



## THE ASSESSMENT OF BIOACCUMULATION

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### ABSTRACT

Bioaccumulation of chemicals in biota may be a prerequisite for adverse effects on ecosystems. Since many of these effects can hardly be substantiated even in chronic laboratory scale ecotoxicity tests (e.g. impact of PCBs on hatching success of eggs, impact of DDE on eggshell thickness of birds or multi-generation effects) bioaccumulation constitutes an ecological risk by itself which has to be considered in an overall risk assessment of a chemical.

A concept of assessing the risk of bioaccumulation is presented which proceeds by a stepwise refinement taking into account physico-chemical data and all available information from bioaccumulation studies as bioconcentration factor (BCF), elimination half-life time ( $CT_{50}$ ), organ specific bioaccumulation, and bound residues. The proposed classification of bioaccumulation into 4 risk assessment categories does not only consider the BCF, but also the complexity of bioaccumulation processes and may be applied to all organic chemicals under different legislations using the same stringent principles.

Moreover, consideration of the complexity of bioaccumulation will also lead to a better understanding of biomagnification processes, i.e. enrichment of chemicals via food-webs, in ecosystems.

**Keywords:** bioaccumulation, bioconcentration, biomagnification, bioaccumulation potential, biomagnification potential, bioavailability, biosorption, ecotoxicology, hazard assessment, risk assessment

### 1. INTRODUCTION

Chemicals can be accumulated in organisms via the direct uptake from surrounding medium (e.g. water, pore-water) by gills, skin etc. or by ingestion of particle-bound chemicals (bioconcentration or bioaccumulation) as well as via food chain following various pathways along different trophic levels (biomagnification). These processes will not always manifest themselves in direct adverse effects, e.g. mortality, but complex phenomena can occur, e.g. reduced fertility, which constitute a risk potential for humans and environment.

It should be noted that even without detectable acute or chronic effects in standard ecotoxicity tests bioaccumulation and biomagnification should be regarded as hazard criteria in themselves, the more so because some effects may only be recognized in a later phase of life, are multi-generation effects or manifest only in higher members of a food-web, e.g. impact of PCBs on hatching success of eggs [1], impact of DDE on eggshell-thickness of birds [e.g. 2, 3], which never could be foreseen from common tests in the past.

Chemicals are subjected to different legislative regulations according to their intended use categories, e.g. industrial chemicals, pesticides, biocides, detergents. Although most of them provide testing of bioaccumulation for deriving a bioconcentration factor (BCF), the environmental significance of bioaccumulation processes is not equally acknowledged.

Hence, under Council Directive 92/32/EEC and Commission Directive 93/67/EEC of the European Communities [4, 5] the risk assessment of new chemicals with regard to protection of humans and environment is of main concern. In the Technical Guidance Document for Ecotoxicity Testing of New Substances [6] the principles of the assessment concept are laid down. Within this concept bioaccumulation is one of the key parameters which will trigger further testing and - depending on data on exposure and adverse effects - allow a refined overall risk assessment.

With respect to German Plant Protection Act [7] the same prerequisites and indications trigger a bioaccumulation study from the very beginning because there is a direct discharge of highly toxic substances into the environment.

A BCF cut-off value of 5,000 for pesticides which should be banned was proposed on the Bilthoven workshop 1992 on the evaluation of pesticides [8] and may be considered as a step into the right direction. It has been also included in the assessment concept of Klein et al. [9] and is discussed for the uniform principles of pesticide assessment according to Council Directive 91/414/EEC of the European Communities [10]. However, this value does not reflect the complexity of the bioaccumulation process and in certain cases might lead to overestimation of bioaccumulation.

Therefore, BCF-values as such are not sufficiently meaningful if used exclusively. Instead, effective body burden concentrations in target tissues or organs along with co-occurring adverse effects, such as mortality, sublethal, or chronic effects, are of much higher significance. The principles how to evaluate the results from bioaccumulation studies for deriving risk assessment for the environment should be uniform for all chemical substances and apply to all respective legislations. From these reasons more refined assessment of bioaccumulation should additionally take into account complex depuration kinetics, uncompleted elimination (bound residues), degree of metabolization, and tissue concentrations. This is more appropriate since it will lower the risk of under- and overestimation of bioaccumulation.

Based on experiences of regulation of organic chemicals under different national and international legislations a concept of assessing the risk of bioaccumulation is presented which proceeds by a stepwise refinement taking into account physico-chemical data and all available information from bioaccumulation studies as bioconcentration factor (BCF), elimination half-life time ( $CT_{50}$ ), organ specific bioaccumulation, and bound residues.

## 2. BIOACCUMULATION POTENTIAL

Bioaccumulation studies are laborious and require animal testing. Therefore, as an initial step it was internationally agreed to use a simple screening method for assessing the probability of bioaccumulation from a minimal set of (physico-chemical) data: the determination of bioaccumulation potential (BAP). Bioaccumulation potential could be understood as a qualitative (not quantifiable) indicator of a risk of bioaccumulation in living organisms due to the physico-chemical and structural properties of a substance.

### 2.1. Indications of Bioaccumulation Potential

There are several indications of bioaccumulation potential.

Until now, bioaccumulation potential has been estimated only on the basis of *n*-octanol/water partition coefficient in its logarithmic form ( $\log P_{OW}$ ). It is easily available and requires no expensive or animal testing. If measured values cannot be made available,  $\log P_{OW}$  can be calculated from the chemical structure of a substance as a first approach. This approach assumes that accumulating organic substances are hydrophobic, can freely diffuse through cell membrane, and are only enriched in the lipid-fraction of organisms. Therefore, partition equilibrium of a substance between *n*-octanol and water is regarded as a model of bioaccumulation.

On the other hand, the correlation between *n*-octanol/water partition coefficient (calculated as  $\log P_{OW}$ ) and the bioconcentration factor (calculated as  $\log BCF$ ) which expresses the enrichment of a substance in an organism (e.g. fish) relatively to outside concentration of the substance (e.g. in surrounding water) has been proved to be very poor for many types of chemicals. It cannot be expected that the *n*-octanol/water partition coefficient generally is a good model of bioaccumulation behaviour of organic chemicals because it does not take into consideration many factors influencing bioaccumulation in organisms, including e.g.:

- phenomena of active transport,
- the influence on the diffusion behaviour through cell membranes,
- metabolism in organisms and accumulation behaviour of metabolites,
- accumulation in specific organs and tissues (also by adsorption onto biological surfaces like gills, skins),
- special structural properties (e.g. amphiphilic substances, dissociating substances leading to multiple equilibrium processes),
- uptake and depuration kinetics, remaining plateau of the substance or of metabolites after depuration.

*n*-Octanol should simulate the lipid-fractions in organisms, but it is doubtful whether *n*-octanol is a sufficient surrogate of biota lipid. However, other possibilities for deposition and accumulation of substances and their metabolites exist in living organisms apart from lipid storage.

A special problem is the measurement of  $\log P_{OW}$  of ionizable substances because this may lead to multiple partition equilibria. The new test guidelines for  $\log P_{OW}$  measurement (cf. e.g. Annex to Commission Directive 92/69/EEC of the European Communities No. A.8 [11] or OECD Guideline for Testing of Chemicals No. 107, Draft [12]) determines that  $\log P_{OW}$  measurements should be made on ionizable substances only in their non-ionized form (free acid or free base), thus allowing to determine maximum lipophilicity of a tested substance. Therefore, the pH-value of an appropriate buffer chosen for  $\log P_{OW}$  measurement must be at least one pH unit below (free acid) or above (free base) pK-value. Other measurements of  $\log P_{OW}$  are not valid with regard to assessment of bioaccumulation potential.

Despite of these limitations it is internationally accepted that  $\log P_{OW}$  values greater than or equal to 3 indicate that the substance has the potential to bioaccumulate.

*Surface active substances*, like tensides and many pesticides, may also have the potential to bioaccumulate even if their  $\log P_{OW}$  values are  $< 3$ . Surface activity is measured as surface tension of a solution of a substance in water. If a substance shows a surface tension of  $\leq 50$  mN/m at a concentration  $\leq 1$  g/l, it is considered to be surface active; this has to be taken as an indication of bioaccumulation potential.

**Adsorption** onto biological surfaces (e.g. gills, skin) may also lead to bioaccumulation and uptake of substances via food chain (see chapter 4. biomagnification). Therefore, high adsorptive capacity can be regarded as an additional indication of bioaccumulation and biomagnification potential.

A further indication of bioaccumulation potential is given for analogues of substances known to have the potential to bioaccumulate in living organisms.

## 2.2. Mitigating Aspects

There are certain physico-chemical and biological phenomena which might exclude bioaccumulation potential for a distinct substance even with a  $\log P_{OW} \geq 3$ .

**Hydrolysis:** Because uptake of a chemical may be very fast as is exemplified in Figure 1, uptake rate and hydrolysis half-life time have to be related for substances which are predominantly emitted directly into aquatic compartments. If the half-life time of hydrolysis for such a substance is less than 1 h, it is assumed that hydrolysis proceeds quicker than the uptake by organisms. No indication of bioaccumulation potential is assumed in this case. However, it may be necessary to check the hydrolysis products for their bioaccumulation potential.

**Biodegradation** has to be considered, also. However, the uptake rates of bioaccumulation can be significantly faster than biodegradation and bioaccumulation can occur even though the substance is readily biodegradable. This has to be assessed carefully on a case-by-case basis.

It has been accepted that for *super-lipophilic substances* with a  $\log P_{OW} > 6$  and with a molecular weight of  $> 600$  to  $700$  D or a molecular cross section larger than  $0.95$  nm a bioaccumulation cannot be expected.

Recently Geyer et al. [13] have shown, in contrast, that the BCF for such substances have been underestimated and may be considerably higher if bioaccumulation is tested within the limits of water solubility, i.e. without the use of solubilizers. Additionally, there is a hazard potential due to biosorption of highly lipophilic compounds. Biosorption is defined as sorption onto biological surfaces, e.g. skins, gills, mucous membranes of the gastrointestinal tract. Biosorption may cause toxic effects on the organism under consideration and may even lead to biomagnification (see chapter 4.). Therefore, the above mentioned criteria cannot be regarded as mitigating aspects.

## 2.3. Assessment of Bioaccumulation Potential

Summing up, the above mentioned points indicate or rule out bioaccumulation potential at the following conditions:

If a substance

- has a  $\log P_{OW} \geq 3$  or
- belongs to a class of substances known to have the potential to bioaccumulate in living organisms (e.g. surface active or highly adsorptive substances) or
- indicates the potential for bioaccumulation from structural features

and if

- there are no mitigating aspects (e.g. see hydrolysis above),

it is classified with "*indication of bioaccumulation potential*" in a first approach. If the potential to bioaccumulate can definitely be excluded, it will be classified as sharing "*no indication of bioaccumulation potential*"

Stable transformation products from abiotic (hydrolysis, photolysis, photooxidation) or biotic degradation processes (biodegradation, metabolization) have also to be checked for their possible bioaccumulation potential.

For chemicals showing an indication of bioaccumulation potential a bioaccumulation study might be necessary immediately for further refinement of hazard assessment, depending on exposure situation and ecotoxicological data on a case-by-case decision.

For chemicals showing no indication of bioaccumulation potential no bioaccumulation study has to be performed at this initial step of hazard assessment.

However, since assessment of bioaccumulation potential based on the above indications is of limited predictive value, bioaccumulation studies must verify the estimated potential for substances which may exert an environmental hazard, e.g. by the tonnage released into the environment. This is considered e.g. for new substances according to Council Directive 92/32/EEC of the European Communities [1] or to German Chemicals Act [14] when production volume exceeds 1 t/a.

## 3. BIOACCUMULATION

The existing standardized and internationally harmonized guidelines for bioaccumulation testing (OECD Guidelines for Testing of Chemicals No. 305 A - E<sup>1</sup> [15]) only examine the bioaccumulation in a water/fish system.

When measuring bioaccumulation behaviour true bioavailability of the substance under concern is a crucial parameter for valid results. Underestimation of bioaccumulation might result if the concentration in fish is not related to the true bioavailable concentration in water, as is stressed upon by Geyer et al. [13] as well as Kristensen and Tyle [17] and which will be considered in the up-dated OECD Guideline for Testing of Chemicals 305 [16]. Therefore, bioaccumulation behaviour should always be tested within the range of water solubility of the substance.

Criteria for the assessment of bioaccumulation are the BCF in the whole fish and the elimination or depuration expressed as half-life clearance time ( $CT_{50}$  i.e. the time needed to reach 50 % removal) as well as organ specific accumulation and uncompleted elimination leading to bound residues.

Methodologically, the tests derive from either a static or a dynamic principle. As both test principles reflect realistic exposure pathways (continual diffuse input resulting in relatively constant environmental concentrations and single release from a point source followed by decreasing environmental concentration), they are assumed to be of equal value and provide the same BCF for substances within a certain lipophilicity range (up to  $\log P_{OW} \approx 5$ ). Information on the course of elimination kinetics, however, can only be obtained from a dynamic test based on a two- or more compartment fish model.

The BCF is calculated from the steady-state concentrations in fish and water or from the quotient of the uptake and elimination rate constants.  $CT_{50}$  is calculated from the elimination-curve in substance free water after a certain time of exposure.

<sup>1</sup> An up-dating proposal for OECD Guidelines for Testing of Chemicals No. 305, which combines the former guidelines 305 A - E, is under discussion [16].

In experimental studies the complexity of bioaccumulation processes makes it necessary to clarify and to take into account all measurable processes influencing bioaccumulation, such as

- metabolism/transformation,
- organ-specific accumulation (reversible/irreversible),
- incomplete elimination (bound residues),
- bioavailability of the chemical (binding to particulate and dissolved fractions),

as well as criteria which are difficult to quantify, such as

- intra- and interspecies variance,
- conditioning factors,
- developmental stages.

The two measured bioaccumulation criteria BCF and  $CT_{50}$  are each divided into four assessment categories, covering the range of experimental data (see Table 1).

As can be seen from Table 1 the assessment categories of the bioaccumulation criteria BCF and  $CT_{50}$  are equally taken into account in the overall assessment leading to one of four bioaccumulation assessment categories.

A more negative classification may be necessary in the overall assessment if e.g. there is an indication of organ specific bioaccumulation or of uncompleted elimination leading to bound residues forming a plateau, thus raising the risk of biomagnification significantly. In this case-by-case assessment various aspects have to be considered. Two shall be picked out:

■ *bi- or multiphasic elimination kinetics*

$CT_{50}$  usually is determined from the elimination curve of the first few days assuming a first order kinetic. Therefore, bioaccumulation risk will be underestimated for substances showing an elimination kinetic with an order higher than 1 if  $CT_{50}$  only is regarded.

■ *plateau formation*

This aspect can also only be assessed case-by-case. If residues of a chemical or its metabolites remain in tissues or organs over a time which exceeds the period of time of long-term ecotoxicity tests even a low plateau raises the risk of biomagnification. Similarly, if a chemical or its metabolites show very low no observed effect concentrations (NOEC) in long-term ecotoxicity or toxicity tests even a plateau of as low as 10 % may indicate an unacceptable bioaccumulation or biomagnification risk.

These examples stress the necessity of an overall assessment of bioaccumulation behaviour which may lead to a more negative classification than BCF and  $CT_{50}$  alone indicated.

The different bioaccumulation assessment categories reflect various degrees of concern. They could be used to derive triggers for demanding more conclusive ecotoxicity testing as well as assessment factors which are applied to the lowest NOEC from long-term ecotoxicity tests in order to calculate a predicted no-effect concentration. In the overall hazard assessment this predicted no-effect concentration is then compared with the predicted environmental concentration.

With regard to testing and assessment strategy the bioaccumulation categories should lead to the following consequences:

*Category I:* No immediate concern with regard to bioaccumulation.

<i>Bioconcentration Factor (BCF)</i>		
BCF Category	Assessment Category	Comment
< 30	I	low BCF
30 - 100	II	moderate BCF
100 - 1,000	III	high BCF
> 1,000	IV	very high BCF

<i>Elimination</i>		
$CT_{50}$ Category	Assessment Category	Comment
< 3 days	I	rapid elimination
3 - 10 days	II	delayed elimination: short term bioaccumulation
10 - 30 days	III	slow elimination: medium term bioaccumulation
> 30 days	IV	insignificant elimination: long term bioaccumulation

*Overall Assessment of Bioaccumulation*

The categories of the bioaccumulation criteria BCF and  $CT_{50}$  are equally taken into account in the overall assessment of bioaccumulation as follows:

$$\frac{\text{BCF category} + CT_{50} \text{ category}}{2}$$

The result of this calculation will lead to one of four bioaccumulation assessment categories. If the resulting quotient lies between two categories, the higher is taken. If elimination data are not available, then only the BCF category can be used.

Overall Assessment Category	Assessment Factor	Comment
I	1	no concern (until further data)
II	2	indication of risk potential
III	5	cause for concern
IV	10	high risk (recommendation for risk reduction)

In the overall assessment a more negative classification may be made if there is an indication of organ specific bioaccumulation or of uncompleted elimination leading to bound residues forming a plateau which would raise the risk of biomagnification significantly.

Table 1: Classification of Bioconcentration Factor and Elimination and Overall Assessment of Bioaccumulation

*Category II or III:*

For chemicals in these categories the risk of biomagnification and secondary poisoning becomes important. On a case-by-case basis it has to be decided whether immediate further testing may be necessary or whether a higher production volume or changes in the use patterns can be awaited. In this decision the category of bioaccumulation, the calculated risk from the indirect effects assessment, data from prolonged (eco)toxicity tests, and exposure data have to be taken into account. Further testing should include tests for chronic effects, e.g. full life cycle tests, preferably together with residue analysis, and testing for other more complex (e.g. genetic, physiological, histopathological) endpoints and multi-generation tests.

To obtain a more comprehensive picture of bioaccumulation, biosorption and biomagnification as well as further aspects such as the impact of highly adsorptive substances on terrestrial and benthic organisms have to be considered. Therefore, bioaccumulation studies on these species may be necessary at this stage.

*Category IV:*

Chemicals in this bioaccumulation category possess a very high risk of bioaccumulation and biomagnification under environmental conditions. For these chemicals it may be necessary to propose specific recommendations for risk reduction.

Two examples of bioaccumulation of pesticides in fish will elucidate the problem and prove the applicability of the assessment concept presented above:

- A DDT analogue insecticide reveals a BCF > 5,000 and insignificant elimination. The occurrence of the detrimental metabolite DDE is to be expected. BCF and depuration will both lead to the worst classification (each category IV: very high BCF and long-term bioaccumulation) and result in the overall assessment category IV: *high risk of bioaccumulation* (recommendation for risk reduction).
- A herbicidal aromatic nitro compound also reveals a BCF of  $\geq 5,000$  but is partially metabolized as well as completely eliminated during the depuration phase within a few days. Although the BCF triggers the classification in category IV (very high BCF), the depuration can be classified in category II (short-term bioaccumulation), which will result in the overall assessment category III: *cause for concern*.

In both cases the proposed cut off value for registration purposes of BCF 5,000 [cf. 8, 9] is reached. In the first case, a persistent metabolite and insignificant depuration strengthen the high risk of bioaccumulation. Therefore, a refusal of registration of this pesticide may be justified.

In the second case, the pesticide is discharged by the quick and complete depuration. This must be taken into account in the final decision-making together with other toxicological and ecotoxicological data. A refusal of registration has not necessarily to be considered.

Finally it must be emphasized again that uncompleted excretion and remaining levels of bound residues should reasonably be considered as key parameters determining biomagnification routes in ecosystems.

#### 4. BIOMAGNIFICATION POTENTIAL

Biomagnification is the transfer of chemical substances via food-web passing different trophic levels and resulting in residues which are particularly detrimental for organisms in terminal positions within food-webs, e.g. dolphins, seals, crocodiles, humans [cf. e.g. 18, 19].

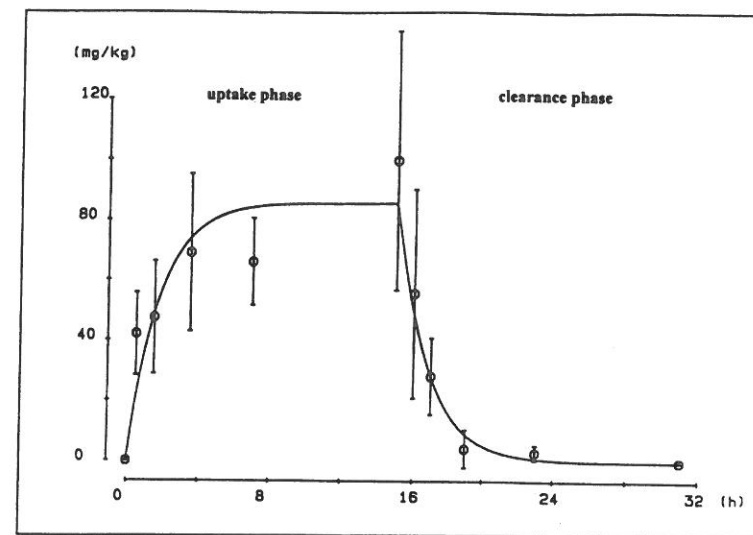


Figure 1: Rapid uptake of 2-t-butylphenol by zebra fish (*Brachydanio rerio*): steady-state concentration within 5 h (taken from Butte et al. [23]) (wet weight basis)

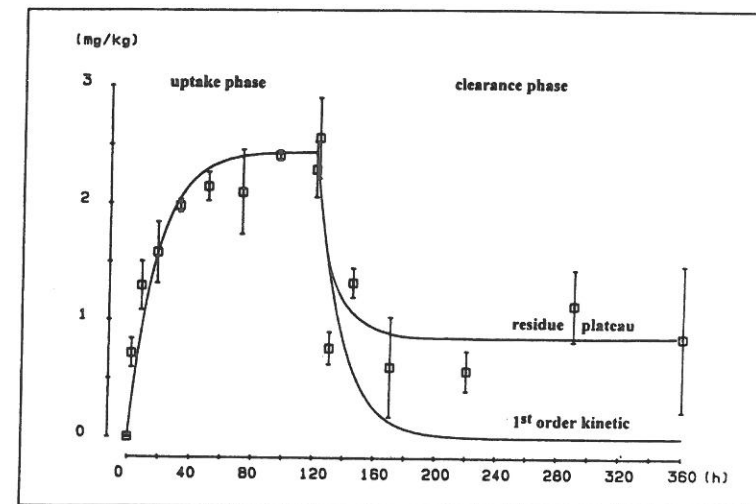


Figure 2: Bioaccumulation of pentachlorophenol by the oligochaete *Tubifex tubifex*: uncompleted elimination and residue plateau (taken from Butte and Rinderhagen [24]) (wet weight basis)



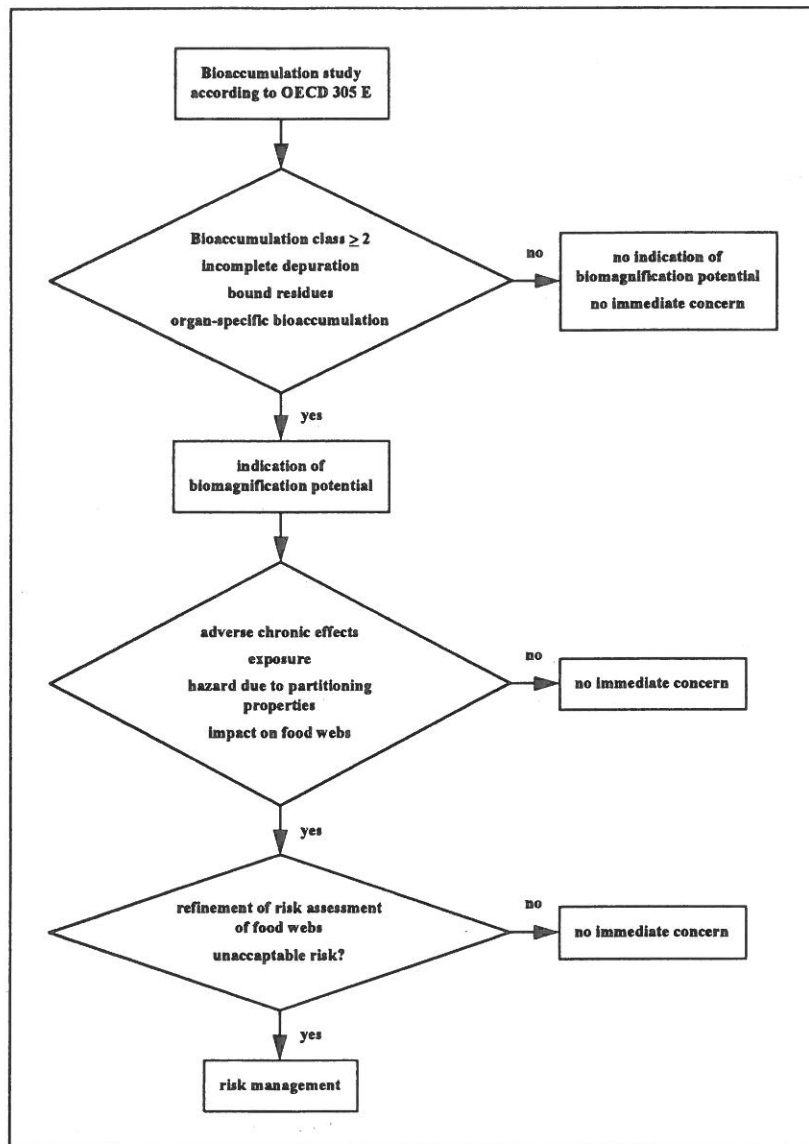


Figure 3: Decision scheme for hazard assessment of biomagnification

A special aspect of biomagnification is the concept of "secondary poisoning" which is concerned with toxic effects on higher members of a food chain. Secondary poisoning results from ingestion of organisms at different trophic levels that contain accumulated substances (indirect exposure). A strategy for the assessment of the potential for secondary poisoning has been developed e.g. by Romijn et al. [20, 21] and has become part of the assessment of new substances in the European Communities [cf. 6, 22]. In this concept the predicted chemical concentration in food of higher organisms is compared with the mammalian toxicity of the chemical as an indication of possible effects on birds and mammals.

Consequences of biomagnification may manifest themselves in a variety of different biological endpoints, e.g. mortality, but also in more complex phenomena, such as reduced fertility, behavioural anomalies, etc. Even without detectable acute adverse effects biomagnification is a hazard criterion per se, the more so since some effects may become obvious only in a later phase of life.

Prerequisite for biomagnification is the bioaccumulation/biosorption of chemicals either by direct uptake from the aquatic or terrestrial environment (via water, pore-water) or by the uptake of particle-bound chemicals and concentration in the organisms respectively (e.g. microorganisms, algae, invertebrates, vertebrates). Furthermore, there is convincing evidence (cf. e.g. examples mentioned above) that non-metabolized or metabolized residues, which are not excreted completely, may be transferred to the next trophic stage. An example of uncompleted excretion is shown in Figure 2 where a considerable residue level is measured in the bottom dwelling worm *Tubifex*.

Biomagnification of a substance can hardly be measured in laboratory testing systems. Therefore, the possibility that a chemical might bioaccumulate - the biomagnification potential (BAP) - has to be considered as an initial step. The flow scheme in Figure 3 gives guidance on how to conduct assessment of biomagnification in a tiered system taking exposure scenarios and toxicological as well as ecotoxicological effects into consideration.

Generally, accumulation, depuration kinetics, and bound residues are the key criteria for a biomagnification potential. If there are strong indications of such residues, further tests including more sophisticated investigations, e.g. of organ-specific concentrations, may become mandatory.

Prior to the final environmental risk assessment of biomagnification, adverse toxicological/ecotoxicological chronic effects and refined exposure assessment must be considered.

## 5. CONCLUSIONS

The presented criteria and schemes for the assessment of bioaccumulation behaviour of organic chemicals are based on the experiences with assessment of chemicals under different legislations (e.g. German Plant Protection Act and German Chemicals Act) and are partially integrated e.g. into the assessment strategy of new substances under Commission Directive 93/67/EEC of the European Communities.

This concept considers bioaccumulation as a complex process including all available information from a bioaccumulation study in an overall assessment of bioaccumulation behaviour of a substance and therefore enables the assessment of bioaccumulation behaviour of all kinds of organic chemicals using the same principles. It emphasizes that bioaccumulation may constitute a potential risk per se because bioaccumulation as well as biomagnification processes and adverse long term effects may occur in ecosystems which generally cannot be verified even in chronic laboratory scale ecotoxicity testing.

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## ORGANIC HALOGEN COMPOUNDS, EOX, IN MUSSELS FROM A CLEAN LAKE AND A PULP MILL RECIPIENT

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### Abstract

The extractable organic halogen (EOX) residue of duck mussels, *Anodonta anatina*, from the unpolluted Lake Höytiäinen, Finland, was found to be 690 µg/g of lipids. The residue was characterized by analyzing chlorinated phenols, hydrocarbons and fatty acids. These compound classes accounted only for 1.9% of the EOX.

Mussels from the same lake were incubated in a lake receiving pulp and paper mill effluents for 12 months to study the bioaccumulation of organochlorine compounds. The EOX of these mussels was 2045 µg/g lipids and only 1.1% of the residue was explained by the low molecular weight compounds. Thus, the majority of EOX remains undefined. However, potential differences in composition are suggested by the relative proportion of defined compounds and the differences among the measured compound classes. These halogen compounds may be of natural origin or reaction products of anthropogenic compounds with humic substances. The presence of such compounds should be taken into account when evaluating the accumulation of pulp mill effluent-related EOX in mussels.

No difference was found in the EOX between male and female mussels caged close to a pulp mill, but, on dry weight basis, mussels infected by a digenean parasite had higher EOX concentration than healthy females.

**Keywords:** Extractable organic halogen, EOX, chlorophenols, chlorinated hydrocarbons, chlorinated fatty acids, *Anodonta anatina*, *Pseudanodonta complanata*, pulp mill effluent, parasites

### Introduction

The presence of anthropogenic organic halogen compounds in the nature is widely considered as one of the major environmental problems of our time. The effects of DDT and its metabolites on populations of predator birds is probably the best known example of the effects of organohalogen com-