphthalene	1.2	2.3	11.2
Acenaphthylene	1.4	12.5	19.7
Acenaphthene	1.4	1.6	16.3
Fluorene	1.6	2.7	16.0
Phenanthrene	6.1	2.3	8.5
Anthracene	1.9	2.6	11.2
Fluoranthene	4.4	2.3	46.8
Pyrene	3.3	1.6	52.5
Benzo(a)anthracene	2.0	3.7	168
Chrysene	4.5	10.2	115
Benzo(b)fluoranthene	138	31.6	55.1

Benzo(k)fluoranthene	4.3	36.2	258
Benzo(a)pyrene	166	30.4	88.0
Indeno(1,2,3-cd)pyrene	126	36.9	96.6
Dibenzo(a,h)anthracene	40.2	30.2	368
Benzo(g,h,i)perylene	11.9	47.5	65.5

C2. PCBs

PBC-126 is environmentally relevant (Wassenberg and di Giulio, 2004).

C2.1 Water

Individual PCBs Concentrations

Table 20 - Volumetric concentrations of individual PCB congeners expressed as pg/L at the three different depths (Axelman *et al.*, 2000).

PCB	12 m	40 m	91 m
Particle bound			
52	0.25	0.4	0.2
101	0.6	0.7	0.8
118	0.4	0.5	0.4
153	1..1	2	0.8
105	0.1	0.25	0.2
138	4	5	2
180	3.5	5	0.7
Dissolved			
52	7.5	9	3
101	7.5	10	4
118	2	4	0.75
153	5	7	2
105	0.7	0.9	0.3
138	5	8	1
180	1.2	5	0.9

Table 21 - Barent Sea (Artic Sea) water PCBs (maximum) concentration (Borga and di Guardo, 2005).

PCB	Water pg/L
28	0.24
52	0.24
101	0.17
105	0.06
110	0.12
118	0.18
138	0.26
149	0.0
153	0.09
180	0.21

Total PCBs Concentrations

Table 22 - Concentration levels (maximum) of PCBs in water samples from Western Mediterranean (Tolosa *et al*., 1997).

	Water phase	Conc ng/L	Compound
Estuarine			
Rhone	Bulk sample	38	
Ebro	Dissolved	0.64	Aroclor 1260
		2.4	Aroclor 1254
Ebro	Particulate	2.7	Aroclor 1260
		3.9	Aroclor 1254
Var	Dissolved	1.75	Aroclor 1254
Var	Particulate	2.6	Aroclor 1254
Coastal			
French coast (Marseille)	Bulk sample	0.002	1254
Sete-Monaco	Bulk sample	29	Phenochlor DP.5
Monaco	Microlayer	42	1254
Monaco	Dissolved	<0.5	1254
Monaco	Particulate	1.1	1254
Languedoc-Provence-Cote Azur	Bulk sample	<2	Phenochlor DP 5/6
Corsica	Bulk sample	<2	Phenochlor DP 5/6
Barcelona	Dissolved	0.06	Σ28,52,101,118,138,180
Barcelona	Particulate	0.17	Clophen 60
Ebro	particulate	0.035	Clophen 60
Open Sea			
Western Basin	Bulk sample	4.5	Phenochlor DP 5/6
Liguro-Provencal (surface)	Dissolved	1.9	Aroclor 1254
Liguro-Provencal (surface)	Particulate	4.6	Aroclor 1254
Liguro-Provencal (profile)	Dissolved	13.4	Aroclor 1254
Liguro-Provencal (profile)	Particulate	17.7	Aroclor 1254
Liguro-Provencal (basin)	Bulk sample	<2	Phenochlor DP 5/6
Western basin	Bulk sample	0.024	Kanechlors300,400,500, 600
Catalan sea (profile)	Particulate		Clophen 60
Catalan Sea	Dissolved	0.05	Σ28,52,101,118,138,180

C2.2 Sediment

Individual PCBs Concentrations

Table 23 - Sediment concentration, maximum, (ng/g) from different water depths (Alborean sea) (Dachs *et al.*, 1996).

PCB	250 m	500 m	750 m
28	1.5	2	2.85
52	2.5	2.60	4.2
101	0.9	2	3.1

118	2.2	1.9	2.9
153	2.6	1.9	3.8
138	1.85	1.75	2.6
180	1.5	1.0	1.5

Table 24 - Maximum concentrations (ng/g dw) in sediments of the Ariake Sea (Nakata *et al.*, 2003).

PCB	Tidal flat	Coastal water
105	0.1	0.96
118	0.51	2.6
156	<0.05	0.18
77	0.009	-
126	<0.01	-
16	<0.01	-

Table 25 - Sediment cores PCBs from the Baltic proper and Gulf of Finland (Jonsson , 2000).

PCB	ng/g dw
167 (Bornholm basin)	15
169 (Gdansk bay)	15
170 (Lithuania)	22
171 (East Gotland deep)	59
178 (West Gotland Deep)	47
180 (N. Baltic Proper)	95
182 (Central Gulf of Finland)	12
187 (Inner gulf of Finland)	18

Table 26 - PCBs in Singapore's coastal marine sediments, maximum concentrations (Wurl and Obbard, 2005).

PCB	ng/g dw
28	31.9
31	8.9
33	14.3
44	13.3
49	10.7
53	11.2
70	13.3
74	13.9
87	13.3
118	13.6
128	13.1
138	13.9
153	21.9
206+208	41

Table 27 - Maximum concentrations of dioxin like-PCBs in coastal sediments (pg/g dw) (Eljarrat *et al.*, 2005).

PCB	pg/g dw
81	50.8
77	193
126	25.9
169	9.26
105	4065
114	320
118	5442
123	1159
156	2149
157	173
167	233
189	179

Table 28 - Concentration, maximum, of PCBs in Baltic sediments (ng/g dw) several depths (0-2 cm) (Konat and Kowalewska, 2001).

PCB	ng//g dw
28	56.2
52	28.54
101	28.74
118	16.19
153	13.01
138	9.96
180	11.93

Total PCBs concentrations

Table 29 - PCB laminated cores (ng/g dw), from offshore and archipelago areas of the NW Baltic sea (Jonsson *et al*., 2000).

	NW Baltic proper	N Baltic Proper	S Baltic Proper	NE gulf of Finland
PCBs	20	39	2.4	60

Table 30 - Maximum concentrations of PCBs in surface sediments of various marine environments-literature data (Konat and Kowalewska, 2001 and Tolosa *et al.*, 1997).

Area (n. PCBs)	ng/g dw
Baltic	
Gulf of Bothnia (12)	6.5
Baltic proper(12)	11.0
Arkona basin (23)	5.4
Oder river estuarine (23)	26.3
North Sea	

Humber Plume (12)	19.7
Scheldt Estuary (13)	200
Mediterranean	
Coast	
Tunisian coast	0.5
Coast of Alicante (10)	2.9
Coast of France	15850
Coast of Greece	775
Italian coast	3200
Rhone Estuary-Fos Gulf	416
Gulf of Lions	780
Nice	1165
Monaco	61
Central Tyrrehian coast	410
Continental shelf Ebro	6
Barcelona	483
Tarragona	122
Valencia Coast	25
Tiber estuary	770
Tiber offshore	73
Naples bay	3200
Naples offshore	170
Sicily	82
Tunisian Sea	1.1
Algerian Sea	323
Open sea	
Alguero-provencal Basin	9
Liguro-Provencal basin	33
Tyrrhenian Sea	1.3
Gibraltar sill and Sicilian –Tunisian sill	0.8
Adriatic	
Venice Lagoon	185
Venice coastal	2203
Venice gulf	9.69
Open sea	332
Atlantic Ocean	
Dominican coast (21)	41.9
Artic ocean	
Chucki Sea	0.14
Pacific Ocean	
Gulf of Alaska	2
Bering sea	0.13
Coastal USA	1000
South China Sea	
Hong Kong	9.75 (wet weight)
Canadian lakes	39

Table 31 - Sediment PCBs concentration, maximum, in Wurl and Obbard, 2005.

PCB	ng/g dw
Osaka Bay, Japan	24.0
Hong-Kong	97.9
Masan bay Korea	41.4
North coast of Vietnam	66.4
Minjiang river estuary, China	57.9
Daya bay, China	11.2
Yangtze Estuary, China	19.0
Singapore	32.9

In Spanish coastal sediments Eljarrat *et al*. (2005) reported a concentration between 0.3 and 75 pg/g dw.

In Swedish lakes (Insjon and Lunsjon) PCBs concentration in the sediment was reported to be 17 ng/g (Soderstrom *et al.*, 2000).

In the USA, PCBs (1, 2, 13, 4, 6, 8, 9, 16, 18, 19, 22, 25, 28 ,52, 44, 56, 66, 67, 71, 74, 82, 87, 99, 110, 138,1 46, 147, 153, 173, 174, 177, 179, 187, 180, 194 ,195, 199, 203, 206) in Lake Michigan, surficial sediments was 40 ng/g dw and in Lake Huron 20 ng/g dw (Song *et al.*, 2005).

C2.3 Water and Sediment

Individual PCBs Concentrations

Table 32 - Lake Ontario PCBs maximum concentrations (Oliver and Niimi, 1988).

PCB	Water pg/L	n/g dry weight Bottom sediment	ng/g dry weight Bottom sediment
8	18		
28+31	46	17	
18	72	4.3	
22	6.7	2.0	
16	3.4		
26		0.1	
33	14	0.5	
17	9.7	0.5	
25		0.4	0.3
24+27		0.4	0.2
32	1.4	0.6	1
66	31	46	27
70+76	45	23	25
56+60+80	26	33	19
52	63	25	15
47+48	41	12	3.4
44	50	23	12
74	10	2.7	4.6
49	24	11	5.8
64	9.7	9.4	4.0

42	3.7	4.7	2.6
53	5.9	0.5	0.5
40	6.2	3.1	1.4
41+71			0.8
46		0.7	0.3
45		1.1	0.2
101	130	27	19
84	19	21	15
118	34	15	21
110	55	37	25
87+97	26	20	17
105	14	10	12
95	52	14	12
85	14	9.8	5.6
92	14	9.1	6.9
82	4.7	2.9	2
91	40	5.7	3.6
99	14	7.2	4.7
153	50	25	23
138	28	15	15
149	34	20	14
146	7.3	6.7	3.4
141	8.6	7.4	5.1
128		4.9	6.2
151	2.7	307	1.7
132	45	11	6.6
156		2.1	2.3
136	16	0.7	2.1
129		1.4	0.8
180	27	13	13
187+182	18	8.4	7.8
170+190	7.2	10	8.4
183	4.4	3.1	3.8
177	3.0	2.5	3.6
174	3.2	4.1	3.7
178		1.7	1.2
171		1.9	2.2
185		1.0	0.5
173		1.6	0.4
203+196	6.8	8.2	6.8
201		7.2	5.7
194	7.8	3.7	3.7
195		1.2	1.7
205		1.6	1.3
206		4.8	4.2
207		1.0	0.4
209		9.4	7.6

Table 33 - PCB (maximum) concentrations in Minjiang River estuary, China (Zhang *et a*l., 2003).

PCB	ng/L Surface water	ng/L Pore water	ng/g dw sediment
1	10.2	86.95	1.86
5	4.01	26.59	0.29
29	292.0	3605	1.14
28	404	1670	2.26
52	302..0	1324	11.91

49	91.32	666.1	10.92
47	155.0	639.9	8.33
97	165.0	750.4	1.87
101	117	352.3	2.66
154	480	1221	5.30
105	97.3	265.8	0.61
171	52.5	158.6	4.82
77	135	564.6	9.10
118	253.6	789.9	2.95
169	127.2	156.9	1.22
153	41.17	167.6	0.54
138	357	352.1	4.21
187	52.5	124.4	2.23
200	304	306.4	1.91
204	350	71.24	1.09
180	44.05	422.1	0.97

Total PCBs Concentrations

Table 34 - PCBs (maximum) concentrations at 3 different depths (three Swedish lakes) (Berglund *et a*l., 2001).

Lake	1 cm ng/g dw	15 cm ng/g dw	22.5 cm ng/g dw	Water Dissolved ng/m3	Water particulate ng/m3
Sovdesjon	20	25	40	6	156
Finsjasjon	80	30	70	23	135
Mien	60	50	10	23	5

PCBs concentration varies with type of lake, eutrophic to ologotrophic (Berglund *et al*., 2001)

C3. PBDEs

Wit (2002) reported that BDE-47 is the predominant PBDE in environmental samples collected from areas affected by general pollution.

The tetra and penta brominated compounds are perhaps of most concern since they tend to remain available in the environment, whereas the deca-brominated tend to partition into soils and sediments (Martin *et al.*, 2004).

The acute toxicity of PBDEs are low (Eljarrat *et al*., 2005).

C3.1 Water

Individual PBDEs Concentrations

Table 35 - PBDEs concentrations for the Netherlands water (pg/L) (Hites, 2004).

BDE	47	99	153	209
Netherlands	1.00	0.5	0.1	0.40

Table 36 - Maximum PBDEs concentrations (µg/L) for several world areas (Palm *et al*., 2002).

	Mono BDE	Di BDE	Hexa BDE	BDE 209
USA, industrial rivers	202700			
Japan		0.01	<0.04	<2.5

C3.2 Sediment

Zegers *et al.* 2003, reported that from sediment cores in Western Europe, BDE-47, 99 and specially 209 were present as major compounds. BDE- 28, 100, 153 and 154, were regularly found at lower concentrations. While BDE-75 and 85 were detected occasionally. And BDE-71, 77, 138, 183 and BDE-190 were never detected.

BDE 209 was the major PBDE detected, followed by, 47, 99 and 100, in Spanish coastal sediments (Eljarrat *et al*., 2005).

And Zhu and Hites (2005) found BDE-153, a major contaminant in lakes.

Song *et al.*, 2004, found that in Lake superior (USA) sediments the most common PBDEs found were: 47,85,128,60,99,100,153,154,183 and 209.

Penta-BDE and tetra-BDE, are the most biologically and environmentally active, and consequently the most hazardous PBDE congeners (Martin *et al.*, 2004).

Individual PBDEs Concentrations

Table 37 - PBDEs concentration in the Cinca River sediment (a tributary of the Ebro river, Spain) (Eljarrat *et al*., 2005a).

BDE	ng/g dw
47	0.2
100	0.1
118	0.3
154	2.9
153	7.8
183	22.8
209	39.9

Table 38 - Environmental concentrations of PBDEs in UK river sediments (Wit, 2002).

BDE	ng/g dw
47	368
99	898
71	366
79	1405
83	399

Table 39 - Concentration of flame retardants in sediment from the Scheldt estuary, The Netherlands (Verslyke *et al.*, 2005).

Flame retardants	ng/g dw
BDE	
29	0.7
41	4.40
66	0.3
71,75,77	<0.1
85	0.
99	4
100	1.7
119	<0.1
138	0.1
153	1.9
154	1
190	<0.1
209	1650
TBBPA	<0.1
HBCD	71

Table 40 - Concentrations of PBDEs in coastal sediments from Spain (Eljarrat *et al*., 2005).

BDE	ng/g dw
28+33	0.3
47	0.13
66	0.09
77	0.03
100	0.19
99	0.22
118	3.35
154	0.11
153	0.32
183	1.22
209	132.10

Table 41 - Concentration (maximum) of PBDEs in marine sediments from industrialized areas in Japan (Choi *et al*., 2003).

BDE	pg/g dw
47	312.4
28	96.2
99	304.2
100	33.5
154	84.3
153	120
183	660

Table 42 - Concentration of individual BDE (maximum) in surface sediments from Hong Kong Coastal waters (Liu *et al*., 2005).

BDE	ng/g dw
3	7.79
15	1.69
28	5.50
47	1.88
60	0.19
85	0.5
99	8.46
100	0.16
138	1.19
153	5.36
154	2.45
183	14.3
197	11.7
207	11.24
209	2.71

Table 43 - PBDEs river and coastal sediment concentrations, maximum, in Portugal (Lacorte *et al.*, 2003).

BDE	Coastal ng/g dw	River ng/g dw
7	0.05	0.08
11	0.01	
12+13	0.26	0.31
15		0.29
30	0.16	
32		0.13
17		0.16
25		0.04
28+3		0.19
75		1.36
71		17.68
49	0.25	

47	0.45	9.91
100	0.16	0.57
99	0.39	1.64

Table 44 - Surface lake water (Michigan, USA) sediment maximum concentration for several PBDEs congeners (Zhu and Hites, 2005).

BDE	ng/g
47	*
99	*
153	0.052
209	315

* Other congeners were detected in low concentrations.

Table 45 - Maximum sediment concentrations (ng/g dw) of several PBDEs around the world (Hites, 2004 and Palm *et al*., 2002).

Location	Type	Mono BDE	Di BDE	47	99	100	153	154	209
Baltic Sea	Core			0.288	0.176	0.056			2.63
Norway	Core			0.145	0.208	0.070	0.040	0.048	146
UK	Estuary			4.80	6.50				27.9
US	Lake			1.37	3.70	0.63	1.76	1.60	
Korea	Marine			1.14	1.33		0.39	0.41	
Denmark	Marine/fresh			0.16	0.23	0.10	0.04		71.0
Japan		<120	<13	31		28			21
Sweden	Rivers			56.6	14.9	13.7			23.3
UK	Rivers			8.47	14.9				22.0
Netherlands	Rivers			1.10	0.0				
Portugal	Rivers			0.39	0.40	0.24			

Total PBDEs Concentrations

Zhu and Hites (2005) reported, for lakes Michigan and Erie – USA, a total PBDE surface concentration of 320 ng/g dw.

Table 46 - Sediment concentration (maximum) from the Pearl River Delta and China Sea (Mai *et al.,* 2005).

Location	PBDE (total except 209)	209
River	95	7400
Estuary/marine	42	145

Table 47 - PBDE concentrations (ng/g dw) in sediment (maximum) from world rivers and coastal zones (Mai *et al.*, 2005).

Location	ΣPBDEs	BDE-209
Pearl river Delta		
Zhujiang river	49.3	3580
Donjjiang river	94.7	7340
Xijiang river	0.6	77.4
Macao coast	41.3	149
Pear river estuary	21.8	119.9
South China Sea	4.5	9.1
North America		
USA	52.3	
Europe		
UK	1270.8	3190
Netherlands	17.6	510
Sweden	50	7100
Portugal	20	
Spain	34.1	132
Denmark	0.53	21.5
Asia		
Korea	33.8	
Japan	352	11600
China		
Qingdao nearshore	5.5	

Table 48 - Sediment of Lake Superior (USA) (Song *et al*., 2004).

	ng/g dry mass
ΣPBDE	2
BDE 209	18

Song *et al.*, 2005, reported the total concentration of PBDEs (28,47,66,85,99,100,153, 183,) in Lake Michigan surficial sediments of 1.7 to 4 ng/d dw and 1 to 1.9 ng/g dw in Lake Huron. BDE 209 was present in higher concentrations (100 ng/g dw Lake Michigan and 35 ng/g dw in lake Huron). The higher concentrations of PBDEs were found at the surface.

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