The Assessment of Bioaccumulation

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Bioaccumulation and biomagnification of chemicals in biota may be a prerequisite for adverse effects in individuals, species, and ecosystems. From disastrous events posed by xenobiotic chemicals, e.g. PCBs, Dioxins, DDT etc. it must be concluded retrospectively that such impacts cannot be avoided and predicted sufficiently with existing hazard and risk assessment strategies. Even sophisticated testing for chronic effects cannot rule out a possible risk of retarded effects completely. Since adverse effects as a consequence of bioaccumulation may become obvious long after a chemical's release and recovery may be retarded if not even hampered, authorities concerned with notification and registration of chemicals need a conceptual approach how to minimise risks posed by dangerous substances. Different concepts for the assessment of bioaccumulation (USA, Canada, Japan, Netherlands, ECETOC and EU) are critically discussed and compared regarding their precautionary principles. The risk assessment for bioaccumulation presented here is more comprehensive than the EU Technical Guidance Document (TGD) for new and existing substances. It gives guidance how to proceed stepwise from testing bioaccumulation, ranking of results, decision-making on the basis of triggered ecotoxicological tests and finally to an assessment of risks for bioaccumulation and biomagnification. Going beyond the scope of existing concepts this approach takes into account the complexity of bioaccumulation processes including uptake and depuration kinetics, bioconcentration factor, metabolism, and bound residues, relating these data to critical body burden concentrations. The risk assessment of biomagnification is driven by the outcome of the bioaccumulation assessment. If following the refined risk assessment recommended by the TGD an uncertain risk of biomagnification in ecosystems cannot be ruled out, the application of an unsafety factor of 10 on the final PEC/PNEC is proposed for discussion.

Keywords: Assessment, Bioaccumulation, Biomagnification, Concepts, Secondary Poisoning.

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Abbreviations

APEO	alkylphenolethoxylate; alkylphenol polyglycol ether
BAF	bioaccumulation factor
BAP	bioaccumulation potential; indicator of a risk of bioaccumulation in living organisms due to the physico-chemical and structural properties of a substance
BCF	bioconcentration factor
BM	biomagnification
BMP	biomagnification potential
CBB_{food}	critical body burden concentration for food in the organisms
CEPA	Canadian Environmental Protection Act
CMC	critical micelle concentration
ct_{50}	half-life clearance time, i.e. the time needed to reach 50% removal
DDD	a main metabolite of DDT
DDE	a main metabolite of DDT
DDT	dichlorodiphenyltrichloroethan
ECETOC	European Chemical Industry Ecology & Toxicology Centre
EU	European Union
HCB	hexachlorobenzene
HCH	hexachlorocyclohexane
α, β, γ and δ-HCH	isomeres of hexachlorocyclohexane
γ-HCH	Lindane
K_{OC}	partion coefficient organic carbon/water
K_{OW}	n-octanol/water partition coefficient; synonym of P_{OW}
LAS	linear alkylbenzene sulfonate
LC_{50}	lethal concentration for 50% of a population
log K_{OC}	logarithmic form of K_{OC}
log K_{OW}	K_{OW} in its logarithmic form
log K_P	logarithmic form of partition coefficient for a compartment, e.g. sediment. K_P is the product of K_{OC} and the weight fractions of organic carbon solids for the respective compartment
MITI	Ministry of International Trade and Industry Japan
MW	molecular weight
NOEC	no observed effect concentration
NOEL	no observed effect level
OECD	Organisation of Economic Co-operation and Development
OTS	Office of Toxic Substances
PAH	polycyclic aromatic hydrocarbons
PCB	polychlorinated biphenyls
PCDD	polychlorinated dibenzo-*p*-dioxins
PCDF	polychlorinated dibenzofurans
PCP	pentachlorophenol
PEC	predicted environmental concentration

PEC/PNEC	ratio triggering tests and risk reduction measures, respectively
PEC_{oral}	predicted environmental concentration of the prey; product of a NOEL and tiered safety factors and conversion factors
PEC_{reg}	predicted regional environmental concentration
PEC_{water}	predicted concentration of a substance in water, exposure concentration
pH	negative common logarithm of the hydrogen activity
pK	negative common logarithm of the constant for a chemical reaction at equilibrium
pKa	acid exponent; negative common logarithm of the acid constant
PNEC	predicted no effect concentration
$PNEC_{oral}$	predicted no effect concentration of the predator
P_{OW}	n-octanol/water partition coefficient; synonym of K_{OW}
QAT	quarternary ammonium compounds
QSAR	quantitative structure activity relationship
R48	danger of serious damage to health by prolonged exposure
R60	may impair fertility
R61	may cause harm to the unborn child
R62	possible risk of impaired fertility
R63	possible risk of harm to the unborn child
R64	may cause harm to breastfed babies
SAR	structure activity relationship
T	toxic
T+	very toxic
T_{95}	time to reach 95 % of the steady state concentration
TBT	tributyltin
TCDF	tetrachlorodibenzofuran
TGD	Technical Guidance Document
TSCA	Toxic Substances Control Act
UBA	Umweltbundesamt (German Federal Environmental Agency)
US EPA	Environmental Protection Agency of the United States
Xn	harmful

1 Bioaccumulation

1.1 Significance of Bioaccumulation for Risk Assessment of Chemicals in the Environment

Enrichment of chemical compounds in organisms, i.e. the bioaccumulation, is a fundamental strategy along evolutionary processes which may act as a driving force towards a selective advantage among competing species, e.g. in situations of limited resources.

Whereas species developed selective uptake mechanisms for naturally occurring beneficial substances, avoiding strategies for the uptake of unwanted substances causing detrimental effects often do not exist. This is particularly true for xenobiotics.

It may be assumed that during evolution the time for adaptation towards naturally existing substances was long enough at least for creating avoidance strategies empirically, but not sufficient to adapt to xenobiotics, e.g. halogenated organics enter food webs and ecosystems as well as any other chemical substance.

Bioaccumulation in organisms may have different consequences:

- selective advantage for species
- building up of a depot and neutral behaviour without causing adverse effects
- reversible, transitory effects, e.g. activation of detoxification systems such as enzyme induction, metabolism, biotransformation, inactivation, depuration
- bioaccumulation in organs/tissues inducing adverse acute, subacute, chronic or unknown long-term effects in individuals, populations, species and ecosystems

The latter phenomena – bioaccumulation and biomagnification of xenobiotics leading to irreversible adverse effects in biota and ecosystems – are subjects of concern and integral parts in legislative and administrative regulations.

Since there is not always conformity about the definitions of the terms bioaccumulation and biomagnification, they are briefly defined in the following:

Bioaccumulation is the uptake of chemicals in organisms from the surrounding medium (water, pore water) by gills, skin, etc. or by ingestion of particle-bound chemicals. However, the distinction between the exclusive uptake of the truly dissolved phase and other fractions (colloidal, dispersed, emulgated) is not clearly definable.

Bioaccumulation is quantitatively expressed by the **bioaccumulation factor** (BAF), the ratio of the concentration reached in the organism under steady state condition and the concentration of the surrounding medium. This factor can be related to the whole organism or tissues and organs thereof on a wet, dry or lipid weight basis depending on the context.

The terms **bioconcentration** and **bioconcentration factor** (BCF) as defined by OECD guidelines should be limited to laboratory test systems (e.g. OECD Guideline No. 305 [1]), where the uptake of a chemical is nearly exclusively re-

stricted to the soluble fraction and any other uptake routes can be neglected e.g. by minimizing the particle-bound fraction of suspended matter.

But even under controlled laboratory conditions a certain uptake of adsorbed fractions onto food may occur.

Generally all fractions must be considered potentially bioavailable regardless of the route of uptake.

Biomagnification is generally defined as the process of bioaccumulation along food chains or more precisely – within food webs – following various pathways on different trophic levels. However, this process must not necessarily end up in a magnification, leading to a stepwise increase with highest concentrations in organisms being in terminal positions of food webs e.g. whales, crocodiles, humans.

More frequently, there is a transfer of a chemical or its metabolites over several trophic levels which, although often not on a spectacularly high concentration level may cause long-term effects e.g. DDE.

Hence, the term biomagnification should express the transfer of a chemical or its metabolites within several trophic levels which may lead to a stepwise increase of the concentration level, if no metabolisation and depuration exist.

After the detrimental toxification events a few decades ago caused by the magnification of inorganic/metallo-organic chemicals, e.g. mercury compounds, there was increasing scientific interest to examine principles and extent of such processes for all chemicals released into the environment.

Whereas fate and effects of the most important metallo-organic and inorganic chemicals – the number and volume of which are smaller compared to organic chemicals – is relatively well known, we feel that there is still a considerable lack of knowledge about the risk of bioaccumulation/biomagnification processes for the bulk of organic chemicals.

Hence, predominantly organic xenobiotic chemicals are focused on in the following, inorganic/metallo-organic chemicals only in cases where relevant risk aspects are of concern.

Until now most risk assessment approaches for bioaccumulation and ecotoxicological processes relate to aquatic systems due to easier test performance and test systems available. However, risk assessment schemes and risk management concepts for all environmental compartments are urgently necessary.

1.2 Overview on Bioaccumulation Processes in Ecosystems

1.2.1 *Predictability Versus Reality*

From chemical structure, partitioning behaviour, fate and exposure of a chemical many bioaccumulation processes may be predicted with sufficient exactness and confirmed by monitoring data.

However, considerable events have been experienced in the last decades due to unknown intrinsic properties of chemicals, physiological responses of species and ecosystemically complex interdependencies which were beyond any

imaginative power, e.g. the reduced eggshell-thickness of bird eggs caused by DDE [2] or the estrogenic effects mediated by organochlorines [3] or APEOs [4, 5] leading to endocrine disruption and reproductive impairment in organisms.

There is common agreement that lipophilicity of a chemical is the major prerequisite for bioaccumulation in organisms. Hence most of the current **Quantitative Structure Activity Relationships** (QSARs) are based on the **octanol/water partitioning coefficient** (P_{OW}) whereby octanol is used as a surrogate for the compartment fat in an organism. It is further generally assumed that the application of such correlations allows for a prediction of the presumptive BCF. P_{OW} is also called $\mathbf{K}_{OW}$ and since K_{OW} has become more common it is referred to in the following.

However, such correlations are applicable only under the premises, that lipophilicity and hydrophilicity are inversely proportional, but not in cases, where chemicals are either insoluble both in octanol and water or soluble in any ratios, both possibilities resulting eventually in a low K_{OW} and thus not predicting a bioaccumulation potential.

Also not considered by these models are bipolar chemicals, e.g. surface active chemicals like detergents and chemicals with certain nitrogen structures like water soluble bipyridinium compounds and quarternary ammonium compounds which inspite of a low K_{OW} and high water solubility may be bioaccumulated considerably and therefore incorrectly assessed.

Prediction of biomagnification potentials are also doubtful. After disastrous intoxication events arising from bioaccumulation of metallo-organic compounds across food-webs in the sixties and seventies it was expected that organics show the same behaviour. First investigations of simple food-chain relationships and compilation of monitoring data on concentrations and effects in ecosystems led to the premature conclusion that biomagnification of organic chemicals is overestimated and plays only a role for a few highly lipophilic compounds [6].

However, meanwhile it is evident, that not only substances like DDT, HCB, PCBs and PCDD, but also less lipophilic substances like Lindane (γ-HCH) with a log K_{OW} of 3.63 are candidates for biomagnification, although laboratory results indicate no biomagnification potential, proven by complete depuration [7]. Also the occurrence of synthetic musk derivatives in humans and biota [8, 9, 10] was surprising and far beyond any expectation in the light of the relatively small amounts placed on the market.

In the following a short selection of paradigmatic bioaccumulation and biomagnification processes compiled from literature and supplemented by results of research and development projects of Umweltbundesamt (UBA) is presented demonstrating the complexity of bioaccumulation processes in ecosystems and the difficulties of predicting accumulation and long-term effects from simple generic test and risk assessment strategies.

1.2.2
Bioaccumulation, Biomagnification, and Long-term Effects of Organochlorines

Apart from well documented bioaccumulation and biomagnification processes of highly lipophilic chemicals, the capacity of enrichment of the rather moder-

ate lipophilic γ-HCH (log K_{OW} 3.63) in ecosystems is remarkable demonstrating that not only the degree of lipophilicity but also the degree and position of chlorination and particularly the elimination pathways determine the potential of biomagnification.

During the 1987/88 mass mortality of bottlenose dolphins along the Atlantic coast γ-HCH was among the nine of the most frequently detected pesticides [11].

However, comparing biomagnification efficiencies and residues of top predators with the same diet, e.g. fish eating tuna fish and dolphins, there are data suggesting that not the predator status per se, but the lack of branchial elimination pathways of mammals as compared to the elimination potential of gill-breathing fish may explain the higher residues and bioaccumulation/magnification potential in marine mammals [12].

This is in conformity with investigations on the bioaccumulation and transfer of γ-HCH, PCBs and DDTs in pike (*Esox lucius*) [13]. Lipids and concentrations of contaminants in hard roe were 10 times higher as compared to muscle suggesting that the transfer via roe is an important elimination pathway for the individual and a prerequisite for persisting residues in the offspring.

γ-HCH residues have also been found in water, sediments, eggs of pelicans and eels, the main pelican prey. Data suggest a biomagnification with a factor of 1.8 between eel and pelican eggs. The log BCFs/BAFs for eel and pelican eggs were 3.33 and 3.58, respectively related to water, i.e. nearly as high as the log K_{OW} for γ-HCH, making evident the risk of underestimating the bioaccumulation from laboratory investigations [14].

But also in terrestrial food-webs γ-HCH is often present when residues of organochlorines in biota are reported. Systemic impact of pesticides was investigated in a terrestrial food-chain based on plant (cabbage) – host (*Pieris brassicae*, *Lepidoptera*) – endoparasitic beneficials (*Apanteles glomeratus*, *Pteromalus puparum*, *Hymenoptera*). Compared to Parathion which was metabolized and excreted along the food-chain, Lindane despite of a relatively low acute toxicity revealed a high chronic food-chain toxicity mainly by prevention of metamorphosis in the endoparasitic wasp population. Although depuration amounted up to 80%, a high pupal mortality occurred [15].

Evidence for the decline of the cattle egret (*Bubulcus ibis*), feeding predominantly on insects in agricultural areas, caused by DDE and γ-HCH is reported by Mullié et al. 1992 [16].

Reduction of breeding success, eggshell strength, and of migration and breeding behaviour of the great tit (*Pares major*) was evidenced by laboratory experiments in a three-step food-chain based on oak-leaf, caterpillar and great tit. Apart from PCB 153 which was detected in all samples, a remarkable amount of γ-HCH was detected in 86% of all samples [17]. Results on eggshell thickness and population dynamics are in agreement with the assumption of negative population effects in the great tit suggesting effects during the early stages of the developing bird.

Summarizing it can be stated that not only highly lipophilic but also moderate lipophilic organochlorines like Lindane exhibit a considerable potential for bioaccumulation/biomagnification in all environmental compartments.

1.2.3
Bioaccumulation of Non-lipophilic Chemicals

Predictions of bioaccumulation potentials for water soluble non-lipophilic chemicals applying generic QSARs based on log K_{OW} may underestimate the true bioaccumulation capacity in certain cases:

Listed in the so-called Japanese MITI – list [18] are several chemicals which despite of a high water solubility, relatively low log K_{OW}, show a considerable bioaccumulation. Three N-containing chemicals may exemplify this (Table 1).

A high tendency for adsorption onto organic carbon (humus) which may be bioavailable for soil/sediment ingesting terrestrial organisms e.g. earthworm, was demonstrated also for the N-containing pesticides [19] (Table 2).

The herbicide Paraquat is bioaccumulated and adsorbed in snails more than 200 fold [21] and induces significant tadpole mortality resulting from tadpole feeding on Paraquat-contaminated plant material [22].

A quarternary ammonium compound used as reference substance in electrophotographic toners with a log K_{OW} between 2 and 3, a water solubility >100 mg/l and a surface tension <40 mN/m was accumulated up to a BCF >300. Steady state was not reached before 6–8 weeks. QSARs would have predicted a BCF <50 [23].

Obviously molecules containing reductive nitrogen tend to bind to negatively charged sites of molecules, e.g. mucopolysaccharides, due to free electrons resulting in positive loadings of N independent of the log K_{OW} of the substance. Equilibrium for such substances is reached late and depuration is often retarded.

Table 1. BCFs of well water-soluble chemicals

Chemical name	CAS No	Water solubility in mg/l	48 h LC_{50} fish in mg/l	BCF
Basic green-4	569–64–2	>1000 (log K_{OW} –0.17)	0.32	36–91 (20 µg/l) 44–75 (2 µg/l)
4-Vinylpyridine	100–43–6	>10,000	1.57	58–96 (20 µg/l) 48–96 (2 µg/l)
4-(N,N-Dimethyl-amino-1,2 dithio-lan	1631–58–9 1631–58–9	>2000 >2000	0.207 0.207	29–59 (1.56 µg/l) 40–64 (0.156 µg/l)

Table 2. Examples for N-containing and well water-soluble pesticides with the tendency of high adsorption

Group	Trade name	log K_{OW} [20]	Water solubility in g/l [20]
QATs	Chlormequat (chloride)	–1.58	950
Diazines	Chloridazon	1.2	0.34
Bipyridylium	Diquat	–4.6	7–8
	Paraquat	–4.5 – –4,7	620

Also anionic surfactants, e.g. the well water-soluble LAS, are accumulated reaching BCFs >100. Long-chain homologues are accumulated more than 1000 fold. The BCFs are an order of magnitude higher than expected from the log K_{OW} [24].

Concluding from these results it can be stated that the log K_{OW} in certain cases is an inadequate descriptor predicting the BCF or BAF, respectively. Surface activity and structural properties together with the intended use category of a chemical which may give indications on a bioaccumulation potential must also be considered when applying QSARs.

1.2.4
Bioavailability of Chemicals for Bioaccumulation

In many publications released on bioavailability during the last years there are still assumptions to be found that a chemical can only be accumulated either by uptake of the truly dissolved fraction or by ingestion of contaminated food, and that sediment-bound fractions are not longer bioavailable.

In a study on sediment-associated hydrophobic organic contaminants from the Great Lakes it was shown that the contaminants were accumulated by benthic organisms exposed to whole sediment, pore water, elutriates and aqueous medium making use of different uptake strategies whereby the BAFs for aqueous extracts of sediment-associated chemicals indicated a much lower bioaccumulation as compared to whole sediment [25].

Bioavailability of sediment-associated hydrocarbons is also demonstrated in a five-compartment steady-state food-web model including fish and a benthic amphipod. Uptake by ingestion of sediment-associated chlorinated hydrocarbons with log K_{OW} >5 was more significant than the uptake via interstitial and overlying water, respectively, in this amphipod-sculpin food-web of Lake Ontario [26].

Adsorption and bioaccumulation of PAHs and pesticides were investigated in sediment and the benthic-feeding bivalve *Corbicula fluminea*.

Bioaccumulation factors of DDT, DDD, and particularly of DDE in *Corbicula* were greater than predicted values from the K_{OW}. The bioaccumulation factors for the hydrophobic pesticides were one order of magnitude higher than values generally obtained in laboratory studies under equilibrium conditions [27].

In a 10-days bioassay the earthworm (*Lumbricus terrestris*) and fathead minnow (*Pimephales promelas*) accumulated significant amounts of PCBs when exposed to Great Lake sediments [28].

Tubificids (*Tubifex tubifex*, *Limnodrilus hoffmeisteri*) accumulated sediment-associated γ-HCH and HCB in a laboratory test system up to a factor of 4 and 7, respectively, related to sediment concentrations [29].

The oligochaete *Lumbriculus variegatus* accumulated sediment-associated pyrene rapidly [30]. Although not significantly accumulated itself, sediment bound polydimethylsiloxane influenced the uptake kinetics of benzo(*a*)pyrene, resulting in a lower bioaccumulation factor as compared to the uptake of benzo(*a*)pyrene alone [31].

Even after 5 years PCDDs remained bioavailable to freshwater mussel and crayfish exposed to contaminated sediments [32].

A large fish kill observed in the river Tajo in Spain was caused by the lipophilic resin dehydroabietic acid which was associated to suspended matter. Toxicity could be dropped and regained by filtration and resuspension, respectively. The toxicity front moved downstream more slowly than the water body in conformity with the retarded distribution of suspended matter [33].

Although a sharp distinction between uptake routes via water, pore water, colloids, suspended solids and sediment is not always possible, these few examples clearly demonstrate the general bioavailability of sediment-associated fractions. However, a prediction on the extent of bioavailability is limited.

1.2.5 *Overestimation and Underestimation of Bioaccumulation*

Metabolisation, distribution, and excretion are major detoxification processes. Hence, BCFs may be lower than expected from log K_{OW} as exemplified for benzo(*a*)pyrene [34].

However, enzyme induction may be hampered by high exposure concentrations e.g. of the insecticide Chlorpyrifos resulting in a retarded depuration kinetic [35]. Consequently bioaccumulation would be underestimated when applying laboratory derived low-exposure depuration kinetic constants in high exposure scenarios.

Bioaccumulation of superlipophilic substances may be overestimated. Experiments with PCB congeners revealed that obviously not the molecular weight but size and steric factors of molecules may reduce the bioaccumulation of very hydrophobic compounds [36]. Log K_{OW}/log BCF correlations could be described by a 2nd order polynom showing maximal BCFs dependent on the degree of chlorination and log K_{OW} and decreasing BCFs at further increasing log K_{OW}, hydrophobicity and degree of chlorination.

Also disperse dyestuffs with low water solubilities show no or a bioaccumulation lower than expected mainly due to their large molecular size and reduced bioavailability owing to their very low water solubilities [37].

Conversely, methodological shortcomings such as testing bioaccumulation of superlipophilic chemicals in concentrations far above their true water solubility by means of solubilisers may result in low BCFs from the ratio of concentrations in fish/water and insufficient time to gain a steady state, respectively, thus underestimating the bioaccumulation. Testing within the true water solubility without solvent carriers and calculating the BCF on the basis of kinetic rate constants result in values in agreement with current QSARs [38, 39].

Significantly different BCFs for chemicals existing in isomeric structures were reported for α, β, γ, and δ-HCH [7] and for insecticidal pyrethroids with higher BCFs up to a factor of 8 for the cis-isomers [40]. Whereas the BCFs of HCH were dependent on different depuration rate constants, the higher BCFs of the cis-isomers of the pyrethroids could only be explained by greater uptake rate constants, since the depuration rates were similar.

Among PCBs, PCDDs, and PCDFs the degree of chlorination and the chlorine position of the molecule will greatly influence the bioaccumulation behaviour, e.g. the BCFs between the coplanar tetrachlorobiphenyl congener No. 77 and the ortho-substituted congener No. 54 differ by a factor of 32 [41].

Beside isomeric differences causing varying BCFs also enantioselectivity and chiral discrimination of optically active chemicals may influence the degree of bioaccumulation. Organ-specific ratios of enantiomers of α-HCH and α-, β-, and γ-HCH isomers were detected in brain and other tissues of neonatal northern fur seals (*Callorhinus ursinus*) revealing surprisingly high ratios of the two α-HCH enantiomers (1.8 to 28) which were discussed in context with the different health status of the seals [42]. Existence of enantioselectivity and specific transport systems point out that bioaccumulation processes may be decisively governed by small submolecular differences leading to results far from predictability.

From residues in biota and surface waters monitored in the field, BAFs can be estimated and compared with laboratory-generated data. Field BCFs were higher by a factor of 50 for α-chlordane and 220 for DDE [43].

Due to the presumption that only undissociated molecules can penetrate membranes and that uptake through aqueous pores is limited, dissociating substances are generally considered to have no essential bioaccumulation potential. However, the pH may influence the bioaccumulation patterns decisively.

Bioaccumulation of dissociating pentachlorophenol in northern pike in acidified lakes (pH ~ 5.8) was nearly twice as high as in alkaline lakes (pH average 8.1) [44]. This may be relevant when assessing the risk of bioaccumulation processes in areas with serious acidification, e.g. Southwest Sweden.

Bioaccumulation studies with 5-chloro-2-(2,4-dichlorophenoxy)-phenol dissociating within a pH range of 5.8 to 8.8 demonstrated that at pH 8.8, where a high degree of dissociation (~ 88 % dissociated molecules) is present, body concentrations and BCFs measured in zebra fish were similar compared to those at pH 5.8 even though uptake and depuration rates were considerably lower. Beside the uptake of undissociated molecules by diffusion through the membrane the permeation of dissociated molecules through gap-junctions is discussed [36].

Predictions of bioaccumulation in plants according to models based on log K_{OC} are doubtful considering the different uptake routes, types of plants and soils, lipid content and translocation processes in plants.

Investigations on the soil-plant relationships for root crops and the soil-borne part of foliar contamination revealed different uptake and translocation processes in plants which only in part can be explained by the physico-chemical properties of the chemicals [45]. Bioconcentration factors were in several cases much higher than predicted from the K_{OW}.

Bioaccumulation in plants by foliar uptake resulting from partitioning between soil-air-plant may be the main uptake route also for more lipophilic substances with theoretically low vapour pressure and high degree of chlorination. BCFs for PCDDs in plants were 2 – 3 orders of magnitude higher than could be expected from their log K_{OW} [46]. Hence air to leaf transfer of gaseous organics may be a key process for bioaccumulation in plants and the primary step towards a magnification in ecosystems.

Also temperature can influence bioaccumulation and sorption processes significantly resulting in increased bioaccumulation with raising temperature as demonstrated for green-algae [47].

Concluding from these selected examples, overestimation of bioaccumulation potentials due to methodological shortcomings and lacking scientific knowledge may be embarrassing but so far without consequences, underestimation, however, may imply a serious risk when applying wrong prediction in risk assessment approaches.

Since it can be assumed that even with sophisticated scientific work wrong predictions of bioaccumulation potentials cannot be avoided, risk potentials must be countered by precautionary principles, e.g. safety factors.

1.2.6 *Sublethal and Indirect Effects by Bioaccumulation*

Surface active substances already in low concentrations i.e. in the range of µg/l may cause sublethal effects with a broad spectrum of actions.

Although controversially discussed whether a lowered surface tension is responsible for toxic effects, tensides may have an impact on chemoreceptors leading e.g. to disturbed orientation of food-searching fish, on functional disruption of cell membranes, on enzyme induction, and embryogenesis [48].

Bioaccumulation of tributyltin (TBT) compounds which have a broad biocidal action and are used as antifoulants is by far underestimated when estimated using the log K_{OW} varying between 3.2 and 3.8 for the different compounds. BCFs as high as 133,000 for mussels (*Mya arenaria*) and 100,000 for snails (*Nucella lapillus*) have been reported [49, 50].

Clear evidence exists between bioaccumulation of TBT compounds in very low concentrations and the imposition of male sexual characters on female snails (imposex) which is a worldwide observed phenomenon and already used as bioindicator. Sublethal concentrations in the range of ng/l are discussed inducing histopathological malformation in the female gonadal system and leading to complete sterility of the marine mollusks *Littorina littorea* and *Hydrobia ulvae* [51].

A correlation between planar PCB concentrations in eggs, enzyme activities, occurrence of deformities and reproductive success in double-crested cormorants (*Phalacrocorax auritus*) is reported as a consequence of environmental contamination [52]. Bill deformities (> 50% of investigated chicks) were significantly greater at Lake Michigan than in other nesting colonies in the other less contaminated Great Lakes or Canada.

Sublethal effects such as cytological alterations in the liver ascribable to the primary acute toxic mechanism of acetylcholin esterase inhibition were observed in rainbow trout exposed to the insecticide Disulfoton in concentrations well below such producing any macroscopically visible effect [53]. Disulfoton has a short half-life time in water and a moderate BCF of about 400. Even if this indistinct mode of action is interpreted as an adaptive/compensative rather than degenerative phenomenon, this example may reveal basic mechanisms on an ultrastructural level demonstrating potential long-term effects also by substances with an acute toxic mode of action.

1.2.7
Compartment-crossing Transfer of Accumulated Chemicals

Apart from the transfer of sediment-associated chemicals via benthic organisms to benthos-feeding fish, there exist further transfer routes enhancing mobility and distribution of contaminants and leading to a compartment-crossing transfer from sediments to other food-webs.

By diurnal migrations of the epibenthic freshwater shrimp *Mysis relicta* substantial amounts of accumulated sediment-associated PCB congeners were transferred into the pelagic food-web thus coupling the benthic and pelagic zones [54].

A transboundary transport of contaminants from sediments to air and terrestrial ecosystems occurs by the emergence of insects, mainly diptera.

Laboratory experiments showed that 0.2% to 2.1% of total sediment contaminant content are exported annually by emerging insects which had accumulated sediment-sorbed *2,3,7,8*,-TCDF [55].

Midge larvae (*Chironomus decorus*) which accumulated the pesticide transchlordane in a whole life cycle laboratory exposure assay over the course of a 50 day study, transferred 82.6% of the contaminant during metamorphosis to the adult insects, whereas 11.4% was left behind in the shed exuviae [56].

Since emergence events often occur synchronically over a short time interval due to the season, high quantities of contaminants may be available e.g. for midge-eating birds thus enhancing the risk of quickly reaching a critical body burden.

1.2.8
Bioaccumulation, Critical Body Burden and Effects

The bioaccumulation, although a risk factor per se, cannot be assessed without consideration of effects, since enrichment of chemicals in or on organisms or tissues thereof is an necessary prerequisite independent of the mode of action.

With regard to the amount of chemicals accumulated, not the relative amount of accumulated substance, expressed as BCF or BAF, is decisive, but the internal concentration level may cause effects after reaching a critical threshold, either unspecific (e.g. narcotic) or specific (e.g. neurotoxic).

The relationship between bioaccumulation and effects has first been described by Kobayashi et al. 1979 [57], further investigated and confirmed by Mc Carty, 1986 [58], and formulated as the concept of "lethal body burden" as a toxicological endpoint by Sijm et al. 1993 [59]. This internal whole-body concentration in millimoles per kilogram at time of death or immobilization is the product of BCF and steady state LC_{50} which has a constant value for certain groups of closely related compounds, e.g. phenols, with respect to a certain end point and the mode of action. This concept was first verified for narcotic substances with an unspecific mode of action, but probably seems to be applicable also for substances with other modes of action.

Since it is evidenced that the BCF is not a characteristic property of a chemical, respectively an organism, but may depend on the concentration tested and

other factors influencing the uptake and depuration kinetics, a more complex strategy for the assessment of bioaccumulation is suggested [60, 61]. Combining this approach with the lethal body burden concept allows for the decision whether an already reached body concentration is of concern and how far it is away from becoming critical for an organism at a given exposure concentration if no depuration system exists avoiding a further increase.

Considering longterm effects the depuration has a direct influence on the time-dependent toxicity. Species with the ability of elimination will reach an equilibrium for the internal concentration and also an ultimate LC_{50}, whereas the LC_{50} in species that are not capable to eliminate e.g. cadmium, may reach values near to zero. For these species the time to reach the lethal body burden is decisive. Taxonomically related species appear to have comparable accumulation patterns, but lethal body burdens may differ. The authors conclude, that knowledge of the accumulation pattern is indispensable for the evaluation of a species‘ sensitivities to toxicants [62].

Lethal body burdens were also used to estimate the toxicological susceptibility of a species [63]. As an alternative to the LC_{50}, which expresses both the bioaccumulation potential and its intrinsic toxicity, the lethal body burden is more appropriate to reflect the intrinsic properties of a chemical and to explain species susceptibility to toxicants.

Moreover, beside the time-dependent toxicity for an individual organism there is the risk of a transfer of not eliminated body burden from females to the offspring via roe [44], bird eggs [14, 16] and lactation [12].

Incomplete depuration and non-eliminated residues of pentachlorophenol (PCP) were also observed in a bioaccumulation study with the benthic oligochaete *Tubifex tubifex*. Although the body burden concentration of approximately 9 µmol/l was low, residue concentration of parent PCP during the depuration phase remained on a plateau of approximately 3.7 µmol/l [60].

Also in fish (*Leuciscus idus*) a retarded depuration of PCP has been observed resulting in residues on a low, but detectable concentration level [64].

It is a reasonable assumption that non-eliminated body burdens are the main prerequisites for biomagnification in food-webs.

1.3
Scope of Risk Assessment of Bioaccumulation

Drawing conclusions from the cited examples revealing unexpected and non-predicted effects one might assume that with our current risk assessment schemes we are doing the mistakes today which we will become aware of tomorrow.

As experienced and demonstrated for certain chemicals, e. g. PCBs, it must be recognized that bioaccumulation/magnification processes may be phenomena lasting over decades and inducing effects even after release into the environment had been stopped years before and residues in almost all compartments of the environment have declined [65]. Remediation measures are limited to curative activities only. This is particularly true for such highly bioaccumulating and persistent substances unknown as yet.

Hence, for providing a better protection of environment and man we need approaches for a future-oriented risk assessment covering that part of risk which obviously never can be determined ultimately.

To gain more insight into the causal relationships interdisciplinary investigations including food-biology, physiology, biochemistry, immunobiology, pharmatoxicology, neurotoxicology, genetics etc. should be performed. To encounter non-predictable effects by risk assessment strategies, precautionary principles such as the use of appropriate uncertainty factors should be included and measures of risk management and risk reduction implemented.

2 Assessment Concepts of Bioaccumulation

2.1 Criteria for a Bioaccumulation Assessment Concept

In contrast to the assessment of bioaccumulation potentials based on QSARs or specific indications, the measurement of bioaccumulation has to consider all relevant criteria described in the following. Existing concepts for the assessment of bioaccumulation should be critically judged with regard to the consideration of these criteria.

2.1.1 *Test Organisms*

With the choice of test organisms a far-reaching decision is made concerning the test design and the assessment of data gained. Because of the intra- and interspecies variations it is not possible to transfer the results from one species to another. Therefore it is not only necessary to have representative species for at least all relevant environmental compartments such as fresh/marine water, sediment and soil, but also adequate assessment approaches when uptake routes are different e.g. fish and sludge-worm, respectively.

2.1.2 *Uptake Routes*

Principally substances can be taken up from the surrounding medium (water, sediment, soil, air), via food or through body surfaces. For an adequate assessment of bioaccumulation it has to be considered which uptake routes or which combination of them are relevant for a specific substance **and** species. All uptake routes mentioned are possible e.g. for fish, but in combination with substance specific properties like molecular size and shape, charge or surface activity some routes may be excluded in favour of others.

2.1.3
Metabolism

Depending on species and chemical accumulated, metabolism may differ in specificity and extent leading ideally to complete depuration. However, this mechanism cannot be regarded as a mitigating property in general, since uptake may be faster than metabolism and metabolites may be stable and not being eliminated still causing adverse effects. Therefore metabolites should be identified and their quantity measured.

2.1.4
Persistence

Another important factor for an integrated approach of assessing bioaccumulation is the persistence/degradation of a substance in environmental compartments. Like metabolism, degradation cannot be regarded generally as a mitigating property because uptake may be faster than degradation. Therefore persistence/degradation have to be integrated in an appropriate way into an assessment concept.

2.1.5
Precautionary Principles and Trigger Values

Although bioaccumulation is not necessarily a prerequisite for adverse effects, unpredictable risk potentials must be encountered by adequate risk management strategies. Therefore, when assessing the risk of bioaccumulation, two aspects have to be considered:

- the **qualitative** assessment of bioaccumulation defining precautionary principles and characterizing risk potentials,
- the **quantitative** evaluation of data on bioaccumulation defining trigger- or cut off-values on the basis of bioaccumulation categories for further testing or administrative measures according to the respective environmental legislations.

2.1.6
Monitoring Data

Monitoring data on biota indicating adverse effects or alterations in food-webs resulting from bioaccumulation/biomagnification are of utmost value and should be integrated in an overall risk assessment scheme with highest priority supporting and refining the final risk assessment.

2.2
Key Parameters for the Assessment of Bioaccumulation

Parameters for the assessment of bioaccumulation are:

- the BCF in the whole fish and in parts thereof, such as fillet, viscera or carcass.

The BCF alone should be considered critically. It does not reflect the complexity of the bioaccumulation process. Which BCF is reached depends largely on the test organism and the test method, so that the height of the BCF is relative and consequences for individuals are not directly related to this value. For example a relatively low BCF may be harmful when a lethal body burden is already reached or the substance is not eliminated and hence transferred into the food-web.

– organ specific accumulation, reversible as well as irreversible.

This may give rise to special effects (for example behavioural alterations possibly adverse to an individual), which cannot be related easily to a relative low BCF in the whole organism. These effects may also be expressed in a later phase of life or in the following generation.

– the elimination or depuration expressed as half-life clearance time (ct_{50} i.e. the time needed to reach 50% removal).

From the half-life clearance time it can be seen how long a substance remains in an organism no longer exposed to this substance. A short half-life clearance time may be a real mitigating property for even a high BCF, a long half-life clearance time may be, however, an incriminating factor for a low BCF.

– uptake routes and elimination kinetics.

The uptake/elimination may be bi- or multiphasic, i.e. with different velocities at the beginning and the end. Therefore a kinetic description of uptake/elimination is needed to reflect the complete uptake and depuration process.

– incomplete elimination/ plateau formation.

An incomplete elimination of a substance or its metabolites gives rise to bound residues, which may form a plateau in tissues or organs over time and raise the risk of adverse effects (if the substance or its metabolites show a low **No Observed Effect Concentration** (NOEC)) or of biomagnification.

– information on metabolism especially with regard to stable metabolites.

Likewise, stable metabolites may remain in the organism possibly causing adverse effects or may be transferred to higher trophic levels, hence raising the risk of biomagnification.

2.3 Indications of Bioaccumulation Potential

Bioaccumulation studies are laborious and require animal testing. Therefore, as an initial step of a testing strategy it was internationally agreed to use a simple screening method for assessing the hazard that a substance might accumulate in organisms, from a minimal set of (physico-chemical) data and the knowledge of its chemical structure: the determination of **bioaccumulation potential** (BAP). Bioaccumulation potential may serve as a qualitative, or to a limited extent as a quantitative, indicator of a risk of bioaccumulation in living organisms due to the physico-chemical and structural properties of a substance.

There are several possible indications of a bioaccumulation potential which are discussed below. Most of the criteria can only be applied to organic, hydrophobic substances. Only a few can also be used for polar organic or for inorganic substances.

2.3.1 *n-Octanol-water Partition Coefficient*

Bioaccumulation potentials are generally estimated on the basis of the n-octanol/water partition coefficient in its logarithmic form (log K_{OW}). It is easily available and does not require expensive animal testing. If measured values are not available, log K_{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 membranes, 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 K_{OW}) and the bioconcentration factor (calculated as log BCF) has been proved to be poor for some types of chemicals. It cannot be expected that the n-octanol/water partition coefficient generally is a sufficient model of bioaccumulation behaviour of organic chemicals because it does not take into consideration 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, residue plateau of the substance or of metabolites after depuration.

A subtle problem is the log K_{OW} measurement of ionisable substances because this may lead to multiple partition equilibria. The new test guidelines for log K_{OW} measurement (cf. e.g. Annex to Commission Directive 92/69/EEC of the European Communities No. A.8 [66] or OECD Guideline for Testing of Chemicals No. 107 [67]) suggest that log K_{OW} measurements should be performed with ionisable 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 K_{OW} measurement must be at least one pH unit below (free acid) or above (free base) pK-value. Other measurements of log K_{OW} are not valid with regard to assessment of bioaccumulation potentials.

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

2.3.2 *Fat Solubility*

Fat solubility may also give an indication of a bioaccumulation potential assuming the same partitioning equilibrium and membrane diffusion processes for bioaccumulation as derived from the octanol solubility. Since fish lipids cannot be considered as a uniform compartment, the partitioning between water and the different lipid fractions have to be taken into account [68]. For polar membrane lipids (phospholipids) octanol is an appropriate surrogate, whereas nonpolar storage lipids are better represented by hexane. Hence, high fat solubility signalizes a high probability of a bioaccumulation potential, particularly with respect to storage fat, e.g. in adult fish.

2.3.3 *Surface Activity*

Surface active substances, like tensides and many pesticides, may also have the potential to bioaccumulate even if their log K_{OW} values are < 3. Surface activity is measured as surface tension of a solution of a substance in water [69]. If a substance has a surface tension of ≤ 50 mN/m at a concentration ≤ 1 g/l, i.e. is surface active, it may be bioaccumulated itself or enhance the bioaccumulation of other chemicals present. A low **Critical Micelle Concentration** (CMC) also may indicate facilitated uptake and alteration of membrane fluidity.

2.3.4 *Adsorption*

Adsorption onto biological surfaces (e.g. gills, skin) may also lead to bioaccumulation and uptake of substances via food chain (see section 3 Biomagnification). Therefore, high adsorptive capacity (log $K_P \geq 3$) can be regarded as an additional indication of a bioaccumulation potential. This aspect may be of relevance for metallo-organic, organic or polar compounds, e.g. dye-stuffs.

2.3.5 *Structural Features*

A further indication of bioaccumulation potential is given for analogues of organic or inorganic substances known to have the potential to bioaccumulate in organisms. The same is true for substances which contain nitrogen, e.g. amines, pyridinium compounds, which accumulate higher than expected from their log K_{OW} (e.g. herbicide Paraquat, log K_{OW} – 4.6, BCF > 200).

2.3.6 *Mitigating Aspects*

Certain physico-chemical, biological, and structural criteria might exclude a bioaccumulation potential for a distinct substance even if it exhibits an indica-

tion for a bioaccumulation potential, i. e. log $K_{OW} \geq 3$. Because uptake of a chemical may be very fast as is exemplified in figure1, 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.

Ready biodegradability of a chemical is commonly considered as a mitigating aspect, however, uptake rates of bioaccumulation may be significantly faster than biodegradation as is shown in Fig. 1. Hence bioaccumulation might occur even though the substance is readily biodegradable. This has to be assessed carefully on a case-by-case basis considering kinetic information on both processes.

It may be necessary to check the products of abiotic and biotic degradation for their bioaccumulation potential.

2.4 Existing Assessment Concepts of Bioaccumulation

Bioaccumulation potential is assessed differently in national and international regulations. The assessment category may even differ in a certain country for

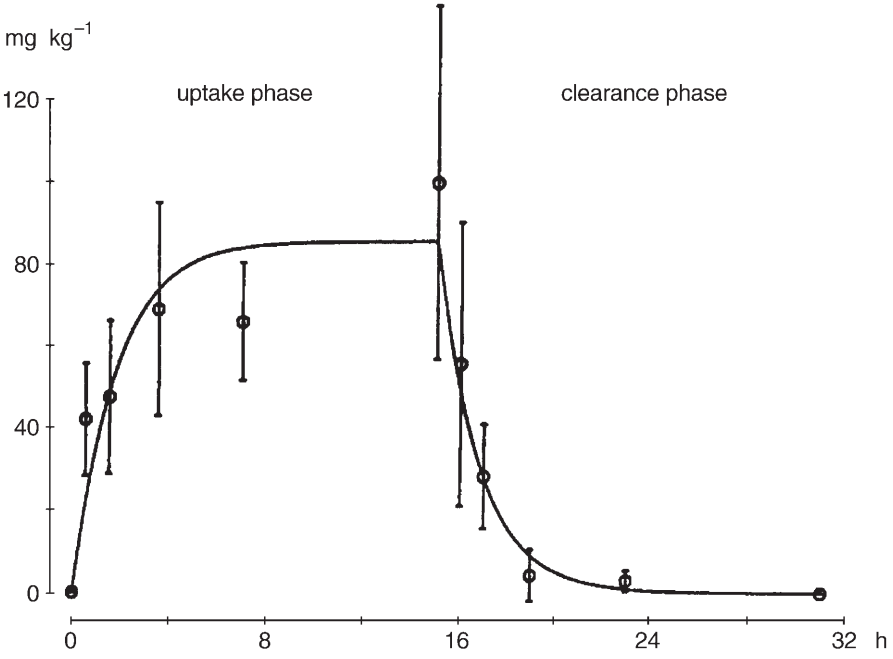


Fig. 1. Rapid uptake of 2-t-butylphenol by zebra fish (*Brachydanio rerio*): steady-state concentration within 5 h (taken from [64]) (wet weight basis)

different groups of chemicals. Furthermore, the indication of bioaccumulation potential for a certain substance may be used for different purposes, e.g.:

- for classification and labelling,
- for deciding on the test duration of ecotoxicological tests, with respect to the steady-state concentration,
- as trigger for bioaccumulation testing.

Table 3 and the following text present a short overview of the regulations and proposals for criteria and trigger for indications of bioaccumulation potential in different countries so far available or known, respectively.

2.4.1 *USA*

legal scope: **Environmental Protection Agency** (EPA), Toxic Substances Control Act (TSCA), new and existing chemicals [78]
criteria: "sufficient" toxicity in the lower TIERS, or indications of chronic effects, or uptake and effective persistence
trigger: log K_{OW} ≥3.5
consequences: bioaccumulation study

Within the EPA the **Office of Toxic Substances** (OTS) is responsible for implementing the **Toxic Substance Control Act** (TSCA). The OTS has developed the following approach:

1) identification of appropriate ecological endpoints,
2) a tier-testing scheme for estimating impacts on such endpoints,
3) ecotoxicological testguidelines,
4) models and techniques for estimating ecotoxicity from chemical structure (SAR/QSAR),
5) hazard assessment factors for establishing chemicals concentration of environmental concern,
6) risk assessment methodologies characterizing the risk by including hazard (ecotoxicity) exposure data.

The tier-testing scheme has four tiers (I-IV) of toxicity testing with aquatic and terrestrial organisms. On TIER III the bioaccumulation is included gaining importance for further decisions.

Bioaccumulation testing at TIER III is conducted if there is "sufficient" toxicity in the lower TIERS, or indications of chronic effects or uptake and effective persistence (based on half-lives in water, soil and plants) could be shown.

A degradation half-life in water ≥ 4 d and log K_{OW} ≥ 3.5 would trigger a bioaccumulation study at this TIER.

Other indications e.g. surface tension are not mentioned, no further explanations are given concerning derivation of the half-lives and no guidance is given concerning the decision of testing bioaccumulation in fish and/or oyster.

QSAR is only described/used for estimating toxicity.

In the evaluation of bioaccumulation data the BCF is the only criterion used in the assessment resulting in three categories:

Table 3. Different National and International Regulations and Proposals for Indication of Bioaccumulation Potential

State/union	Regulation	Criterion and Trigger	Result, Consequence	Reference
Germany	Chemicals Act (new and existing chemicals)	cf. EU (new and existing chemicals)	see EU	[70]
	Plant Protection Act (pesticides)	log $K_{OW} \geq 3$	bioconcentration study in fish	[71]
EU	67/548/EEC (all chemicals)	log $K_{OW} \geq 3$	Classification and labelling (indication of bioaccumulation potential)	[72]
	93/67/EEC (new and existing chemicals)	log $K_{OW} \geq 3$ or highly adsorptive or belongs to a class of substances known to have a potential to accumulate in living organisms or indications from structural features and no mitigating properties (cf. 2.4.4)	Indication of bioaccumulation potential SAR estimation of BCF for assessment of secondary poisoning which may trigger a bioaccumulation study Trigger of aquatic long-term ecotoxicological tests	[73]
	91/414/EEC (pesticides)	log $K_{OW} \geq 3$	Bioconcentration study in fish	[74]
Canada	Toxic Substances Management Policy (new and existing chemicals)	log $K_{OW} \geq 5$	Bioaccumulation study (cf. 2.4.3)	[75]
	Pesticides	log $K_{OW} \geq 3$ og K_{OW}: 2–6	Bioconcentration study in fish Raises concern about potential bioaccumulation	[76]
Japan	Chemicals Substance Control Law (new and existing chemicals)	Not readily biodegradable and log $K_{OW} \geq 3$	Bioconcentration study in fish (cf. 2.4.2)	[18, 77]
The Netherlands	Pesticides	log $K_{OW} \geq 4.3$	Bioconcentration study	[76]
USA	Toxic Substances Control Act (new and existing chemicals)	$8 > \log K_{OW} \geq 3.5$ and MW ≤ 1000, effective persistence	Bioconcentration study (cf. 2.4.1)	[78]
	Pesticides	log $K_{OW} \geq 3$	Bioconcentration evidence	[76]

high: BCF ≥1000
medium: BCF ≥100<1000
low: BCF <100

Elimination behaviour, the formation of a plateau of residues or persistent metabolites are not mentioned.

In the assessment process of new substances so called assessment factors are used, ranging from 10, or 100 to 1000. They have to be understood as "uncertainty factors" and they are used only with toxicity test results.

No factor for a bioaccumulation risk is mentioned.

2.4.2 *Japan*

legal scope: Chemical Substances Control Law, new and existing chemicals [18]
criteria: non-biodegradability
trigger: log $K_{OW} \geq 3$
consequences: bioaccumulation study

Biodegradability, bioaccumulation and toxicity are basic criteria for regulating chemical substances in Japan under the Chemical Substances Control Law. Chemical substances are not subject to regulation when they have high biodegradability, low bioaccumulation and low toxicity in general.

In any case it is generally assumed that chemicals have a low bioaccumulation when their BCFs are less than 1000 in the bioaccumulation test OECD 305 C [79]. However, the final decision is drawn after a review in the Judgement Committee considering also additional factors. In principle, the bioaccumulation test is applied to non-biodegradable chemicals. If a test substance is altered to another chemical substance in the biodegradation test, the bioaccumulation test is conducted with the altered substance until the BCF reaches equilibrium.

Two concentration levels are tested; if the BCF is shown to be concentration-dependent, more than two levels are tested.

Generally non-polar substances with a log $K_{OW} \geq 3$ have to be tested. Dissociating substances also have to be tested if their log K_{OW} is < 3 and the criteria for weak acids (pKa-pH < 1.7) and weak bases (pH-pKa < 1.7) have to be applied.

Testing is also indicated if the substance reveals other properties, e.g. hydrolysis and QSARs are not applicable. If rapid transformation occurs, the transformation products have to be considered.

2.4.3 *Canada*

legal scope: **Canadian Environmental Protection Act** (CEPA), new and existing chemicals [75]
criteria: persistence
trigger: log $K_{OW} \geq 5$
consequences: bioaccumulation study

Key objectives of the Canadian Toxic Substance Management Policy are virtual elimination from the environment of toxic substances that result from human activity and that are persistent and bioaccumulative (referred to as Track 1 substances).

Criteria for selection of Track 1 substances are:

- Persistence given as half-life for air (≥ 2 d), water and soil (≥ 182 d), sediment (≥ 365 d),
- Bioaccumulation with BAF or BCF ≥ 5000 or log K_{OW} ≥ 5.0
- Toxicity[1],
- Predominantly anthropogenic[2].

Expert judgement and the weight of scientific evidence will be used in determining whether these criteria are met.

Substances not meeting all four criteria are so-called track 2 substances for which a life-cycle management is demanded to prevent or minimize release into the environment.

Concerning bioaccumulation lipid content of the organisms should be considered for a better comparability of data.

Only BCF or BAF and log K_{OW} are used, no other parameters, e.g. elimination from the organisms, are used. However, BCF and BAF are considered as environmentally more relevant than K_{OW}, and bioavailability of the substance has to be considered particularly when BAF is determined. Field data (i.e. BAF) are preferred over laboratory data (e.g. BCF).

There is no guidance on test guidelines and no concept on testing strategies for other compartments than water.

2.4.4
European Union

legal scope: 1) Commission Directive 93/67/EEC for new and existing substances [80], Technical Guidance Document [73]
2) Council Directive 91//414/EEC for pesticides [74]
Criteria ad 1): log K_{OW} ≥ 3 **or** highly sorptive **or** belongs to a class of substances known to have a potential to accumulate in organisms **or** indications from structural features **and** no miti-

[1] Defined in the Canadian Environmental Protection Act, Sec. 11: "a substance is toxic if it is entering or may enter the environment in a quantity or concentration or under conditions (a) having or that may have an immediate or long-term harmful effect on the environment; (b) constituting or that may constitute a danger to the environment on which human life depends; or (c) constituting or that may constitute a danger in Canada to human life or health."

[2] On the basis of expert judgement, the concentration of the substance in any environmental medium is due largely to the quantities of the substance used or released as a result of human activity relative to contribution from natural sources.
Elements and naturally occurring inorganic compounds are not candidates for virtual elimination.

gating properties such as hydrolysis ($dt_{50} < 12$ h) or molecular mass > 700
Criteria ad 2): log $K_{OW} \geq 3$

Concerning new notified chemicals risk potentials and testing requirements depend on the quantity placed on the market. On the base set level (> 1 t/a < 100 t/a) the bioaccumulation potential is assessed according to the criteria listed above. If one of the criteria is met, the substance will be classified with "indication of bioaccumulation potential" in a first approach. If a potential to bioaccumulate can definitely be excluded, it will be classified as showing "no indication of bioaccumulation potential".

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

The bioaccumulation potential is used for three purposes:

- as indicator of a risk for possible adverse long-term effects in ecosystems caused by bioaccumulation,
- as trigger for a bioaccumulation study according to Council Directive 92/32/EEC [81] and German Chemicals Act [70], respectively. A bioaccumulation potential defined by a log $K_{OW} \geq 3$ or the other criteria in combination with mammals/bird toxicity indicate a risk of secondary poisoning and may trigger a bioaccumulation study already on the base set level. On level 1 (> 100 t/a < 1000 t/a) or at 10 t/a depending on ecotoxicological data a bioaccumulation study is mandatory if an indication of a bioaccumulation potential was assessed.

Depending on the result of the study and the risk assessment taking into account exposure and ecotoxicological data (PEC/PNEC), further tests, e.g. bioaccumulation with other organisms may be required on level 2 (> 1000 t/a).

- classification and labelling according to Council Directive 92/32/EEC.

Concerning existing chemicals all available data on bioaccumulation in biota are considered and assessed by expert judgement case by case.

Deficiencies of the risk assessment of the TGD are:

- only log K_{OW} and BCF are considered,
- bioaccumulation is not integrated into the risk assessment scheme and ecotoxicological testing strategy.

Concerning pesticides a bioaccumulation study is mandatory if the active ingredient of a pesticide has a log $K_{OW} \geq 3$.

If the BCF is > 1000 or > 100, respectively, depending on biodegradation and ecotoxicological data, no registration may be granted. However, the complexity of bioaccumulation, particularly the formation of possible bound residues is generally not considered for those hazardous pesticides passing cut-off values.

2.4.5
European Chemical Industry Ecology & Toxicology Centre (ECETOC)

legal scope:	ECETOC-Concept [82] based on Commission Directive 93/67/EEC, new and existing chemicals
criteria:	high persistence, toxicity, negligible metabolism
trigger:	log K_{OW} between 5 and 8
consequences:	bioaccumulation study

In this concept a distinction is made between bioconcentration and bioaccumulation. Bioconcentration is defined as the net result of uptake, distribution, and elimination of a substance in an organism due to water-borne exposure, whereas bioaccumulation includes all routes of exposure including food.

Bioaccumulation is not regarded as 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. If no measured BCF data are available, SAR relationships are recommended. K_{OW} is preferred but other relationships based on water solubility and molecular connectivity indices may also be applied. Bioaccumulating substances are characterized by high persistence, toxicity, negligible metabolism and a log K_{OW} between 5 and 8. However, they are only of concern when widely distributed in the environment. Hence the bioaccumulation potential is regarded as an exposure-related parameter in risk assessment. Molecular volume (molecular weight well above 700), low lipid solubility, low bioavailability, rapid biotransformation and structural features are considered as mitigating aspects.

The risk assessment is driven by the key criteria:

- environmental exposure,
- possible uptake.

For substances which reach a steady-state body burden within the organism during the toxicity test, direct effects of bioconcentration are included. Hence a PNEC derived under this condition is regarded as appropriate for use in risk assessment. However, for substances which are taken up and depurated very slowly by fish, the steady-state body burden concentration may not be reached during the toxicity test. Hence, it is recommended to consider the time to reach steady-state (recommended is T_{95}, i. e. time to reach 95 % of the steady state concentration) when calculating the **Predicted No Effect Concentrations** (PNECs) for such substances.

If exposure and uptake is possible, bioaccumulation potential is integrated in the ECETOC assessment concept in two ways:

- T_{95}, calculated from K_{OW} is used to select an appropriate duration of aquatic ecotoxicological tests.
- A calculated BCF greater then 1000 is used as trigger for an assessment of secondary poisoning applying the value of this BCF then to estimate a PEC_{oral} (cf. 3.2.3).

If exposure and uptake are not assumed, further assessment is not necessary. Deficiencies are summarized in the context of biomagnification (cf. 3.2.3).

2.5 Proposal for a Comprehensive Assessment Concept of Bioaccumulation

Resuming the criteria, trigger values, and deficiencies in the fore-mentioned concepts, an attempt is made for a comprehensive concept of risk assessment of bioaccumulation as follows:

Criteria for the assessment of bioaccumulation should be 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 incomplete elimination leading to bound residues.

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, k_1 and k_2. Ct_{50} is calculated from the elimination curve in substance free water after a certain time of exposure.

The complexity of bioaccumulation processes makes it necessary to take into account all measurable processes influencing bioaccumulation, such as

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

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

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

Since the degree of elimination of an accumulated chemical is decisive with regard to a possible transfer to higher trophic levels, BCF and half-life time of depuration, ct_{50}, are equally taken into account resulting in 4 respective assessment categories covering the whole range of experimental results, as is shown in Table 4.

The combination of the BCF and ct_{50} will lead to 4 averaged overall assessment categories characterizing the degree of concern.

A more restrictive classification may result in the overall assessment if e.g. there is an indication of organ specific bioaccumulation or of incomplete 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 of them pointed out below:

- 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} is regarded only.

Table 4. Classification of Bioconcentration Factor and Elimination and Overall Assessment of Bioaccumulation

Bioconcentration Factor (BCF)

BCF range	Assessment Category	Comment
< 30	I	low BCF
30 – 100	II	moderate BCF
100 – 1000	III	high BCF
> 1000	IV	very high BCF

Elimination

ct_{50} range	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.

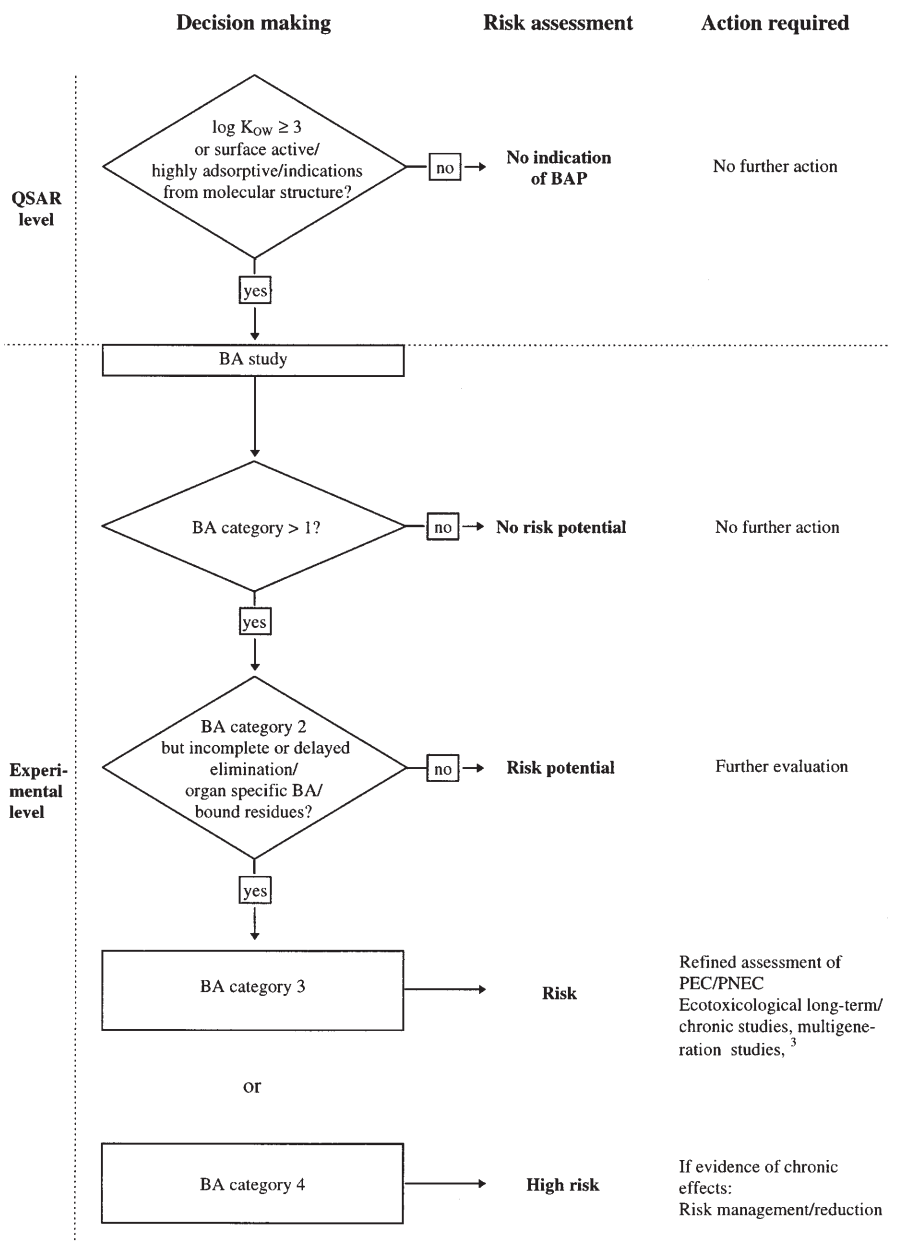
Overall Assessment Category	Comment
I	no concern
II	indication of risk potential
III	cause for concern
IV	high risk (recommendation for risk reduction)

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.

– 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 period which exceeds the duration of long-term ecotoxicity tests, even a plateau as low as 10% of the total ammount of accumulated substance raises the risk of biomagnification.

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

The different bioaccumulation assessment categories reflect various degrees of concern. The flow-scheme in figure 2 gives guidance how these categories



[3] testing for complex i.e. genetic, physiological, histopathological endpoints, endocrine disruptions etc., field studies.

Fig. 2. Risk assessment strategy of bioaccumulation

could be used to assess the risk of bioaccumulation and trigger more conclusive ecotoxicological tests.

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.

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 with these species may become 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.

3 Biomagnification

3.1 Significance of Biomagnification for Risk Assessment of Chemicals in the Environment

Biomagnification (BM) is the transfer of chemical substances via food-webs passing different trophic levels and resulting in residues which may be detrimental for organisms in terminal positions within food-webs, e.g. dolphins, seals, crocodiles, humans (cf. [6, 17]).

A biomagnification potential (BMP) is indicated if within a food-web the concentration of a chemical or its metabolites in an organism is higher than in its food as major source of uptake.

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. [83, 84] and has become part of the assessment of New and Existing Chemicals in the European Communities [73, 80] (see Fig. 3). 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.

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. micro-organisms, 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. A part from the BCF the consideration of bound residues are of main concern when conducting and evaluating a bioaccumulation study.

Biomagnification of a substance can hardly be measured in laboratory testing systems existing so far. Therefore, the possibility that a chemical might bioaccumulate – the biomagnification potential (BMP) – has to be considered as an initial step. The flow scheme in Fig. 4 (cf. 3.3) 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.

3.2 Existing Assessment Concepts of Biomagnification

3.2.1 *USA*

There is no special concept for biomagnification, but US-EPA applies so-called "food chain multipliers" which account for bioaccumulation starting at log K_{OW} of 4.0 [78].

3.2.2 *European Union*

The EU risk assessment approach involves bioaccumulation, biomagnification and secondary poisoning, i.e. the indirect intoxication along a short food-chain water → fish → fish-eating bird or mammal (see Fig. 3).

Secondary poisoning is indicated if the concentration in fish (PEC_{oral} of the prey) reaches a level exceeding the threshold for adverse effects in most sen-

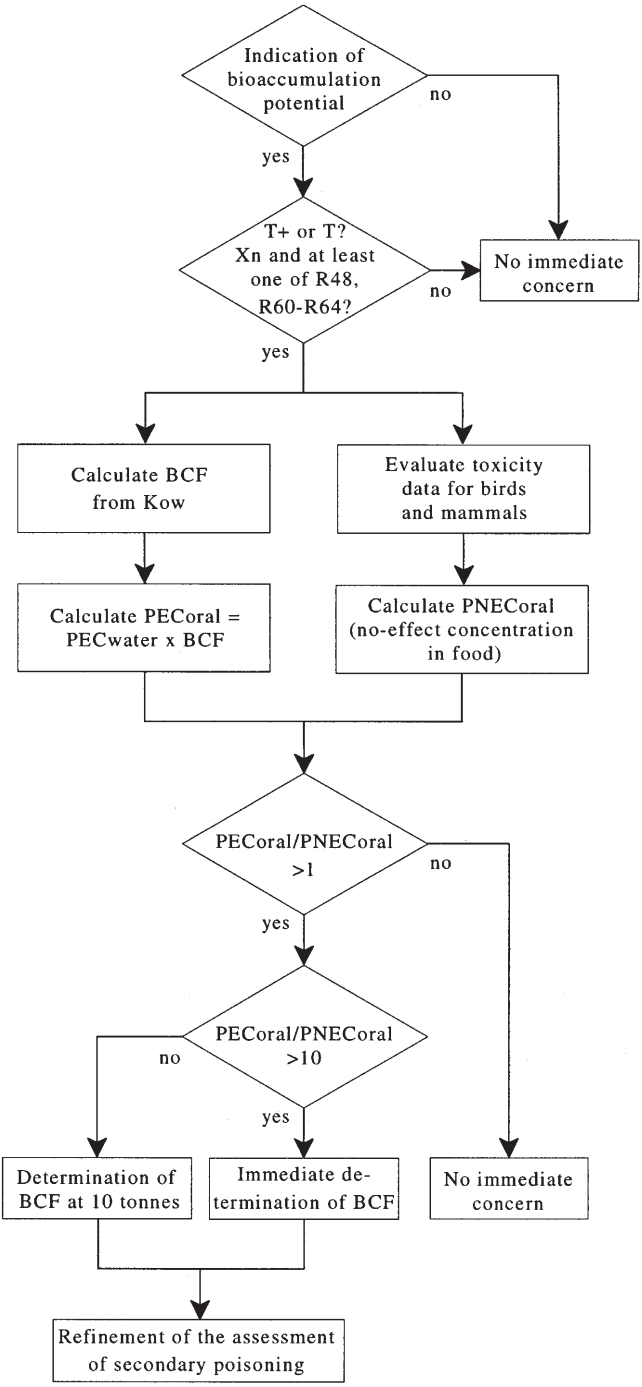


Fig. 3. Strategy for the risk assessment of secondary poisoning

sitive fish-eating birds or mammals ($PNEC_{oral}$ of the predator), i.e. **PEC_{oral}/ $PNEC_{oral}$** >1 (Predicted Environmental Concentration/Predicted No Effect Concentration).

PEC_{oral} is the product of exposure concentration (PEC_{water}) and the BCF in fish (modeled or measured). The $PNEC_{oral}$ is the product of a **No Observed Effect Level** (NOEL) in dietary toxicity tests with animals representative for fish-eating birds or mammals and tiered safety factors. The latter taking into account interspecies variations, subchronic to chronic toxicity extrapolation, laboratory data to field impact extrapolation and conversion factors.

When $PEC_{oral}/PNEC_{oral}$ ratio exceeds 1, a concern is signalized triggering further tests to refine the data and risk reduction measures, respectively.

Other food-chain models are not excluded, but no guidance for other compartments and species is given.

Summarizing, the EU concept for biomagnification and secondary poisoning is based solely on PEC/PNEC ratios which imply clear toxicologically defined endpoints of predators.

Generally the EU concept has the following short-comings and deficits:

- risk assessment only based on PEC/PNEC philosophy,
- no safety factors for unforeseeable effects,
- no guidance for other compartments (marine, sediment, terrestrial),
- the secondary poisoning concept is only a limited aquatic food-chain model,
- no consideration of aquatic, sediment associated and terrestrial food-webs.

3.2.3
European Chemical Industry Ecology & Toxicology Centre (ECETOC)

In this concept the biomagnification is integrated in an overall risk assessment scheme. If the calculated BCF is >1000 as the outcome of an initial assessment of bioaccumulation potential (cf. 2.4.5), the risk assessment of secondary poisoning is triggered. Dietary uptake by aquatic organisms is considered only if the BCF of prey organisms is >1000 corresponding to a log K_{OW} of 4.3. If this criterion is met, a $PEC_{oral}/PNEC_{oral}$ assessment for predators is conducted and refined if considered necessary.

Referring to the EU Technical Guidance Document the ECETOC concept criticizes that the risk assessment for the secondary poisoning concept is initiated at log $K_{OW} > 3$. This approach would thus overestimate the risk of chemicals of „little“ relevance (i.e. already with log $K_{OW} \geq 3$) and underestimate the risk of chemicals in the log K_{OW} range of 4.5 – 8 with higher lipophilicity where dietary uptake is more significant for biomagnification.

Further it is suggested that the PEC_{oral} should be based rather on actual body burden concentration than on log K_{OW} regression, since elimination may significantly reduce the body burden thus overestimating the magnification. Unfortunately other potential end points are not discussed as well as consequences resulting from body burden concentration.

Deficiencies of ECETOC concept:

- underestimation of biomagnification for substances with lower log K_{OW} values,
- risk assessment solely based on the PEC/PNEC approach,
- no consideration of bound residues,
- only consideration of known (acute toxic) effects,
- no safety factors to counter unforeseeable effects,
- persistent, lipophilic and toxic substances (fish/mammals) are not considered in the three case studies presented.

3.2.4 *Van Leeuwen and Hermens*

The biomagnification has been modelled by Van Leeuwen and Hermens (1995) [85] taking into account uptake of food and ingestion of sediment, e. g. by sediment dwelling organisms.

The BMP does not consider the BCF alone but also information regarding kinetics of uptake, metabolism and elimination in the organism.

Risk characterization of BM is based on exposure and effect assessment (PEC/PNEC) defining risk quotients. Effect assessment is mostly based on acute, less frequently on subacute or chronic tests, i.e. well defined ecotoxicological end points.

After performance of an uncertainty analysis the probability of the occurrence of defined and known effects is identified and quantified.

Secondary poisoning, i. e. the indirect intoxication via a short food chain, e. g. fish → fish-eating bird or mammal is not only related to increased mortality, but may consider also fitness parameters and more subtle effects, e. g. impact on eggshell thickness.

Although unforeseeable effects can never be ruled out completely by any risk assessment strategy, the PEC/PNEC approach, in our opinion, does not sufficiently counter the risk of unexpected effects, even by application of uncertainty factors on the final PNEC.

3.2.5 *Cowan et al.*

An integrated approach for environmental assessment of new and existing substances is presented by Cowan et al (1995) [86] which specifically evaluates persistence and bioaccumulation of a substance in order to assess the potential for direct and indirect effects on species in aquatic, sediment and terrestrial environments.

This concept is based on the assessment of bioconcentration, dietary pathways, potential for bioaccumulation and effects resulting in 4 tiers of concern.

A measured BCF > 1000 signalizes the potential of dietary exposure for aquatic organisms and the ratio $PEC_{oral}/PNEC_{oral}$ is calculated. If the ratio exceeds 1, long term ecotoxicological studies are demanded. Risk management is indicated, if after a refined assessment which includes monitoring data the ratio is still > 1.

This approach exceeds conventional risk assessment concepts, but aims obviously only at mortality rather than at long-term/chronic effect in ecosystems.

3.3 Proposal for a Comprehensive Assessment Concept of Biomagnification

The main principles of a risk assessment strategy for biomagnification are shown in the flow-scheme in Fig. 4.

Basic prerequisite of a risk assessment for biomagnification is a valid comprehensive bioaccumulation study taking into account uptake and depuration kinetics, organ-specific distribution and accumulation, metabolic activities and conjugation products, bound residues and, preferably, critical body burden concentrations for subtle end points e.g. chromosomal aberrations.

However, there is a considerable variability of these parameters among species and transmission of data from one species to another is generally not possible.

Not or only partially metabolized chemicals, classified in a bioaccumulation category ≥ III (cf. 2.5), which persist in individuals and may be transferred to further generations or trophic levels, respectively, signalize an indication of biomagnification potential.

In turn, lack of these incriminating criteria may as a first approach lead to the conclusion that there is no immediate concern for a risk so far and no further action is required for the moment.

Monitoring data on the environmental fate of pollutants in terrestrial and aquatic compartments and the occurrence in biota may give decisive indications on biomagnification processes.

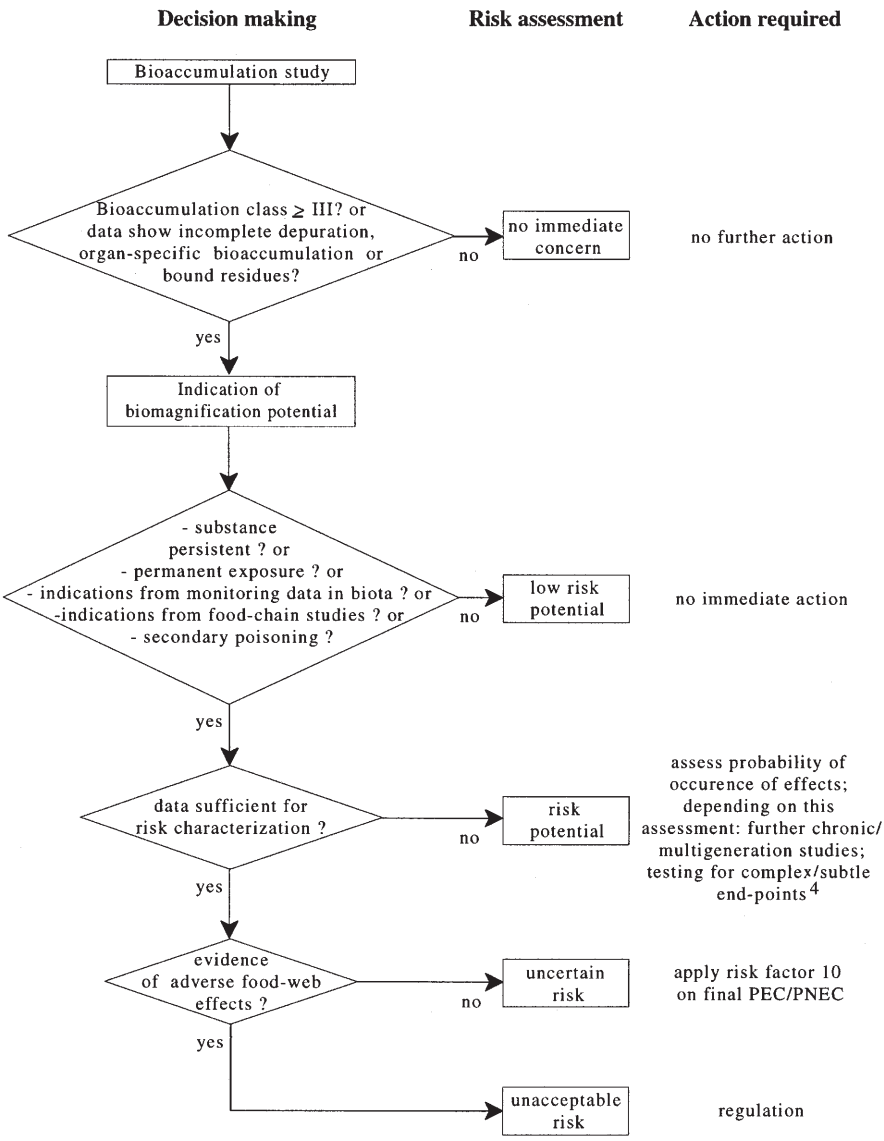
Such indications may also be derived from laboratory scale food-chain-studies. Additionally, if permanent exposure is anticipated or proven or if the predicted regional environmental concentration (PEC_{reg}) is in the same order of magnitude as the **critical body burden** (CBB_{food}) concentration for food in the organisms, a risk of biomagnification exists and the probability for the occurrence of effects must be assessed, provided data are sufficient.

If such indications are not recognizable on this level the risk of a biomagnification potential is low. Immediate action is not required but further data should be gathered for a refined assessment.

Is a risk characterization not possible on this level due to lacking data more sophisticated laboratory studies have to be performed comprising chronic multigeneration studies and investigating, e.g. genetic, physiological, histopathological and endocrinological endpoints.

The outcome of such studies may confirm the suspicion revealing adverse effects or – in case of non-visible effects – may lead to the conclusion that an uncertain and unforeseeable risk remains, which should be countered by the application of a risk factor of 10 on the final PEC/PNEC.

A clear evidence of adverse effects in food-webs means an unacceptable risk for ecosystems and should result in risk management and reduction/regulation measurements.



[4] genetic, physiological, histopathological, endocrine disruptions etc.

Fig. 4. Risk assessment strategy of biomagnification

4 Deficits and Development of Guidelines

By now there is only one internationally standardized test system for testing the bioaccumulation in fish: OECD guideline 305: Bioconcentration: Flow-through Fish Test (1996) [1].

Beside recommended fresh water species including bottom feeding fish, cold-water and warm-water fish, various estuarine and marine species have been used in different countries.

The US EPA has additionally adopted bioaccumulation tests with oysters or fresh water clams and suggests also bioaccumulation studies with crustaceans, e.g. daphnia, shrimps or crayfish, or insect nymphs, e.g. mayfly.

Since in the EU inventory of test guidelines there is no one other than the bioaccumulation test on fish mentioned, and test results of fish cannot be transferred e.g. to invertebrates, there is an urgent need for representative species of different trophic levels and compartments, respectively.

Particularly for the environmental compartments soil and sediment no guidelines are available. With regard to sediment organisms a Draft Guideline for Testing Bioaccumulation in Tubificids (sediment ingesting sludge-worm) has been submitted to the OECD by the UBA in 1997 as the outcome of an "**Research & Development**" (R & D) project. Based on these results the development of a short food-chain model test system was initiated consisting of sediment – Tubifex → fish → fish-eating bird.

Also for the terrestrial compartment no test system exists until now. Within the framework of the OECD Chemicals Program a test system investigating the bioaccumulation in earthworms was scheduled in 1997 by an R & D project also sponsored by the UBA.

Like for the aquatic compartment, food-chain model test systems should also be developed for the terrestrial compartment as well as for marine and estuarine environments thus considering the most important environmental compartments for an overall comprehensive risk assessment for ecosystems.

5 Conclusions

- The risk assessment of bioaccumulation by environmental authorities should not be based on QSARs alone since many chemicals do not obey commonly applied correlations. Whereas overestimation of bioaccumulation may be irrelevant to real environmental conditions, underestimation of risks may have serious consequences for ecosystems.
- Risk assessment of bioaccumulation solely based on the BCF is insufficient and may be misleading. Instead, a tiered risk assessment strategy of bioaccumulation and biomagnification is proposed taking into account the complexity of bioaccumulation processes integrating equally the key parameters BCF/BAF and depuration half-life times for deriving four classes which characterize the risk of bioaccumulation and if necessary trigger further ecotoxi-

cological tests. Incomplete depuration and the occurrence of bound residues are additionally considered as incrimination factors.
- The critical body burden concentration (i. e. the internal concentration in tissues or organs above which effects may be induced) is finally the decisive parameter of bioaccumulation with regard to effects. This ecotoxicological endpoint is more meaningful than conventional EC_x-values defining external concentrations. Having knowledge of the internal threshold concentration for a specific endpoint, the safety margin for the risk resulting from the difference between the concentration already reached and the concentration inducing effects, can be defined (see contribution of Sijm and Hermens, this volume).
- BCFs and BAFs as numerical values should not equally be used in risk assessment approaches. BAFs of organisms related to sediment or soil concentrations are of course usually lower than BCFs of aquatic organisms for which the main uptake route for moderately lipophilic substances is predominantly via water. However, regardless which uptake routes are involved a terrestrial or sedimental BAF >1 is considered as relevant regarding a significant bioaccumulation.
- Even exonerating results from sophisticated chronic bioaccumulation and biomagnification studies, if ever conducted for each relevant chemical released into the environment, cannot completely rule out the risk for adverse long-term effects. Hence, if for the risk assessment of biomagnification a risk for ecosystems is indicated, the application of an additional safety factor of 10 on the final PEC/PNEC according to the TGD is proposed. If as a consequence of the risk assessment strategy an unacceptable risk for ecosystems is shown, restrictions and bans, respectively, should be considered.

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