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The Role of Bioaccumulation in Environmental Risk Assessment: The Aquatic Environment and Related Food Webs

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CONTENTS

SUMMARY

In this report the assumptions and equations used to determine bioconcentration and bioaccumulation of substances are reviewed and a possible approach for the integration of these processes into a risk assessment is discussed. The approach has been evaluated with the use of different bioaccumulation models for several representative substances with different properties and use patterns.

A distinction is made between bioconcentration and bioaccumulation. Bioconcentration is defined as the net result of the uptake, distribution, and elimination of a substance in an organism due to water-borne exposure, whereas bioaccumulation includes all routes of exposure (i.e. air, water, soil, food). Biomagnification is defined as accumulation and transfer of substances via the food web. The report describes the critical mechanisms underlying these processes with a brief description of some of their implications of on environmental exposure and effects. It also addresses methods for predicting and determining the potential of substances to bioconcentrate or bioaccumulate.

It was concluded that bioaccumulation of a substance into an organism is not an adverse effect or hazard in itself. Bioconcentration and bioaccumulation may lead to an increase in body burden which may cause toxic effects due to direct (water) and/or indirect (dietary) exposure. Bioaccumulative substances characterised by high persistence and toxicity, negligible metabolism and a log K_{ow} between 5 and 8 may represent a concern when widely dispersed in the environment. Therefore, when appropriate, the potential of a substance to bioaccumulate in the aquatic environment should be included as an exposure related parameter in risk assessment. Biomagnification, resulting in an increase of the internal concentration in organisms at succeeding levels in the trophic chain, is not as widespread as commonly believed; it has only been demonstrated for a very limited number of substances.

The potential of a substance to bioaccumulate is related primarily to its lipophilicity. A surrogate measure of lipophilicity is the n-octanol-water partition coefficient (K_{ow}), which is correlated with bioconcentration potential. Therefore, K_{ow} is often used as predictor in quantitative structure-activity relationships (QSARs) for bioconcentration factors (BCF) of organic non-polar substances. Such QSARs, however, are not universally applicable. While predictions of BCF in aquatic organisms for lipophilic, nonionic substances undergoing minimal metabolism or biotransformation may be satisfactory, there are exceptions, and the equations to predict BCF are best used only within the chemical class for which the QSAR was developed. Substances with a low lipid solubility, substances with a molecular weight well above 700, or substances which are considered as highly lipophilic will not be taken up as predicted from simple QSARs for BCFs. Non-linearity of BCF

versus log K_{ow} for highly lipophilic substances has been demonstrated. Similarly deviations have been reported also for other chemical classes such as surface-active, ionisable and polar substances.

When biotransformation of the substance by the organism occurs, elimination may increase significantly thus reducing bioconcentration. The major drawback of the various models proposed for the prediction of bioconcentration and bioaccumulation is that they assume no active biotransformation. In addition, specific physico-chemical properties of the substance may reduce availability and possibly exclude uptake. Therefore, when available, measured BCF values based on the analysis of parent substance should be used rather than predicted values.

A step-wise approach is recommended to integrate bioaccumulation in an environmental risk assessment scheme for substances which are widely distributed in the environment due to wide dispersive use and effective intermedia transfer, and which potentially can be taken up by biota. Substances which are persistent, bioaccumulative and exhibit negligible metabolism will be selected by this scheme for a more detailed evaluation.

For those substances which reach a steady-state body burden within the organism during the toxicity test, direct effects of bioconcentration are included, and thus the PNEC derived from this testing is appropriate for use in risk assessment. However, for lipophilic substances which are taken up and depurated very slowly by fish, the steady-state body burden may not have been achieved during the test. Hence, environmental effects assessments should consider the "time to reach steady-state" in evaluating PNEC values for these substances. In a preliminary assessment, it is recommended to evaluate the time to reach 95% of steady-state (T_{95}).

It is concluded that dietary uptake by aquatic organisms is significant only if the substance has low water solubility, high lipid solubility and is slowly metabolised or eliminated by the prey organism. Initially, the BCF may be estimated as described above. When the predicted BCF value is above 1,000 (corresponding to a log K_{ow} of 4.3) a PEC/PNEC assessment for predators is made and refined as deemed necessary.

The EU Technical Guidance for environmental risk assessment requires that bioaccumulation and secondary poisoning be assessed when the substance has a log K_{ow} of 3 or more. In this scheme, the approach does not explicitly include the dietary pathway at lower tiers and may underestimate the body burden of prey organisms for substances with higher lipophilicities. The EU method could be overconcerned with substances of little relevance for secondary poisoning, while underestimating actual exposure for substances in the log K_{ow} range of 4.5 to 8 which are potentially the most critical for dietary exposure.

Bioconcentration and bioaccumulation models will generally overestimate the potential for bioaccumulation and therefore may trigger unjustifiable concerns about transfer of substances up the food web. Additional work is needed to provide further insights into the limitations and uncertainties of QSARs used at the screening stage in the bioaccumulation assessment. Validation and/or reformulation of these QSARs for a wider range of chemical classes is needed if these QSARs are to be used with confidence in the risk assessment process. Furthermore, additional work is needed to incorporate both knowledge and prediction of metabolic processes to account for its influence on bioaccumulation. The development of empirical QSARs for metabolism would aid in the prioritisation, assessment and regulation of apparently persistent and bioaccumulative substances.

1. INTRODUCTION

The development of environmental risk assessment procedures for substances has received considerable attention from regulators, academia and industry (EEC, 1992a; EEC 1993a; ECETOC 1993; OECD 1994a). An iterative and tiered approach in data gathering, evaluation, and decision making has been generally adopted (EEC, 1993b; EEC, 1994; OECD, 1994a). The risk assessment process essentially consists of a stepwise and iterative comparison of the Predicted Environmental Concentration (PEC) with the Predicted No Effect Concentration (PNEC) for the substance and compartment of interest, with each iteration improving the PEC and PNEC estimates.

The environmental exposure can be estimated if it is known how and in what quantity a substance enters the environment and how it is subsequently distributed and transformed in these receiving compartments (i.e. air, water, soil). The effect of transport and transformation processes on the distribution and concentration of substances in the different environmental compartments may be predicted by using mathematical models (OECD, 1989a; OECD, 1991; Braat *et al*, 1991; ECETOC, 1992; ECETOC, 1994c; RIVM, VROM, WVC 1994), assessed in experimental laboratory simulation models, or measured in actual environmental compartments if specific analytical techniques are developed for the substance of interest. An environmental exposure assessment typically yields predicted or measured concentrations on the local or regional scale.

The potential for environmental effects due to exposure to a substance can be estimated if the intrinsic hazardous properties of the substance have been determined, and if it is known how these effects can be extrapolated to the field, i.e. how populations, communities or ecosystems are affected (ECETOC, 1993; OECD, 1994a). It is important to account for potential effects on biota which may be expressed only after some time.

The properties of certain substances - resistance to biotic or abiotic degradation resulting in high persistence, lack of metabolism by organisms and tendency to accumulate in lipid - have led to their widespread dispersion in the environment and elevated concentrations in aquatic organisms. Some substances, such as DDT/DDE (causing egg-shell thinning) and co-planar PCBs (causing mink reproductive failure) have elicited unexpectedly subtle forms of toxicity. The challenge facing industry and regulators is to screen for the potential of other substances to bioconcentrate and/or bioaccumulate and elicit toxic effects after prolonged exposure.

Bioconcentration and bioaccumulation may therefore be of concern for lipophilic substances as both direct and indirect (secondary poisoning) toxic effects may be observed upon chronic exposure. Bioconcentration is defined as the net result of the uptake, distribution, and elimination of a

substance in an organism due to water-borne exposure, whereas bioaccumulation includes all routes of exposure (i.e. air, water, soil, food). Biomagnification is defined as accumulation and transfer of substances via food webs, resulting in an increase of the fat-adjusted internal concentration in organisms at succeeding levels in the trophic chain.

It is generally assumed that the potential to bioaccumulate is related primarily to lipophilicity. A surrogate measure of lipophilicity is the n-octanol-water partition coefficient (K_{ow}), which can be correlated with bioconcentration (Mackay, 1982; Chiou, 1985). Therefore K_{ow} is often used as a predictor in Quantitative Structure Activity Relationships (QSARs) for bioconcentration factors (BCF) of organic non-polar substances. Since most BCF QSARs are based on K_{ow}, regulatory trigger values to determine the potential for bioaccumulation are primarily derived from this single property. It has been proposed that values of log K_{ow} greater than or equal to 3 indicate that a substance has the potential for significant bioaccumulation (EEC, 1993b), triggering the requirement for a determination of a so-called secondary poisoning assessment.

The setting of log K_{ow}, BCF and BAF (= bioaccumulation factor) trigger values to determine the potential for bioconcentration, bioaccumulation and particularly biomagnification must be based on scientific evidence to ensure that the 'real' problem substances are tackled and unnecessary animal testing is avoided. Bioconcentration and bioaccumulation have generally been considered in a risk assessment based on specific "cut-off" values (e.g. log $K_{ow} > 3$), but were not integrated in an overall tiered risk assessment scheme. These approaches do not consider factors including actual release pattern, distribution and fate of substances in the environment and their potential to be metabolised by aquatic organisms.

To further explore when and how bioaccumulation should be incorporated into a risk assessment, the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) formed a Task Force with the following Terms of Reference:

- discuss the processes involved in bioaccumulation and describe the methods for its measurement and prediction on the basis of physico-chemical properties;
- assess the importance of bioaccumulation in environmental risk assessment of aquatic ecosystems and recommend possible physico-chemical and bioconcentration trigger values to be used in consideration of additional work, and
- recommend alternative approaches to assess bioaccumulation for substances which cannot be addressed by the generic regulatory approach.

The objective of the Task Force was to review the assumptions and equations used to determine bioconcentration and bioaccumulation of substances and to recommend some practical approaches to the use of this information in risk assessments.

The potential of a substance to bioconcentrate and bioaccumulate should be assessed and, when appropriate, accounted for in a risk assessment. The critical mechanisms underlying bioconcentration and bioaccumulation will be summarised in Section 3, with a brief description of some of the implications of the processes on environmental exposure and effects. Section 4 will discuss methods for determining the potential to bioconcentrate and bioaccumulate, both from measurement and prediction. Section 5 will provide guidance and suggestions in the assessment and interpretation of the Predicted No Effect Concentration (PNEC) when prolonged exposure may possibly elicit true effects. In addition, guidance is provided in Section 5 for the assessment and interpretation of the potential increasing Predicted Environmental Concentrations (PEC). Finally, conclusions and recommendations are summarised in Section 6.

2. BACKGROUND

On 30th April 1992 the European Council adopted the 7th Amendment of Directive 67/548/EEC (EEC, 1992a). This came into force on 31st October 1993. Article 3.2. of this Council Directive requires that risk assessment be carried out in accordance with principles laid down in a Commission Directive on Risk Assessment of New Substances (93/67/EEC). The specific guidance on how to conduct an exposure and effect assessment, and risk characterisation is described in Technical Guidance Documents (EEC, 1993b). This guidance is to be used in conjunction with the Risk Assessment Directive (93/67/EEC).

The purpose of these Technical Guidance Documents is to assist the notifier and assessor in the risk characterisation and, if necessary, in deciding on what further testing would be required and its timing. The Guidance Documents for New Chemicals call for a classification of "Indication of Bioaccumulation Potential" when a substance:

- has a log K_{ow} > 3; or
- is highly adsorptive; or
- generates a surface tension of < 50 mN/m at a concentration < 1 g/l in water; or
- belongs to a class of substances known to have a potential to accumulate in living organisms; or
- there are indications from structural features;
- and there are no mitigating properties, e.g. hydrolysis or fast biodegradation.

If the K_{ow} cannot be experimentally determined, it has been suggested that this value be calculated from the chemical structure in order to estimate a bioconcentration factor (BCF). The Technical Guidance documents point out that log K_{ow} - BCF QSARs do not account for phenomena of active transport, non-linear uptake and depuration kinetics, metabolism in organisms, changes in the behaviour of diffusion through cell membranes, affinity due to specific interactions with tissue components and special structural properties (e.g. amphiphilic substances or dissociating substances that may lead to multiple equilibrium processes) (EEC, 1993b). In the absence of specific information on the above processes, the well-established regression equations based on log K_{ow} (i.e.

Mackay, 1982) will be used for conservative estimates of the potential for bioconcentration and bioaccumulation.

Depending on the intrinsic toxicity of substances classified as exhibiting a bioaccumulation potential, further testing and evaluation for secondary poisoning will be requested.

Similarly, the EC Council Regulation 793/93/EEC on the evaluation and control of the environmental risks of existing substances requires competent authorities to evaluate the risks to man and environment of existing substances (EEC, 1993a). This regulation was adopted on March 23rd 1993, and came into force on 4th June 1993. It was amended by a Commission Regulation (No. 1488/94) describing the principles of risk assessment of Existing Substances to man and the environment and by another set of Technical Guidance documents (EEC, 1994). This Directive refers to a large extent to the Commission Directive on Risk Assessment of New Substances and its respective Technical Guidance documents.

3. PROCESSES INVOLVED IN BIOCONCENTRATION AND BIOACCUMULATION

3.1. INTRODUCTION

The bioconcentration factor (BCF) is determined as the concentration of parent substance in whole fish (C_f) at steady-state divided by the mean concentration of the substance during the exposure period in the water phase (C_w); and/or as the ratio between the uptake rate constant (k_1) and depuration constant (k_2), assuming first order kinetics.

Uptake may occur via passive diffusion, facilitated diffusion or active transport mechanisms (Schmidt-Nielson, 1979). Uptake of xenobiotics by aquatic organisms occurs via passive diffusion across the gill membranes (Rand and Petrocelli, 1985) or via uptake from the gut. Factors including water chemistry, biological variables and chemical properties of the substance may impact uptake. Distribution throughout the body may lead to different tissue concentrations, although for many substances these levels may be similar when normalised for lipid content. For neutral, poorly-metabolisable substances, elimination will occur predominantly via passive elimination across surfaces such as the gill.

Aquatic organisms have a variety of metabolic systems which act to remove xenobiotics, such that elimination is often considerably more rapid than that predicted for passive diffusion of the parent substance. Phase I and Phase II enzyme systems are of key importance to the active removal of lipophilic substances from fish. These enzymes react with, or biotransform, the parent lipophilic substance, rendering it more hydrophilic and amenable to further metabolism and eventual elimination from the body. The most frequently studied Phase I enzymes are the P-450 dependent monooxygenases and the flavin monooxygenases which "activate" xenobiotics. Phase II enzymes commonly conjugate such metabolites or substances which do not require activation.

Actual measured BCF or BAF values may be orders of magnitude lower than predicted values because metabolism is not taken into account in the prediction. Hence, additional work is needed to incorporate both knowledge and prediction of metabolic processes of lower vertebrates and invertebrates into models of bioconcentration.

Accumulation of a substance by an organism is not in itself a toxic effect. However, this process may have an impact on organisms in the environment in two ways. First, the body burden may increase to toxic levels (e.g. critical body residue, McCarty and Mackay, 1993) due to direct

exposure. Second, dietary sources may cause food web transfer to be a significant exposure route, thus adding to direct exposure and increasing the body burden to higher levels. When appropriate, the potential of a substance to bioconcentrate and bioaccumulate should be assessed and taken into account.

3.2 UPTAKE OF SUBSTANCES IN AQUATIC ORGANISMS

Uptake may be defined as the process of absorption into an organism which for aquatic species includes gill uptake, ingestion, and cutaneous absorption. For substances with lipophilicity up to a log K_{ow} of about 5, gill uptake is most important for aquatic organisms, whereas cutaneous uptake is regarded as a minor process, especially for animals with a high volume-to-surface-area ratio (Lien and McKim, 1993). Additional uptake may occur via the gut which may become significant for substances with higher lipophilicity. The uptake rate constant (k_1) is the mathematically determined value that is used to define the uptake of a substance by exposed organisms, usually reported in units of ml/(gxh) (OECD, 1981b). An understanding of substance uptake requires a basic knowledge of relevant mechanisms of membrane transport, additional barriers regulating gill uptake, and certain environmental, chemical and biological factors. It must be noted that adsorption onto an organism may also play a role for dietary uptake (see Section 3.2.4).

3.2.1 Mechanisms of Uptake

Most substances cross membranes by simple diffusion, mainly through the lipid domain of membranes. However, small hydrophilic substances will diffuse through aqueous pores (Benz *et al*, 1980). Concentration gradients provide the driving force for diffusion processes. Ionised organic acids and bases usually have low lipid solubility, hence, their passive uptake is mainly restricted to the aqueous pores and is limited. Other mechanisms of transport have been identified, some of which are discussed below.

Active transport systems are characterised by four different criteria. Active transport systems:

- move the substance against an electrochemical or concentration gradient;
- saturate at high substrate concentration;
- are selective for certain structural features of the substance, hence it has the potential for competitive inhibition between substances to be transported by the same transporter;

- require expenditure of energy.

An example of active transport is the ATP-driven uptake of K^+ by the Na-K ATPase.

Facilitated diffusion is a carrier-mediated transport system process that saturates at high substrate concentration and is selective for certain chemical structural features. However, this mechanism does not move substances against an electrochemical or concentration gradient, and it does not expend energy. An example of facilitated diffusion is glucose uptake in the intestine.

In fish, a receptor-mediated uptake mechanism has been identified for the uptake of vitellogenin (lipophosphoglycoprotein) by oocytes of coho salmon (*Oncorhynchus kisutch*) (Stifani *et al*, 1990).

Pinocytosis is an uptake mechanism in which aggregates of molecules are inserted in the membrane and are secreted on the opposite side. An example of pinocytosis is the uptake of lipid vesicles or liposomes.

Charged molecules may combine with lipophilic ligands to form uncharged apolar complexes that can more readily cross the cell membrane. An example is the increased uptake of Cd^{2+} in the presence of xanthate (Block and Pärt, 1986).

3.2.2 Uptake Models

Identification of the rate limiting step in the uptake kinetics of substances by fish directly from water is an area of increasing research which has shown that the uptake of substances by fish is controlled by:

- water flow through the gills;
- diffusion through aqueous diffusion layers at the gill epithelium;
- permeation through the gill epithelium;
- the rate of blood flow through the gills;
- the binding capacity of blood constituents.

The uptake rate constant k_1 for small fish is between 5 and 200 ml/(gxh); the k_1 increases linearly with K_{ow} until a plateau is reached (Saarikoski *et al*, 1986). Plateau values for k_1 in fathead minnow are around 60 ml/(gxh) (Sijm *et al*, 1993a).

Several models have been proposed to describe and explain the biphasic relationship between log K_{ow} and uptake rate constants. A membrane permeation model, initially described by Flynn and Yalkowsky (1972), was used by several authors (Gobas *et al*, 1986; Gobas and Mackay, 1987; Clark *et al*, 1990). Gobas *et al* (1986) suggested that the observed relationship between k_1 and log K_{ow} is due to a change from membrane-controlled to diffusion layer-controlled kinetics. The thermodynamically driven model of Gobas and Mackay (1987) indicates that kinetics of uptake and release of substances with log $K_{ow} > 3$ are primarily controlled by fish aqueous-phase transport, whereas kinetics for substances with log $K_{ow} < 1$ are primarily controlled by fish lipid-phase transport.

Barber *et al* (1988) proposed a model which considered both biological attributes of fish and physico-chemical properties to determine diffusive exchange across gill membranes. This model could predict excretion rates, gill uptake efficiencies and BCF-values for non-metabolisable organic substances. The model of Hayton and Barron (1990) indicates that for highly lipophilic substances blood flow can limit xenobiotic accumulation by controlling absorption, or limiting distribution into storage tissues. Erickson and McKim (1990a) indicated that water flow through the gill may be an important rate limiting barrier to xenobiotic uptake.

An alternative to the interpretation of the gill as the main barrier in uptake of substances by fish was presented by Streit et al (1991). Their modelling of ventilation extraction efficiency of teleost fish gills showed that the enhancement of mass transfer across the gill barrier is attributed to a rapid and effective binding of substances with plasma proteins.

All potentially rate limiting barriers were combined in the model of Erickson and McKim (1990b). Their analysis suggests that gill exchange can be understood and predicted on the basis of fundamental physiological and morphological variables. Hence, any parameter that influences the actual rate limiting barrier, either directly or indirectly, may affect the uptake kinetics of a substance. This may cause deviations from linearity in the relationship between log BCF and log K_{ow}.

3.2.3 Factors Influencing Uptake

Ionised substances do not readily permeate membranes, hence, aqueous pH can influence substance uptake. Saarikoski *et al* (1986) showed that the rate of absorption of phenols in the guppy was independent of pKa as long as the pH in water was low enough to keep the acid non-ionised.

Above the Pka, the rate of absorption was exponentially related to pH, as was the degree of ionisation. These authors concluded that some uptake of ionised substances could take place in fish and proposed an empirical relationship to correct for ionisation. When corrected for ionization, the log BCF of eight phenols was highly correlated with log K_{ow} (Saarikoski and Viluksela, 1982).
Loss of membrane permeability is expected for substances with a considerable cross-sectional area (Opperhuizen *et al*, 1985; Anliker *et al*, 1988) or long chain length (> 4.3 nm) (Opperhuizen, 1986a). Loss of membrane permeability will result in total loss of uptake. Opperhuizen (1986b) identified a limit on the uptake of substances with an effective cross section > 0.95 nm in guppy (*Poecilia reticulata*), whereas Anliker *et al* (1988) identified a cross-sectional limit of > 1.05 nm in goldfish (*Carassius auratus*). The influence of molecular weight (MW) on bioconcentration is due to an influence on the diffusion coefficient of the substance, thereby reducing uptake rates constants (Gobas *et al*, 1986).

Black and McCarthy (1988) showed that substances bound to dissolved organic material (DOM) do not diffuse across the gill membrane, thus, only unassociated dissolved substance appears to be available for uptake by fish gills. Hence, presence in water of DOM or particulate matter reduced the bioavailability of substances (McCarthy and Jimenez, 1985). As shown by Schrap and Opperhuizen (1990), presence of sediment suspension reduced accumulation of five chlorobenzenes in guppy, with a larger reduction for the more lipophilic substances. Since binding of lipophilic substances to organic material increases with increasing log K_{ow} (Karickhoff, 1981), bioconcentration of lipophilic substances will be affected by the presence of sediment or DOM in water.

Environmental parameters influencing the physiology of the organism may also affect the uptake of substances. For instance, when the oxygen content of the water is lowered, fish have to pass more water over the gills in order to meet respiratory demands. Oxygen and endrin uptake efficiencies across gills of brook trout (*Salvelinus fontinalis*) decreased at low oxygen content of the water, while the total uptake rate for endrin increased because ventilation volume increased more than efficiencies dropped (McKim and Goeden, 1982). There may be a species dependency since Opperhuizen and Schrap (1987) did not observe an influence of a low oxygen content of the water on the uptake rate constants of chlorobenzenes in guppy.

Sijm *et al* (1993a) showed that temperature influenced uptake rate constants of lipophilic substances as determined by the isolated perfused gills of rainbow trout. The most pronounced effect was observed for hexabromobenzene. Hence, they concluded that the rate-limiting step in uptake seemed to be diffusion, either through the aqueous diffusion layer or through the membrane, and not ventilation or blood flow-rate. *In vivo*, an increase in water temperature increased accumulation of substances in rainbow trout (Reinert *et al*, 1974), guppy (Opperhuizen *et al*, 1988) and mosquito fish

(Murphy and Murphy, 1971). In contrast, Black *et al* (1991) did not find any consistent effect on uptake efficiencies by gills of rainbow trout after an acute reduction in temperature.

Saltwater acclimatisation of guppy resulted in an increase of the BCF of α-hexachlorocyclohexane (Canton *et al*, 1975; 1978). This increase was attributed to apparent differences in osmoregulation in freshwater and seawater teleosts, with fish acclimated to saltwater showing a much higher oral uptake due to active drinking of water (Canton *et al*, 1978). In contrast, accumulation of pentachlorophenol (PCP) in seawater-acclimated killifish (*Oryzias latipes*) was much lower than in freshwater killifish, due to a lower uptake rate and higher elimination rate in the saltwater-acclimated fish (Tachikawa *et al*, 1991).

Body size may correlate with variables affecting the limitations on uptake imposed by flows of water and blood through the gills, diffusion barriers, and substance binding relationships (Barber *et al*, 1988; Hayton and Schultz, 1991). Indeed, an influence of fish size on uptake rate constants has been observed (Saarikoski *et al*, 1986; Sijm *et al*, 1993b; Clark *et al*, 1990), and estimates for the constants of a relationship between uptake rate constant and body weight were obtained (Saarikoski *et al*, 1986; Sijm *et al*, 1993b).

3.2.4 Adsorption

Adsorption of organic substances to outer surfaces may contribute to dietary uptake, particularly if prey organisms possess a high surface-area to volume ratio (i.e. unicellular organisms). Distinction between adsorption and absorption (actual uptake into the organism) is difficult in experimental and field situations. Adsorption is not expected to disturb the linear relationship between BCF and log K_{ow} significantly since the amount that can adsorb is limited by the surface area available. Without making a distinction between absorption and adsorption, the logarithm of the BCF of substances for the green alga (*Selenastrum capricornutum*) was linearly related with the log K_{ow} (Casserly *et al*, 1983).

3.3 DISTRIBUTION

Distribution of a xenobiotic within the body following uptake is a key determinant in eliciting a toxic effect: target tissues or organs must receive a certain dose. Diffusion, as described for uptake, is a fundamental process, but will be physiologically significant over short distances only (e.g. diffusion across membranes) (Schmidt-Nielsen, 1979). Transport across larger distances will be convective or via mass movement. Blood circulation, for example, is important and the degree of perfusion of different tissue types may influence both initial concentrations within individual organs and

elimination rates once the external exposure has ceased. Thus, for poorly-perfused tissues such as muscles, concentrations will initially be lower than for other tissues, equilibrium will be reached later (if at all), and depuration will be slower. Physiologically-Based Pharmacokinetic (PBPK) models originally developed for biomedical applications may be useful for modelling differences in tissue concentration in aquatic and terrestrial wildlife species (Barron *et al*, 1990).

For the persistent, poorly metabolised, lipophilic substances most often studied in relation to bioaccumulation, tissue distributions appear to reach a "steady-state" in which, on a lipid-adjusted basis, the concentrations are similar (e.g. Owens *et al*, 1994). Due to partitioning relationships, organisms with elevated fat content tend to accumulate higher concentrations of lipophilic substances at steady-state. Body burdens are therefore often higher for "fatty" fish such as eel. In addition, lipid "pools" may act as repositories of highly lipophilic substances. Starvation or other physiological changes may change the lipid balance and release such substance, perhaps resulting in some cases in delayed impacts (NERC, 1971). On the other hand, for substances subject to active metabolism, great differences in tissue distribution may be evident even during the period of "steady-state" exposure. Thus, the concentration of parent substance may be greater in less metabolically-active tissues, while concentrations of metabolites increase due to biotransformation at the site of action (e.g. liver) or in the organs involved in the elimination process (kidney, gall bladder) (e.g. Oikari and Holmbom, 1986).

3.4 ELIMINATION

3.4.1 Introduction

The rates of uptake and elimination determine the net accumulation of a substance in the organism (Spacie and Hamelink, 1982). The elimination rate constant k_2 is defined as the rate of loss per unit time. Elimination may occur via passive mechanisms or via active routes. Removal of parent substance by biotransformation with subsequent elimination of metabolites can also be considered an active elimination process. In the latter case, enzymatic detoxification systems biotransform lipophilic substances which would otherwise accumulate in the body, generally into more water-soluble substance which may be further metabolised prior to elimination. The impact of metabolism is quite clear for some substances, such as some polycyclic aromatic hydrocarbons (PAH), where despite high exposures, only trace levels can be observed in fish tissue due to rapid metabolism and excretion (Varanasi, 1989). It is less well known that even relatively slow metabolic activity can dramatically shift the balance towards elimination (e.g. of dioxins, De Wolf *et al*, 1992). Despite a lack of sophisticated models to predict metabolism by lower vertebrates and invertebrates,

the potential for metabolism should be considered whenever assessing the potential of an organism to bioaccumulate a substance.

Additional modes of elimination, less commonly considered, include molting processes (e.g. arthropods) and egg deposition (Rand and Petrocelli, 1985). These may be important processes for the removal of contaminant for the individual; in the case of maternal transfer, it can lead to questions of toxicity to early life stages.

3.4.2 Passive Elimination

Passive elimination processes are the mirror-image of uptake processes by passive transport (see Section 3.2.1) and again will occur via externally-exposed surfaces such as the gut or gill. As with uptake, simple diffusion may be the rate limiting step, however, blood flow may also be limiting.

Substances will accumulate in organisms if the rate constants for the processes of uptake are larger than those for the processes which control the elimination. During steady-state or equilibrium the concentration of this substance in the organism will then be higher than that in the surrounding environment.

Opperhuizen (1991) has shown that log k_2 is in an inversely proportional relationship with log K_{ow} up to a value of about 6, within approximately one order of magnitude. The rate constant for elimination tends to decrease with increasing lipophilicity for organic substances which are not significantly biotransformed. Above a log K_{ow} of 6, log k_2 approaches a plateau.

3.4.3 Biotransformation (Metabolism)

When biotransformation occurs, k_2 increases significantly.

Fish are able to metabolise many different classes of xenobiotics and some of the enzymes catalysing these reactions have been identified and characterised. A metabolite, which is the product of a biotransformation reaction, has different physical and chemical properties than its parent substance. Bioaccumulation can be decreased by altering a substance to a more hydrophilic derivative. For instance, pentachlorophenol is excreted from goldfish predominantly as the glucuronide conjugate, reducing the BCF of pentachlorophenol significantly.

In general, two types of biotransformation reactions are observed in aquatic organisms, these have been classified as phase I and phase II reactions.

Phase I reactions are the first phase of metabolism, which unmasks or adds reactive functional groups, and involves oxidation, reduction or hydrolysis. Phase II reactions are referred to as conjugation. In these reactions xenobiotics or their metabolites are conjugated to other substances such as sulphate or glucuronic acid (Sijm and Opperhuizen, 1989).

Some xenobiotic substances possess the requisite functional groups for direct metabolism by the conjugative or Phase II enzyme systems, while others are metabolised by an integrated process involving prior action of the so termed Phase I enzymes (Appendix B).

3.4.4 Role of Metabolism and the Effect on Bioaccumulation

Several cases of the influence of metabolism on the measured BCF have been reported in the literature. Some examples of measured bioconcentration factors for a range of substances are given in Table 1 where they are compared with those derived from their calculated log K_{ow} and the Mackay (1982) equation.

A combination of enzyme systems known to interact with endogenous substances (e.g. β-oxidation of fatty acids and synthetic surfactants) and enzyme systems thought to have evolved to deal primarily with xenobiotics (e.g. hydroxylation of PAHs) may be employed. With rare exceptions, the resulting metabolites are more hydrophilic, such that they are excreted more rapidly than parent substance. Therefore, biotransformation is a crucial mechanism for reducing the bioconcentration and bioaccumulation of substances within aquatic organisms.

From Table 1 it is apparent that significant discrepancies exist between measured and calculated BCF values which become more pronounced with increasing log K_{ow}. As described previously (Section 3.4.3 and Appendix B) a large number of biotransformation enzymes/pathways have evolved which allow lower vertebrates such as fish and to a lesser extent invertebrates to metabolise a wide range of substances. For such substances physico-chemical parameters such as log K_{ow} cannot reliably be used to predict the actual potential to bioconcentrate and bioaccumulate. In the tiered risk assessment scheme this prediction represents a conservative upper limit at the screening level. Caution is required for substances with a high predicted potential to bioaccumulate since biotransformation may substantially decrease actual bioconcentration factors. Further details on the prediction and measurement of BCF are given in Section 4, whereas the tiered use of predicted versus measured BCF is described in Section 5.

Table 1: Some Examples of the Influence of Biotransformation on the Bioconcentration Factor (BCF) of Substances in Fish (references below)

Substance	Species	log K_{ow} [a]	BCF exp. [b]		BCF calc. [c]	Biotrans-formation [d]	
Bromacil	*Pimephalis promelas*	2.11	< 3	[1]	6	A	[1]
Diuron	*Pimephalis promelas*	2.86	2	[1]	35	A	[1]
Fenitrothion	*Mugil cephalus*	3.01	30	[2]	49	A	[2]
Diazinon	*Pimephalis promelas*	3.41	39	[3]	123	A	[3]
Diazinon	*Brachydanio rerio*	3.41	302	[3]	123	A	[3]
2,4-D butoxyethyl ester	Channel catfish Bluegill sunfish	3.5	2-14 6-21	[4]	162	A	[5]
4-Chloroaniline	*Poecilia reticulata*	1.93	13	[6]	4	A	[6]
2,3,4-Trichloroaniline	*Poecilia reticulata*	3.58	45	[7]	182	A	[7]
3,4,5-Trichloroaniline	*Poecilia reticulata*	3.58	93	[7]	182	A	[7]
Benz[*a*]acridine	*Pimephales promelas*	4.61	107	[8]	1,950	A	[9]
Dibenz[*a,h*]acridine	*Pimephales promelas*	5.78	79	[9]	28,800	A	[9]
Pentachlorophenol	*Oncorhynchus mykiss*	5.06	195	[10]	5,500	A B	[11] [12]
Benzo[*a*]pyrene	*Lepomis macrochirus*	6.12	490	[13]	63,100	A	[13]
Deltamethrin	*Oncorhynchus mykiss*	6.2	203	[14]	75900	A	-
2,8-Dichlorodibenzo-*p*-dioxin	*Carassius auratus*	6.23	670	[15]	81,300	B	[15]
Di-2-ethylhexyl-phthalate	*Cyprinodon variegatus*	8.71	630	[16]	24,547,000	B	[17]
Dodecylbenzene	*Lepomis macrochirus*	8.33	25	[18]	10,232,930	-	-

a Values calculated with CLOGP version 3.54 (Streit *et al*, 1991)
b Experimentally determined bioconcentration factor based on wet weight
c Bioconcentration factor (on a wet weight basis) calculated with the equation given by Mackay: log BCF = log K_{ow} - 1.32 (Erickson and McKim, 1990b).
d Experimental evidence for biotransformation of a substance is either by the identification of biotransformation products (A) or by an increase of the bioconcentration factor upon inhibition of biotransformation processes (B).

References

1. Call *et al* (1987). 2. Takimoto *et al* (1987). 3. Keizer *et al* (1991). 4. Rodgers and Stalling (1972). 5. Huckle and Millburn (1990).6. De Wolf *et al* (1994). 7. De Wolf *et al* (1993). 8. Southworth *et al* (1980). 9. Southworth *et al* (1981). 10. Kukkonen and Oikari (1988). 11. Stehly and Hayton (1989a). 12. Stehly and Hayton (1989b). 13. Spacie *et al* (1983). 14. Muir *et al* (1986).15. Sijm and Opperhuizen (1988). 16. Karara and Hayton (1984). 17. Karara and Hayton (1988). 18. Werner and Kimerle (1982).

3.4.5 Extrapolation of Biotransformation Data

A wealth of information is available on the potential of different types of organisms to eliminate xenobiotics. The following discussion provides some information on the utility and potential limitations of such data for the study of biotransformation in fish and the implications of biotransformation on bioconcentration or bioaccumulation.

Extrapolation from Microbial Activity to Eukaryotic Metabolic Activity

Vertebrate metabolism has several fundamental biochemical pathways in common with those of bacteria (Lehninger, 1976). Thus, if a substance plays a role in the natural biochemical cycles, it may be incorporated into energy cycles in both bacteria and higher organisms. However, while bacterial consortia may completely metabolise or co-metabolise many substances back to biomass, CO_2, methane, etc., vertebrates generally only biotransform xenobiotics and eliminate their metabolites. Complete mineralisation of xenobiotics is rare for eukaryotes. However, complex detoxification systems unique to eukaryotes have evolved, some apparently as the result of gene duplication (Nebert and Gonzalez, 1985; Nelson and Strobel, 1987; Heilmann *et al*, 1988), to metabolise lipophilic xenobiotics. Thus, extrapolation from microbial activity alone may underestimate the potential of eukaryotes, particularly vertebrates, to biotransform and thus eliminate xenobiotics.

Extrapolation from Mammalian Activity to Lower Vertebrates

As summarised in Appendix B, lower vertebrates and invertebrates maintain many of the same systems to biotransform xenobiotics which are present (and more thoroughly studied) in mammals. These range from similar basic intermediary processes for fatty acid metabolism which may come into play in the breakdown of surfactant materials, to the Phase I and Phase II enzyme systems (George, 1994; Stegeman and Hahn, 1994). In fact, results from mammalian safety studies which are generally more detailed concerning metabolic capacity could be used as a first indication of the potential of fish to metabolise a substance, especially if the pathways have been elucidated. A list of known enzyme systems present in lower vertebrates and in invertebrates can be made, and major chemical classes identified. It is interesting to note that the reverse exercise - use of fish experimental data to extrapolate to mammals - has been suggested for more effective screening of the carcinogenic potential of substances (NTP, 1992).

Extrapolation from In Vitro Studies

Finally, extrapolation from *in vitro* information may be useful prior to full bioconcentration testing. Because the liver is a key organ involved in biotransformation, use of fish hepatic preparations to screen for activity could be useful. Such *in vitro* screening systems could be easily adapted from investigational use to examine the potential for aquatic organisms to biotransform xenobiotics.

3.5 BIOCONCENTRATION AND BIOACCUMULATION

3.5.1 Bioconcentration and Body Burden and an Introduction to Critical Body Residue

The body burden is the total amount of substance an animal has taken up from all sources over time and retained in the body. Critical Body Residues (CBR) can be defined as the concentration of a substance in an organism at the time of death (or any other biological endpoint). They have been advocated as suitable replacements for exposure-based toxicity criteria, e.g. LC_{50}, NOEC, EC_{50} (Landrum *et al*, 1992; McCarty and Mackay, 1993).

With exposure-based toxicity criteria the aqueous concentration is regarded as a surrogate for the concentration at the receptor or target site in the organism. Before equilibrium between exposure concentration and target-site concentration is reached, an increase in exposure-time will result in a decrease of the exposure-based toxicity criterion. After reaching the equilibrium, the exposure-based toxicity criterion will not change further. In the case of LC_{50} values the equilibrium value is defined as the incipient LC_{50} (Sprague, 1969). Using the incipient LC_{50}, the CBR can be calculated from Equation 1:

$$CBR = BCFx\,LC_{50} \qquad (Eq.1)$$

where $BCF=C_f/C_w$, $LC_{50} = C_w$ and C_f = CBR (McCarty and Mackay, 1993).

A calculated value of approximately 2 mmol/kg for the CBR of substances with a lethal narcosis effect was obtained by combining empirical relationships (QSARs) between BCF and K_{ow} and between LC_{50} and K_{ow} (McCarty *et al*, 1985; McCarty, 1986). This calculated value was independent of log K_{ow}. Measured data for acute exposures indicate that tissue residues between 2 to 6 mmol/kg yield 50% mortality for small fish and invertebrates. Tissue residue at death of less than 0.5 mmol/kg indicate a specific mechanism of action, whereas a residue between 0.5 and 2 mmol/kg is indeterminate with respect to mechanism of action (McCarty and Mackay, 1993).

For non-lethal endpoints lower values can be expected. For instance, using scope for growth in mussels as the endpoint gave a measured CBR value of 4 μmol/kg (Donkin *et al*, 1989).
Some factors may influence the determination of the CBR. An influence of exposure concentration on CBR has been demonstrated for several substances, including substances with a lethal narcosis effect (De Bruijn *et al*, 1991; Pawlisz and Peters, 1993; De Wolf *et al*, 1994). Pawlisz and Peters also showed an influence of body weight on CBR and an apparently higher CBR for very hydrophilic substances. Using model calculations, Lassiter and Hallam (1990) predicted that increasing body fat is a variable influencing the toxic response.

Some of the present limitations to CBR use were discussed by Landrum *et al* (1992) and can be summarised as:

- the need for research to establish threshold tissue concentrations;
- the need to expose a range of taxa to ensure that sensitive species are included;
- data requirements to handle proportionality issues between whole-body and target concentrations.

The CBR concept has a potential for future use in environmental risk assessment since it relates external exposure to internal exposure through the bioconcentration or bioaccumulation factor. This has been further elaborated in Appendix C.

3.5.2 Bioaccumulation and the Aquatic Food Web

Numerous laboratory and field studies indicate that food web transfer in the aquatic environment is significant at higher trophic levels (secondary, tertiary consumers) only when poorly metabolised, nonpolar substances have a lipophilicity above a log K_{ow} of 4.5 to 5.0 (e.g. Bruggemann *et al*, 1982; Thomann, 1989; Barber *et al*, 1991; Thomann *et al*, 1992; McCarty and Mackay, 1993). Thus, it is not surprising that the substances of major environmental concern are usually persistent, poorly metabolisable, often highly toxic, and have calculated/measured BCF values in fish of tens of thousands.

Examples include polychlorinated biphenyls (PCB) as one of the major classes of substances of concern. Many PCB congeners have calculated and experimentally determined log K_{ow} values in the range of 6 to 8. Calculations using standard QSARs would result in very high BCF. For 2,2',5,5'-tetrachlorobiphenyl, with an experimentally determined log K_{ow} of 6.1 (Bruggeman *et al*, 1982), such

calculations result in BCFs of 63,000 (Mackay) or 26,000 (Veith and Kosian)[1]. For comparison, some measured BCF values for 2,2',5,5'-tetrachlorobiphenyl and biphenyl are 18,200 and 440, respectively (Hawker and Connell, 1989). 'Environment Canada' has concluded that biphenyl is not of concern, while the PCB would be (Environment Canada, 1994). Similarly, the US-EPA has derived so-called "food chain multipliers" which account for bioaccumulation starting at log K_{ow} of 4.0; a table with such multipliers is based on the experience gained in the Great Lake Studies for the protection of wildlife (US-EPA, 1993a-d).

It is very unlikely that dietary exposure is important for nonpolar substances having a lipophilicity below log K_{ow} of 4.5 or 5.0. On the basis of these observations, a conservative value for examination of dietary contributions would correspond to a BCF of 1,000, or a log K_{ow} of 4.3. Below this range, substances are unlikely to contribute significantly to dietary exposure (e.g. Bruggemann *et al*, 1982; Thomann, 1989; Barber *et al*, 1991; Thomann *et al*, 1992; McCarty and Mackay, 1993) in the aquatic environment, even for top predators, and they should reach steady-state rapidly, as well as depurate quickly from the organism once exposure has ceased. For poorly metabolisable substances characterised by a log K_{ow} of 4.3, the time needed to eliminate 95% of the substance (CT_{95}) is calculated at 6 days and the half-life to eliminate 50% (CT_{50} is calculated at 1.4 days (OECD, 1994b). Thus, for such substances bioaccumulation should not be an environmental problem.

Transfer in the Food Web

Early observations of a stepwise increase in biota residues of persistent chlorohydrocarbons with increasing trophic level (Hunt and Bischoff 1960, Woodwell *et al*, 1967) resulted in concern on transfer and magnification of hazardous substances within the food web. Although frequently cited these observations were later shown to be based on data of unclear quality, neglected the direct uptake from water and were based on some biological misinterpretations (Streit, 1992).

Later research showed that magnification within the aquatic food web is not a general rule. But there is a continuing debate in the literature on the relative contribution of water and food to contaminant residues in aquatic organisms.

[1] Note that the resulting predicted BCF diverge for elevated K_{ow} values.

Uptake and Accumulation Mechanism

Two models have been proposed to explain biomagnification of persistent organic substances (Gobas *et al,* 1993): the first model assumes that intestinal absorption of lipophilic organic substances from the gastrointestinal tract into the organism's tissues is predominantly through passive diffusion. The second model assumes that biomagnification occurs in the organism's tissues by metabolisation of lipids into energy, causing the absorbed and non-metabolisable substances to be "left behind" but at a higher concentration and fugacity. From their experimental data obtained with goldfish the authors concluded that passive diffusion is the predominant driving force for gastrointestinal uptake of lipophilic organic substances, and that magnification occurs in the gastrointestinal tract as a result of food digestion. They also concluded that for very lipophilic (log K_{ow} >6) non-metabolisable substances with insignificant gill elimination this will lead to biomagnification factors ranging typically from three to five. If elimination through metabolic transformation and/or gill excretion is much faster than faecal elimination (usually log K_{ow} <6), biomagnification factors are <<1 resulting in no significant biomagnification.

Uptake of substances via food is influenced by the growth efficiency (body weight gain per weight of food consumed) and the uptake efficiency of contaminants (Hamelink and Spacie, 1977). With growth efficiencies of fish being around 8 % any dietary uptake efficiency which is greater than 8 % will result in an accumulation of residues in the absence of metabolism and elimination. A large range of dietary uptake efficiencies have been reported: for various chlorinated hydrocarbons, Hamelink and Spacie (1977) reviewed values ranging from 9 to 68%; Opperhuizen (1991) reported values of approximately 50% and being mainly determined by the digestibility of the food; Gobas *et al* (1993) analysed dietary uptake rates for several organochlorines in goldfish and reported values ranging from 26 to 66%. Gobas *et al* (1986) showed after a compilation of available literature that, for substances with a log K_{ow} up to approximately 7, the uptake efficiency from food seems to be constant (between 35 and 55%). For substances with a log K_{ow} exceeding 7, they noted that uptake efficiency from food fell with increasing log K_{ow}.

A large number of feeding experiments were reviewed by Connell (1988). He reported transfer efficiencies and biomagnification factors for several one-step experimental food webs resulting in biomagnification factors being usually below 1 (ranging from 0.007 to 1.7). He concluded that both mechanisms (direct uptake from water and uptake from food) vary in importance with organisms in various groups (e.g. trophic levels) depending on a variety of factors (e.g. age of organisms, feeding rates, metabolic rates, other chemical components in the food), but in general bioconcentration is of greater significance. Furthermore he summarised that biomagnification is most likely to occur with

persistent substances having log K_{ow} values > 5 and with organisms having long lives as in the case of top predators.

Compared to the ventilation rate of fish (2,000 ml/(gxd)) feeding rate is very low (0.02 g/(gxd)). Thus uptake from food contributes significantly if the concentration of the pollutant in food is five orders of magnitude higher than the concentration in water (Opperhuizen, 1991). The often reported fact that biomagnification is more important for large aquatic predators was explained by this author mainly due to the decrease of ventilation volumes and not due to a food web effect.

Since uptake of substances in the gut mainly occurs by diffusion, biological membranes must be crossed. Uptake from food may therefore also be strongly reduced due to hindered membrane permeation for very large molecules (see Sections 3.2.3 and 4.5.1).

Modelling Food Web Transfer

Given the potential importance of food web transfer to accumulation and impacts of contaminants at higher levels of the food web, much effort has been expended to model available data and predict future trends. Models range from single-compartment, no growth, first-order depuration models (Spacie and Hamelink, 1985) to complex assemblages of proposed trophic levels with increased detail within each level. Some of these models focus predominantly on the chemical's properties (e.g. Mackay, 1992; Gobas *et al*, 1993), although growth dilution and other biological processes may be incorporated. A recent US-EPA model called the Food and Gill Exchange of Toxic Substances model (FGETS) provides a breakdown of the contribution from food and water, and a summed BAF. Within this model, several scenarios can be examined, from simple laboratory water and/or dietary exposures to simulated food webs. For example, it predicts that for a low molecular weight substance with a log K_{ow} of 5.5, the diet would comprise only 6% of the source for a small plankton feeder (final BAF of 29,000), while it would comprise a majority of the source (56%) for a larger predator of the plankton feeder (final BAF also higher, 48,000). Although metabolism has not yet been included, this example illustrates that not only substance properties, but also the position of an organism in the food web, will influence the importance of dietary pathways. FGETS incorporates species-specific information on factors such as growth, ventilation and feeding rates as well as environmental variables such as temperature (Barber *et al*, 1991). Thus, adaptations of the model to fit the ecological or environmental variables at a specific target site are possible.

Thomann (1989) developed a model for calculating the concentration of organic substances in a simple generic aquatic food web. He concluded that food web effects are not significant up to log K_{ow} of around 5. For log K_{ow} of 5-7, observed field concentration factors in top predators indicate

significantly (1-2 orders of magnitude) increased levels above calculated values. In addition, he observed that for log K_{ow} > 7, food web effects are sensitive to the substance assimilation efficiency and phytoplankton BCF. Some aspects of Thomann's model have been incorporated into the US-EPA's Food Web Multiplier method in the Great Lakes Water Quality Initiative (US-EPA, 1993a-c).

Most of the data used to generate hypotheses and design and validate bioconcentration and food web transfer models were generated for poorly-metabolised, persistent, nonpolar organic substances. While these substances deserve special scrutiny, this does not indicate that all other substances with similar lipophilic characteristics pose a similar environmental concern. It is important to also evaluate the persistency, uptake and biotransformation.

Appendix E provides a comparison of results of the bioaccumulation models from Thomann (1989), Clark *et al* (1990) and Barber *et al* (1991). A significant contribution of biomagnification to the bioaccumulation factor only occurs at log K_{ow} greater than 5. At the highest trophic level bioaccumulation through biomagnification according to the presented models is smaller than one order of magnitude, if the lipid content of fish and its feed is similar. If the lipid content of the feed is 3 times lower than that of the fish, biomagnification occurs. This is in line with the findings of Clark *et al* (1988).

It should be noted that the models tend to overestimate the potential of substances to bioconcentrate and to transfer up the food web. Thus, attempts to understand, predict, and incorporate processes such as volume exclusion, metabolism, and others into these models should continue.

Effect of Biotransformation on Food Web Transfer

Movement of xenobiotics through aquatic ecosystems is affected by a number of processes which include biodegradation/biotransformation, bioaccumulation and trophic transfer (Reynoldson, 1987 and references therein). Trophic transfer can be defined as the movement of contaminants through the food web which may result in biomagnification.

As discussed previously (Section 3.4.4), metabolism of lipophilic substances will strongly influence the degree to which they may bioconcentrate. Similarly biomagnification will depend on the extent the substance in question is biotransformed as it moves through the food web. The importance of metabolism in biomagnification can be illustrated by a comparison of two classes of lipophilic xenobiotics, polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB). Porte and Albaigés (1993) analysed the bioaccumulation patterns of PAH and PCB in invertebrates and fish in the Western Mediterranean. They observed that PAH were metabolised along the food web

unlike PCB which exhibited higher bioaccumulation in fish. Clements *et al* (1994) also found that although *Chironomus riparius* accumulated fluoranthene and benzo[*a*]pyrene, the levels of these contaminants were generally low in bluegill (*Lepomis macrochirus*) that were fed contaminated chironomids. Van der Oost *et al* (1991) demonstrated that PCB residues increased from roach → eel → pike obtained from Dutch lakes, but levels of PAH were similar in all three species.

It is recognised that metabolism of substances such as PAH is generally slower in invertebrates than in vertebrates (James, 1989a; 1989b, see also Appendix B). Within the former, higher invertebrates (Arthropods, Echinoderms and Annelids) have greater metabolic capacity than lower invertebrates such as molluscs. Molluscs are known to accumulate PCB, PAH and pesticides (Hawker and Connell, 1986 and references therein) and consumption by man in certain instances may cause health effects.

Most food webs whether marine or fresh water, pelagic or benthic terminate in vertebrates which comprise the upper portions of the pyramids of production and consumption. Therefore in most food webs xenobiotics that can be metabolised by fish (or other aquatic vertebrates) will generally not be biomagnified during trophic transfer. However, biomagnification of recalcitrant substances such as chlorinated organics can occur. Rowan and Rasmussen (1992) showed that variation in residues of PCB and DDT in Great Lakes fish (Canada) did not reflect environmental concentrations of these contaminants. They demonstrated that in certain lakes which possessed shorter and more direct food webs, fish acquired lower body residues of these substances. These authors concluded that the important factors which appeared to determine the partitioning of persistent xenobiotics included fish lipid content, trophic position and trophic structure of the food web.

Finally, although it is established that certain classes of substances (i.e. PCB, polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF)) are generally extremely lipophilic and persistent, some members within these groups can be metabolised by higher vertebrates. Elskus *et al* (1994) found that in the Winter flounder (*Pleuronectes americanus*) PCB congeners with adjacent meta, para-unsubstituted carbon atoms (i.e. 2,2',5,5'-tetrachlorobiphenyl) could be biotransformed. Similarly Opperhuizen and Sijm (1990) and Sijm *et al* (1993b) observed that several congeners of PCDD and PCDF were rapidly eliminated by fish and concluded that bioconcentration and biomagnification could not be described or predicted from the octanol/water partition coefficients. This again underlines the importance of biotransformation on the bioaccumulation of contaminants.

Field Data on Transfer in the Food Web (Examples)

The latest and probably most comprehensive review of biomagnification potential of contaminants in aquatic ecosystems was published by Suedel *et al* (1994). They summarised literature on contaminant trophic transfer (or lack thereof) from laboratory and field experiments. Whenever possible results were expressed quantitatively in terms of trophic transfer coefficient (concentration of a contaminant in consumer tissue divided by the concentration of contaminant in food). In a second step they compared these values and estimates of overall potential contaminant trophic transfer through aquatic food webs with data from aquatic food web models. They analysed data on metals (arsenic, cadmium, chromium, copper, lead, mercury, methyl mercury, nickel, selenium, tin, vanadium, zinc) and organic substances (atrazine, DDT/DDE, dieldrin, hexachlorocyclohexane, kepone, PCB, PAH, TCDD and its derivatives, toxaphene). For other organic substances, lack of information precluded assessing the relative importance of biomagnification for these contaminants. Results do not allow a clear decision on the general importance of food web transfer. But some general conclusions were drawn by these authors:

- the majority of substances evaluated (both organic and metals) do not biomagnify in aquatic food webs;
- for many of the substances examined, trophic transfer does occur but does not lead to biomagnification in aquatic food webs;
- DDT, DDE, PCB, toxaphene, methyl mercury, total mercury and arsenic have the potential to biomagnify in aquatic systems;
- the lipid fraction of predators directly influences biomagnification potential of lipophilic substances;
- the food web model reviewed (Thomann, 1989) provided similar estimates for most of the organic substances examined (log K_{ow} values between 5 and 7) with model predictions falling within the range of values of all substances except dieldrin;
- even those substances for which evidence for biomagnification is strongest show considerable variability and uncertainty regarding the magnitude and existence of food web biomagnification in aquatic systems.

These conclusions are in agreement with other literature not reviewed above:

Thomann *et al* (1992) investigated an amphipod-sculpin food web in Lake Ontario. They reported that the observed BAF for amphipod and sculpin was about one order of magnitude higher than calculated, and almost entirely due to transfer from the sediment, as opposed to uptake from the water route.

Owens *et al* (1994) found a large difference in lipid-adjusted levels of TCDD/TCDF between fish species inhabiting the same river each. Direct water-column bioconcentration alone could not account for lipid-adjusted differences between species. They hypothesised that body burdens are determined by food uptake and that different dietary niches provide different uptake routes and exposures. This hypothesis was supported by stomach analyses of the different fish species.

Clements *et al* (1994) investigated the accumulation of polycyclic aromatic hydrocarbons by the sediment dweller *Chironomus riparius* from sediments and transfer of these contaminants to fish (bluegill sunfish). In experiments where bluegill were exposed to Benzo[*a*]pyrene from water, direct contact with sediments and chironomids, each source contributed to total body burden. But the authors concluded that transfer from chironomids to bluegill sunfish was highly inefficient probably due to metabolism of these substances within the fish.

Porte and Albaigés (1993) determined hydrocarbons and polychlorinated biphenyls in tissues of marine organisms (mussels, crabs, benthic and pelagic fishes). They found clear evidence that PCBs were magnified through the trophic level whereas, on the contrary, hydrocarbons were increasingly metabolised.

Several studies have shown good agreement between laboratory BCF and field BAF values. For example, Romijn *et al* (1991) compared laboratory BCFs of lindane, dieldrin, Cd, Hg, PCB153, PCB118 with measured field residues in fish and observed a good fit between the calculated and measured concentrations in fish. Gunkel (1981) showed for the pesticide atrazine that transfer of the contaminant in the aquatic food web is of no importance and residues in the aquatic biota did not increase with their trophic levels. The mechanism of lipophilic pesticide bioaccumulation in aquatic environments is no longer considered to be primarily the result of food web effects, at least not in aquatic communities and for trophic levels below mammals and birds (Streit, 1992).

Conclusions

It can be concluded that significant food web transfer and subsequent biomagnification is not a general phenomenon in the field. However, food web transfer may significantly contribute to the body burden of aquatic organisms in the case of highly persistent, non-metabolisable and highly lipophilic substances. For this group of substances secondary poisoning should be assessed if chronic exposure of aquatic biota is likely to occur.

4. DETERMINATION OF BIOCONCENTRATION AND BIOACCUMULATION

4.1 INTRODUCTION

In the previous section the importance of bioconcentration and bioaccumulation has been outlined. This section will focus on the determination of these by measurement and prediction. As originally the bioconcentration factors (BCF) were actually measured, this will be dealt with first. Then attention will be paid to the prediction of BCF based on more easily accessible physico-chemical or structural parameters.

On the basis of the information provided a general appraisal will be given for the determination of BCF of substances that can be performed by the methods described. However, there are substances that exhibit physical or chemical properties that complicate the determination or estimation of the BCF using the methods described. These so called "Problematic Properties" are the subject of the final part of this section.

4.2 MEASUREMENT OF BIOCONCENTRATION AND BIOACCUMULATION

4.2.1 Outline of Methodology

A number of test guidelines for the experimental determination of bioconcentration in fish have been documented and adopted; the most generally applied being the OECD test guidelines (OECD, 1981) and the ASTM standard guide (ASTM, 1993). This section will therefore focus mainly on accepted methods within the regulatory context, and discuss the proposed new OECD guideline on bioconcentration.

There are no internationally recognised guidelines on the experimental determination of bioaccumulation and biomagnification.

Bioconcentration

The objectives of the OECD and ASTM test guidelines are similar. The bioconcentration of a substance in fish or bivalve mollusc (ASTM, 1985) is measured during a period of exposure to this substance dissolved in water. The bioconcentration factor (BCF) is then determined as the

concentration of parent substance in whole fish (Cf) at steady-state divided by the mean concentration of the substance during the exposure period in the water phase (C_w); and/or as the ratio between the uptake rate constant (k_1) and depuration rate constant (k_2), assuming first order kinetics. Kristensen and Tyle (1991) reported that the OECD test guideline 305E or the flow-through test method has been most widely applied. The revised draft OECD (1994b) guideline 305 "Bioconcentration: flow-through fish test" consolidates and will replace previous Guidelines 305 A to E. The new guideline describes a procedure for characterising the bioconcentration potential of substances in fish under flow-through conditions. Although flow-through test regimes are preferred, semi-static regimes are allowed, provided that the validity criteria on mortality and maintenance of test conditions are met. The "validity of the test" in general has now been captured in the revised guideline as specified in a number of test conditions (OECD, 1994b), which include that:

- the concentration of the test substance in the chambers was maintained within ± 20% of nominal values during the exposure phase;
- the concentration of total organic carbon in water was less than 10 mg C/l (± 20%) and lipid variation, if determined, less than ± 20% (1 standard deviation) of the mean;
- the concentration of dissolved oxygen did not fall below 60% saturation;
- the temperature variation was less than ± 1°C;
- the pH value did not vary by more than ± 0.5 pH units within each test chamber (especially important for test substances which ionise).

Further, mortality or other adverse effects/disease in both control and treated fish should normally be <10% at the end of the test. When the test is extended over several weeks or months, death or other adverse effects in both sets of fish should be less than 5% per month and not exceed 30% overall.

The test consists of two phases: the exposure and post-exposure phases. During the exposure phase, separate groups of fish of one species are exposed to at least two different concentrations of the test substance. They are then transferred to a medium free of the test substance for the post-exposure or depuration phase. A post-exposure phase is necessary unless uptake of the substance during the exposure phase has been insignificant (i.e. there is no statistical difference between the concentration of test substance in test and control fish). The concentration of the test substance in/on the fish (or specified tissue thereof) is followed through both phases of the test. In addition to the two test concentrations prescribed in the revised OECD test guideline, a control group of fish is

held under identical conditions in the absence of the test substance, to relate possible adverse effects observed in the bioconcentration test to a matching control group and to obtain background concentrations of test substance.

A 28-day exposure phase is obligatory unless a steady-state has been reached within this period. The test guideline details an approach for estimating the time to steady-state for lipophilic non-metabolisable substances. The time to reach 95% of the steady-state concentration is approximated by:

$$T_{95} = \frac{3.0}{k_2} \qquad (Eq.2)$$

where k_2, the depuration rate which can be predicted using the log K_{ow} according to:

$$k_2 = (-0.414x \log K_{ow}) + 1.47 \qquad (Eq.3)$$

This T_{95} value is less than 5 days for substances with a log K_{ow} of < 4. Normally, measurements are continued during the exposure phase until a plateau or steady-state of the concentration of test substance in fish has been established. Only for very lipophilic substances the time to reach steady-state could be greater than 28 days. If a steady-state is not achieved within 28 days, the first option is to initiate the post-exposure phase and to calculate the kinetic bioconcentration factor, BCF_K as the ratio of k_1 and k_2 assuming first order kinetics. Alternatively, the exposure period may be extended, taking further measurements until equilibrium is reached, up to a maximum period of 60 days. After this the post-exposure period begins. The post-exposure period consists of transferring the fish to the same medium but without the test substance, in a clean vessel. The duration of the post exposure period should allow a 95% reduction of the body burden. Normally this is achieved when a post exposure period twice the duration of the exposure period is applied. However, this period may be adjusted when appropriate.

The bioconcentration factor (BCF_{ss}) is calculated as the ratio of concentration in the fish (C_f) and in the water (C_w) at equilibrium.

The BCF is expressed as a function of the total wet weight of the fish. However, for special purposes, specified tissues or organs (e.g. muscle, liver), may be used if the fish are sufficiently large and an appropriate analytical method is available. Since, for many organic substances, there is a clear relationship between the potential for bioconcentration and lipophilicity, there is also a corresponding relationship between the lipid content of the test fish and the observed

bioconcentration of such substances. Thus, to reduce this source of variability in test results, particularly for those substances with high lipophilicity, it is recommended that bioconcentration be expressed in relation to lipid content in addition to the whole body weight BCF. When possible, the lipid content should be determined on the same biological material as is used to determine the concentration of the test substance.

Bioaccumulation and Biomagnification

Internationally recognised guidelines for the measurement of bioaccumulation and biomagnification are lacking. The objective of a bioaccumulation study is to determine the concentration of parent substance in whole organism at "near" steady-state when exposed to all possible routes of exposure (i.e. air, water, soil, food). Dietary uptake can be determined experimentally by properly designed feeding studies; uptake rates as well as body burdens can be measured. Studies which incorporate food-only and food plus water-borne exposures can be utilised to determine the relative contribution of the different exposure pathways in the final BAF for fish.

Micro- and mesocosm systems are used to validate extrapolations of laboratory single species toxicity data to the real environment. The systems can also be used to provide some information on exposure or accumulation and transfer of substances via the food web. Defining a mesocosm study requires a fundamental understanding of adequate structure and function, knowledge of spatial and temporal variability, and the influence of external environmental factors (such as storm events, changes in weather patterns, etc.) on system stability. The mesocosm should be structurally complex to include relevant aspects of ecosystems such as trophic interactions, nutrient cycling, and primary production. Under these conditions and within the limitations of the test, mesocosms can allow direct measurement of the fate and effect of the substance upon prolonged exposure to the ecosystem.

Finally, field monitoring studies can be used to derive a "field BAF". The US-EPA has suggested such values should supersede BCF predictions or laboratory BCF determinations. Field results will take into account such crucial factors as bioavailability, kinetics, and absorption; but such studies must be carefully designed and executed to obtain useful results (US-EPA, 1993d).

4.2.2 Appraisal

Parent Substance

An appropriate analytical method, of known accuracy, precision and sensitivity, for the quantification of the substance in the test solutions and in biological material must be available, together with details of sample preparation and storage. If these are lacking it becomes impossible to determine a true BCF. The use of radio-labelled test substances can facilitate the analysis of water and fish samples. However, unless combined with a specific analytical method, the total radioactivity measurements potentially reflect the presence of both parent substance and possible metabolite(s). For the determination of a true BCF it is essential to clearly discriminate the parent substance from possible metabolites. This can be accomplished using selective clean-up procedures, and/or specific determination of the parent substance.

Methods

Experimental conditions of the individual test guidelines (OECD 1981; ASTM 1993; OECD 1994b) are different in respect to the dynamics of the test (static, semi-static or flow-through method), calculation of BCF, need for a depuration phase, medium/feeding requirements, number of test substance concentrations, chemical sampling requirements (type and frequency) and minimum duration of the uptake phase (*vide supra*).

The validity of the first order kinetic model to (i) predict the duration of the uptake phase and (ii) calculate the kinetic bioconcentration factor (BCF_K) is an issue. In addition, questions about the bioavailability of the test substance in relation to partitioning to suspended organic and inorganic matter have not yet been fully addressed.

The experience from the ring test of the OECD test guideline 305E (OECD 1981b) between 13 different laboratories showed relatively small deviations between BCF_K and BCF_{ss} (Kristensen and Nyholm, 1987). For example, the results for lindane indicate a BCF = 450 ± 170 based on whole body weight, and BCF = 11,000 ± 250 based on lipid content. Kristensen and Tyle (1991) concluded that the selection of either BCF_{ss} or BCF_K based on 1st order or 2nd order kinetics as the preferred calculation method would not affect data quality or precision. It is more important to improve the reproducibility of the test results by specifying the type and frequency of sample analyses and to account for the fraction of the substance which is actually bioavailable for direct uptake by the organism.

Resources

Decision-making on the need to proceed in risk assessment from screening to confirmatory to investigative research programmes is based on a stepwise or tiered evaluation of the value of PEC/PNEC (e.g. ECETOC, 1993). The initial evaluation of bioconcentration in fish (and possibly other animals) needs to be made before proceeding to more investigative test systems, such as feeding studies, microcosms, mesocosms and field testing. Dietary exposure only becomes relevant at log K_{ow} > 4.5 - 5 (e.g. Bruggemann *et al*, 1982; Thomann, 1989; Barber *et al*, 1991; Thomann *et al*, 1992, McCarty and Mackay, 1993), which clearly reduces the need for such test systems.

The introduction of overly conservative trigger factors for the assessment of the potential for bioconcentration, bioaccumulation, and possibly biomagnification can lead to unjustified animal testing contradictory to the spirit of Council Directive 86/609/EEC. It is therefore important to recognise the different aspects which determine the economics and number of animals in bioconcentration and bioaccumulation testing. The development of screening tests such as use of semi-permeable membranes to establish maximal bioconcentration of substances should be further explored in this context as an intermediate step between K_{ow} and BCF measurement.

The primary advantages of the steady-state plateau method are its conceptual simplicity and the fact that the BCF is experimentally determined based on measured concentration data. However, since some extremely lipophilic substances may require a long exposure phase and because of the complicating factors of bioenergetics, growth and lipid deposition, it may not be feasible to demonstrate steady-state or 'near' steady-state. The advantages of the kinetic approach are that it requires less test material, shorter exposure time, less animals and is generally less expensive. The validity of the simple first order kinetic model has been demonstrated (e.g. Bishop and Maki, 1980), and it is generally accepted that adequate information can be obtained using short-term exposures. However, there may be classes of substances for which first order kinetics do not apply.

It is only necessary to proceed to more investigative test systems such as feeding studies, microcosm, mesocosm and field testing when (i) aquatic exposure and bioconcentration factors indicate some level of concern in the secondary poisoning assessment, and (ii) bioaccumulation through food is thought to be relevant (e.g. log K_{ow} > 5). The number of animals, analyses, and costs need to be carefully evaluated in the experimental design of the study.

4.3 PREDICTION OF BIOCONCENTRATION AND BIOACCUMULATION

4.3.1 Introduction

The experimental determination of some substance properties like BCF and BAF is a time consuming and costly exercise (see Section 4.2). Therefore, in the past methods have been developed that correlate easily obtainable physical or chemical parameters with the BCF. Such Quantitative Structure-Activity Relationships (QSARs) are normally developed on the basis of measured BCF of a number of similar substances (Suter II, 1993).

QSAR estimates often are the first step in evaluating the properties of substances. However, in the assessment of BCF the use of a QSAR is often the only step taken in its determination. Besides QSARs there are also qualitative relationships. The latter looks at the substance under consideration in a qualitative manner and can provide essential information for the selection or the availability of appropriate QSARs.

Generally, QSARs can be developed on the basis of measured physical properties or on the structure of the substance. The properties on which the QSARs are based are called the descriptors, as these have the capacity of describing the property that is to be calculated. Examples of both types of QSARs will be discussed in this section.

Firstly, QSARs for the determination of BCF based on experimentally derived descriptors will be discussed. Many of these have been reported in the literature. The descriptors will be discussed with respect to their nature, their derivation, the range of practicality and possible other restrictions. Secondly, descriptors that are calculated will be discussed in a similar manner.

4.3.2 Descriptors

Experimentally Derived Descriptors

The following discussion summarises those descriptors more frequently used or cited, for estimating bioconcentration. Of these, the octanol-water partition coefficient, water solubility and surface tension are required in the base set in support of the notification of new substances (EEC, 1992b).

Octanol-water Partition Coefficient

The octanol-water partition (K_{ow}) of a substance is widely used for estimating its potential for bioconcentration. This arises from the ability of octanol to act as a satisfactory surrogate for lipid in fish tissue. Highly significant relationships between log K_{ow} and the solubility of substances in cod liver oil and triolein exist (Niimi, 1991). Triolein (1,2,3,-tri[cis-9-octadecenoyl]glycerol) is one of the most abundant triacylglycerols found in freshwater fish lipids (Henderson and Tocher, 1987).

There are a number of methods available for the measurement of K_{ow}. The classical method is the shake-flask approach (OECD, 1981a), which is often used as a reference. This involves shaking the substance in water and octanol, and after equilibration, determining the concentrations of the substance in the two immiscible phases (Niimi, 1991).

The method is restricted to log K_{ow} values up to 5. This is caused by limitations of the sampling methodology and/or the sensitivity of the analytical method.

Alternatives to this method include the generator column approach (DeVoe *et al*, 1981) or the slow stirring method (Brooke *et al*, 1986). The former method involves coating an inert material, normally chromosorb-W or glass beads, with the substance in octanol, and packing a glass column with this mixture. Water is then pumped through the column, and the concentration of the substance in the water determined. The range of this method is considerably wider than the shake-flask method, and has been applied to substances with log K_{ow} values as high as 8.5 (Woodburn *et al*, 1984; Doucette and Andren, 1987). The slow-stirring method as described by Brooke *et al* (1986), and applied by De Bruijn *et al* (1989), involves the equilibration of the substance between octanol and water held in a large flask, with a large area of surface contact between the phases, and slowly stirring the water, ensuring no emulsions are formed. The water is then sampled via a tap in the bottom of the flask thus preventing octanol contamination. Using this method values of log K_{ow} over 8 have been measured.

A general criticism of these methods is that they are not applicable to surface-active or ionisable substances and are also restricted to single pure substances.

Another technique for experimentally estimating the log K_{ow} of substances is based on reverse-phase high performance liquid chromatography (RP-HPLC). HPLC columns packed with C_{18} hydrocarbons bound onto silica are used, with a mobile solvent phase of methanol and water (OECD, 1989b). The test substance partitions between the solvent and hydrocarbon phases, and elute from the column in proportion to their log K_{ow}, water soluble substances first.

The partition coefficient may be correlated with the capacity factor (k), calculated from Equation 4:

$$k = \frac{t_r - t_0}{t_0} \qquad (Eq.4)$$

where t_r is the average retention time of the substance and t_o is the dead time, i.e. the average time a solvent or unretained molecule needs to pass through the column. By injecting reference substances of known log K_{ow}, a correlation between k and log K_{ow} is obtained, and from this the log K_{ow} of the unknown substance is derived. An advantage of the method is that more than one substance can be run at the same time, i.e. mixtures, as long as separation on the HPLC column is achieved.

Practical limitations restrict the method to substances with a log K_{ow} within the range of 1-6. As written the OECD (1989b) method has an accuracy, compared to the shake-flask technique of ± 0.5. However, this accuracy may be improved through the use of structurally similar substances.

A technique for measuring the log K_{ow} of ionisable substances, based on potentiometric titration has been described by Avdeef (1991). This technique relies on the changes to the Pka of a substance in water when octanol is added.

A number of relationships correlating log K_{ow} to bioconcentration have been developed, and some of these are given in Table 2.

Table 2: Regression Equations for Estimating BCF in Fish from log K_{ow} (Equation of the form log BCF = a + b log K_{ow})

a	b	n	log K_{ow} range	Chemical class	r	Reference
0.12	0.54	8	2.6 - 7.6	Various	0.95	Neely *et al* (1974)
-0.7	0.85	55	1.0 - 5.5	Various	0.95	Veith *et al* (1979)
-1.32	1.00	44	0.03 - 4.7	Various	0.97	Mackay (1982) *
-0.63	1.02	11	3.4 - 5.5	Chlorobenzenes	0.99	Oliver and Niimi (1983)
-0.4	0.79	122	1.0 - 6.9	Various	0.93	Veith and Kosian (1983) *
0.61	0.89	18	0.9 - 6.7	Aromatics	0.95	Chiou (1985)

* most frequently encountered relationships

Water Solubility

Water solubility (S) is an alternative to K_{ow}, for predicting bioconcentration in that it is inversely correlated to a substance's solubility in octanol. Thus substances with high solubilities are less likely to partition into lipids, and hence have a low bioconcentration potential.

As with log K_{ow}, there are a number of alternative approaches available for the measurement of the solubility of a substance in water. The most frequently used methods will probably be based on the OECD 105 method (OECD, 1981), and the generator column approach as described by DeVoe *et al* (1981). Within the OECD method both approaches are described, and a brief description is given below. Neither of these methods are particularly suited for volatile substances, and the flask method is probably unsuitable for surface-active substances.

In the flask method, an excess of the substance is stirred slowly in water at a temperature higher than that at which the determination is required. After a suitable period of time, the flask is transferred to a water bath at the required temperature, and after 24 hours samples of the water are analysed for the substance after centrifugation. It is usual for a number of flasks to be used, and they are sequentially transferred to the test temperature after 24, 48 and 72 hours. The method is normally limited to solubilities in excess of 1 mg/l due to sampling and analytical limitations.

The generator column method is basically as described above for log K_{ow}. The main difference being that the substance is dissolved in a volatile solvent, e.g. hexane, mixed with the support, and the solvent is then evaporated. Water is then pumped through the column, and normally after an initial surge of substance, an equilibrium is set up at the saturated concentration. It is also normal with this method to run the experiment a number of times at differing flow rates. In this way the contact time of the water with the substance is varied, and it may be established if a true equilibrium has been set up. The method is suitable for substances with solubilities less than 1 mg/l.

Although not as extensively used as log K_{ow}, correlations of log S with bioconcentration factors have been developed. Three such are given below in Table 3.

Table 3: Regression Equations for Estimating BCF in Fish from Water Solubility (S)
(Equation of the form log BCF = a + b log S)

a	b	n	S (mg/l)	Chemical class	r	Reference
2.79	-0.564	36	0.001 - 50,000	Various	0.7	Kenega and Goring (1980)
3.03	-0.44	29	0.002 - 210,000	Various	0.8	Davies and Dobbs (1984)
2.02	-0.47	107	0.0002 - 36,000	Various	0.87	Isnard and Lambert (1988)

Interfacial Tension

The possible use of interfacial tension to act as a predictor of the potential for a substance to bioconcentrate, arises from the tendency of some molecules to concentrate at the interface between phases, e.g. water and lipid, or onto surfaces.

When two immiscible phases are in contact with each other, the interface possess free surface energy. This surface energy is equal to the interfacial tension and arises due to the attraction of unlike molecules at the boundary between the two liquids. The unit is N/m. If one of the liquids contains a substance that will partition between the two phases, it may also concentrate at the surface between the phases. This is particularly true for surface active substances. Such behaviour would lead to a change in the interfacial tension, which could be measured.

The method is prone to a number of problems, mainly due to the need to keep the apparatus used scrupulously clean. The interfacial tension of a mixture will be extremely dependent on the presence of other substances and parameters such as pH. The interfacial tension changes with temperature, and so it is important that it be determined at a constant temperature. It is normal to determine the interfacial tension several times, and only to take the result obtained when equilibrium appears to have been reached.
Other than for surface tension (see below), there is little data available on the interfacial tension of many substances. Therefore, it is not surprising that there are no published data on correlations of this property with bioconcentration. However, there are some data suggesting that interfacial tension may be related to lipophilicity, as reported by Hoffman (1990).

In the absence of any such clearly developed empirical relationships, the use of this criterion for the prediction of the potential bioconcentration is questionable.

Surface Tension

The surface tension of a substance is a special sub-set of the general property of interfacial tension, with the two phases being air and water.

When a pure substance is dissolved in water, the reduction in surface tension is a measure of the surface activity of that substance. The surface tension of a solution of the substance at a particular concentration is normally expressed in N/m. The method as described in OECD test guideline 115 (OECD, 1981) involves the measurement of the surface tension of pure water and solutions of the substance. The surface tension is determined by measuring the force required to pull a platinum ring of known diameter, held horizontally in the water, free of the surface film.

As dust and other airborne substances affect the measurements it is important to cover the test apparatus during the course of the study. As with interfacial tension (see above) it will be extremely dependent on the presence of other substances and on parameters such as pH.

EEC (1993b) suggests that surface active molecules may have a potential for bioconcentration. However, to date there have been no published correlations linking surface activity with bioconcentration.

As with interfacial tension, in the absence of such correlations, it is recommended that this property should not be used for assessing the potential of substances to bioconcentrate.

Critical Micelle Concentration

The critical micelle concentration (CMC) is the concentration of a substance at which micelles are formed. As such, the CMC of a substance may provide an alternative method of expressing that substance's lipophilicity. Hence it may also be able to indicate the potential of a substance to bioconcentrate.

It is measured by monitoring the change in surface tension of an increasing concentration of the substance in water (Mukerjee, 1979). Above the CMC the surface tension will no longer alter.

As may be seen in Table 4 the CMC decreases with increasing alkyl chain length in nonionic surfactants, and there is also a slight decrease with decreasing ethylene oxide chain length.

Table 4: Critical Micelle Concentration of Nonionic Surfactants

Alkyl chain length	Ethylene oxide chain length	CMC (mol/l)
C9	EO8	3×10^{-3}
C10	EO8	1×10^{-3}
C11	EO8	3×10^{-4}
C12	EO8	7.2×10^{-5}
C13	EO8	2.7×10^{-5}
C14	EO8	9×10^{-6}
C15	EO8	3.5×10^{-6}
C12	EO5	6.5×10^{-5}
C12	EO6	6.8×10^{-5}
C12	EO7	6.9×10^{-5}
C12	EO8	7.2×10^{-5}

A good review of the effect of the structure of surfactants on CMC is given by Rosen (1978). The data presented confirm that the CMC decreases with increasing alkane chain lengths, although care in interpretation of the data is needed, even within homologous series.

As with both interfacial tension and surface tension, this property is dependent on the presence of other substances in water, pH and temperature. This is particularly true for ionic surfactants for which the CMC decreases with increasing water hardness and/or ionic strength.

A review by Tolls and Sijm (1993) on the bioconcentration of surfactants showed that there was some evidence of increasing bioconcentration with decreasing CMC. However, the data set used included non-steady-state studies, and the authors were unable to conclude that a clear relationship did in fact exist.

QSARs Based on Calculated Descriptors

Octanol/Water Partition Coefficient

The octanol-water partition coefficient K_{ow} was discussed in Section 4.3.2 together with some QSARs that relate this physico-chemical property with the BCF. However, there are reliable computerised

systems available that estimate log K_{ow} values on the basis of the molecular structure (CLOGP, release 3.51; LOGKOW, 1994; Suter II, 1993).

The principal method is the Fragment Constant Method developed by Hansch and Leo (Leo *et al*, 1971; Hansch and Leo, 1979). The detail of this method is clearly outlined in Lyman *et al* (1982, Ch.1). The log K_{ow}, sometimes referred to as log P, is calculated according to Equation 5 by the addition of atomic or group fragment factors (f_i) and structural factors (F_j).

$$\log K_{ow} = \sum_{i,j} (f_i + F_j) \qquad (Eq.5)$$

Fragment factors describe the contribution of a single atom or group of atoms, and structural factors provide the correction for the influences that the individual fragment factors have on each other. The algorithm used has been shown to be reliable up to a log K_{ow} value of about 5 (CLOGP; Banerjee and Howard, 1988). The verification of the algorithm above this value is hampered by the lack of reliable measured data.

The primary information required to calculate a log K_{ow} by applying this method is the knowledge of the exact molecular structure of the substance under consideration. When the exact molecular structure is not used an incorrect value will be estimated (Nabholz *et al*, 1993). To calculate reliably the log K_{ow} for a substance the values for f and F must be known. These constants are normally derived empirically by comparing substances with measured log K_{ow} values. Where a substance contains fragments that have no reliable values for the factors, this method cannot be used.

The method also gives an incorrect log K_{ow} when a group fragment value is not equal to the sum of its atomic fragment values. A good example of this is the ethoxy-moiety. When its contribution to a log K_{ow} value is calculated on the basis of the atoms, a value of approximately +0.25 is obtained. Treating this fragment as a group, the contribution to the calculated log K_{ow} turns out to be -0.2 (Lodge *et al*, 1992). Roberts (1991) derived a log K_{ow}-increment value of -0.10 for this moiety in non-ionic surfactants.

Before applying this method a qualitative assessment should take place to confirm that the calculated log K_{ow} has any real meaning for environmental risk assessment. The first question to be answered is whether a substance will exist as such in the aquatic environment. Isocyanates, for example, will not be present in the aquatic environment because they hydrolyse easily forming polyureas and CO_2 (Brockhagen and Grievesson, 1983). Therefore, whilst a log K_{ow} can be calculated, neither bioconcentration nor accumulation can occur.

Secondly the calculated log K_{ow} of a substance should not be used when either the physico-chemical properties make it inappropriate (see Section 4.5) or when this property cannot be measured, because one never can be certain that the constants used are correct (Nabholz *et al*, 1993). For these substances the calculated log K_{ow} has no real physical meaning. Examples of these are surface active substances and substance that ionise in water. However, the number that is generated using this methodology may provide a new empirical descriptor for a QSAR that calculates other properties of the substance (Roberts, 1991).

In 1988, Banerjee and Howard published a method in which log K_{ow} was derived from the activity coefficients (γ) of the substance under consideration. These are calculated for an octanol solution, γ_o, and a water solution, γ_w, by means of the UNIFAC-approach. In this approach the solute and the solvent are dissected into their functional groups and the activity coefficient for each group is calculated (Fredenslund *et al*, 1975). A description of the UNIFAC method is given in Lyman *et al* (1982, Ch. 2).

The relation between log K_{ow} and the activity coefficients, γ_o and γ_w was empirically derived and is expressed in Equation 6 (Banerjee and Howard, 1988). For a set of 75 substances the correlation coefficient (r) for this equation is 0.98.

$$\log K_{ow} = -0.40 + 0.73x \log\gamma_w - 0.39x \log\gamma_o \qquad (Eq.6)$$

The main advantage of the activity coefficient method over the Hansch and Leo algorithms is that it provides more reliable estimates for log K_{ow} values > 5.

The third method for calculating K_{ow} described here is based on the parachor (P).

The simplest explanation of the parachor is that it is a measure of the molecular volume. Its relation to solubility is found in the "Hole-Theory" (Briggs, 1981). In this theory it is assumed that to dissolve a molecule must make a hole in the solvent matrix. Thus the larger the molecule the lower the solubility unless there are interactions between the solute and the solvent. An example of these interactions is the hydrogen bond. The parachor which can be established precisely based on the molecular weight, the liquid density, the vapour density and the surface tension, is often used for the calculation of the solubility of a substance in various solvents.

The empirical derivation of P based on the molecular structure is comprehensively described by Quayle (1953).

Briggs (1981) derived a relationship between the parachor and log Kow based on a set of 26 substances (Equation 7). The correlation coefficient (r) is 0.95.

$$\log K_{ow} = 0.011P - 1.2n - 0.18 \qquad (Eq.7)$$

where P is the parachor and n represents a correction value for each hetero-atom or functional group present.

Water Solubility

In this section methods will be mentioned by which the water solubility (S) can be calculated.

The method most frequently applied uses log K_{ow} as the descriptor (Lyman *et al*, 1982, Ch. 11). These methods will not be discussed here, because when the log K_{ow} is available this should be used for the calculation of the BCF.

Also the activity coefficients derived by the UNIFAC approach (Fredenslund *et al*, 1975) provide a reliable algorithm for the calculation of S (Banerjee, 1985). However, as log K_{ow} also may be calculated by this approach, this is preferred in the estimation of the BCF.

As described above the parachor is a convenient descriptor for the estimation of the solubility of a substance in various solvents including water.

McGowan (1954) derived the following empirical relation between log S and P:

$$\log S = 1 - 0.0135P - 1.2n - (0.01T_m - 0.25) \qquad (Eq.8)$$

where T_m is the melting point. The other variables are similar to those in Eq. 7.

Molecular Connectivity Index

The molecular connectivity index (χ) is a non-empirical parameter that is derived from the molecular structure only (Gutman and Trinajstic, 1973). As a non-empirical parameter, it is by definition more accurate, having zero intrinsic error (Sablic, 1987).

Although χ is a non-empirical parameter, the relationship that correlates it to the BCF is an empirical one. Sablic and Protic (1982) published a non-linear QSAR derived from 20 measured BCF (Eq. 9). The correlation coefficient (r) for this set was 0.971.

$$\log BCF = -0.171({}^{2}\chi^{v})^{2} + 2.253({}^{2}\chi^{v}) - 2.392 \qquad (Eq.9)$$

In this equation ${}^{2}\chi^{v}$ is the second order valence molecular connectivity index. Later Sablic (1987) published a refined QSAR derived from a data set of 84 substances of various classes, e.g. chlorinated hydrocarbons, substituted phenols and alkyl and alkenyl benzenes (Eq. 10). The correlation coefficient (r) for this equation is 0.966.

$$\log BCF = -0.160({}^{2}\chi^{v})^{2} + 2.124({}^{2}\chi^{v}) - 2.131 \qquad (Eq.10)$$

The fit between calculated and measured values is striking. Therefore, this method has a large potential for reliably estimating the BCF of new substances, especially of those which are outside the confidence range of the methods described above. However, the method is still under investigation and more work is required. Especially, it is necessary to check whether this QSAR is valid for classes of molecules that have not yet been taken into account during its derivation. However, this QSAR appears to be broadly applicable and when properly validated it may prove to have few limitations in its use.

Furthermore, it is noteworthy that a number of QSAR studies using χ as the descriptor have provided promising results in the field of drug development (Sablic, 1987).

4.4 APPRAISAL OF METHODS

When a properly measured BCF or BAF is available, preferably obtained according to the new OECD method 305 (OECD, 1994b), this result should always supersede one which is derived by a calculation.

When no measured values are available the BCF should preferably be derived from the log K_{ow}. When this property is used for the estimation of the BCF, an appropriately measured value has preference over calculated values. It must be pointed out that for each additional calculation step, error and uncertainty is introduced. Furthermore, the selection of the QSAR for the calculation of the BCF has to take into account all relevant information on the nature of the substance.

In all other cases the BCF may be derived using one of the other descriptors. When this is done the selection of the descriptor and the appropriate QSAR has to take into account all available information on the substance under consideration.

4.5 PROBLEMATIC PROPERTIES

As described in Section 4.3, there are a number of methods for predicting the bioconcentration potential of substances in fish other than the direct experimental determination of the bioconcentration factor (BCF). In most cases these have been developed using data obtained with non-ionised organic substances. These approaches do not calculate the BCF directly, but predict the BCF value via some physico-chemical property of the substance, e.g. log K_{ow}.

There are certain physico-chemical properties which can make prediction of BCF or its measurement difficult. These "problematic properties" may be defined as those that either :

- lead to substances not bioconcentrating in a manner consistent with their other physico-chemical properties, e.g. steric hindrance, *or*

- make the use of descriptors used inappropriate e.g. surface activity making log K_{ow} inappropriate for surface active substances.

In the following such physico-chemical properties are discussed in more detail.

4.5.1 Molecular Volume

Above certain molecular dimensions the potential for a substance to bioconcentrate decreases. This is possibly due to steric hindrance of the passage of the substance through gill membranes or membranes in the gut (see Section 3.2.3 above). It has generally been accepted that a cut-off limit of 700 for the molecular weight should be used (e.g. EEC, 1993b).

4.5.2 Low Solubility in Lipids

A number of substances which on the basis of their low aqueous solubility would be predicted to have a high bioconcentration potential, have subsequently been found to have low bioconcentration. This is thought to be due to their low solubility in lipids (Anliker and Moser, 1987; Banerjee and Baughman, 1991). The latter were able to improve the predictions of bioconcentration potential by including in the model a term describing the substance's solubility in octanol.

4.5.3 Availability

To bioconcentrate a substance needs to be soluble in lipids, present in the water and available for transfer across fish gills. Thus properties which affect this availability will alter the actual bioconcentration of a substance when compared with the prediction. For example, ready biodegradable substances may only be present for short periods of time in the aquatic compartment, and concentrations may be such that exposure becomes insignificant. Similarly, volatility and hydrolysis will reduce the concentration and time a substance is available for bioaccumulation. A further important parameter that may reduce a substance's (bio)availability is adsorption, either to particulate matter or surfaces in general. This may also interfere during the measurement of the BCF or other physical chemical properties making the determination of the BCF or appropriate descriptors difficult. Examples of reduced bioconcentration resulting from interactions with humics or other dissolved organic matter have been reported (Landrum *et al*, 1985; McCarthy, 1983).

A substance that forms micelles or aggregates may also bioconcentrate to a lesser extent than would be predicted from simple physico-chemical properties (Opperhuizen, 1986a).

Highly lipophilic substances, with log K_{ow} greater than 6, show non-linearity of bioconcentration factors versus log K_{ow} (Könemann and van Leeuwen, 1980; Connell and Hawker, 1988). The latter, for example, derived a non-linear function based on chlorohydrocarbons, which showed a maximum bioconcentration corresponding to log K_{ow} of 6.7.

4.5.4 Molecular Structures with Difficult Features

One approach to predicting a substance's bioconcentration potential via its structure involves the calculation of the log K_{ow} (see Section 4.3) using fragment values or connectivity indices. Many substances have a complex structure with either a number of complex interactions or groups for which there are no applicable fragment values. Such substances will clearly not follow previously

observed relationships. For such substances there needs to be an alternative process for assessing their potential to bioconcentrate. This may be an area for further work.

4.5.5 Biotransformation

There are a number of substances which have been shown to be rapidly transformed in the organism thus leading to lower than expected bioconcentration. This was discussed in Section 3.4.3 and summarised in Table 1.

4.5.6 Active Uptake

Finally, although not a specific physico-chemical property, there are substances that may be taken up by specific mechanisms (see Section 3.2.1). For such substances there are currently no relationships to account for active interactions or to predict the BCF.

5. THE INFLUENCE OF BIOACCUMULATION ON THE ASSESSMENT OF RISK

5.1 INTRODUCTION

For most substances the simple PEC/PNEC quotient method used in conventional tiered assessment approaches will be sufficient to determine potential environmental effects. However, bioconcentration and bioaccumulation may be of additional concern for lipophilic, persistent and poorly metabolisable substances. Analysis of both direct and indirect (dietary) toxicity should be carried out for these substances, especially if falling into the Use Category 4 (wide dispersive use) (EEC, 1993a). Modelling using well-established regression equations can provide conservative estimates of the potential for bioconcentration and bioaccumulation. These can then be fed back in to the basic risk assessment procedure (e.g. ECETOC, 1993) for a more comprehensive evaluation of fate and effects.

The potential for bioaccumulation of some substances has focused most research interests on a narrow range of substances such as DDE, polychlorinated dioxins and co-planar PCBs (e.g. Woodwell et al, 1967; Thomann, 1989; Suedel et al, 1994). Testing within the conventional risk assessment framework may not have triggered concerns about these substances because cumulative exposure and bioaccumulation would not have been considered. It should be noted that these substances are poorly-metabolised, persistent, nonpolar organic substances which are broadly dispersed in the environment, possibly resulting in exposure of concern particularly to species with a long life-span at the top of the food chain.

Various regulatory agencies have sought ways to deal with such substances which have the potential for serious impacts. However, these attempts have generally been outside a risk assessment framework and set up specific "cut-offs" for use or restriction of substances without consideration of their actual use, fate and effect patterns.

Bioconcentration or bioaccumulation is not an adverse "effect" or a hazard in itself (Weber et al, 1993). It does not reflect toxicity (McKim and Schmieder, 1991) and should be considered as a "pure" fate and exposure related process (Kristensen and Tyle, 1991). Thus, triggers for testing based solely on BCF or log K_{ow} cannot be scientifically defended. But it should be clear that as persistence and the potential to bioaccumulate increase, greater scrutiny of the substance is necessary.

The potential to bioaccumulate can affect interpretation of PEC and PNEC, and two particular issues must be addressed:

- is the duration of a standard toxicity test long enough for the substance to approach or achieve steady-state within the organism and elicit a toxic response which might occur in the environment?

- can transfer within the food web lead to toxic levels in predator organisms and do dietary sources (prey organisms) substantially supplement the exposure of predators?

This section presents an addition to conventional methods which incorporates bioconcentration and bioaccumulation into the conventional iterative process to establish and compare increasingly realistic estimated PEC and PNEC values for a substance (e.g. ECETOC, 1993 and references therein).

5.2 EXPOSURE ASSESSMENT

The principal questions are: (i) will there be environmental exposure to the substance and: (ii) is uptake possible for the substance of interest? (Figures 1 and 2). If exposure or uptake do not occur, further assessment is not necessary.

The predicted substance characteristic of bioaccumulation would therefore be considered of 'no concern' if the substance is non-persistent or present only at very low levels for very short periods of time. This implies that if the substance does not enter the environment due to closed production or processing system and/or effective removal during use, disposal and treatment (e.g. in a wastewater treatment plant), and/or b) is removed from the receiving environment at a very rapid rate, such that exposure for lengthy periods is unlikely, the assessment would not be needed. Only if substances are present and bioavailable in the water phase for a sufficiently long time, the potential for bioaccumulation needs to be evaluated. According to the EEC (1993b), a substance with a hydrolysis half-life of less than 12 hours would be unlikely to bioconcentrate. Similarly, rapid biodegradation and/or photodegradation may significantly affect the exposure concentration. It is therefore important to assess the concentration of the substance in both space and time to evaluate its potential to expose aquatic organisms.

Estimation of the release of a substance to air, water and soil is the first step in the exposure assessment and provides the basic input for environmental modelling. The importance of this release estimation and the use of appropriate fate and distribution models has been extensively

discussed by ECETOC (1993; 1994a; 1994b). It is important to acknowledge differences between point sources - either single or multiple- and diffuse sources. In addition, the nature, frequency and duration of the emissions may differ according to local prevailing conditions, and should be accounted for in the initial steps of an exposure assessment. When emission of the substance occurs via a wastewater treatment plant (WWTP), suitable models can be used to estimate 'steady-state' concentrations in the aquatic effluent stream, in the gaseous waste stream, and in the solid sludge streams. The fraction released into air, water and soil will be subject to further transformation and transport processes. Intermedia transport and the role of transformation processes such as biodegradation, hydrolysis and phototransformation has been extensively discussed by ECETOC (1993; 1994b). ECETOC (1994c) has developed a simple computer tool - HAZCHEM - to assist the risk assessor in the estimation of intermedia distribution and prediction of concentrations in different environmental compartments.

Similarly, most regulatory authorities use Mackay Level III multi-media models. These calculate steady-state non-equilibrium concentrations in the various environmental compartments using specific volumes and advection rates. The homogenous partitioning, transport and reaction of the substance among completely mixed compartment volumes is not always attained in reality and hence the values obtained for the PEC represent so-called average steady-state concentrations. However, it provides valuable information on the environmental compartments of concern and will identify substances with a tendency to bioaccumulate. Similarly, if no partitioning to the water compartment occurs for extremely volatile substances, bioaccumulation in aquatic food webs would be unlikely. A local release estimation is the basis for the calculation of a predicted environmental concentration near the emission site ($PEC_{initial}$) and - after application of a local environmental fate model - a predicted local environmental concentration (PEC_{local}). Although not realistic, for the screening bioaccumulation assessment, a local PEC estimate can be used as a worst case exposure level. If the assessment warrants further refinement, the spatial area and duration of exposure should be further investigated.

5.3 UPTAKE ASSESSMENT

If the substance does enter the environment, it needs to be established if the substance can enter the organism that resides in the aquatic compartment of interest. It is important therefore to evaluate the molecular dimensions, since steric hindrance is known to play an important role in the potential passage of the substance through gill membranes, or other tissue membranes (see Sections 3.2 and 4.5). Factors such as solubility and (bio)availability (see Section 4.5) will also need consideration prior to entering the risk assessment scheme described below.

Substances with a low lipid solubility, molecular weight well above 700, or substances which are considered as highly lipophilic will not be taken up as predicted from simple QSARs for BCF. Non-linearity of BCF versus log K_{ow} for highly lipophilic substances has been demonstrated, and similar deviations have been reported for surface-active, ionizable, and polar substances (Bintein *et al*, 1993). A major drawback of the various models proposed for the prediction of bioconcentration and bioaccumulation is that they assume no active biotransformation of the substance by the organism. However, even low rates of biotransformation can lead to a significant reduction of actual accumulation of substance (see Section 3).
Because predictions based on structural information which provide physico-chemical, topological, or other parameters currently do not include predictions of biotransformation or metabolism potential, actual measured BCF or BAF values may be orders of magnitude lower than predicted values. Additional work is needed to quantitatively incorporate both knowledge and prediction of metabolic processes of lower vertebrates and invertebrates into models of bioconcentration.

If the substance does enter the environment but a) does not enter the organism and/or b) is rapidly eliminated from the receiving organism, the predicted bioaccumulation potential would be considered of 'no concern'.

5.4 INCORPORATION OF BIOCONCENTRATION INTO ENVIRONMENTAL RISK ASSESSMENT

In this section, direct exposure from the surrounding medium (water) will be considered, that is, bioconcentration. As described above, properties of the substance and its use patterns must be evaluated to assure that this additional assessment is required (Figure 1).

An issue is whether the duration of a standard toxicity test is long enough for the substance to reach steady-state and elicit a toxic response which might occur in the environment if the exposure to the substance is continuous. Body burdens are key to toxic response (McCarty and Mackay, 1993), especially for substances with a non-specific mode of action. For those substances which reach steady-state in the organism within the duration of the toxicity test (e.g. fish 96 hour LC_{50} test), direct effects of bioconcentration are included in the outcome of the toxicity test. Therefore, the PNEC derived from such testing is appropriate and can be used in the PEC/PNEC assessment. However, for a substance which is taken up and depurated very slowly by the organism (e.g. substance with a half-life of many weeks; Bishop and Maki, 1980), the elevated steady-state body burden which could be achieved in the environment will not be reached during an acute or short-term chronic test.

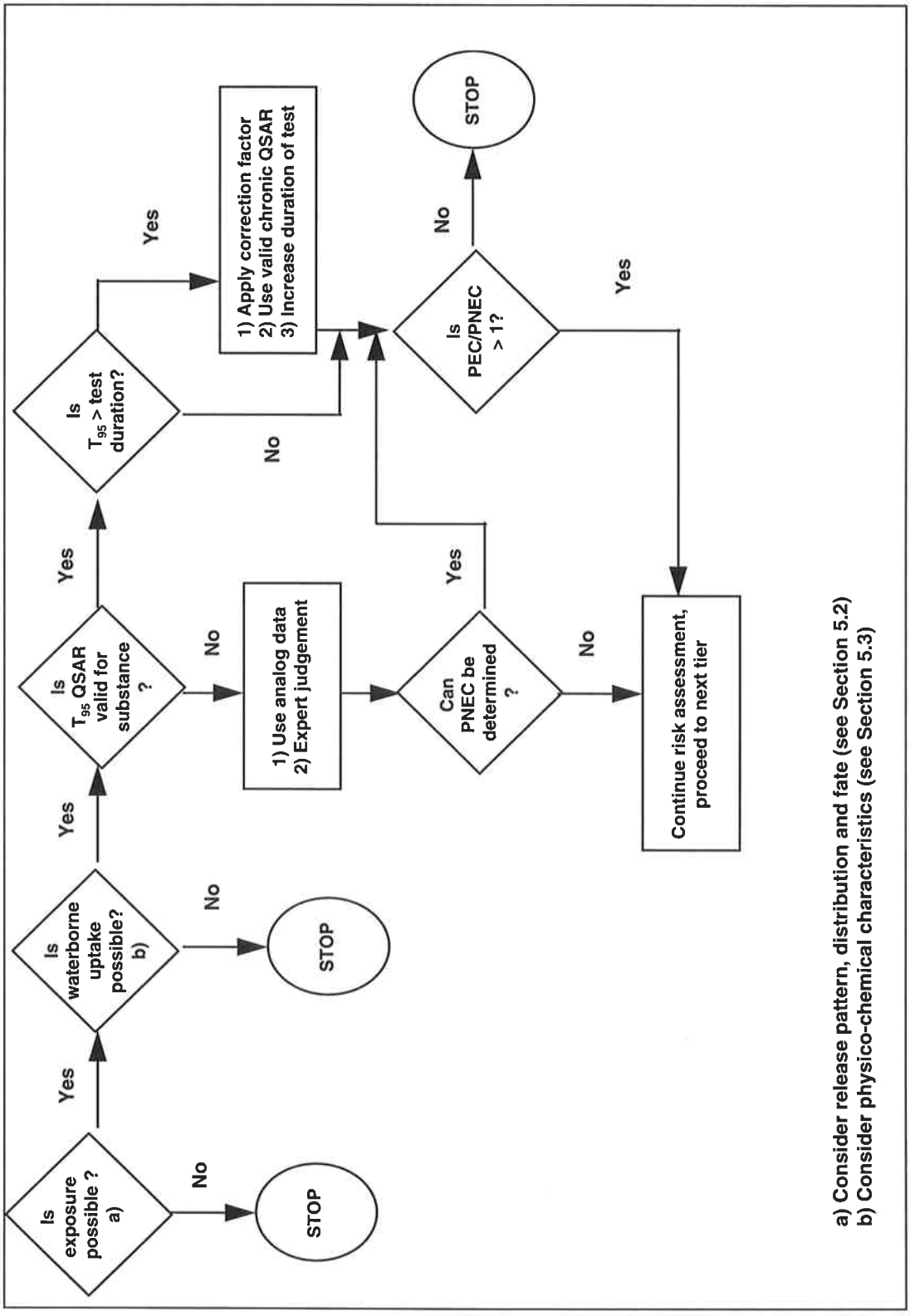


Figure 1: Decision Flow Diagram for Integration of Bioconcentration in a Tiered Risk Assessment Scheme.

The prediction of a no effect concentration should therefore consider the "time to steady-state" in evaluating PNEC values. This can be incorporated directly during the execution of the test by evaluating the asymptote of the measured effect parameter over time (e.g. 48h, 72h, 96h), or by predicting the time to steady-state. The time to steady-state is operationally defined as the T_x, the time it takes to achieve x% of the steady-state concentration in the organism. For example, the T_{80} or T_{95} have been used; this report will work further with the T_{95}, the time to reach 95% of the steady-state concentration in the organism (OECD, 1994b). The T_{95} can be determined directly by measuring the time to reach a plateau or steady-state concentration given a constant exposure concentration, or predicted using a calculated or observed elimination rate constant.

In the preliminary assessment, the T_{95} can be estimated for a substance using an established QSAR (OECD, 1994b). If such a QSAR is not appropriate for the substance, e.g. high molecular weight and/or rapidly metabolisable substances, alternative methods such as examination of data from analogous substances could be used either to estimate a T_{95} or to assess directly the appropriateness of the toxicity test result. If such additional methods are not available, a PEC/PNEC calculation may contain greater uncertainty, and it is recommended to proceed to the next tier in the risk assessment.

When the QSAR is appropriate, the T_{95} can be estimated. If this T_{95} is greater than the duration of the toxicity test (see examples, Table 5), several options exist. First, the assumptions used in calculating the T_{95} may be reevaluated. Second, the short-term result could potentially be "adjusted" to account for achieving only a fraction of the total body burden (Parkerton, 1993). Such a correction factor would take into account the theoretical % body burden achieved during the test. This value can be calculated for fish according to $Cf/Cf,s = 1 - e^{(-k2t)}$, where k_2 is also estimated based on the log K_{ow} (OECD, 1994b). Third, if available, an appropriate chronic QSAR for one or more aquatic species could be applied. It should be noted that use of such a chronic QSAR presumes that the chronic data are based on steady-state values; the validity of the chronic QSAR must therefore be carefully examined before application. Fourth, the duration of the toxicity test may be extended, with the above screening calculation used to indicate the extent of additional time needed.

If in fact a BCF test has been performed, the experimental T_{95} can be used to reevaluate the toxicity data and to confirm the appropriateness of the derived PNEC value.

Thus, for direct toxicity, an assessment of the time to reach steady-state (operationally defined here as T_{95}) in relation to the duration of the testing performed should be made to confirm the appropriateness of the PNEC value derived from the testing. The options outlined above may then be applied to improve our understanding of the potential of the substance to elicit effects under realistic exposure conditions. Based on an empirical data base which has been used to derive

K_{ow}/T_{95} relationships for fish (OECD, 1994b), this non-achievement of steady-state within a short period (≤ 3 - 4 d) and thus need for additional assessment may occur for substances with a log K_{ow} > 4 (Table 5). For these lipophilic substances, toxicity testing and interpretation of results may be difficult[2]. This is mainly due to operational problems related to solubility and dosing.

Table 5: Calculated T_{95} Values and Elimination Half-life ($T_{1/2}$) for Various log K_{ow} Values (OECD, 1994b)

T_{95} (days)	log K_{ow}	$T_{1/2}$ (d)
2.0	3.1	< 1
2.9	3.5	< 1
4	3.9	1
12	5.0	3
30	6.0	7

A similar concern for (non-)achievement of steady-state may be present for invertebrates. However, the regression equation used above (OECD, 1994b) should not be applied to invertebrates as it was derived from bioaccumulation testing of small fish. Measured invertebrate data sets on analog substances should be used in conjunction with expert judgement to evaluate whether the toxicity endpoint can be used with confidence within the risk assessment.

5.5 INCORPORATION OF BIOACCUMULATION INTO ENVIRONMENTAL RISK ASSESSMENT

In this section, the potential contribution of dietary sources in addition to simple bioconcentration is examined. Two main elements must be considered. First, when is food web transfer likely to be significant? Second, if food web transfer is significant, then how can potential toxic effects at higher levels of the food web be determined?

Initially, properties of the substance and its use patterns must be evaluated to ensure that this additional assessment is appropriate (Figure 2). The primary issues of environmental exposure of the substance and possible uptake have been discussed in Sections 5.2 and 5.3.

[2] If the LC_{∞} is undeterminable, reference is made on how to test sparingly soluble substances (ECETOC, Monograph, in preparation).

Dietary uptake by aquatic organisms will only be significant for a small subset of substances released to the environment. Dietary exposure does not begin to be significant even for persistent non-ionic substances until lipophilicity reaches an intermediate range between log K_{ow} of 4.5 to 5.0 (e.g. Bruggemann *et al*, 1982; Thomann, 1989; Barber *et al*, 1991; Thomann *et al*, 1992, McCarty and Mackay, 1993). In order to be conservative in this assessment, but not encompass the much larger number of substances which have negligible potential to bioaccumulate via the food web in the aquatic environment, a BCF of 1,000, which corresponds to a log K_{ow} of 4.3, was proposed as a starting point[3] (Section 3.5). Below this value substances are unlikely to contribute significantly to dietary exposure in the aquatic environment, even for top predators, and they should reach steady-state rapidly and depurate quickly from the organism once exposure has ceased.

The iterative approach outlined below (Figure 2) attempts to address the potential for bioaccumulation and "indirect" (dietary) toxicity. Initially, the BCF is estimated. When the BCF value is above 1,000, a PEC/PNEC determination for predators is made and refined as deemed necessary through screening, confirmatory and investigative phases. The incorporation of "oral" PEC and PNEC values into the aquatic risk assessment provides an opportunity to assess dietary exposure and toxicity to fish and to fish-eating birds and mammals. It should be noted that while assessment of oral routes is necessary to assure safety of persistent, bioaccumulative, toxic substances, standard methods are not available and the assessment would likely proceed in a case-by-case, research-oriented fashion.

Some organisms may be exposed via ingestion due to physical adsorption of a substance onto the surface of their prey. Adsorption is not explicitly described by food web models. However, two points can be made. First, the "BCF" values reported for algae (which given their surface area to volume ratio are the most important in terms of relative adsorption) are likely due to internal accumulation and adsorption (Casserly et al, 1983). Second, adsorption is not of primary importance at higher trophic levels. Adsorption may be important for the proximal predator, but actual uptake into that organism will be needed for transfer to the next trophic level.

[3] The contribution of diet to the total exposure for fish and thus a significant difference between BCF and BAF is only expected when log K_{ow} is between 4.3 and around 8. Above 8, BCF values drop rapidly due to low bioavailability, low lipophilicity, or other reasons. Individual assessment using expert judgement may be needed.

5.5.1 Prediction of Bioaccumulation Potential

Initially, a BCF is predicted by empirically-derived QSARs and used as a surrogate for bioaccumulation potential (Figure 2). Most BCF QSARs are based on log K_{ow}. This property provides good predictions of fish BCF for lipophilic, nonionic substances undergoing minimal metabolism or biotransformation within the organism. The equations should only be used to predict BCF for substances within this class. The suitability of an individual QSAR (Lyman *et al*, 1982) should be evaluated prior to use by comparing the properties of the substance with those used to derive the QSAR. The most commonly encountered QSARs for the log K_{ow} to BCF relationship are: BCF = 0.048 x K_{ow} (Mackay, 1982), and log BCF = (0.79 x log K_{ow}) - 0.4 (Veith and Kosian, 1983)[4]. Any bioaccumulation assessment scheme should be flexible enough to employ the most appropriate BCF predictors for a certain type of substance, once such predictors are developed and validated (see Section 4.3.2).

When the calculated BCF is greater than 1,000, the assessment of a "predator" PEC/PNEC should be undertaken (Figure 2). Prior to this step, the predicted BCF may be reassessed. For example, if a predicted log K_{ow} was utilised, the log K_{ow} may be measured. Similarly, as metabolism can greatly influence actual bioaccumulation (Section 3.4.4), an *in vitro* metabolism screen could be applied to assess the potential for substantial metabolism if additional information indicates this may be an important pathway. Metabolically competent fish hepatocyte cultures or fish homogenate preparations could be used to establish the potential for biotransformation. For example, the metabolism of benzo[*a*]pyrene by fish has been extensively investigated in both cell cultures and subcellular fractions (review by Varanasi *et al* in Varanasi, 1989). Although standardised techniques are not yet available, many of the procedures used are modified from those developed for mammalian systems. Generation of more polar metabolites, for example, would indicate that parent substance could be lost more rapidly than expected, leading to a lower BCF. As an alternative, the BCF could be directly determined experimentally.

[4] As noted in Chapter 3, these two regressions diverge for elevated K_{ow} values where the Mackay equation tends to overestimate BCF.

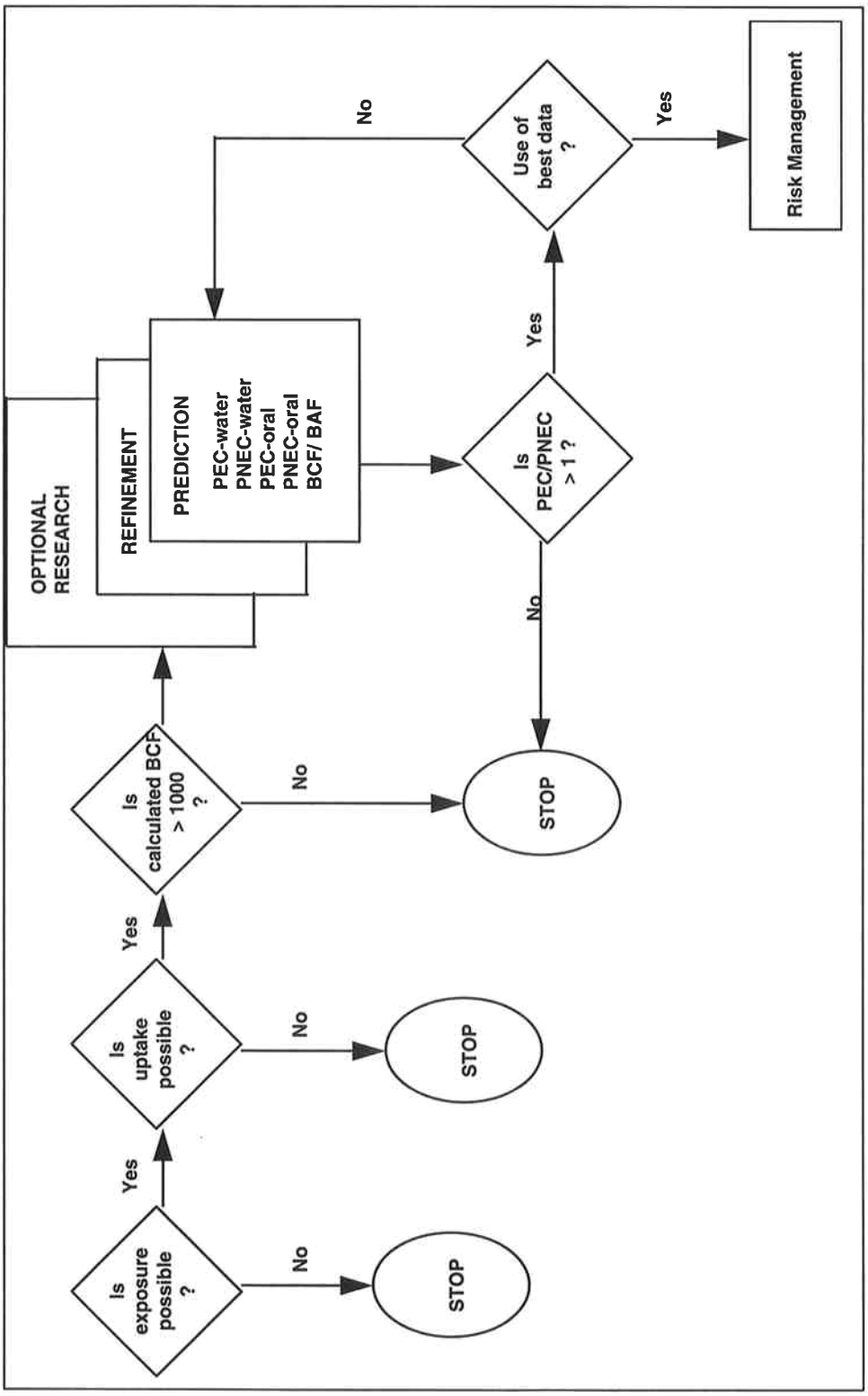


Figure 2: Decision Flow Diagram for Integration of Bioaccumulation in a Tiered Risk Assessment Scheme.

5.5.2 Refinement of Bioaccumulation Factor

In the next phase, BAF values can be calculated using food web transfer models and used with the conventional PEC_{water} to determine a body burden or PEC_{oral} (BAF x PEC_{water} = PEC_{oral}). This information, along with a $PNEC_{oral}$ estimate, would be used to establish an initial PEC/PNEC for predators. The most commonly used is the US-EPA FGETS (see Section 3.5.2) which has been partially validated with PCB as model substances. Further improvements with the incorporation of metabolism QSAR at different trophic levels are to be expected.

The most appropriate PEC_{Water} value used to obtain the PEC_{oral} will depend on the use and release patterns of the substance, as well as the species being exposed (e.g. home range, foraging zone). The PEC_{oral} can be used in two ways.

First, the body burden value can be compared to available toxicity information as a check that the critical body residue or CBR (McCarty and Mackay, 1993) is not exceeded due to dietary plus water-only exposure. Thus, potential impacts on the "prey" organism itself are re-examined, this is a second check (see Section 5.2) of the potential for impacts at lower levels of the food web (e.g. herbivorous fish). While aquatic toxicity testing could be used to determine a CBR, such testing generally does not include dietary exposures. Thus, for a given water concentration ("PEC"), higher body burdens in the organism may be achieved if the diet contributes significantly to the overall dose. This higher body burden may approach or exceed the CBR.

Second, and critical to the secondary poisoning question, the PEC_{oral} or concentration in the prey can be multiplied by the feeding rate for a given predator to obtain a daily dose ingested. This value may then be compared to a $PNEC_{oral}$, a diet-based toxicity value. The $PNEC_{oral}$ may be estimated or measured. Initially, other data (e.g. analogous substances dosed to similar species) could be used to estimate the $PNEC_{oral}$. Top predators (e.g. fish-eating birds or mammals) would be the primary focus. Mammalian data may be available from human safety testing, but extrapolation from lab rodent data to fish-eating birds or mammals is difficult. Two possible approaches are described below.

The US-EPA has suggested a series of extrapolation factors to deal with this need (US-EPA, 1993a). The most significant items for this inclusion here are the use of a chronic NOAEL (which may be estimated from subchronic data if necessary) and a species sensitivity factor (SSF) which may range from 1 to 0.01. Examples of this approach are the Great Lakes Wildlife Values for DDT, mercury, TCDD and PCB that have been proposed by the EPA (US-EPA, 1993b).

The EC Technical Guidance for environmental risk assessment of new substances applies assessment factors of 100, 30, and 10 to dietary 28 d, 90 d or chronic NOAEL values to derive a $PNEC_{oral}$ (EEC, 1993b). In this scheme, the PEC_{oral} is calculated from the $PEC_{regional}$ multiplied by the (calculated) BCF. Because this approach does not explicitly include the dietary pathway at lower tiers, it may underestimate the body burden of prey organisms for substances with higher lipophilicities (Figure 2). It also initiates the assessment at a log K_{ow} of 3. The combination of these two factors suggests that this method would be overconcerned with substances of little relevance for secondary poisoning, while underestimating actual exposure for substances in the log K_{ow} range of 4.5 to 8 which are the most critical to dietary exposure.

5.5.3 Optional Research in Considering Bioaccumulation

If the initial predator PEC/PNEC or risk quotient is not favourable, several options exist. The PEC_{oral} and/or $PNEC_{oral}$ could be further refined to reduce uncertainty in the assessment and to provide further safety reassurance. An iterative procedure is envisioned, in which the refined predator PEC/PNEC would result in either no further concern (PEC/PNEC <1) or a decision that risk management measures would be necessary for that substance.

In a refinement of PEC_{oral}, several options exist. Which of the options described below or other options are followed will depend largely on the use pattern and intrinsic substance characteristics.

- For example, the BCF may be experimentally determined. If the measured BCF is below that initially predicted, it is likely that the substance was not bioavailable (e.g. too large, sparingly water soluble) or was metabolised once taken up.

- In refining the PEC_{oral}, any of the methods used to reassess the predicted water concentration could be employed (ECETOC, 1993). This includes the use of local and regional multimedia models as described in HAZCHEM (ECETOC, 1994c).

- Additionally, well-designed monitoring programs could improve our understanding of the substance's behaviour in and impacts on the environment. Thus, field monitoring of appropriate biota (and water conditions) could confirm or refute model predictions of the PEC_{oral} or prey body burden, if the substance was already in use. Recommendations for the design of such field studies have recently been made (US-EPA, 1993d; UNEP/FAO, 1993).

In the refinement of the $PNEC_{oral}$, several options exist:

- Results of mammalian testing programmes should be considered to determine the $PNEC_{oral}$. Appropriate application factors should be introduced depending on the duration of the tests (acute vs. chronic).

- Potential food webs and target species for the substance can be identified given its fate and distribution in the environment. This may allow the discrimination between mammalian and avian target species. Focused feeding studies could be performed.

It must be pointed out that the use of food web models may still suffer from the lack of knowledge in (i) feeding frequency of predators on contaminated prey, (ii) bioavailability of the substance in food and water, (iii) estimates of metabolism in predators, (iv) dietary uptake efficiency, (v) diet and lifespan. The variability in these factors must be taken into account on a case-by-case basis.

6. CONCLUSIONS AND RECOMMENDATIONS

The main objective of the work described in this report was to review the assumptions and equations used to assess and integrate bioaccumulation in an environmental risk assessment. The approach recommended in this report applies to aquatic ecosystems and related food webs.

6.1 BIOACCUMULATION ASSESSMENT

It is concluded that bioaccumulation of a substance into an organism is not an 'effect' or hazard in itself. Bioconcentration and bioaccumulation will result in a body burden which may or may not lead to toxic effects. Therefore, when appropriate, the potential of a substance to bioaccumulate in the aquatic environment should be included as an exposure related parameter in the risk assessment.

Bioaccumulative substances, widely dispersed in the environment and which are known to be of concern are characterised by high persistence, toxicity, negligible metabolism and a log K_{ow} between 5 and 8. Biomagnification - defined as accumulation and transfer of substances via the food web, resulting in an increase of the internal concentration in organisms at succeeding levels in the trophic chain - is not as widespread as commonly believed. Biomagnification has only been demonstrated for a very limited number of substances.

The potential to bioaccumulate is related primarily to lipophilicity. A surrogate measure of lipophilicity is the n-octanol-water partition coefficient (K_{ow}), which is correlated with bioconcentration. Therefore, log K_{ow} is often used as predictor in quantitative structure activity relationships (QSARs) for bioconcentration factors (BCF) of organic non-polar substances.

For the physico-chemical descriptors listed in the EC Technical Guidance documents, only BCF QSARs based on log K_{ow} have been developed and validated. While this property may provide good predictions of fish BCF for lipophilic, nonionic substances undergoing minimal metabolism or biotransformation within the organism, the equations are best used only to predict BCF for substances within the class for which the QSAR was developed. Thus, the suitability of the individual QSAR should be evaluated prior to use by comparing the properties of the substance with those used to derive the QSAR.

Substances with a low lipid solubility, substances with a molecular weight well above 700, or substances which are considered as highly lipophilic will not be taken up as predicted from simple quantitative structure activity relations (QSARs) for bioconcentration factors (BCF). Non-linearity of

BCF versus log K_{ow} for highly lipophilic substances has been demonstrated. Similarly deviations have also been reported for other chemical classes such as surface-active, ionisable and polar substances.

Measured BCF values - based on parent substance - should when available be used instead of predicted values, because of the potential for biotransformation to reduce BCF. The major drawback of the various models proposed for the prediction of bioconcentration and bioaccumulation is that they assume no active biotransformation of the substance by the organism. Such metabolism can effectively prevent significant bioaccumulation. In addition, specific physico-chemical properties of the substance may reduce availability and possibly exclude uptake to such an extent that actual bioconcentration is negligible.

6.2 INTEGRATION OF BIOACCUMULATION IN A RISK ASSESSMENT

A step-wise approach is recommended to integrate bioaccumulation in an environmental risk assessment for substances which are widely distributed in the environment due to wide dispersive use and effective intermedia transfer, and which potentially can be taken up by biota. In practice, substances which are persistent, bioaccumulative, and exhibit negligible metabolism will be evaluated in this scheme.

For those substances which reach steady-state within the organism within the duration of the toxicity test, direct effects of bioconcentration are included, and thus the PNEC derived from this testing is appropriate for use in the risk assessment. However, for lipophilic substances which are taken up and depurated very slowly by fish, the steady-state body burden may not have been achieved during the test. Hence, environmental effects assessments should consider the "time to reach steady-state" in evaluating fish PNEC values for these substances. In a preliminary assessment, it is recommended to evaluate the time to reach 95% of steady-state (T_{95}). However, it must be pointed out that the T_{95} relationship was developed for classical lipophilic substances and therefore may overestimate the time needed to reach steady-state for other substances.

It was concluded that dietary uptake by aquatic organisms will be significant only if the substance has low water solubility, high lipid solubility and is slowly metabolised or eliminated by the prey organism. Initially, the BCF may be estimated as described above. When the BCF value is above 1,000 - if predicted this would correspond to a log K_{ow} of 4.3 - a PEC/PNEC assessment for predators is made and refined as deemed necessary on a case-by-case basis.

The recommended approach to integrate bioaccumulation in the environmental risk assessment has been evaluated for several representative substances with different properties and use patterns. This framework deals properly with actual "problem" substances, and avoids unjustified animal testing (Appendix E provides results of case-studies).

6.3 AREAS FOR FURTHER RESEARCH

Additional work is needed to provide further insights in the limitations and uncertainties of quantitative structure activity relationships (QSARs) used in the bioaccumulation assessment. Validation and/or reformulation of these QSARs for a wider range of chemical classes is needed if these QSARs are to be used with confidence in the risk assessment process.

Additional work is also needed to incorporate both knowledge and prediction of metabolic processes. The development of empirical QSARs for metabolism would aid in the prioritisation, assessment and regulation of apparently persistent and bioaccumulative substances.

The bioaccumulation and food transfer models will overestimate the potential of substances to bioconcentrate and to transfer up the food web. Thus, attempts to understand, predict and incorporate processes such as volume exclusion, metabolism, and others into these models should continue. Incorporation of biotransformation into bioaccumulation models is seen as an important next step for the verification and validation of these models.

APPENDIX A. GLOSSARY OF TERMS

Bioaccumulation

The net result of the uptake, distribution and elimination of a substance due to all routes of exposure.

Bioconcentration

The net result of the uptake, distribution and elimination of a substance due to water-borne exposure.

Biomagnification

The accumulation and transfer of substances via the food web (e.g. *algae* - invertebrate - fish - mammal) due to ingestion, resulting in an increase of the internal concentration in organisms at the succeeding trophic levels.

Bioaccumulation Factor (BAF)

The ratio of the steady-state concentration of a substance in an organism due to all routes of exposure vs. the concentration of the substance in water.

Bioconcentration Factor (BCF)

The ratio of the steady-state concentration of a substance in an organism due to water-borne exposure vs. the concentration of the substance in water.

Bioavailability

The ability of a substance to interact with the biosystem of an organism. Systemic bioavailability will depend on the chemical or physical reactivity of the substance and its ability to be absorbed through the gastrointestinal tract, respiratory tract or skin. It may be locally bioavailable at all these sites.

Depuration

The removal of the substance from an organism. The rate of depuration is expressed by its half-life or the time needed to eliminate 50% of the substance in a non-contaminated medium. This term is often referred to as the depuration time (DT_{50}).

Secondary Poisoning

The product of trophic transfer and toxicity.

Poikilothermic Organisms

Organisms the body temperature of which is widely dependent on the temperature of the surrounding environment and muscular activity.

Hydrophilicity

Property of substances which dissolve in water or which are able to absorb or to bind water.

Lipophilicity

Property of substances which dissolve in lipids or which are able to absorb or to bind to lipids.

Hydrophobicity

Property of substances which are unable to absorb or to bind water, and therefore difficult to dissolve in water.

Fugacity (from Jørgensen, 1990)

Fugacity may be considered a measure of 'escaping tendency' from the phase. When phases are at equilibrium, their fugacities are equal.

Fugacity, f (Pa), is related to concentration by the following equation:

$$f = \frac{C}{Z} \qquad (Eq.11)$$

C= concentration (mol/m^3)

Z= fugacity capacity (mol/m^3 Pa)

Equilibrium involves:

$$\frac{C_a}{C_w} = \frac{Z_a}{Z_w} = K_p \qquad (Eq.12)$$

a = air

w = water

K_p= partitioning coefficient

Transfer rates (N) between two phases by diffusion:

$$N = D \cdot \Delta f \qquad (Eq.13)$$

D = diffusion coefficient

Δ_f = difference in fugacity

D = K_p *x* Z

APPENDIX B. ENZYME SYSTEMS INVOLVED IN BIOTRANSFORMATION OF XENOBIOTICS

In general, two types of biotransformation reactions are observed in aquatic organisms, these have been classified as phase I and phase II reactions (see Section 3.4.3). They are described in this appendix in greater detail.

B.1 PHASE I REACTIONS

Oxidative metabolism involving molecular oxygen is the initial enzymatic process in the biotransformation of a majority of lipophilic organic xenobiotics and is catalysed by monooxygenases. The general scheme for such reactions is:

$$RH + NADPH + O_2 + H^+ \rightarrow ROH + NADP^+ + H_2O$$

where RH = the substrate
ROH = the hydroxylated product

These oxidative processes are the main ones involved in Phase I. Hydroxylation of carbon atoms is catalysed by the cytochrome P450-dependent monooxygenases (or mixed-function oxidases, MFO), and hydroxylation of N- or S- heteroatoms is catalysed by the flavine monooxygenases (FMO). Other Phase I enzymes are redox enzymes (e.g. alcohol and aldehyde dehydrogenases), which alter the oxidation state of a carbon atom.

Alternatively, pre-existing functional groups can be set free by a number of hydrolytic enzymes, including epoxide hydrolases, esterases, and amidases.

B.1.1 Cytochrome P450 in Vertebrates

The cytochromes P450 comprise a large family of haeme proteins which catalyse many types of seemingly disparate, primarily oxidative reactions. Their substrates include both endogenous substances as well as xenobiotics.

Table B.1: P450 Reactions and Representative Xenobiotic Substrates

Type of oxidation	Representative xenobiotic substrate
Aromatic hydroxylation	Benzo[*a*]pyrene
Aliphatic hydroxylation	n-Hexane
Epoxidation	Aflatoxin B1
N-Dealkylation	Aminopyrine, ethylmorphine, carbaryl
O-Dealkylation	7-Ethoxyresorufin
S-Dealkylation	Methylmercaptane
N-Oxidation	Aniline, amphetamine
S-Oxidation	Aldicarb
P-Oxidation	Parathion
Desulphuration	Parathion
Oxidative dehalogenation	Halothane
Reductive dehalogenation	Hexachlorobenzene, carbon tetrachloride

Some types of P450 reactions and representative xenobiotic substrates are shown in Table B.1. Cytochrome P450 forms, identified and classified on the basis of their gene sequences, are numerous (possibly about 100 in one species). Some cytochrome P450s are induced by xenobiotics. For example, PAH, polychlorinated biphenyls (PCB), chlorinated dioxins and furans, ethanol, ketones, β-naphthoflavone and phthalates are inducers (Stegeman and Hahn, 1994).

Fish have been less studied than mammals, but strong similarities have been found. Concentrations of cytochrome P450 based on microsomal liver protein in fish are similar to those in birds and mammals (Sijm and Opperhuizen, 1989), including marine mammals (Watanabe *et al*, 1989; White *et al*, 1994). Fish P450s that are proven members of several mammalian subfamilies have been described.

Studies of the metabolism of xenobiotics by fish demonstrate the following:

- PAH metabolism in fish is strongly induced by polynuclear and planar halogenated aromatic hydrocarbons. The rates of *in vitro* metabolism of PAH are rapid in most fish exposed to inducers (Stegeman, 1981).

- Halogenated aromatic hydrocarbons including PCB and dioxins are metabolised more slowly than PAH in fish, even in animals that are induced (Sijm and Opperhuizen, 1989).

- PCB congeners with unsubstituted meta or para positions may be metabolised more rapidly than others, but rates are still low (White and Stegeman, 1994).

B.1.2 Cytochromes P450 in Invertebrates

In general, the levels of cytochromes P450 in non-mammalian vertebrates approach and in some cases exceed those commonly seen in mammals, while the levels in invertebrates are lower (Stegeman and Kloepper-Sams, 1987). Inducibility of P450 forms in invertebrates is not well elucidated.

Some generalisations can be drawn (Stegeman and Hahn, 1994):

- the rates of xenobiotic metabolism in many invertebrate groups are substantially lower than in vertebrates (2 to 3 orders of magnitude lower in molluscs for PAH);

- the rates of PAH metabolism in crustaceans are intermediate to those in molluscs and fish.

Therefore, invertebrates such as molluscs, with low capacities for biotransformation of many substrates, accumulate higher steady-state levels of many xenobiotics.

B.1.3 Flavoprotein Monooxygenases (FMO) in Vertebrates

The FMOs are not inducible by xenobiotics and are membrane-bound. These enzymes carry out N-oxidations or S-oxidations (more generally heteroatom-oxidations) with a wide range of aliphatic amines and sulphides, thiols, phosphines and others. Any nucleophilic structure that can be oxidised by a peroxide appears to be a potential substrate for FMO.

Typical examples of reactions catalysed by FMOs are given in Table B.2 (from Jacoby and Ziegler, 1990).

In fish, some FMO-like activities similar to those presented in Table B.2 were detected (Augustsson and Strom, 1981). The studies by Schlenk and Buhler (1990, 1993) in rainbow trout also showed immunochemical cross-reactivity between antibodies to either pig or rabbit FMO and proteins in rainbow trout liver or gill. Multiple forms of FMO have been detected in fish liver.

Table B.2: Examples of Reactions Catalysed by Flavoprotein Monooxygenases

Substrate	Product	Examples of substrates
R_3-N	R_3-NO	Trimethylamine; N,N-dimethylaniline; morphine
R_2-NH	R_2N-OH → R_2N→O	N-methylaniline; desipramine; nortryptyline
R_2-N-NH_2	R-N=N-R	1,1-dimethylhydrazine; 1-methyl-1-phenylhydrazine
RSH	RSOH → RSSR → RS(=O)SR	Dithiothreitol; benzylmercaptan
R-S-R	R-S(=O)-R → R-S(=O)$_2$-R	Dimethylsulphide; sulindac sulphide; cimetidine
R-N=CSH-N-R_2	R-N=CSOH-N-R_2 → R-N=CSO_2H-N-R_2	Thiourea; methimazole; propylthiouracil
R-C(=S)-S^-	R-C(=S)-SO^-	Dithiobenzoate; N,N-diethyldithiocarbamate
R_3-P	R3-P=O	Triphenylphosphine; phonophos
RB(OH)$_2$	ROH + B(OH)$_2$	Butylboronic acid

B.1.4 Flavoprotein Monooxygenases in Invertebrates

In invertebrates, a number of aromatic amines, e.g. 2-acetylaminofluorene (AAF), are metabolised by FMO by N-hydroxylation (Miller and Miller, 1981). Molluscan hepatopancreas preparations were found to catalyse N-hydroxylation of AAF. The absence of carbon monoxide inhibition of AAF activation by the molluscan preparations indicated that this reaction was catalysed by the non-haeme FMO. Kurelec (1985), Kurelec and Krca (1987) and Schlenk and Buhler (1990) found FMO in invertebrates. As P450 activity is relatively low, FMOs are important in the metabolism of xenobiotics in invertebrates.

B.1.5 Non-Oxidative Reactions

Little is known about hydrolytic reactions in fish compared with oxidative reactions. They are catalysed by esterases, amidases and epoxide hydrases, and occur in liver, kidney, and plasma (Lech and Vodicnik, 1985). These enzymes catalyse hydrolysis of amides, phosphates, carbamates, esters and alkene or arene oxides (Bend and James, 1978).

Enzymes which catalyse reduction reactions have been identified in mammals and comprise (Bend and James, 1978):

- dechlorinative reductase;
- ketone reductase;
- alkene oxide reductase;
- arene oxide reductase;
- N-oxide reductase;
- nitro reductase;
- azo reductase.

Dechlorination does occur in fish, but at a very low rate (Bend and James, 1978). Azoreductases were identified in the primitive fish, the elasmobranchii, while both azo and nitro reductases were found in less primitive fish, the teleosts. In a comparative study, it was shown that mammals have slightly higher nitroreductase activities than fish (Hitchcock and Murphy, 1967).

B.2 PHASE II REACTIONS: CONJUGATION

Conjugation reactions have been reviewed by George (1994). They are major pathways for solubilisation, inactivation, and subsequent excretion of both endogenous and xenobiotic organic substances. They are catalysed by three main families of enzymes: UDP-glucuronosyltransferases (UDPGTs), sulphotransferases, and glutathione S-transferases. Conjugation also serves to solubilise endobiotic molecules for transport.

In fish, the major pathway for conjugation of electrophilic substances is conjugation with glutathione, while for nucleophilic substances, conjugation with glucuronic acid is the major route. Sulphation is a competing pathway to glucuronidation, but it is only effective at very low substrate concentrations. Another pathway, acetylation, is largely confined to organic xenobiotics containing an amino group such as aminobenzoic acid, while amino acid conjugation may be important for carboxylic acids, including chlorinated phenoxyacetic acids (2,4-D and 2,4,5-T) and carboxylic acids such as the pyrethroid insecticides.

B.2.1 Glucuronidation

Mammalian UDPGTs catalyse the transfer (conjugation) of glucuronic acid from the high energy nucleotide UDP-glucuronic acid to a wide variety of acceptor substrates to form O-glucuronides (alcohols, phenols, and carboxylic acids), N-glucuronides (carbamates, amides and amines), S-glucuronides (aryl mercaptans and thiocarbamates), and C-glucuronides (1,3-dicarbonyls).

As with most enzymes that exhibit a broad specificity for structurally diverse substances, multiple isoenzymes are found. Several isoenzymes appear to show a fairly narrow substrate specificity, while others display a degree of overlapping specificity. The UDPGTs display differential induction by xenobiotic substances.

While liver is quantitatively the most important site for glucuronidation of xenobiotics, significant activity has also been detected in kidney, gills and intestine of several species. Thus, both mammals and fish exhibit a tissue-specific expression of different UDPGT activities, indicating a multiplicity of UDPGT isoforms.

As in mammals, glucuronidation is clearly an extremely important pathway for detoxication and excretion of xenobiotic substances in fish. Glucuronide conjugates have been detected in bile, urine, and various tissues (Clarke *et al*, 1991). The xenobiotics so conjugated include an extensive range of phenols and phenolic metabolites of PAH. The majority of these conjugates are O-glucuronides, although N-glucuronides of a carbamate insecticide and a phenylthiazole have also been identified in rainbow trout (Clarke *et al*, 1991).

B.2.2 Sulphation

The mammalian sulphotransferases (ST) are cytosolic enzymes that conjugate hydroxyl groups of polyaromatic substances, aliphatic alcohols, aromatic amines, and hydroxylamines with activated 3'-phosphoadenosine 5'-phosphosulphate to form a sulphate monoester (sulphonate or ethereal sulphate).

In mammals, 4 classes of sulphotransferases have been identified, displaying specificities for phenolic steroids, hydroxysteroids, bile salts, and xenobiotics such as phenols, catechols and hydroxylamines.

High ST activity toward phenolic substances has been reported in a number of fish species (Layiwola and Linnecar, 1981). However, quantitatively, sulphates have been shown to be very minor

metabolites of PAH such as naphthalene and BaP (Varanasi and Gmur, 1981; Morrison *et al*, 1985; Varanasi, 1989).

Glucuronidation is the more important excretory pathway in salmonids and pleuronectid flatfish, where aryl ST activity is low. However, further metabolic studies using isolated hepatocytes and characterisation of aryl ST are required before the functional significance of sulphation in fish can be truly assessed.

B.2.3 Glutathione S-transferase Activity

The glutathione S-transferases (GST) have been identified in molluscs, crustaceans, insects, fish, amphibians, and mammals. They are believed to play major roles in :

- irreversible binding of reactive electrophilic xenobiotics, such as azo dyes and PAH;
- catalysis of glutathione (GSH) conjugation with electrophilic centres in a variety of compounds (e.g. epoxides) as the first step in the formation of the excretory metabolites, the mercapturic acids. They can replace hydrogen, chlorine or nitro groups.

There are many GSTs in rats and they display a diversity of activities with xenobiotic substrates.

Activity toward the model substrate chlorodinitrobenzene (CDNB) has been demonstrated in all fish species so far examined. The activity in fish is comparable to that in mammals. Extrahepatic GST activity has been demonstrated in a number of species as well as hepatic activity. The expression of a varied spectrum of relative activities in the different tissues suggest that, as in mammals, different isoenzymes are expressed in a tissue-specific manner (George, 1994).

B.2.4 Acylation

Acylation is the conjugation of carboxylic acids with amines. The reaction is catalysed by an N-acetyltransferase and either the amine or the carboxylic acid can be a xenobiotic. Three conjugates have been found which can react with xenobiotics in this way: acetate, taurine and glycine. Examples are given in Table B.3.

Table B.3: Conjugates which can be Acylated with Xenobiotics

Substance	Matrix	Conjugate	Reference
Aniline	whole body	acetyl	Metcalf *et al* (1973)
Benzoic acid	whole body	glycine	Metcalf *et al* (1973)
2,4-D	urine	taurine	Stalling and Huckins (1978)
Phenylacetic acid	urine	taurine	Stalling and Huckins (1978)

B.3 MECHANISMS OF BIOTRANSFORMATION

The following sections on PAH, pesticides and surfactants will treat the mechanisms of biotransformation in more detail.

B.3.1 PAH Metabolism and the Effect on Bioaccumulation

PAHs have been reported in field-sampled marine animals, and concentrations vary widely with the site of collection and with species (Stegemann, 1981). These data show that PAH can accumulate in marine animals, but also that effective elimination may occur when the organisms are removed from the source of contamination. Differences between fish, crustaceans and molluscs have been observed in the concentrations of benzo[*a*]pyrene (BP) (in teleost fish and crabs below 10 µg/kg dry weight and in molluscs up to 780 µg/kg dry weight (Neff, 1981)). This clearly implies differences in factors controlling the steady-state concentration of such substances. These differences are shown hereafter to be due to physiological differences related to metabolism of PAH.

There is abundant evidence from mammalian studies that oxidative metabolism of PAH can result in the formation of reactive metabolites responsible for toxic effects. Studies on the metabolism of PAH clearly indicate that arene oxides, and especially diol epoxides, are important in carcinogenicity of these substances (Gelboin *et al*, 1972; Sims and Grover, 1974).

Metabolites of Benzo[a]pyrene (BP) Formed In Vitro

Most of the studies concerning metabolites of PAH formed by aquatic species have dealt with hepatic metabolism of BP by fish. Comparable results imply that formation of oxygenated PAH metabolites in marine fish occurs by the same mechanisms as in mammals. Arene oxides first formed by cytochrome P450 are either hydrated to diols by epoxide hydrolase (EH) or rearranged to form phenolic derivatives. Quinones probably represent further, non-enzymatic oxidation products.

Various pathways have been observed as described in the scheme below (Stegemann, 1981):

---(P450)---> [?] ------> 1-OH ------> 1,6-quinone

---(P450)---> [?] ------> 3-OH -----> 3,6-quinone

---(P450)---> 4,5-oxide ---(EH)---> 4,5-diol

---(P450)---> 7,8-oxide ---(EH)---> 7,8-diol -----> 7-OH

---(P450)---> 9,10-oxide ---(EH)--> 9,10-diol ----> 9-OH

6,12-Quinone has also been found, but its origin is unclear as no 6-OH has been detected.

Profiles of metabolites formed by tissue microsomes of several aquatic species are compared in Table B.4. All species tested exhibited extensive metabolism. However, the amount and distribution of metabolites differ according to the species.

Metabolism In Vivo

The pharmacokinetics of PAH in marine species are less well known than are the *in vitro* aspects of metabolism. A number of environmental factors (e.g. temperature) have been found to influence the disposition of aromatic hydrocarbons in aquatic species (Collier *et al*, 1978). Induction of cytochrome P450 also affects the disposition of aromatic hydrocarbons *in vivo*, at least in fish (Statham *et al*, 1978).

Metabolism of some PAH in induced animals could result in a higher steady-state of toxic products (arene oxides, phenolic derivatives). However, these products can be detoxified by conjugating enzymes and epoxide hydrolases (Oesch, 1973). Conjugated derivatives are the dominant species of PAH excreted in bile by some fish (Solbakken *et al*, 1980).
Epoxide hydrolase and glutathione transferase activities have been studied with a number of alkene and arene oxide substrates in hepatic and extrahepatic tissues of species representing several phyla of aquatic animals (Bend *et al*, 1977; James *et al*, 1979; Tate and Herf, 1978; Balk *et al*, 1980). The levels of hepatopancreatic microsomal epoxide hydrolase activity in crustaceans are generally higher than those in fish liver. On the other hand, cytosolic glutathione transferase activities are generally higher in fish species.

Table B.4 : Microsomal Metabolites of Benzo[*a*]pyrene (BP)

Species	Treatment	Metabolites (%)						Reference
		3-OH-BP	9-OH-BP	Quinones	4,5-Diol	7,8-Diol	9,10-Diol	
Mammal								
Rat	Control	35	13	34	6	4	8	Pezzuto *et al* (1978)
	Phenobarbital	30	8	28	23	3	7	
	3-Methyl cholanthrene	36	6	19	6	14	18	
Fish								
Little Skate (*S.erinacea)*	Control	50	18	4	4	15	9	Bend *et al* (1979)
	1,2,3,4-Dibenz-anthracene	32	17	26	1	13	11	
Scup (*S.chrysops),*	Control	14	3	27	-	33	22	Stegemann and Woodin (1980)
S.versicolor	3-Methyl cholanthrene	55	11	16	-	12	6	Stegemann (1981)
Sheepshead	Control	12	12	15	5	21	22	Little and James (1980)
	3-Methyl cholanthrene	19	9	11	2	22	12	
Southern flounder	Control	12	12	12	3	27	26	Little and James (1980)
	3-Methyl cholanthrene	17	3	9	2	32	26	
Fundulus heteroclitus	None	29	5	27	1	20	19	Stegemann (1981)
Trout (*Salmo trutta)*	None	33	2	14	1	27	22	Ahokas *et al* (1976)
Fundulus parvipinnis	None	45	7	8	3	20	18	Puffer and Van Hofe (1980)
Crustacean								
Barnacle	None	58	19	1	-	14	7	Stegemann and Kaplan (1981)

Changes in the proportions of these activities (monoxygenase, epoxide hydrolase, glutathione transferase) probably contribute to patterns of PAH metabolism in different species, and therefore to final PAH levels in these species.

Metabolism of PAH may greatly influence bioaccumulation. For example, the metabolism of BP has an important influence on BCF in *Lepomis macrochirus* (see Section 3.4.4, Table 1): the experimentally determined BCF is 490, rather than 63,100 calculated from the log K_{ow}.

B.3.2 Pesticide Metabolism and the Effect on Bioaccumulation

Pesticides are a chemically heterogeneous group of molecules which can be defined as substances or mixtures of substances intended for preventing, destroying or controlling any pest. Depending on the target organisms pesticides can be subclassified e.g. as insecticides, acaricides, herbicides, fungicides, rodenticides.

Metabolism of pesticides in mammals and fish is extensively investigated before registration. Most higher organisms exhibit significant capacities for metabolism of pesticides, based in general upon hepatic microsomal mono-oxygenase systems, which are inducible and employ cytochrome P450 (Phillips, 1993). Enzyme systems which metabolise pesticides are essentially the same as for other substances and xenobiotics and are summarised in Table B.5.

Typically, biotransformation reveals or introduces polar functional groups into the chemical structure of the pesticide, rendering it less lipophilic and therefore more readily excretable. Thus, the depuration rate of the polar metabolite is enhanced with respect to the uptake rate of the parent molecule. The effects of metabolism can significantly reduce the correlation between predicted and observed BCF values (Huckle and Millburn, 1990). Some examples of the influence of biotransformation on the bioconcentration factor (BCF) of pesticides in fish are presented in Table 1.

Enzyme induction after exposure to xenobiotics may also have an effect on the rate and extent of biotransformation and hence bioconcentration of pesticides in fish. Nevertheless, there is still a need for further research in this area.

In the following a few examples of metabolism of pesticides are reported and its impact on bioconcentration is evaluated.

Table B.5: Enzyme Systems which Metabolise Pesticides (from Phillips, 1993)

Enzyme system	Location	Substances metabolised
Phase I reactions		
Mixed function oxidases	Microsomes, notably from vertebrate liver and insect fat body	Many liposoluble pesticides
Phosphatases	Present in nearly all tissues and subcellular fractions of species	Organophosphorus insecticides and "nerve gases"
Carboxyesterases	In most tissues of insects and vertebrates	Malathion and malaoxon
Epoxide hydrase	Microsomes, particularly in the mammalian liver	Dieldrin, heptachlor and arene epoxides
DDT dehydrochlorinase	Virtually all insects and vertebrates	p,p'-DDT and p,p'-DDE
Phase II reactions		
Glucuronyl transferases	Mainly in microsomes; widespread in vertebrates other than fish and insects	Substances with labile hydrogen, including hydroxylated metabolites
Glutathione-S-transferases	Vertebrate livers and insects	Chlorinated substances, e.g. γ-HCH; also some epoxides

Organophosphates

The organophosphorothionate insecticides can undergo biotransformation leading to activated products, e.g. the oxidation of the P=S group to the P=O derivative, and also to metabolites which possess reduced or negligible activity as acetylcholinesterase inhibitors.

In a study to determine lethal body burdens of four organophosphorus pesticides in the guppy (*Poecilia reticulata*), de Bruijn *et al* (1991) have observed that the bioconcentration factor of chlorothion at lethal exposure levels was a factor of 10 higher than the bioconcentration at sublethal levels. The authors concluded that this dependence on aqueous exposure levels of the administered substance may be caused by effects on the biotransformation processes.

In a bioaccumulation study with malathion (log K_{ow} = 2.75), pinfish (*Lagodon rhomboides*) were exposed for 24 hours to 75 µg/l malathion in flowing seawater. Various organs of pinfish were analysed for malathion, malaoxon, the monocarboxylic acid (MCA) and the dicarboxylic acid (DCA) of malathion. Malathion and malaoxon were not found in any organ, whereas MCA and DCA were detected in relevant concentrations. Thus, pinfish very rapidly convert malathion to mono- and

diacids, the greatest concentrations of which were found in the gut (Cook and Moore, 1976). This finding illustrates the value of analysing tissues for metabolites, as well as for the parent substance, in monitoring the exposure of fish to pesticides following field applications (Edwards and Millburn, 1985).

The fate of fenitrothion was studied in the euryhaline fish, killifish (*Oryzias latipes*) and mullet (*Mugil cephalus*) (Takimoto *et al*, 1987). The major reaction in killifish appears to be hydrolysis and that in mullet demethylation. The insecticide was rapidly absorbed and when the fish were transferred to fenitrothion-free water the body burden of both radioactivity and the parent substance was rapidly cleared, with biological half-lives of less than 0.5 day for killifish. In mullet, the concentration of fenitrothion also decreased rapidly, but the half-life of ^{14}C was between 2.4 and 3.8 d. For further details see Table 4.

Organochlorines

Routes of biotransformation of organochlorines vary, dechlorination and oxidation reactions, however, predominate (Hutson and Roberts, 1985).

The organochlorine insecticide dichloro diphenyl trichloroethane (DDT) together with its main metabolite dichloro diphenyl dichloroethene (DDE) is the classic example of a persistent substance. The adverse effects of DDT include the accidental killing of fish, birds and other non-target species. Furthermore, DDT has become widely distributed in the environment. The fate of DDT in fish illustrates that, for poikilothermic animals, the rates of substance uptake and metabolism are linked to the temperature of their environment. Thus fish can rapidly take up high concentrations of DDT from the water, and the uptake increases with temperature. DDT is metabolised by dehydrochlorination to the persistent and insecticidally inactive DDE (Huckle and Millburn, 1990). An alternative route of biotransformation for DDT is reductive dechlorination to DDD, which can undergo further metabolism via several intermediates to DDA, 1,1-bis-(4-chlorophenyl) acetic acid. This carboxylic acid is conjugated with the amino acid taurine in the southern flounder (*Paralichtys lethostigma*) and then excreted in the urine (James, 1978; 1987).

A comparison of the bioaccumulation of DDT and several of its structural analogues was performed in a model ecosystem, consisting of algae, snails, mosquito larvae and mosquito fish (*Gambusia affinis*) (Kapoor *et al*, 1973). Substances studied were different methoxy, ethoxy, methyl and methylthio derivatives of DDT. The partition coefficients log K_{ow} of the substances were similar, in the range of 2.89 to 4.47, but the substances exhibited significantly different bioaccumulation; the experimentally determined BAF values based on parent substance were in the range 5.5 [2-bis(p-

methylthiophenyl)-1,1,1-trichloroethane] to 84,500 (DDT). While DDT appeared to be stable in mosquito fish, the structurally related analogues were in some cases extensively degraded. The presence, for example, of the CH_3S moiety in 2-(p-methoxyphenyl)-2-(p-methylthiophenyl)-1,1,1-trichloethane, which is subject to sulphoxidation, provides a biodegradable centre and effects a significant reduction of BCF when compared with DDT itself (310 and 84,500, respectively).

Pyrethroids

Synthetic photostable pyrethroids are used as insecticides on a variety of crops. Their metabolism in most vertebrate species is largely dependent upon the cis/trans conformation of the substituent groups at C-1 and C-3 of the cyclopropane ring. Thus, the cis-isomers (in which the C-1 carboxyl group and the C-3 dichlorovinyl group are on the same side of the cyclopropane ring) are preferentially metabolised by aromatic hydroxylation, whereas the trans-isomers (C-1 and C-3 substituents on opposite sides of the ring) are better substrates for ester cleavage (Hutson, 1979). Trout liver microsomes metabolise the cis- and trans-isomers of cypermethrin at much slower rates than liver microsomes from mouse and quail (Edwards *et al*, 1987). Trout has a low capacity for ester cleavage, using primarily aromatic hydroxylation, followed by glucuronidation. By contrast, the mouse has abundant esterase activity, particularly for the readily hydrolysed trans-isomers, and the quail rapidly metabolises both cis- and trans-cypermethrin by ester cleavage. Therefore species differences in the rates and routes of cypermethrin biotransformation are important factors in its selective toxicity (Huckle and Millburn, 1990). Even in fish, metabolism of pyrethroids is rapid. A case example on the assessment of bioaccumulation of cypermethrin is included in Appendix E.

Bioaccumulation studies with fish have shown that pyrethroids have low bioconcentration factors, far less than predicted from correlation between log K_{ow} and BCF (L'Hotellier and Vincent, 1986). For deltamethrin, the authors report a log K_{ow} of 6.2, estimated by reverse-phase HPLC. Muir *et al*, 1986 have exposed rainbow trout to 100 ng/l concentrations of *cis*-cypermethrin, *cis*-permethrin, fenvalerate and deltamethrin in flow-through systems for four days. Exposure was carried out with three water types: filtered lake water (12 mg/l DOC), humic acid solution (6.5 mg/l DOC) and distilled water (0.5 mg/l DOC). The equilibrium bioconcentration factors in distilled water were 123 (fenvalerate), 203 (deltamethrin), 300 (*cis*-permethrin) and 344 (*cis*-cypermethrin). In humic water even lower bioconcentration factors in the range from 27 to 58 were reported by the authors.

Herbicides

Most herbicides undergo extensive biotransformation and environmental degradation (Huckle and Millburn, 1990). For example, Call *et al* (1987) have measured uptake and elimination of the herbicides bromacil (K_{ow}=75.2, (from Tomlin, 1994)) and diuron (K_{ow}=700, (from Tomlin 1994)) in fathead minnows. The BCF values experimentally determined were < 3.2 and 2.0, respectively.

The butoxyethyl ester of 2,4-D exhibits a low water solubility (12 mg/l) and has a log K_{ow} of 3.5 (Reinert and Rodgers, 1987). Theoretical prediction of BCF based on water solubility or log K_{ow} gives values of 408 and 162, respectively (Huckle and Millburn, 1990). The experimentally determined whole body BCF values were between 2 an 14 in channel catfish and between 6 and 21 in bluegill sunfish. The ester readily undergoes hydrolysis to the free acid and is rapidly eliminated from these fish (Rodgers and Stalling, 1972).

B.3.3 Surfactant Metabolism and the Effect on Bioaccumulation

Surfactant Structure

Surfactants are characterised by combining a lipophilic and a hydrophilic moiety in the same molecule. Four classes can be recognised namely, anionic, cationic, nonionic and amphoteric surfactants. Although these classes possess quite different hydrophilic groups, the lipophilic part usually consists of an alkyl chain or alkyl chains of various lengths. There is evidence that the lipophilic groups of surfactants are metabolised by a mechanism also utilised in the breakdown of fatty acids.

Fatty-acid Metabolism

Metabolism of free fatty acids via β-oxidation is known to take place both in mitochondria and peroxisomes (for reviews see: Schulz; 1991, Guzmán and Geelen, 1993). Additionally fatty acids may enter a minor metabolic route in which they undergo ω-oxidation and are biotransformed to ω-hydroxymonocarboxylic acids (Hardwick *et al*, 1987; Christensen *et al*, 1991) which can be further metabolised to dicarboxylic acids. Although only of minor importance in fatty acid breakdown, this route is of obvious importance to surfactant biotransformation. The process of β-oxidation is ubiquitous with many similarities between prokaryotic and eukaryotic systems (Black and DiRusso, 1994). ω-oxidation has been found in mammals (Guzmán and Geelen, 1993), fish (Miranda *et al*, 1989) and bacteria (Swisher, 1987). There is little data on the occurrence of ω-oxidation in invertebrate species.

Eukaryotic Surfactant Metabolism

Aquatic Invertebrate Species

Published data on surfactant metabolism by invertebrates are limited. Comotto *et al* (1979) found only intact linear alkyl benzene sulphonate (LAS) in *Daphnia* following exposure to radiolabelled surfactant suggesting little or no metabolism. It is not clear if invertebrates like *Cladocera* possess the capacity for ω-oxidation. The metabolism of the nonionic surfactant 4-nonylphenol by the bivalve *Mytilus edulis* was also very limited (Ekelund *et al*, 1990). This may be due to the branched nature of this surfactant or the low capacity for surfactant metabolism in *Mytilus*. McKinnell (1994), however, found that the nonionic surfactant dodecyl tetra(oxyethylene) ether was rapidly metabolised in *Chironomus riparius* to both more polar (possibly shortened alkyl chain) as well as less polar metabolites which were not identified. Therefore little can be said about the capacity for surfactant metabolism in aquatic invertebrates at present as the limited data available are inconclusive.

Fish

Compared to aquatic invertebrates more data on surfactant metabolism are available for fish. There is good evidence that metabolism plays an important role in the elimination of surfactants although in only a few studies metabolites were identified. Some of the published data are summarised in Table B.6.

Apart from these, further evidence of surfactant metabolism in goldfish is provided by research carried out by Unilever (Unilever Research, manuscript in preparation). This work has mainly focused on anionic surfactants but included studies on some nonionics. Although these were not bioconcentration studies they did look at surfactant metabolism using radio-TLC and GC-MS. For comparative purposes some of these studies also looked at metabolites in rat urine. The main findings are summarised in Table B.7.

The above evidence strongly suggests that both anionic and nonionic surfactants are rapidly and extensively metabolised by fish and that the metabolic pathway is likely to be similar i.e. ω- followed by β-oxidation leading to small, water-soluble molecules which are often derivatives of butyric acid for alkyl chains with even numbers of carbon. Propionic acid derivatives would be expected for "uneven" carbon-chains. There is no published data on metabolism of cationics in fish although it may be expected that the same pathways as described for fatty-acids and anionic and nonionic surfactants apply.

Table B.6: Surfactant Metabolism in Fish

Substance	Species	Matrix	Metabolite	Reference
Sodium lauryl sulphate	*Carassius auratus*	Bile	Principal metabolite : butyric acid-4-sulphate * Minor metabolites : C_{10}, C_8 and C_6 intermediates†	Tovell *et al*, 1975
		Water	Principal metabolite : butyric acid-4-sulphate *	
Linear alkyl benzene sulphonate	*Pimephales promelas*	Bile	98 % of ^{14}C recovered as metabolites †	Comotto *et al*, 1979
		Gills	25-75 % of ^{14}C as metabolites †	
		Kidney	75-85 % of ^{14}C as metabolites †	
2-phenyl dodecane	*Oncorhynchus mykiss*	Bile	Principal metabolites (4) including 3-phenyl-butyric acid * Minor metabolites (10) †	Burke *et al*, 1988 and 1991
Dioctyl sodium sulphosuccinate	*Oncorhynchus mykiss*	Bile	Principal metabolites (2) : 85% of recovered ^{14}C † Parent substance : 14 % of recovered ^{14}C †	Goodrich *et al*, 1991
Sodium dodecyl trioxyethylene	*Cyprinus carpio*	Bile	Majority of ^{14}C recovered as metabolites †	Kikuchi *et al*, 1980
Sodium dodecyl tetra(oxyethylene)ether	*Cyprinus carpio*	Bile	> 95 % of recovered ^{14}C as metabolites †	Wakabayashi *et al*, 1987
		Gills	> 85 % of recovered ^{14}C as metabolites †	
		Kidney	> 90 % of recovered ^{14}C as metabolites †	
		Liver	> 85 % of recovered ^{14}C as metabolites †	
		Blood	> 80 % of recovered ^{14}C as metabolites †	

* metabolite identified using GC-MS
† identity of metabolite(s) not known

Table B.7: Surfactant Metabolism in Goldfish and Rat

Surfactant	Species	Matrix	Metabolite(s)
2(p-Benzene sulphonate) n-dodecane	*Carassius auratus*	Water	Principal metabolite: butyric acid-3-benzene p-sulphonate* Minor metabolites: hexanoic and octanoic acid intermediates†
6(p-Benzene sulphonate) n-dodecane	*Carassius auratus*	Bile	Pprincipal metabolites (4): decanoic acid 5 p-benzene sulphonate, undecanoic acid 6 p-benzene sulphonate and dodecanoic acid 6 p-benzene sulphonate†
C12-15 Sulphate (commercial sample)	*Carassius auratus*	Bile	Principal metabolites (3): short chain alkanoic sulphates†
C15 Alkyl sulphate	*Carassius auratus*	Bile	Principal metabolites (3): identity unknown Parent surfactant: 8 % of recovered ^{14}C
		Water	Principal metabolites (4): 82 % of recovered ^{14}C Parent surfactant: 4 % of recovered ^{14}C Free alcohol: 14 % (suggests desulphation)
2-Methyl undecan 1-sulphate	*Carassius auratus*	Bile	Principal metabolites (2): 64 % of ^{14}C recovered, C3 derivative of parent* Minor metabolites: 25 % of recovered ^{14}C† Parent surfactant (11%)
		Water	Principal metabolites (2): 47 % of recovered ^{14}C† Minor metabolites: 52 % of recovered ^{14}C
	Wistar rat	Urine	Principal metabolites (2): 87 % and 13 % of recovered ^{14}C, C_3 and C_5 derivatives of parent *
Dodecyl triethoxy sulphate	*Carassius auratus*	Bile	Principal metabolites (3): 40 % of recovered ^{14}C† Minor metabolites and parent substance: 44 % of recovered ^{14}C
		Water	Metabolites (dissimilar to those in bile), no parent substance
	Wistar rat	Urine	Principal metabolite: 3-ethoxy sulphate of either glycollic or γ-butyric acid (similar to fish "water" metabolite*
Dodecyl triethoxylate	*Carassius auratus*	Water	One major and three minor metabolites†
Dodecyl hexaethoxylate	*Carassius auratus*	Bile	Principal metabolites (3): 70 % of recovered ^{14}C† Minor metabolites (4): 22 % of recovered ^{14}C and 4% $C_{12}EO_6$†
	Wistar rat	Urine	Principal metabolite (1): 51 % of recovered ^{14}C, likely to be 4-hexaethoxy-butyric acid† Minor metabolite (1): 18 % of recovered ^{14}C†
Dodecyl decaethoxylate	*Carassius auratus*	Bile	Principal metabolites (3): 26, 19 and 13 % of recovered ^{14}C† Parent substance: 28 % of recovered ^{14}C
		Water	Principal metabolites (2): 61 % of recovered ^{14}C†
	Wistar rat	Urine	Principal metabolite (1): 52 % of recovered ^{14}C, possibly 4-decaethoxy-butyric acid† Minor metabolite (1): 18 % of recovered ^{14}C†

* Identification confirmed
† Not identified/tentative identification

Mammals

Evidence for ω- and β-oxidation of surfactants is strongest for mammalian species. Most of the work has concentrated on anionics (in rodents) and is reviewed by Black and Howes (1992). It is summarised in Table B.8.

Rat liver microsomes were found to metabolise sodium dodecyl sulphate (SDS) *in vitro* to produce ω and ω-1 hydroxylated products (Stewart *et al*, 1988). Interestingly these authors also found that pre-exposure of rats to SDS increased the biotransformation rate of this surfactant *in vitro*. This suggests that the mixed-function oxidase(s) involved in SDS metabolism can be induced. Black and Howes (1992) suggested that these linear surfactants can only be oxidised to within 3 or 4 carbon atoms of the hydrophile after which the polarity of the metabolite or steric hindrance prevents further oxidative attack.

The nonionics lauryl triethoxylate, lauryl hexaethoxylate and lauryl decaethoxylate were all metabolised in the rat resulting in the formation of ethoxylated ω-hydroxy-carboxylic acids with short alkyl chains (Unilever, unpublished results). There are little or no published data on mammalian metabolism of cationic surfactants but they are also likely to undergo biotransformation via the routes described above.

Table B.8: Metabolism of Surfactants in Rodents

Surfactant	Metabolite	Matrix	Final metabolite
Alkyl benzene sulphonates	2-Sulphophenyl n-dodecane 6-Sulphophenyl n-dodecane	Urine Faeces	Butyric acid-3-benzene-p sulphonate (unconjugated)* Dicarboxylic acids†
Alkyl sulphates	Sodium dodecyl sulphate Sodium undecyl sulphate		Butyric acid sulphate* Propionic acid-3-sulphate, valeric acid-5-sulphate (minor)*
Alcohol ethoxysulphates			Carboxylic acid, ethoxysulphates

* Confirmed identification
† Not identified/tentative identification

Elimination of Surfactants by Aquatic Organisms

Bioconcentration and elimination of surfactants have been reviewed recently (Tolls and Sijm, 1993; Tolls *et al,* 1994). Elimination of surfactants (as total ^{14}C) was generally rapid (3-10 days) in both fish and invertebrates like *Daphnia*. High levels of radioactivity in the gall bladder of fish were seen in a number of studies. This and the metabolic data described previously strongly suggest that surfactant metabolites are secreted into the gallbladder after hepatic biotransformation. They may be reabsorbed from the intestine to undergo further metabolism and finally be excreted via the urine. Sodium lauryl sulphate (SLS) was not excreted via the gills or skin in goldfish (Tovell *et al* 1975). These authors also found that 68% and 38% of SLS (as total ^{14}C) was eliminated from fed and unfed fish respectively. In unfed fish the gall bladder does not empty leading to a large increase in metabolites in this organ which can increase the apparent bioconcentration factor when based on ^{14}C measurements. This is further supported by the data presented in Table B.9 (Unilever Research, manuscript in preparation).

Tolls and co-workers (Tolls and Sijm, 1993; Tolls *et al*, 1994) concluded that a large number of studies underestimated bioconcentration as steady-state was not achieved. These were not further considered. Many of the remaining studies overestimated bioconcentration due to the fact that the experimental fish were not fed during the uptake and elimination phase. All of the remaining studies overestimated bioconcentration as the BCF was based on total ^{14}C recovered from the animal or its tissues. Even so the BCF values were generally low (<500) with the exception of nonionics such as nonylphenol ethoxylates. The true BCF (i.e. based on parent substance only) will thus only be a fraction of these values since metabolism of these substances is rapid and extensive. This also means that prediction of bioconcentration potential for these substances cannot be calculated from physico-chemical parameters alone.

Table B.9: Depuration of Surfactants from Goldfish

Surfactant	Depuration of radiolabel from goldfish*
C_{12} LAS (2-phenyl isomer)	fed fish 99% in 96h, unfed fish 80 % in 96 h
C_{12} LAS (commercial mixture)	fed fish $T_{1/2}$ 12h: unfed fish 84h
C_{11} AS	$T_{1/2}$ 2-3d
C_{12} AS	fed fish 68% in 24h: unfed fish 38% in 96h
C_{12-15} AS	fed fish 97% in 14d: unfed fish 84% in 14d
C_{15} AS	fed fish $T_{1/2}$ 100h: unfed fish $T_{1/2}$ 144h
C_{12} $(EO)_3S$	fed fish $T_{1/2}$ 42h: unfed fish $T_{1/2}$ 90h
C_{12} $(EO)_3$	fed fish 88% in 48h: unfed fish 71% in 48h
C_{12} $(EO)_6$	fed fish $T_{1/2}$ 54h
C_{12} $(EO)_{10}$	fed fish $T_{1/2}$ 30h

* Goldfish were exposed for 5-24 h before transfer to clean water

APPENDIX C. APPROACH FOR INCORPORATING CRITICAL BODY RESIDUES IN THE RISK ASSESSMENT OF A SUBSTANCE

The concentration of a substance in an organism at the time of death (or any other biological endpoint) is defined as the Critical Body Residue (CBR). It is a concept advocated as suitable replacement for exposure-based toxicity criteria. It has a potential future use in environmental risk assessment, since it can be related to external exposure (e.g. PEC) through the bioconcentration factor (BCF or BAF) (see Section 3.5.1).

This appendix discusses incorporation of the CBR concept in the risk assessment of a substance. The main assumption is that exposure to an external concentration is related to the concentration within the organism through the BCF. Hence, the internal effect concentration (CBR) is a function of exposure and bioconcentration.

One of the most important decisions to be made prior to applying the process map as presented in Figure C.1 is the endpoint considered (among others lethal, neurological, immunological, reproductive, biochemical effects). This is one of the key issues for applying CBR in the environment. It should be considered thoroughly, since the available information on CBR is mostly on acute lethal effects. Another important aspect addressed by other ECETOC Task Forces is the estimation and refinement of the PEC (see, for example, ECETOC, 1993; 1994a; 1994b).

Figure C.1 indicates a hierarchy in the use of data, with a preference for measured data over estimated ones. As a starting point of the decision tree a survey of available information is necessary. Estimation takes place only when no reliable, measured data are available. The centre of the decision tree is the evaluation of possible concern, driven by relating CBR to external exposure concentration (PEC) and BCF. Identification of a concern triggers the evaluation of the possibility for further refinement of the data. In case further refinement is not possible, risk management should take place.

At present, the information on CBR is available for substances with a lethal narcosis type of toxicity. Limited information is available for substances with more complex mechanisms of toxicity.

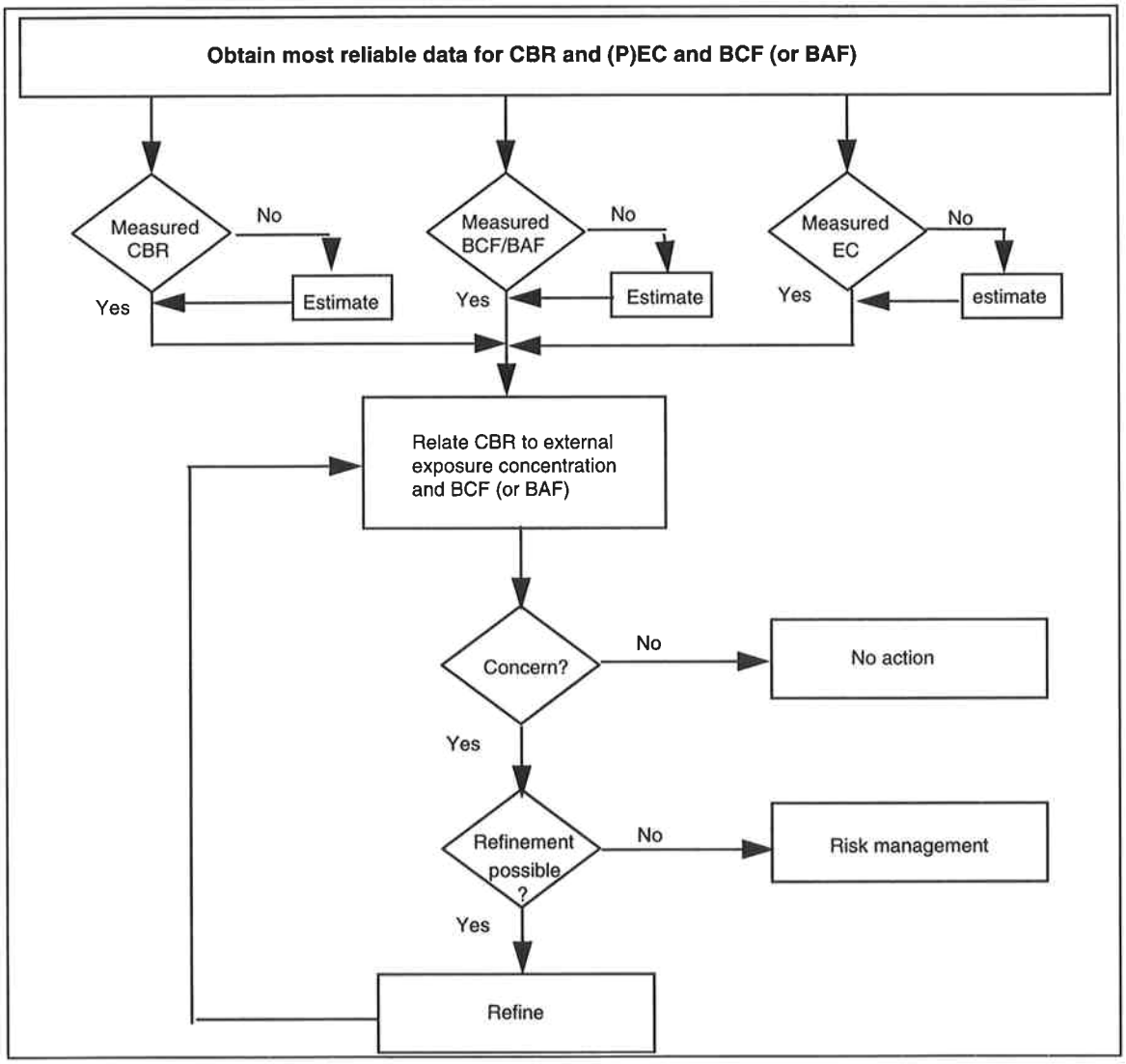
Measurement of CBR gives rise to several problems. There may be an influence of the lag-time between occurrence of the effect and retrieval of the organism from the exposure medium. Also,

exposure time, exposure route, lipid composition and content, and interspecies differences can have an effect. The effect of exposure time will most probably be observed with substances attacking a specific target organ. Hence, use of the CBR approach may prove impossible with relatively complex mechanisms of action. Unanswered questions remain regarding the possibility of extrapolation of CBR values from acute to chronic toxicity, and from chronic toxicity to field situations. The influence of exposure to mixtures of substances remains unclear. The concept of CBR appears promising but many questions need an answer before it can be used in the risk assessment of substances.

Methods for the estimation of the CBR are necessary when no experimental data are available. Classification of a substance according to its mechanism of toxicity allows use of the CBR associated with that mechanism. (Important assumption: substances with the same mechanism of toxicity exhibit similar CBR). If classification is not possible, estimation is not possible. In those instances, measurement of the CBR is the only solution.

As illustrated in this appendix, CBR undoubtedly appears a promising concept with potential for application in environmental risk assessment. Before application in risk assessment becomes feasible, many of the above mentioned questions require an answer. Additional research may provide this further insight in the concept of CBR and its implications.

Figure C.1: Process Map Indicating a Way to Introduce the CBR Concept in the Environmental Risk Assessment of a Substance



APPENDIX D. MODELLING BIOCONCENTRATION AND BIOMAGNIFICATION IN THE AQUATIC FOOD WEB

Three models describing bioconcentration and biomagnification were evaluated (Thomann, 1989; Clark *et al*, 1990 and Barber *et al*, 1991). The three models consider:

- gill uptake and release;
- food intake from the lower trophic level and egestion;
- limited bioconcentration in algae lipids (increase of log K_{ow} >6.5 does not result in increase of the bioconcentration into algae);
- excretion and metabolism;
- food assimilation efficiency;
- growth.

The three models provide different equations for all these biological processes. In order to get some feeling for the extent of similarity between these models, the bioconcentration and biomagnification was calculated for each model. Only food intake and growth were made identical to enable a fair comparison of the predicted absorption via gills and feed and the predicted elimination via gills and intestines between the three models. Therefore we considered the specific food uptake to be related to the sum of the specific respiration and growth rate in all three models:

$$RESP = 0.036 \cdot BW^{-0.2} \qquad [day]^{-1}$$

$$GROWTH = 0.01 \cdot BW^{-0.2} \qquad [day]^{-1}$$

In the simulation presented below, the concentration in the lipid fraction of aquatic organisms is predicted under the assumption, that the substance in the lipids originates from bioconcentration from water and from biomagnification from feed. Furthermore, the lipid fraction is assumed to be similar to octanol considering partitioning of hydrophobic substances between lipid and water respectively octanol and water. Hence, if higher levels in the lipids of aquatic organisms are predicted than based on simple octanol-water or lipid-water partitioning via gill absorption and release, than food-lipid partitioning becomes important.

The simulation is carried out for a series of hypothetical substances with a log octanol-water partition coefficient of 5, 6, 7 and 8. In the first series of simulation the lipid content of algae is set to 1% and that of the organisms from level 2 and higher is set to 5%. In the second series this level is set to 0.5% for algae, 1.8% for invertebrates (level 2) and to 5.4% for fish of level 3 and 4 according to LeBlanc (1995).

D.1 SCENARIO 1

Table D.1: Simulation of Bioaccumulation Factor in Fish Lipids

Log K_{ow}	5
Molecular weight	300
Food assimilation efficiency	0.8
Ratio ingestion/egestion	4
Phytoplankton bioconcentration limited (Yes/No)	Yes
Fixed assimilation efficiency (Yes/No)	Yes
Fraction lipid level 1	0.01
Fraction lipid level 2	0.05
Fraction lipid level 3	0.05
Fraction lipid level 4	0.05
Growth rate level 2	0.01
Growth rate level 3	0.01
Growth rate level 4	0.01
Body weight organism level 2 (g)	0.1
Body weight organism level 3 (g)	10
Body weight organism level 4 (g)	1,000
Metabolic degradation level 2 (d^{-1})	0
Metabolic degradation level 3 (d^{-1})	0
Metabolic degradation level 4 (d^{-1})	0

Table D.2: Estimation of Bioaccumulation in Fish Lipids according to Thomann (1989)

	Trophic level	log (BCF)	log (BMF)	log (BAF)
log K_{ow} = 5	2	4.99	2.65	4.99
	3	4.99	3.35	5
	4	4.99	3.36	5
log K_{ow} = 6	2	5.78	5.34	5.92
	3	5.69	6.07	6.23
	4	5.69	6.38	6.46
log K_{ow} = 7	2	5.67	6.23	6.34
	3	5.72	6.77	6.81
	4	5.72	7.25	7.26
log K_{ow} = 8	2	5.2	6.25	6.29
	3	5.49	6.75	6.77
	4	5.49	7.23	7.24

Table D.3: Estimation of Bioaccumulation in Fish Lipids according to Barber *et al* (1991)

	Trophic level	log (BCF)	log (BMF)	log (BAF)
log K_{ow} = 5	2	5.43	3.85	5.44
	3	5.43	4.97	5.56
	4	5.43	5.09	5.6
log K_{ow} = 6	2	6	5.42	6.1
	3	6.01	6.21	6.42
	4	6.01	6.53	6.64
log K_{ow} = 7	2	6.19	6.11	6.46
	3	6.22	6.77	6.88
	4	6.22	7.19	7.24
log K_{ow} = 8	2	6.21	6.13	6.48
	3	6.24	6.81	6.91
	4	6.24	7.25	7.29

Table D.4: Estimation of Bioaccumulation in Fish Lipids According to Clark *et al* (1990)

	Trophic level	log (BCF)	log (BMF)	log (BAF)
log K_{ow} = 5	2	4.94	3.89	4.98
	3	4.87	4.63	5.07
	4	4.78	4.98	5.19
log K_{ow} = 6	2	5.83	5.33	5.95
	3	5.62	5.96	6.13
	4	5.38	6.33	6.37
log K_{ow} = 7	2	6.32	6.21	6.57
	3	5.91	6.78	6.83
	4	5.54	7.13	7.14
log K_{ow} = 8	2	6.45	6.19	6.64
	3	6.02	6.76	6.83
	4	5.62	7.08	7.1

D.2 SCENARIO 2

Table D.5: Simulation of Bioaccumulation Factor in Fish Lipids

log k_{ow}	5
Molecular weight	300
Food assimilation efficiency	0.8
Ratio ingestion/egestion	4
Phytoplankton bioconcentration limited (Yes/No)	Yes
Fixed assimilation efficiency (Yes/No)	Yes
Fraction lipid level 1	0.005
Fraction lipid level 2	0.018
Fraction lipid level 3	0.054
Fraction lipid level 4	0.054
Growth rate level 2	0.01
Growth rate level 3	0.01
Growth rate level 4	0.01
Body weight organism level 2 (g)	0.1
Body weight organism level 3 (g)	10
Body weight organism level 4 (g)	1,000
Metabolic degradation level 2 (d^{-1})	0
Metabolic degradation level 3 (d^{-1})	0
Metabolic degradation level 4 (d^{-1})	0

Table D.6: Estimation of Bioaccumulation in Fish Lipids According to Thomann (1989)

	Trophic level	log (BCF)	log (BMF)	log (BAF)
log K_{ow} = 5	2	4.99	2.35	4.99
	3	4.99	2.9	4.99
	4	4.99	3.38	5
log K_{ow} = 6	2	5.91	5.16	5.98
	3	5.68	5.67	5.98
	4	5.68	6.15	6.28
log K_{ow} = 7	2	6.08	6.34	6.53
	3	5.69	6.49	6.55
	4	5.69	6.99	7.01
log K_{ow} = 8	2	5.64	6.4	6.47
	3	5.46	6.45	6.49
	4	5.46	6.95	6.96

Table D.7: Estimation of Bioaccumulation in Fish Lipids According to Barber *et al* (1991)

	Trophic level	log (BCF)	log (BMF)	log (BAF)
log K_{ow} = 5	2	5.71	3.83	5.72
	3	5.42	4.79	5.51
	4	5.42	5.05	5.57
log K_{ow} = 6	2	6.39	5.51	6.44
	3	5.98	6.08	6.34
	4	5.98	6.45	6.58
log K_{ow} = 7	2	6.65	6.26	6.8
	3	6.18	6.63	6.76
	4	6.18	7.08	7.13
log K_{ow} = 8	2	6.68	6.3	6.83
	3	6.2	6.69	6.81
	4	6.2	7.14	7.19

Table D.8: Estimation of Bioaccumulation in Fish Lipids According to Clark *et al* (1990)

	Trophic level	log (BCF)	log (BMF)	log (BAF)
log K_{ow} = 5	2	4.94	3.99	4.99
	3	4.9	4.33	5
	4	4.78	4.92	5.16
log K_{ow} = 6	2	5.83	5.42	5.97
	3	5.69	5.72	6
	4	5.38	6.2	6.27
log K_{ow} = 7	2	6.31	6.3	6.6
	3	6.01	6.59	6.69
	4	5.54	6.99	7.01
log K_{ow} = 8	2	6.44	6.25	6.66
	3	6.08	6.61	6.72
	4	5.62	6.98	6.99

Discussion

The bioaccumulation models from Thomann (1989), Clark *et al* (1990) and Barber *et al* (1991) illustrate that a significant contribution of biomagnification to the bioaccumulation factor only occurs at log K_{ow} greater than 5 (Figure D.2).

At the highest trophic level bioaccumulation through biomagnification according to the presented models is smaller than one order of magnitude, if the lipid content of fish and its feed is similar. If the lipid content of the feed is 3 times lower than that of the fish, biomagnification occurs. This is not in full agreement with the opinion of LeBlanc (1995) but in line with the findings of Clark *et al* (1988).

Figure D.1: Illustration of Barber's Model at Different Levels of the Foodchain

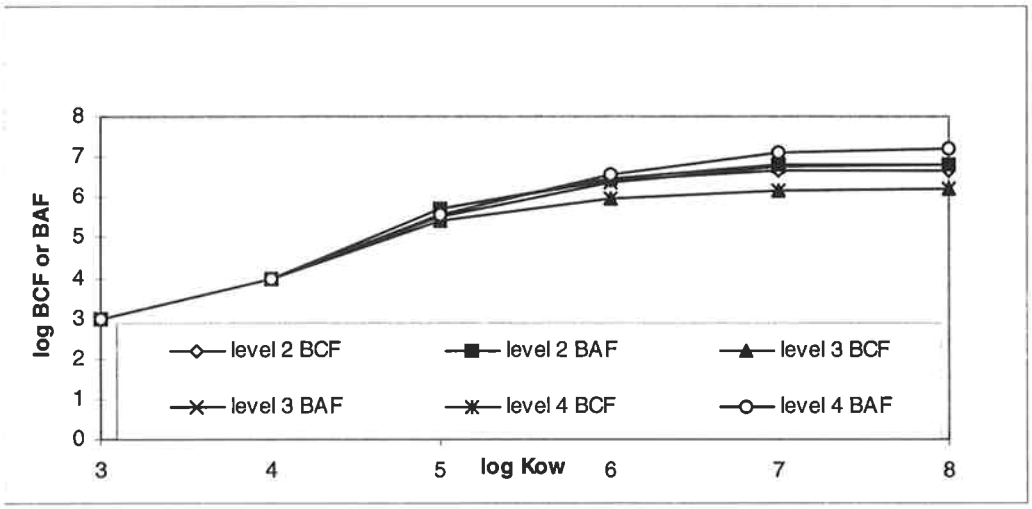
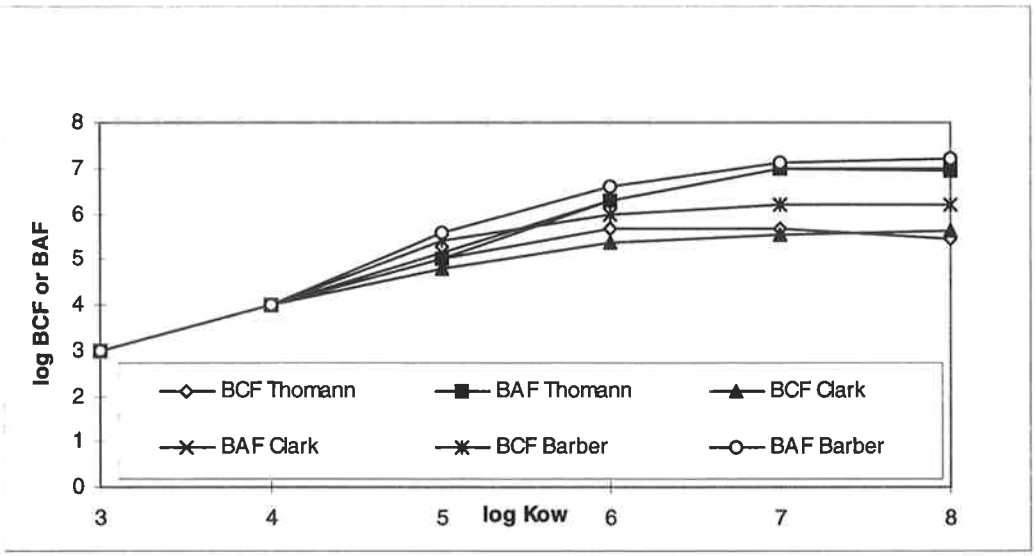


Figure D.2: Comparison of Bioaccumulation Models at Level 4 of the Foodchain



APPENDIX E. CASE STUDIES

E.1 GENERAL PRINCIPLES

An essential element in the development of a Bioaccumulation Assessment Scheme is the ability to evaluate critically its performance. To examine the performance of the proposed assessment scheme a number of substances were chosen which were also reported and discussed in ECETOC Technical Reports 51 and 58 (ECETOC, 1993; 1994a).

These substances represent a wide range of substance types for which an extensive data base on environmental effects and adequate data for environmental exposure assessments were available. The collated data on these substances were used to establish PECs and PNECs within the proposed hazard assessment scheme of ECETOC (1993 and 1994a). This also implies that the ECETOC Application Factors are used to derive the aquatic PNEC.

The substances used in the case histories are: 2,6-di-*tert*-butyl-4-methylphenol (BHT), linear alkylbenzene sulphonate (LAS) and cypermethrin (CYP). A more extensive illustration is also provided for pentabromodiphenylether.

A step-wise evaluation of the scheme was performed by refining both the PEC_{oral} and $PNEC_{oral}$. This implies calculation, refinement and/or measurement of PEC_{water}, BCF/BAF, PEC_{food} for higher organisms, and evaluation of acute, subchronic or chronic mammalian toxicity data of the substance as indication of possible effects on birds and mammals.

E.1.1 Calculation of BCF/BAF

If measured BCF values are not available, the BCF for fish can be predicted from the relationship between log K_{ow} and BCF for fish (on wet weight basis) by using the QSAR of Mackay (1982) (see Section 4.3.2, Table 2). At log K_{ow} > 5, bioaccumulation factors can be predicted as discussed in Appendix D.

E.1.2 Toxicity Data for Birds and Mammals

The results of these tests may be expressed as a concentration in the food (mg/kg) or a dose (mg/kg bodyweight/day) causing no effect.

E.1.3 Calculation of the Predicted No-Effect Concentration in the Food ($PNEC_{oral}$)

The result of the 28-day repeated dose test was used for the assessment of secondary poisoning effects. Extrapolation from this result can give a predicted no-effect concentration in food which should be protective of other mammalian and avian species. Assessment factors used which take into account interspecies variation, subchronic to chronic toxicity extrapolation and laboratory data to field impact extrapolation are taken from the EU Technical Guidance documents.

An indicative assessment factor of 100 (10x10) was applied to the NOEC for the 28 day repeated dose test to derive the $PNEC_{oral}$. Where a 90-day toxicity test is submitted instead of the 28-day test, the assessment factor may be reduced to 30. When chronic studies are available at higher levels, an indicative assessment factor of 10 may be used. Reproduction toxic effects are in this context regarded as chronic effects. The conversion factors are given in Table E.1 below.

Table E.1: Conversion Factors for Toxicity Data (Romijn *et al*, 1991)

	Body Weight (g)	Daily food intake (mg/kg body weight/day)	Body weight/daily food intake*
Canis domesticus	10,000	250	40
Macaca spec.	5,000	250	20
Microtus spec.	25	3	8.3
M. musculus	25	3	8.3
Oryctolagus c.	2,000	60	33.3
R. norvegicus (> 6 weeks old)	200	10	20
R. norvegicus (< 6 weeks old)			10
Gallus domesticus	128.5	64.3	2

* conversion factor to mg/kg food

E.2 CASE EXAMPLE: BHT

Butylated hydroxytoluene (2,6-*bis*(1,1-dimethylethyl)-4-methylphenol) (BHT) is used as the first example to illustrate the approach.

The basic physico-chemical, mammalian and ecotoxicity data used for this assessment are set out in Table E.2. Most of the data used in this example are taken from ECETOC (1993; 1994a) and BUA

Report 58 (1991). This substance can be assumed to satisfy the criteria of a "nonpolar, lipophilic" substance (see Table E.2). An initial assumption of lack of metabolism will be made. As has been seen throughout this report, the K_{ow} drives the predicted BCF and BAF. Based on structure alone, the log K_{ow} is calculated to be 6.2 by the fragment method, while the shake flask method resulted in a measured value of 5.1. Freese *et al* (1979) report a lower log K_{ow} of 4.17. Initial estimates here will use the (intermediate) log K_{ow} of 4.6 used by ECETOC in earlier reports. However, as will be shown below, the uncertainty in log K_{ow} is *not* crucial in the final assessment in this case, as available experimental data override the assumptions based on K_{ow}.

Table E.2 : Data Used for Estimation of PEC and PNEC for BHT

Property	Value
Molecular Weight	220
Melting point	70°C
Density	1.08 g/ml
Water solubility (20°C)	0.4 - 1.1 mg/l
log K_{ow}	4.6*
Lowest 96 h LC_{50} (fish)	64.4 mg/l
Lowest long-term NOEC (fish)	0.49 mg/l
Subchronic LOAEL (rat)	500 mg/kg/d
Long-term NOAEL (rat, 72 weeks)	25 mg/kg/d

* (other values 6.2, 5.1, 4.2 also reported)

In the bioaccumulation assessment, two points are examined:

- the potential for exposure via bioconcentration to result in increased accumulation over time, such that standard laboratory tests do not reflect possible chronic environmental body burdens, and

- the potential for added exposure due to food chain transfer and an assessment of the oral toxicity of the substance to consumers.

First, an assessment of the reliability of the chronic data is made for illustration purposes.

BHT can be considered to fall in the range of substances for which the QSAR for determination of T_{95} is appropriate (see Section 5.4). If the highest calculated log K_{ow} is used to conservatively predict the T_{95}, a time period of greater than 30 days results (Table 5, calc. log K_{ow} >6.0). However, a

microcosm-based bioconcentration experiment showed that organisms reached steady-state concentrations within 7 days (Inui *et al*, 1979). Thus, "correction" or further examination of the chronic data are not needed. We conclude that the aquatic toxicity test data accurately reflect any role which bioconcentration (uptake route being water only) may play in eliciting toxicity.

Table E.3: Result of the Conventional Aquatic Risk Assessment for BHT

	PEC_{local} (mg/ l)	PNEC * (mg/ l)	PEC/PNEC
Tier 1	3.1×10^{-6}	3.2×10^{-1}	10^{-5}
Tier 2		9.8×10^{-2}	3×10^{-5}
Tier 3			

* PNECs calculated from data in Table E.2 and the application factors in ECETOC (1993).

In the "traditional" assessment, the water PEC/PNEC ratio is very low (Table E.3). Further refinements of either PEC or PNEC values are not judged necessary, since PEC/PNEC ratios are $<10^{-4}$.

Second, an assessment of the potential for bioaccumulation to impact higher organisms due to dietary exposure is made.

PEC_{oral}: The PEC_{oral} is dependent on both the PEC_{water} and the potential for bioaccumulation. Initially the PEC_{local} is used for the most conservative exposure estimate. This would reflect organisms which reside exclusively under the most exposed conditions. The calculated log K_{ow} of 4.6 results in a predicted BCF of 2,000. At this BCF, dietary exposure is estimated to contribute significantly to total uptake, such that estimation of a BAF is appropriate. The predicted BAF according to Thomann is about 2,600. According to the FGETS model, the BAF value is about 5,500 (assuming a Great Lakes-type food chain scenario as in Barber *et al*, 1991). The Gobas model provides a BAF value of 3,600. The resulting PEC_{oral} value would then be as high as 0.0173 mg/l (Table E.4).

$PNEC_{oral}$: On the effects side, the acute toxicity of BHT to mammals is minimal (LD_{50} generally > 1,000 mg/kg bw, lowest value 138 mg/kg bw). Several subchronic studies have been performed, with clinical changes generally occurring at 500 mg/kg/day. Detailed analysis of these data was not performed here, as studies of longer duration were also available. For demonstration purposes, a value of 500 mg/kg/day is used in the Tier 1 assessment, with an application factor of 100 as suggested in the EU Technical

Guidance documents. The corresponding dietary PNEC is 100 mg substance per kg food. The Tier 2 PNEC is based on two NOECs from long-term studies: a 76 week study with male F344 rats and a two generational carcinogenicity study. The lower NOEC of 25 mg/kg/day is used below; this corresponds to a dietary PNEC of 50 mg/kg (Table E.4).

Table E.4: Data Used for the Bioaccumulation Assessment of BHT for the Aquatic Food Chain

Phase	Level	PEC_{water} (mg/l)	BCF/BAF		PEC_{oral} (mg/kg food)		$PNEC_{oral}$ (mg/kg food)
			Lowest	Highest	Lowest	Highest	
Screening	Regional	8×10^{-8}	2000	5500			100
	Local	3.1×10^{-6}			0.0062	0.0173	
Confirmatory	Regional		25	25		0.0001	50
	Local						
Investigative	Regional						
	Local	10^{-3} - 10^{-2} *			0.006	0.069	

* In original ECETOC assessments, measured environmental values tended to be higher than estimated ones, presumably due to direct addition of BHT to foods as an anti-oxidant.

E.2.1 Results of the Bioaccumulation Assessment of BHT for the Aquatic Foodchain

Integration and comparison of PEC_{oral} and $PNEC_{oral}$ estimates results in large safety margins (>5,000) at screening level. Because additional data are available, and monitoring information indicate that higher than expected concentrations of BHT can be found in the environment, the assessment is continued.

Two key factors are involved at Tier 2. First, the calculated BAF overestimates the potential for bioaccumulation of this substance. Actual bioconcentration values (Inui *et al*, 1979), are orders of magnitude lower than calculated. These lower values are supported by metabolism data, which indicate that biotransformation is rapid in vertebrates (BUA, 1991). Low BCFs were also determined for invertebrates and algae (Inui *et al*, 1979), such that disproportionate accumulation at lower levels of the food chain is excluded. In addition, the substance is moderately volatile in water, which will result in lower than modelled water concentrations. Using this new information and setting the BAF

equal to the BCF equal to 25, the PEC oral is reduced more than two orders of magnitude. Thus, the $PEC_{oral}/PNEC_{oral}$ at Tier 2 is even lower at 2×10^{-6}, hence a safety factor of about 500,000.

In the case of BHT, additional uses actually result in higher than expected concentrations in food and environmental compartments (ECETOC, 1994a). Thus, measured values in fish and river water are used in Tier 3. Despite these increased concentrations, the $PEC_{oral}/PNEC_{oral}$ still is favourable, at 0.0014. This is true even if a very conservative estimate of the highest calculated fish PEC_{oral}, based on the highest measured river water concentration determined ($0.01 \times 25 = 0.25$), is used for the $PEC_{oral}/PNEC_{oral}$ calculation. The resulting value is 0.005. (Note that the highest measured fish concentration is 0.069 mg/kg, not 0.25 mg/kg).

In conclusion, BHT is determined not to be an environmental problem due to either direct exposure (traditional aquatic risk assessment) or exposure via the food chain. Metabolism appears to play an important role in reducing body burdens of fish (PEC_{oral}) and other organisms in the food chain. If this had not been the case, the higher observed levels of BHT in surface waters could have been expected to lead to significant exposure at higher levels of the food web.

This case study indicates that biotransformation can be a significant removal mechanism for substances besides the better studied PAHs (Section 3).

E.3. CASE EXAMPLE: LAS

Linear alkyl benzene sulphonate (LAS) is used to illustrate the example of a widely used and ready biodegradable surfactant.

In view of differences in fate and effects of alkyl homologs, isomers and/or congeners, it is deemed necessary to define the commercial product. For linear alkyl benzene sulphonate, the alkyl chain distribution ranges from C-10 to C-13 with different phenyl distributions according to the production process. Knowledge on the relative importance of each process allows the calculation of so-called commercial LAS - with its typical mean alkyl chain length of 11.7 and typical isomer distribution. The per capita consumption of LAS is about 1 kg/y or 3 g/d.

The basic physico-chemical, mammalian and ecotoxicity data used for this assessment are set out in Table E.5. Most of the data used in this example are taken from ECETOC (1993; 1994a).

Similarly to the BHT example, the assessment is taken from screening through more refined stages of the assessment, since data are available for this substance.

Table E.5: Data Used for Estimation of PEC and PNEC for LAS

Property	Value
Molecular Weight	347
Melting point	10°C
Density	1.01 g/ml
Water solubility (20°C)	350 mg/l
log K_{ow}	3.0 (calculated)
Lowest short-term $L(E)C_{50}$	4 mg/l
Lowest long-term chronic NOEC (fish)	0.22 mg/l
Acute LD_{50} (rat)	> 2000 mg/kg body weight
Lowest long-term NOAEL (rat)	250 mg/(kg x day)

LAS can be considered to fall outside the range of substances for which the QSAR for determination of T_{95} is appropriate (see Section 5.4). Thus, further examination of the chronic data are not needed. We conclude that the aquatic toxicity test data accurately reflect any role which bioconcentration (uptake route being water only) may play in eliciting toxicity.

Table E.6: Result of the Conventional Assessment for the Aquatic Effect of LAS (ECETOC, 1993)

	PEC_{local} (mg/ l)	PNEC (mg/ l)	PEC/PNEC
Tier 1	118×10^{-3}	23×10^{-3}	5.13
Tier 2	32×10^{-3}	160×10^{-3}	0.2
Tier 3	$5 - 20 \times 10^{-3}$	250×10^{-3} *	0.02 - 0.05

* This value is taken from a recent report on LAS (BKH, 1994).

In the "conventional" assessment, the water PEC/PNEC ratio is below 1 (Table E.6). Local PEC calculations (Table E.6) are about 2 to 3 order of magnitude higher than regional PECs (which are $<0.15\ 10^{-6}$ mg/l).

Second, an assessment of the potential for bioaccumulation to impact higher organisms due to dietary exposure is made.

PEC_{oral}: The PEC_{oral} is dependent on both the PEC_{water} and the potential for bioaccumulation. Initially the PEC local is used for the most conservative exposure estimate. This would reflect organisms which reside exclusively under the most exposed conditions. The estimated log K_{ow} of 3 results in a calculated BCF of 50 according to the QSAR of Mackay. At this BCF, dietary exposure is estimated not to contribute to total uptake, such that estimation of a BAF is not appropriate.

$PNEC_{oral}$: On the effects side, the acute toxicity of LAS to mammals is minimal (LD_{50} generally > 2,000 mg/kg body weight). From all studies, a chronic value of 250 mg/kg/day is proposed.

Table E.7: Data used for the Bioaccumulation Assessment for the Aquatic Foodchain of LAS

Phase	Level	PEC_{water} (mg/l)	BCF/BAF		PEC_{oral} (mg/kg)		$PNEC_{oral}$ (mg/kg)
			Lowest	Highest	Lowest	Highest	
Screening	Regional	$< 10^{-6}$	50				400
	Local	118×10^{-3}			5.9	11.8	
Confirmatory	Regional		50	100			500
	Local	32×10^{-3}			1.6	3.2	
Investigative	Regional						
	Local	$5\text{-}20 \times 10^{-3}$		30		< 1	

E.3.1 Results of the Bioaccumulation Assessment of LAS for the Aquatic Foodchain

Integration and comparison of PEC_{oral} and $PNEC_{oral}$ estimates results in adequate safety margins. Because additional data and monitoring information are available, the assessment is continued. Laboratory studies carried out with ^{14}C labelled LAS indicated that the BCF of LAS 11.6 and 11.7 ranged from 50-70 (Kimerle *et al*, 1981). Concentrations in fish (300 µg/kg) measured in the Tokyo Bay are related to local discharges (Tokai *et al*, 1990). Surface water concentrations averaged around 10 µg/l (Field BCF = 30).

It can be concluded that LAS does not represent a significant hazard for toxicity due to bioaccumulation.

E.4 CASE EXAMPLE: CYPERMETHRIN

Cypermethrin is a synthetic pyrethroid used primarily as an insecticide. It is effective against a wide range of pests and has been used extensively on cotton. Other uses include control of insects for veterinary purposes and as a wood preservative. The active ingredient is a mixture of 8 cis- and trans- stereo isomers of (RS)-α-cyano-3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane carboxylate. The ratio of the cis- to trans- isomers varies from 50:50 to 40:60 in the technical substance. For the purposes of this case study, the technical substance has been assessed.

The basic physico-chemical, mammalian and ecotoxicity data used for this assessment are set out in Table E.8. Reported annual production from 1979 to 1982 was 200 - 340 tonnes. These data are all taken from IPCS (1989).

Table E.8: Data Used for Estimation of PEC and PNEC for Cypermethrin

Property	Value
Molecular Weight	416.3
Melting point	80°C
Density	1.12 g/ml
Water solubility (20°C)	0.009 mg/l
log K_{ow}	6.3
Lowest short-term L(E)C_{50}	0.0003 mg/l
Lowest long-term NOEC *(Pimephales promelas)*	0.00003 mg/l
Field studies based on data described in IPCS (1989)	0.0001 mg/l
Acute NOAEL (rat, 28 days)	7.5 mg kg/(bw x day) or 150 mg/kg diet
Long-term toxicity NOAEL (rat, 90 days)	50 - 75 mg/(kg diet x day)

Table E.9: Result of the Conventional Aquatic Rrisk Assessment of Cypermethrin

Cypermethrin	PEC mg/l	PNEC mg/l*	PEC/NEC
Tier 1	1.7×10^{-7}	1.9×10^{-6}	0.09
Tier 2	8.4×10^{-6}	6×10^{-6}	1.5
Tier 3	$<1 \times 10^{-5}$ **	2.9×10^{-4}	< 0.03

* PNECs are derived from lowest acute and chronic values using the application factors in ECETOC (1993).

** The measured data for the $PEC_{regional}$ has been taken from ECETOC (1994a). The lower value is determined by the limit of detection of the analytical methodology. Therefore, it does not reflect a background concentration.

The $PEC_{regional}$ was calculated with HAZCHEM, assuming a surface of 1 km^2, divided in 100 areas of 10,000 m^2. Every part of 10,000 m^2 was surrounded by a ditch of about 1 meter wide and 0.25 meter deep. On the average a fractional area of water of 0.033 was assumed, resulting into an advective residence time of 240 hours (10 d).

The application rate was 0.1711 kg/h (1500 kg/year for a surface of 1 km^2 or 15 kg/year for a hectare). It was assumed that the application rate on the ditches was 10% of the amount applied to soil and crop.

The bioaccumulation factor in fish was estimated according to Mackay (1981). This factor appeared to be 100000. The bioaccumulation factor was not corrected for metabolism in the fish. However, laboratory studies carried out on rainbow trout have indicated that the bioaccumulation when exposed to concentrations of 0.165 and 0.19 $\mu g\ l^{-1}$ was 1,000 and 1,200 respectively. In these cases the depuration half life was 11 and 9 days respectively.

Under field conditions, in a pond study, cypermethrin take up by in fish from an initial spike equivalent to 1 $\mu g\ l^{-1}$ was rapidly lost as the cypermethrin in the water rapidly disappeared. It was concluded that fish in the wild would be unlikely to contain measurable residues of cypermethrin due to the low levels in water and rapid depuration.

Table E.10: Data used for the Bioaccumulation Assessment of Cypermethrin for the Aquatic Foodchain

Phase	Level	PEC_{water} (mg/ l)	BCF/BAF		PEC_{oral} (mg/kg)		$PNEC_{oral}$(mg/kg)
			Lowest	Highest	Lowest	Highest	
Screening	Regional	$1.7x10^{-7}$	10^5		$1.7x10^{-2}$		1.5
	Local	2.3×10^{-5} *					
Confirmatory	Regional	$1.7x10^{-7}$	1,000	1,200	$1.7x10^{-4}$	$2.0x10^{-4}$	100-150
	Local						
Investigative	Regional	$< 1x10^{-5}$			10^{-2}		
	Local	2.9×10^{-5} **				$3.4\ 10^{-2}$	

* PEC_{local} was calculated with the pesticide module of USES 1.0 or ESPE module (Evaluation System for Pesticides) of Emans *et al* (1992). The value cited refers to the initial value obtained immediately after spraying. Values based on longer-term assumptions - including an overestimation of leaching from soil - was not used.

** Peak concentration, measured immediately after spraying occurred.

E.4.1 Results of the Bioaccumulation Assessment of Cypermethrin for the Aquatic Foodchain

Integration and comparison of PEC_{oral} and $PNEC_{oral}$ estimates results in a significant safety margin at screening level. Upon further refinement, and taking into account potential local worse-case estimates a safety margin remains. Thus cypermethrin does not represent a significant hazard for toxicity due to bioaccumulation within the aquatic foodchain. This is even more evident due to the rapid degradation and metabolism in the field.

E.5 Case Example: Technical Pentabromodiphenylether

Technical pentabromodiphenylether was and may possibly be still used as an additive in epoxy resins, phenol resins, unsaturated polyesters, polyurethane flexible and textiles. The approximate use level was about 300 tons in Germany and 350 tons in The Netherlands in 1988. The substance is no longer used in The Netherlands.

A batch of a technical mixture (De Boer, 1989) of pentabromodiphenylether consisted of:

41.7% 2,4,2',4'-tetrabromodiphenylether,

44.4% 2,4,5,2',4'-pentabromodiphenylether,
7.6% pentabromodiphenylether of unknown structure,
6% hexabromodiphenylethers.

Table E.11: Data Used for Estimation of PEC and PNEC for Technical Pentabromobiphenylether.

Substance characteristics	Tetrabromodiphenylether	Pentabromodiphenylether
CAS number	32534-81-9	40088-47-9
Molecular weight	486	565
Melting point, °C	20	0
Vapour pressure, Pa	7	9.3
Solubility, mg/l	$2 \cdot 10^{-3}$ (estimated)	$2 \cdot 10^{-4}$ (estimated)
log K_{ow} (measured)	6	6.8
log K_{ow} (calculated)	7.2	8.1
Aquatic toxicity	unknown	unknown
LD_{50} (technical product)	6,600 mg/kg	6,600 mg/kg
NOAEL 90 days feeding study	10 mg/kg	10 mg/kg
Chronic NOAEL terrestrial animals	0.33 mg/kg	0.33 mg/kg

The physicochemical properties were measured for the individual components of technical pentabromodiphenylether. The toxicological data were only available for the technical mixture.

The environmental levels of the main 2 congeners of technical pentabromobiphenylethers and its bioaccumulation factors were estimated by means of:

- the local model of HAZCHEM (The USES model could not be used because it estimated levels above water solubility in the water compartment). It was assumed that 0.5% of the annual amount of 175 t of both congeners was lost during processing and was discharged to a local waste treatment plant (0.1 kg/h). This is called Tier 1;

- the regional model of HAZCHEM using The Netherlands as example country. In this case it was assumed that 0.5% of the annual amount of 175 ton of both congeners was lost during processing and was discharged directly into the water compartment (0.1 kg/h). This is called Tier 2;

- the actual levels in the Dutch environment (De Boer and Dao, 1993). This is called Tier 3.

The NOAEL was estimated to be 0.33 mg/kg for aquatic mammals and birds. At a feeding rate of 0.3 kg/kg body weight in the real environment this is similar to a no adverse dietary level of 1.11 mg/kg. This level applies to both tetrabromodiphenylether and pentabromodiphenylether.

On the basis of the bioaccumulation models of Thomann (1989), Clark *et al* (1990) and Barber *et al* (1991) it was judged, that no correction was needed for biomagnification in herring and eels because of the much higher lipid content in the end stages of the food chain. This is in conformity with the observations of LeBlanc (1995).

Table E.12: Levels in Fish and PEC/NEC Ratios for Two Brominated Diphenylethers

	Fish level mg/kg	Safe level in fish (mg/kg) to prevent secondary poisoning	PEC-biota / NEC-biota
Tetrabromo-diphenylether			
Tier 1	0.755	1.11	0.7
Tier 2	0.029	1.11	0.03
Tier 3	0.110	1.11	0.1

	Fish level mg/kg	Safe level in fish (mg/kg) to prevent secondary poisoning	PEC-biota / NEC-biota
Pentabromo-diphenylether			
Tier 1	0.540	1.11	0.5
Tier 2	0.127	1.11	0.11
Tier 3	0.0063	1.11	0.006

It is remarkable that the tissue level in yellow eel in the river Waal was only 6.3 ppb. The reason may be that the increasing molecular weight by five bulky bromine atoms already hampers the uptake of pentabromodiphenylether via the gills and the intestines. In all environmental samples of yellow eel the level of tetrabromodiphenylether appeared to be 10 to 20 times higher than that of pentabromodiphenylether.

E.5.1 Exposure Assessment from Residues of Tetrabromodiphenylether (TeBDE) and Pentabromodiphenylether (PeDBE) in Fish-eating Mammals

Dolphin blubber contained 3 mg/kg PeBDE and TeBDE. The blubber content is estimated to be 25% of the body weight (de Boer, 1994, private communication). The lipid content of blubber is assumed to be 75 %. This means, that the lipid content of PeBDE and TeBDE is about 4 mg/kg.

The biomagnification factor may be directly derived from the halflife of the contaminants in lipid tissue. The half life of TeBDE and PeDBE in perirenal fat of Wistar rats (160-180) appeared to be 25 respectively 42.1 days (IPCS, 1993). For extrapolation of these half lives to a dolphin of 200 kg the metabolic rate should be considered. The metabolic rate of a mammal of 200 kg is estimated to be 6 times lower compared to the rat. This would result in residence times of 216 days for TeBDE and 364 days for PeDBE in lipid tissue. Because these substances are present mainly in lipid tissue, the residence times in lipid tissue are more or less similar for the whole body. So the whole body burden of the contaminant divided by the residence time reveals the daily intake of the mammal. This uptake may be directly compared with the maximum tolerable daily intake. The dolphin's blubber contained 3 mg/kg TeBDE. The total body burden appeared to be 150 mg TeBDE. If the residence time is 216 days, the daily uptake is expected to be 0.70 mg or 3.5 µg/(kg bw x day).

It is assumed that the feeding rate of the dolphin is about 0.1 kg/kg bodyweight. Considering the daily intake of the dolphin is 3.5 µg/kg bw pentabromodiphenylether, the content of the fish consumed by the dolphin is estimated to be about 35 µg/kg. This level may occur as maximum level in herring in the Southern North Sea (de Boer and Dao, 1993).

E.5.2 Hazard Quotient for Fish Eating Mammals

The maximum tolerable daily intake is 330 µg/(kg bw x day). The daily uptake of the dolphin was estimated to be 3.5 µg/(kg x day). This means that the ratio between the predicted environmental dose level and the maximum tolerable dose level is 3.5/330 = 0.01. From this study it can be concluded that the distance between the beginning hazard level and the actual exposure is wide enough to prevent damage to the environment.

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