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Molekularbiologie und
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Literature Study: Effects of Molecular Size and Lipid Solubility on Bioaccumulation Potential

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1. Introduction

The European regulation concerning the registration, evaluation, authorization and restriction of chemicals (REACH) requires demonstration of the safe manufacture of chemicals and their safe use throughout the supply chain [1]. While REACH is based on the precautionary principle, it includes also the aim to reduce animal testing where possible. Waiving of tests under REACH is foreseen for scientific and technical reasons. Studies on bioaccumulation in aquatic species, preferably fish, need not be conducted, if the substance has a low potential for bioaccumulation (for instance a log K_{OW} < 3) and/or a low potential to cross biological membranes; or if direct and indirect exposure of the aquatic compartment is unlikely.

Identification of substances of concern, such as persistent, bioaccumulative and toxic chemicals (PBT) and very persistent, very bioaccumulative chemicals (vPvB) is an important aspect of environmental hazard assessment and required according to Annex XIII of REACH. Regulatory labelling of PBT and vPvB chemicals will require authorization under REACH, and their use may be restricted. However, chemical property data required by Annexes VII and VIII of REACH (chemicals with tonnages greater than 1 tonne and 10 tonnes, respectively) are not sufficient to perform a PBT assessment.

Therefore, reliable and broadly applicable estimation methods will be required to generate necessary data for the PBT assessment under REACH. First screening tiers may comprise tools selecting those compounds that are highly unlikely to be of concern, such that testing may be exempted. With regard to bioaccumulation, waiving criteria based on physico-chemical or structural properties, e.g. molecular size and lipid solubility, are currently discussed. The underlying rationale is that uptake into biota may be limited due to hindered membrane passages of large molecules. Low lipid solubility may prevent membrane permeation as well. The properties, alone or in combination, may cause minor absorption into organisms, hence a low bioaccumulation potential of environmental contaminants.

2. Study Objectives

The study addresses five major issues to rationalize the effects of molecular size and lipid solubility on the bioaccumulation potential of environmental contaminants:

- Do studies on biological membranes support limits of permeability related to size or lipid solubility of the chemicals?

- Do studies on bioconcentration support limits of uptake and accumulation potential related to size or lipid solubility of the chemicals?

- How relevant are active transport mechanisms for the uptake of large organic chemicals?

- Do compound properties related to size or lipid solubility provide guidance in assessment schemes of bioaccumulative chemicals? Which parameters are potentially useful? Can cut-off triggers be defined?

- Do high quality data for superhydrophobic substances provide new insights into relationships between BCF and log K_{OW}?

3. Bioaccumulation Assessment

The accumulation of chemicals in organisms, water bodies, sediment and soil is of major concern for environmental hazard assessment. The uptake of dissolved contaminants into biota occurs mostly by direct absorption, but also along the trophic web. The internal concentration in the body may increase by accumulation to a level causing toxic effects, even if the external concentration remains below the critical limit. Short-time exposure may produce high internal concentrations that persist in the organism much longer than in the surrounding water. Because of their elevated and lasting level in biotic compartments, substances that are accumulated may evoke potentially chronic effects, not only in the organisms directly exposed, but also in species at higher levels in the food chain, including humans. Bioaccumulation is, therefore, an important link between the pollution of surface waters and human exposure to xenobiotic substances.

Accumulation is the general term for any phenomenon associated with increasing the concentration of chemicals in a compartment relative to the surrounding phases. With regard to organisms, the accumulation processes are defined according to the mode of uptake of contaminants:

- Bioaccumulation: uptake from the environment via any possible pathway

- Biomagnification: uptake via the foodweb resulting in increased concentrations at higher trophic levels

- Bioconcentration: uptake from the surrounding phase via absorption, lipid diffusion, etc.

The potential of chemicals to bioaccumulate is generally characterized by the bioconcentration factor BCF, which serves as a measure of the chemicals' concentration in the organism concurrent with ambient concentrations under steady state conditions, e.g. for aquatic environments:

$$\mathrm{BCF} = \frac{\text{concentration of chemical at equilibrium in organism}}{\text{mean concentration of chemical in water}}$$

The standard procedure to determine the BCF in fish is OECD 305 (Bioaccumulation: Flow-through Fish Test) [2]. According to this guideline, BCF is experimentally determined using a

flow-through exposure regime with an initial uptake phase of up to 28 days followed by a depuration phase in clean water. The BCF can be estimated from the ratio C_F/C_W (C_F: concentration of the test chemical in fish at steady state; C_W: concentration of test chemical in the exposure phase (water)) or k_U/k_D (k_U: rate constant for uptake and k_D: rate constant for depuration), provided that 1st order one compartment kinetics apply.

Bioaccumulation is governed by four major processes [3,4]:

Absorption: Uptake of chemical substances from food, water, air, sediment, or soil, by transport across biological membranes into the systemic circulation, e.g. across fish gills, intestine, skin.

Distribution: Circulation of chemical substances throughout the body, binding to plasma proteins or tissue components like fat or bone. The chemical may be distributed to a tissue and elicit a toxic response; other tissues may serve as sink or as temporary depot allowing for slow release into circulation.

Metabolism: Enzymatic transformation of chemical substances: During phase I, a polar group is introduced into the molecule, which increases its water solubility and renders it a suitable substrate for phase II reactions. In phase II, the altered molecule combines with an endogenous substrate and is excreted. Metabolism is often a detoxification mechanism, but in some cases, metabolism may activate the parent compound. Intermediates or final products may cause toxicity (toxification).

Excretion: Elimination of chemical substances: Soluble molecules are removed through renal filtration and passed into urine. Fat soluble chemicals may be conjugated and excreted in bile (faeces). Chemicals with nutritional benefit may be broken down and ultimately exhaled as CO_2. Volatile substances may also be exhaled directly through the lungs.

In addition to metabolism and excretion, dilution by growth is relevant in reducing the chemical concentration in the organism, when the rates of other elimination processes are in the same order of magnitude as the growth rate. Elimination through transfer of chemicals to offspring through gestation or lactation may also be important.

4. Bioaccumulation QSARs

Quantitative structure-activity relationships (QSAR) make use of the fact that bioaccumulation of stable substances is determined by partitioning between aqueous and lipid phases. Estimating bioconcentration factors (BCF) from octanol/water partition coefficients (log K_{OW}) is well established and essentially valid for neutral organics of intermediate lipophilicity ($0 < \log K_{OW} < 6$) [5-8]. Problems occur, if the applicability domains of the QSARs are exceeded.

Chemicals with log K_{OW} > 6 often have measured BCFs lower than calculated from linear QSARs. Apparently, BCFs no longer increase in correspondence with log K_{OW}. A maximum range in log BCF of approx. 6 – 7 for compounds with log K_{OW} 6 – 8 is observed, followed by a plateau or a gradual decrease with further increase in log K_{OW} (Figure 1). The maximum BCF associated with a given lipophilicity can be described by a bilinear worst-case function [8]:

$$\log BCF = 0.99 \log K_{OW} - 1.47 \log (4.97 \times 10^{-8} K_{OW} + 1) + 0.0135 \qquad \text{eq. 4.1}$$

The bilinear curve (eq. 4.1) resumes a linearly increasing part between log K_{OW} 0 and 6, where the empirically postulated coincidence of log K_{OW} and log BCF is reflected by a near-unity slope (0.99) for the 1st-order log K_{OW} term and the intercept of about 0. Maximum log BCF values of approximately 7 are obtained for compounds with log K_{OW} between 7 and 8. Compounds that are more lipophilic are observed to be less accumulating, which corresponds to the negative slope derived for the second log K_{OW} term of the bilinear function.

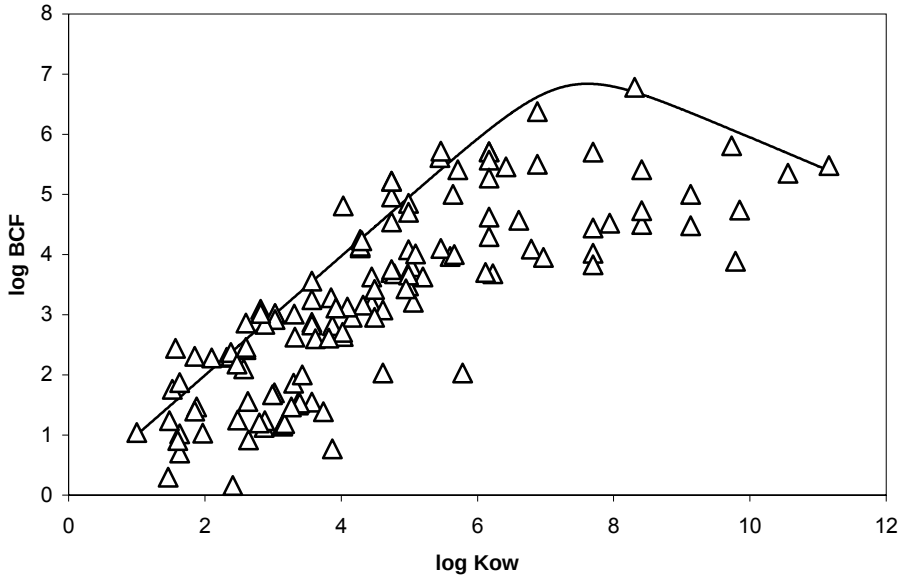


Figure 1. Correlation of log BCF and log K_{OW}. The curve illustrates a bilinear 'worst case' QSAR model (eq. 4.1). Data and QSAR model from Ref. [8].

Investigations of the water to octanol transfer for hydrophobic compounds revealed the rate constants to be essentially independent of log K_{OW} [9], indicating that diffusion in the aqueous phase is the controlling factor for these solutes. For an extended log K_{OW} range, a curvilinear relationship has to be expected between the logarithm of the water to octanol transfer rate constants and log K_{OW}, as has been also found for water to lipid transfer or uptake to aquatic organisms. These qualitative similarities in mass-transfer kinetics in abiotic and biotic partitioning systems suggest analogous control processes for lipid/water and octanol/water systems, but at different solute K_{OW} values and with different magnitudes of rate constants.

Differences in the thermodynamic properties of lipid/water and 1-octanol/water partitioning processes, e.g. enthalpy changes, have been observed for different types of lipophilic chemicals

indicating that no unique log K_{OW}/log BCF relationship can be assumed for all contaminants [10].

Comparing the phase properties of the fish lipids and octanol towards organic chemicals reveals different structures of the lipid phases. Besides storage lipids in some fish species, fish lipid primarily consists of biological membranes in which the molecules are predominantly arranged in bilayers. The lipid phase thus has a distinct structure and restricted spatial dimensions. Because the octanol phase is a bulk phase, presumably with little or no structure, organic solutes may display different activity coefficients and partitioning behaviour in octanol compared to membranes. The loss of a linear correlation between log K_{OW} and log BCF can then be at least partly ascribed to differences in solvent characteristics between natural lipids and octanol. For molecules falling below a certain volume or certain dimensions, octanol reveals a satisfactory surrogate, i.e. the activity coefficients in octanol and fish lipid are approximately equal, whereas for larger molecules this similarity breaks down, and the activity coefficients in the membrane phase are much larger than in octanol. Octanol then is no longer a satisfactory surrogate and log K_{OW} is no longer a linearly corresponding descriptor.

The relatively low solubility of voluminous molecules in membranes as compared to octanol may contribute to the loss in linear correlation between log K_{OW} and log BCF. To compensate for differences in lipid solubility, the inclusion of a term in octanol solubility in log BCF/log K_{OW} relationships has been suggested [11]. Furthermore, membrane/water partition coefficients may be used as a more reliable parameter to estimate and correlate the BCFs of organic chemicals in aquatic organisms [12].

5. Bioaccumulation Data Quality

Several factors may contribute to the substantial variability observed in measured BCF values. Depending on chemical class and testing regime, either increased or lowered values may occur. Graphical representation of relationships between log BCF and log K_{OW} data (Figure 1) illustrates considerable scatter. In the range $6 < \log K_{OW} < 10$, experimental BCF values are often lower than calculated by linear QSARs, but mostly higher than the TGD criteria for P (BCF > 2000) or vP (BCF > 5000) chemicals [6]. BCF data may range over several orders of magnitude for the same compound; e.g. for pentachlorobenzene, BCF values between 900 and 250000 have been reported [8]. Particularly with superhydrophobic chemicals, data quality is frequently questionable.

The evident variability in experimental BCF data may arise from:

- Species sensitivity: bioconcentration of xenobiotics in organisms varies with size, lipid content, age, sex and life span of species.

- Purity of test compounds: very pure substances have to be used, since even small amounts of soluble impurities can cause large errors in measured bioconcentration.
- Attainment of equilibrium: the required time may take several days to weeks.
- Analytical method: difficulties may be related to, e.g., the synthesis of radio-labelled test compounds, detection of metabolites and to reveal organ-specific accumulation.
- Stability of test compounds in water: substances must not degrade during the experiment; losses in concentration of test compounds may also occur by evaporation or adsorption to glassware.
- Surface-active materials: the presence of solubilizing agents alters the bioavailability of test compounds significantly; the apparent increase of the total (but not bioavailable) concentration in the water phase, possibly above water solubility limits for chemicals of low water solubility and high lipophilicity, may cause spuriously low BCF values, i.e. experimental artefacts.
- pH and buffer capacity of the water phase: pH conditions strongly influence the bioconcentration of organic acids and bases.
- Water chemistry: hardness, ionic strength, etc. are determinant especially for the bioconcentration of surfactants.
- Cosolute effects: presence of further organic solutes, as is the case in 'real' waters, may significantly alter the bioconcentration of individual compounds.
- Suspended organic matter: soil and sediment components, e.g. humic acids, may result in decreased bioavailability by serving as a sink compartment due to sorption processes.

Particularly for superlipophilic chemicals, considerable doubts about the validity of experimental BCF values have been addressed. Many experiments have been conducted at concentrations that were orders of magnitude above the water solubility of the test compounds with the help of solvent carriers. However, the total amount of test compound in the aqueous phase is not relevant for accumulation, because only the truly dissolved, i.e. bioavailable, fraction of the chemical can be taken up. As a consequence, BCF values must be far too low, if they are calculated with nominal concentrations of oversaturated aqueous phases. Extrapolations of accumulation data to concentrations below the water solubility revealed substantially higher BCF values than previously reported [13,14] and were later confirmed experimentally [15]. Therefore, valid BCF data for superlipophilic compounds should be determined by a kinetic method at concentrations below their water solubility. Among the possible explanations for the low observed bioaccumulation of chemicals with log K_{OW} > 6, several relate to deficiencies of the biological test data:

- exceeded water solubility of the test chemicals
- decreased bioavailability in the water phase
- non-attainment of equilibrium
- inaccuracies in the estimations/measurements of log K_{OW}
- metabolism/degradation

A variety of formal systems are available for rating the quality of experimental data in terms of accuracy and reliability [16,17], mostly based on the scoring system by Klimisch et al. [18] (Table 1). Different classification systems (Table 2) have been compared by Lepper [19].

Table 1. Scoring system to categorize the reliability of a study according to Klimisch et al. [18].

1	**Reliable without restrictions**: Studies or data...generated according to generally valid and/or internationally accepted testing guidelines (preferably performed according to GLP) or in which the test parameters documented are based on a specific (national) testing guideline...or in which all parameters described are closely related/comparable to a guideline method.
2	**Reliable with restrictions:** Studies or data...(mostly not performed according to GLP), in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable.
3	**Not reliable**: Studies or data...in which there were interferences between the measuring system and the test substance or in which organisms/test systems were used which are not relevant in relation to the exposure (e.g., unphysiologic pathways of application) or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for assessment and which is not convincing for an expert judgement.
4	**Not assignable**: Studies or data....which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature (books, reviews, etc.).

The Good Laboratory Practice (GLP) regulations ensure that test data produced in GLP compliant laboratories meet certain quality criteria. However, many data were generated before current regulatory guidelines and the GLP regulations were introduced. There are several reasons why existing study data may be of variable quality [18], e.g.:

- use of different test guidelines (compared with today's standards)
- inability to characterize the test substance properly (e.g. purity, physical characteristics, etc.)
- use of crude techniques/procedures which have since become refined
- certain information may have not been recorded (or possibly even measured), but that has since been recognized as being important.

Table 2. Comparison of different classification systems [19].

Class	TGD Reliability index (RI)	US EPA	IUCLID
I	I (highly reliable)	high confidence	valid without restrictions
II	II (reliable)	moderate confidence	valid with restrictions
III	III (not reliable)	low confidence	invalid
IV	IV (unknown reliability)	unknown confidence	not assignable

Some study results may nevertheless be valid and robust, e.g., from well-described scientific publications which have been peer-reviewed, if they satisfy a number of criteria addressing the overall scientific integrity and validity of the information in a study, i.e. reliability, with particular emphasis on:

- description of the test substance
- description of the test procedure including exposure period
- data on the test species and the number of individuals tested
- description of measured parameters, observations, endpoints.

Table 3: Checklist for BCF data reliability.

Test Substance Identification (Adequate description of test substance, including chemical purity and identification/quantification of impurities to the extent available)	✓
Full Reference/Citation	✓
Method/Guideline	✓
Controls (If a vehicle is used in the administration of the test substance, vehicle controls should be established and reported.)	✓
Species (Strain, number, gender, age, size/weight, lipid content of organisms)	✓
Temperature	✓
Type of Exposure/Test System (static, flow-through, etc.)	✓
Duration of Exposure (Including time to steady-state)	✓
Dose/Concentration Levels (In water and in organisms at steady state)	✓
Uptake- and Elimination Rates	✓
Statistics	✓
Results	✓
Weight-of-Evidence (Relative to other available BCF data, pooling of studies)	✓

Any data based on a test not providing the information according to the checklist would be considered as less reliable compared to data from a test that is fully in line with the criteria set (Table 3). Irrespective of whether or not data meet the full set of quality criteria, consideration should be given as to whether the data

- are outliers in a large data set for a particular substance,
- fit with what is known about the bioconcentration of other related substances.

A weight-of-evidence analysis can provide additional information about the reliability of reported BCF data: The pooling of several studies, one or more of which may be inadequate by itself, may satisfy collectively the overall requirement for valid data, e.g. similar accumulation (uptake/ elimination rates) at approximately the same dose and time.

6. Factors Affecting Absorption

The uptake from the point of initial exposure to the site of action or storage involves passages through a number of tissues. Every step involves the transfer of the chemical across multiple membranes (e.g. mucosa, capillary wall, cell membrane). The principal architecture of membranes according to the fluid mosaic model is universal, though differentiated by distinct lipid types and structural and functional proteins [20]. Several mechanisms operate to absorb compounds into the body [4]:

Passive diffusion (lipid diffusion): Molecules diffuse across cell membranes into cells, and they can pass between cells, along their concentration gradient.

Ion pair transport: Compensation by ion pair formation enables passive diffusion of charged molecules across membranes.

Filtration: Small molecules with molecular weight (MW) < 100 g/mol can pass through pores within membranes (diameter ~ 0.4 – 0.8 nm), but this process is considered more important for elimination than absorption.

Active transport: Molecules are transported, usually against their concentration gradient, by specific carrier proteins. This route is important for gastrointestinal absorption of essential nutrients. Efflux proteins, such a P-glycoprotein, can shunt molecules out of cells.

Endocytosis: Uptake of dissolved or particulate material into cells can occur by invagination of the plasma membrane and its internalisation in a membrane-bound vesicle. Endocytosis can be segregated into unspecific uptake of extracellular fluids including solutes via mechanisms, which are independent of ligand-binding (pinocytosis), and receptor-mediated endocytosis for selective uptake of, e.g., hormones, growth factors, enzymes, plasma proteins (Clathrin-mediated endocytosis, caveolar endocytosis).

Passive diffusion is the major mechanism of transport for xenobiotics inside organisms [7]. Diffusion is caused by a gradient in concentration of the chemicals. The driving force is the thermal movement of molecules and not the flux of the respective solvent. Fick's first law of diffusion

$$\frac{dQ}{dt} = -DA\frac{dc}{dx} \qquad \text{eq. 6.1}$$

relates the amount of chemical transported per unit time (dQ/dt) to the effective concentration gradient (dc/dx), the area of diffusion interface (A) and the compound-specific diffusion coefficient (D). The latter depends on the size of the molecules (r: radius of molecule/particle), the viscosity of the solvent (η) and the temperature (T) according to:

$$D = \frac{RT}{6\pi\eta r\mathrm{N}} \qquad \text{eq. 6.2}$$

with N denoting Avogadro's number (N = 6.02 x 10^{23} molecules/mol). Ultimately, diffusion yields a homogeneous distribution of compounds within a given compartment. Depending on the phase properties of, e.g., different tissues, different maximum concentrations may result in different compartments. Lipid diffusion involves passive diffusion as well as interphase partitioning. The transport of chemicals between two aqueous compartments, separated by a lipid membrane, depends on their partitioning between the first aqueous compartment and the membrane, their diffusion across the membrane and the consecutive constitution of another

partitioning equilibrium between the membrane and the second aqueous compartment. The respective concentrations are established according to Nernst distributions:

$c_{M1} = P_1\, c_{W1}$ and $c_{M2} = P_2\, c_{W2}$ eq. 6.3

with c_{M1} and c_{M2} denoting the equilibrium concentrations at the membrane surface in contact with the aqueous compartment 1 and 2, c_{W1} and c_{W2} the concentrations in the water phases, and P_1 and P_2 the membrane/water partition coefficients for the partitioning between the membrane and the aqueous compartments 1 resp. 2. The effective concentration gradient ($-dc_M/dx$) across the membrane of width x

$$-\frac{dc_M}{dx} = \frac{(P_1 c_{W1}) - (P_2 c_{W2})}{x} \qquad \text{eq. 6.4}$$

results in an efflux of the compound from the first aqueous compartment

$$-\frac{dQ_1}{dt} = -DF\,\frac{(P_1 c_{W1}) - (P_2 c_{W2})}{x} \qquad \text{eq. 6.5}$$

The pK_a value of the chemical and the pH conditions on both sides of the membrane play an essential role. Differences in pH on both sides of the membrane frequently cause asymmetric distributions of chemicals within an organism and the steady state concentrations, i.e. accumulation, may not be equal in all compartments.

Equations 6.1 – 6.5 rationalize that absorption of diffusible compounds relates to their molecular size and the respective lipid/water partition coefficients. The chemical factors that influence interphase partitioning are solute charge, dipolarity/polarizability, hydrogen bonding capacity and molecular size [20,21].

The role of molecular weight and K_{OW} on absorption of non-ionic organic chemicals changes as chemicals become more hydrophobic (i.e., K_{OW} increases). Membrane permeation rates become increasingly controlled and ultimately dominated by aqueous boundary layer transport rather than phospholipid bilayer permeation. Uptake rate constants and efficiencies increase with increasing K_{OW} for low K_{OW} chemicals (i.e., lipid layer diffusion or convection control) and then become constant once log K_{OW} reaches approximately 3 to 4, as chemical diffusion through aqueous layers dominates the kinetics [22,23]. For high-K_{OW} chemicals, transport in water layers (not lipid layers) controls membrane permeation and uptake kinetics [24].

6.1 Membrane Permeation

Factors that may affect passive transport of substances across cell membranes concern properties of the diffusible substances as well as the cell membrane [25]:

A. Properties of the diffusible substance:

- Concentration gradients of the diffusible substance across the membrane
- Permeability coefficient (or diffusion coefficient)
- Relative lipid/water solubility (partition coefficient)
- Effective diameter
- Electric charge

B. Properties of the cell membrane:

- Total surface area available for diffusion
- Thickness and structure of the membrane
- Electric charge on membrane pores
- Presence of carrier molecules.

The relevance of molecular size and partition coefficients to membrane permeation is generally stated throughout the literature, e.g. [4,6,7,20,25-34]. A variety of membrane models has been established in medicinal and pharmaceutical chemistry. Most complex are membrane isolates from cultured cells featuring different mechanisms of passive and active transport. Metabolism can further complicate the assay outcome. Artificial membranes are phospholipid bilayers that form vesicles (liposomes) or that are immobilized by support on lipophilic filters. Most membrane models mimic the situation in the gastrointestinal tract to screen absorption of pharmaceuticals and drug candidates. Because of differences in composition and architecture of membranes, different permeabilities result between organs. Comber et al. [35] compare tissue capabilities to allow chemicals to passively diffuse through them based on transepithelial electrical resistance (TEER). They indicate similar uptake rates in fish and mammalian intestines. Fish gills, the major route of absorption of waterborne contaminants, are more restrictive and may be similar to the mammalian blood brain barrier.

Membrane isolates from cultured cells: The Caco-2 assay, based on colon carcinoma cell lines, has been widely used for the simulation and prediction of intestinal drug absorption after oral administration. These membranes have useful properties for correlation with *in vivo* data such as enzymatic and transporter systems [20]. Caco-2 monolayers have been used in the prediction of intestinal absorption *in vivo* [36], and to identify pharmaceuticals with potential absorption problems [37]. Passive diffusion of small molecules across Caco-2 cell monolayers via the paracellular pathway is correlated with size [38], but to different extents depending on charge (Figure 2).

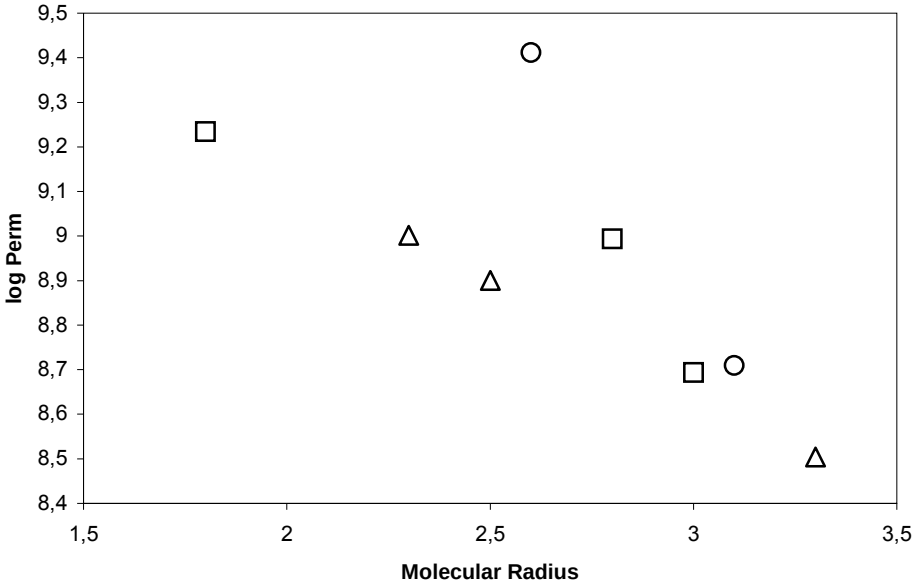


Figure 2. Correlation of transport properties (Perm: apparent permeability coefficient [cm/s]) of metabolically stable peptides and their size (Molecular Radius [Å]), differentiated by net charge: negative (triangles), positive (squares), neutral (circles). Data from Ref. [38].

The data shown in Figure 2 tempt to extrapolate a molecular radius associated with 'zero permeation' in this assay, however, the calculated value of ~1.3 nm is a statistical ghost and not substantiated by experiment. Particularly, the data set is very limited featuring only eight charged peptides without environmental relevance. The small size-related cut-off is easily vanished by uptake rate constants of various large-size antibiotics (Figure 3). Oral bioavailability is excellent for, e.g., Rifampicin (MW 822.96 g/mol) and Rifapentine (MW 877.03 g/mol) [20]. Erythromycin (MW 733.94 g/mol) is also absorbed in the intestines and diffuses across the bacterial plasma membrane to target intracellular receptors. Cyclosporine (MW 1202.64 g/mol), a hydrophobic immunosuppressive agent, is efficiently absorbed orally and plasma concentrations peak within 3-4 hours [24].

Caco-2 monolayers were used to study the dietary uptake of PCBs with very low water solubilities [39]. While PCBs with < 3 chlorine substituents were transported by aqueous/lipid diffusion, more lipophilic PCBs with > 3 chlorine substituents were preferentially transported by triglyceride particles and lipoproteins. Their high affinity to mixed bile salt micelles and membrane lipid vesicles may contribute to overcome resistance of the unstirred water layer adjacent to brush-border membranes of enterocytes. Highly lipophilic compounds may move into the hydrophobic core of bile salt micelles and, protected by the hydrophilic outer layer of the micelles, be assimilated together with dietary lipids. This transport route may significantly contribute to accumulation from food, but not to uptake from the water column.

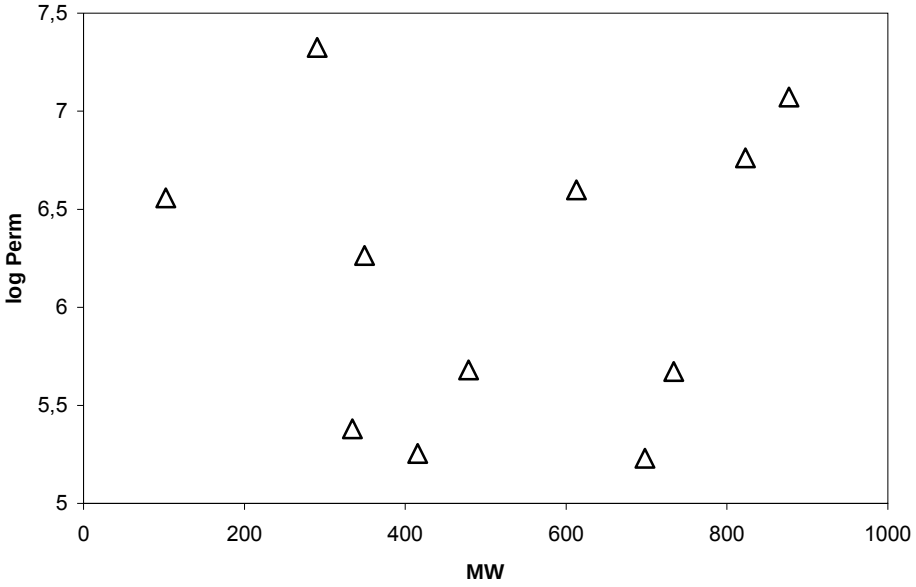


Figure 3. Relationship between absorption in Caco-2 cells (Perm: apparent permeability coefficient [cm/s]) of diverse antibiotics and their molecular weight ([g/mol]. Data from Table 4.3 of Ref. [20].

Alternative membrane models often used are human blood cells, as 'ghost' erythrocytes with cytoplasm contents removed. Closer to aquatic bioaccumulation are cell lines prepared from fish gills for studies of the bioavailability of chemicals [40,41]. The complex three-dimensional morphology of the gill, together with its poor viability under *in vitro* conditions of perfusion and incubation has limited the generation of substantial data bases. Cultured gill epithelium on permeable support may serve as a model for the freshwater fish gill, but its potential has not yet been realized [42].

Liposomes: Vesicles with walls made of phospholipid bilayers contain the main ingredients found in all membranes. Liposomes have been widely used as a more 'biological' alternative partition model compared to 1-octanol bulk phases, e.g. [20,27,43-45]. Liposomes allow for different types of interaction with structured phospholipids related to size/bulk, charge state and hydrogen bonding capacity. The lipid chain ordering affects the selectivity of bilayer membranes for permeant size and shape [46].

Comparison of 'gold standard' experimental values of octanol-water and liposome-water partitioning [27] reveals liposome-water partitioning invariant for very hydrophilic substances, and a significant correlation ($r^2 = 0{,}85$) for substances with log K_{OW} in the range 2 – 5.4 (Figure 4). Due to their nature, liposomes are very well suited to study drug-membrane interactions and absorption kinetics, but they do not provide a means to derive size-related cut-offs for membrane permeability.

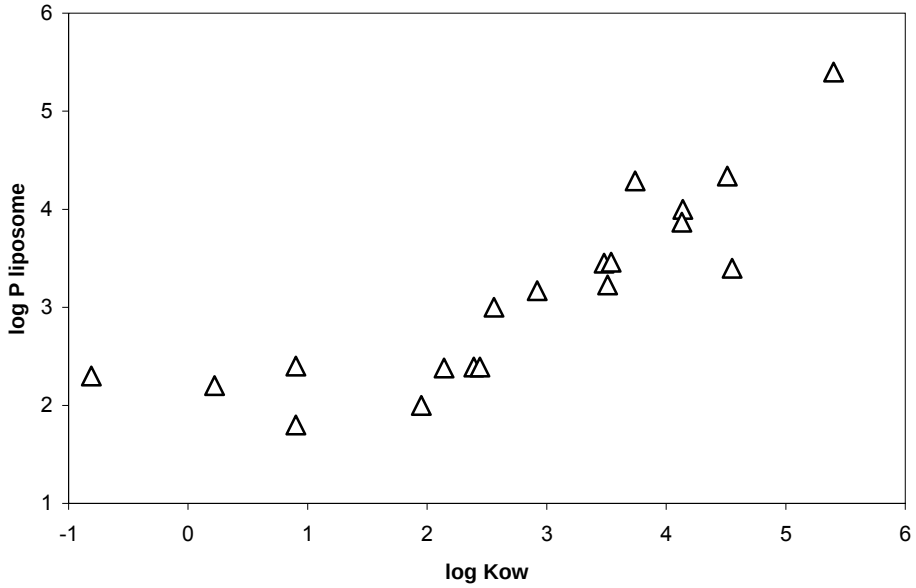


Figure 4. Correlation between critically selected experimental liposome-water partition coefficients and octanol-water partition coefficients. Data from Tables 4.1 and 5.3 of Ref. [27].

Immobilized artificial membranes: Permeability assessments using immobilized artificial membranes (IAM) have been established for profiling drug candidates for oral absorption characteristics and pharmacokinetic properties. Most IAM consist of 2-20 % phospholipids in a dodecane matrix. Because the structure of the dodecane-phospholipid mixture on the support material is not known, they cannot be used to derive size-related cut-offs for membrane permeability.

The usefulness of parallel artificial membrane permeability assays (PAMPA) in pharmaceutical sciences has been recently reviewed [27]. Models of gastrointestinal absorption differ with respect to levels of lecithin membrane components, use of negatively charged phospholipids, pH gradients and artificial sink conditions. Blood brain barrier models are based on salient differences between the properties of the gastrointestinal tract and the blood brain barrier. The structure of filter-immobilized artificial membranes is not known with certainty, assuming a single bilayer per pore. PAMPA allows rapid screening of large numbers of chemicals for drug-membrane interactions and absorption kinetics, but excluding hydrophobic substances with log K_{OW} > 4 under standard conditions. Sparingly soluble drugs can be handled using excipients [47]. A first application of PAMPA to evaluate passive absorption and elimination in small fish [48] is promising, but limited to simple aromatic chemicals and requires further refinement. A current project under way at EAWAG aims to develop an *in vitro*-system for modelling bioaccumulation of neutral, ionisable and metabolically active organic pollutants using PAMPA [49].

In 1997, Lipinski et al. [50] published their ‘rule of 5’ method relating absorption properties of 2245 compounds to molecular weight, hydrogen bonding capacity and lipophilicity. The criteria are computational alerts in early drug discovery and high throughput screening to avoid

development of lead structures with unfavourable pharmacokinetic properties. Drugs are more likely to show poor oral absorption if:

1. Molecular weight > 500 g/mol
2. Σ hydrogen bond donors (expressed as Σ OH + NH) > 5
3. Σ hydrogen bond acceptors (expressed as Σ N + O) > 10
4. log K_{OW} > 5.

The parameter cut-offs do not apply to antibiotics, antifungals, vitamins and cardiac glycosides that have high molecular weights (> 500 g/mol) and excellent oral uptake.

Although focussed on absorption of oral drugs across the intestinal wall, it has been suggested to apply the 'rule of 5' concept to estimate absorption in fish based on similarity in tissue structures among vertebrates [51]. While principal processes-based considerations support this knowledge transfer, comparison of chemical domains is discouraging. Many pharmaceuticals are hydrophilic (log K_{OW} < 0) with a trend for higher MW to correlate with lower K_{OW} [50]. Environmental contaminants are more often lipophilic (log K_{OW} > 3) with higher K_{OW} related to increasing MW. Because of key differences in oral absorption of pharmaceutical drugs and uptake of waterborne environmental contaminants in aquatic organisms, critique has been raised. Pharmaceuticals are often used in solid form (e.g., a pill) and must dissolve to become available within a short time (i.e., the gut transit time). In contrast, environmental contaminants are already dissolved in or sorbed to environmental media, such as water, air or particulate matter, and can provide a source of exposure throughout the animals' lifetime. It is, therefore, important to distinguish between processes controlling dissolution (solubility) and membrane permeation when extrapolating the behaviour of pharmaceuticals to environmental contaminants [24].

Summary: With regard to the study objective **'Do studies on biological membranes support limits of permeability related to size or lipid solubility of the chemicals?'** the literature review revealed:

The available information indicates that large high molecular weight-substances (MW > 1000 g/mol) are able to permeate through membranes and can be efficiently absorbed. No robust evidence is provided to substantiate molecular weight or size cut-offs for membrane permeation that can be applied to assess bioaccumulation. Chemicals of moderate lipophilicity and sufficient solubility in water and membranes diffuse rapidly through aqueous and lipid phases. Large superlipophilic compounds also permeate through membranes, but at slower rates, due to their low solubility in water layers. The slow rate of elimination gives high log K_{OW} chemicals their inherent bioaccumulative potential [52]. Substances that would make bad oral drugs because of minor solubility and slow absorption kinetics, can be very bioaccumulative environmental contaminants.

6.2 Uptake in Organisms – Bioaccumulation Studies

Many papers have been published concerning reduced uptake based on experimental bioconcentration studies. They usually attribute lower BCF-values to reduced uptake via fish gills. Still, under realistic (e.g. field) conditions, substantial accumulation may occur due to uptake with food. The contributions of the different routes of uptake can hardly be discriminated for fish and aquatic invertebrates. Some highly persistent and hydrophobic chemicals are significantly accumulated from food by predatory fish at higher levels in the food chain, but also by gill breathing organisms.

One of the first notions of size-limited uptake in aquatic bioconcentration studies was brought about by Opperhuizen et al. in 1985 [30]. They found that bioconcentration of polychlorinated naphthalenes increased with lipophilicity up to log K_{OW} ~ 6. No further increase was observed at higher log K_{OW} values. BCF values of 0 were reported for hepta- and octachloronaphthalenes, no detectable concentrations were found in fish. The absence of bioconcentration was explained by steric hindrance of absorption due to additional chlorine atoms. Hepta- and octachloronaphthalenes were postulated to be unable to permeate gill membranes, related to the molecular size of these compounds. Based on analyses together with halogenated benzenes, biphenyls and dioxins, a cross-sectional diameter cut-off of 0.95 nm (9.5 Å) was proposed for membrane permeation of molecules.

In 1987, Opperhuizen et al. reported on bioaccumulation of linear and cyclic polydimethylsiloxanes (PDMS) after dietary and aqueous exposure [53]. BCF < 10 was found for oligomers > 12 siloxane units. Molecular weight alone did not explain their reduced absorption, one of the substances that was found in fish has a MW of 1050 g/mol. The cross-sectional diameter cut-off of 0.95 nm did not apply, since the cross-sections of linear PDMS can be considered to be almost identical for all oligomers. In fact, they are smaller than or equal to those of PCBs that bioaccumulate strongly. Instead, a chain length of ~ 4.3 nm was suggested to explain decreased membrane permeation. Lack of accumulation was observed for hydrophobic chemicals with lengths > 5.3 nm [54]. For comparison, reference was made to a study by Hardy et al. [55], where uptake of long chain alkanes in codling was disturbed for alkanes longer than $C_{27}H_{56}$. Limited accumulation was thus observed for alkanes and silicones of similar length ~ 4.3 nm. The suggested mechanistic explanation that long chain molecules may stretch across membranes and simultaneously disturb the polar head groups at both sides of the biomembrane (i.e. the contaminants are located in the membranes), appears to conflict with a lack of analytical detection of the compounds in the fish. Further doubts about maximum diameter cut-offs are founded on studies by Toll et al. [56], who observed uptake in fish of some nonionic surfactants with similar chain length.

Based on studies of limits of bioconcentration in fish, Anliker et al. [57,58] suggested bioaccumulation cut-offs for organic colorants (ionic and non-ionic dyestuffs and pigments) related to solubility in water (S_W < 0.1 mg/l or > 2000 mg/l) and octanol (S_O < 10 mg/l), log K_{OW} (< 3) and molecular size (MW > 450 g/mol and cross section (second largest van der Waals diameter of the molecules, measured on conformations optimised by force field calculations) > 1.05 nm).

They explained low BCF-values of high log K_{OW}-compounds with limited absorption and fat (lipid) storage potential of pigments, indicated by low solubility in n-octanol and large molecular size. The study included 23 disperse dyestuffs, two organic pigments, a fluorescent whitening agent and, for comparison, 16 halogenated aromatic hydrocarbons. Because bioaccumulation experiments were conducted at exposure concentrations in excess of the aqueous solubility of the organic colorants, the BCF-values in these papers have to be regarded with caution and, hence, the derived cut-off criteria.

The influence of lipid solubility on bioaccumulation was investigated by Banerjee and Baughman, 1991 [11]. They attributed low bioconcentration of medium and high molecular weight solutes to the relatively low solubility of these compounds in lipid. They included a term in octanol/lipid solubility into the log K_{OW}/BCF relationship to significantly improve the quality of fit for highly hydrophobic chemicals. The data set of Banerjee and Baughman [11] indicates no substantial correlation between log K_{OW} and log S_O (Figure 5). Chessells et al. [59] demonstrated a decrease in lipid solubility with increasing log K_{OW} for superhydrophobic compounds (log K_{OW} > 6) and suggested that the influence of reduced lipid solubility caused lower bioconcentration of hydrophobic compounds.

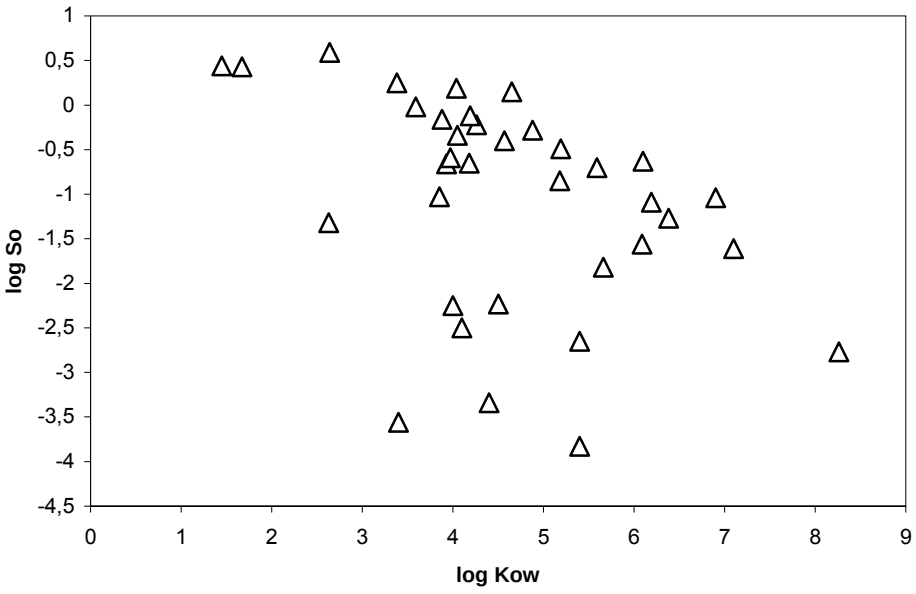


Figure 5. Correlation between octanol-water partition coefficients (log K_{OW}) and solubility in octanol (log S_O). Data from Table 1 of Ref. [11].

Bioconcentration of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in guppies after aqueous exposure to a complex PCDD/PCDF mixture was found different for laterally substituted (2,3,7,8 substituted) congeners that were bioconcentrated, while the non-laterally substituted congeners were not (Loonen et al., 1994 [60]). The main reason for this was attributed to metabolism, however, lower lipid solubility and lower membrane permeability were also considered to have played a role in the reduced BCFs observed. The non-accumulating structures do have effective cross-sectional diameters > 0.95 nm.

Arnot and Gobas, 2003 [52], identified sorption in the water phase as the main reason why accumulation decreases with increasing log K_{OW} for highly lipophilic chemicals. The decline was not due to a lack of biomagnification or steric factors affecting membrane permeation. If accumulation was quantified as the ratio of the concentration in the organism divided by the freely dissolved chemical concentration in water, the BCF of very high K_{OW} chemicals would exhibit values ~10^7 and would not vary with increasing K_{OW}. Transport in water (not lipid) layers controls the uptake kinetics from water and food for high K_{OW} chemicals [24], and dietary uptake efficiency falls with increasing K_{OW} for chemicals with log K_{OW} > 7 as well [61].

Overall, results of various bioconcentration studies appear inconclusive about limited uptake of chemicals with large cross sections (e.g. some relevant dioxin and PBDE congeners) by fish and other species. The cut-off value of 0.95 nm is not generally confirmed. In fact, it has been demonstrated by Dimitrov et al. [62] that many chemicals with effective diameters > 0.95 nm bioaccumulate to substantial extents. Most likely, a simple parameter may not be sufficient to explain, when reduced BCF/BAF occurs.

Dimitrov et al. [62-64] have used molecular weight, size, and flexibility to predict BCF. The approach aimed at discriminating chemicals with log BCF > 3.3 (i.e. B) from those with log BCF < 3.3 (i.e. not B) and chemicals with log BCF > 3.7 (i.e. vB) from those with log BCF < 3.7 (i.e. not vB), respectively. Initially [62], log K_{OW} > 5.5 and maximum diameter D_{max} > 1.5 nm (averaged over flexible conformers) identified compounds with log BCF < 3.3. The superiority of maximum diameter over effective cross-sections of molecules was attributed to multiple orientations of the molecules at membranes. The hypothesis that the effective diameter controls permeability of chemicals assumes a strict spatial orientation of the molecules towards the cell membrane surface in a way that the molecular projection over the membrane does not exceed a certain threshold (e.g. 0.95 nm). The appropriate orientation, however, is prevented by entropy, i.e. by chaotic movement of the molecules. The higher the molecular length (i.e. D_{max}), the smaller are the chances of the molecule reaching the cell membrane in an appropriate angle. Furthermore, Dimitrov et al. [62] speculated that the threshold of D_{max} ~ 1.5 nm may be due to a change in the mechanism of uptake from passive diffusion through the phospholipid bilayer of membranes to facilitated passage by exocytosis and endocytosis for larger molecules. The critical value of ~ 1.5 nm was stated to be comparable with the architecture of cell membranes, i.e. half the thickness of lipid bilayers, indicating the maximum tolerance of cell membranes. A follow-up study by Dimitrov et al. [63] with a wider range of chemicals confirmed D_{max} ~ 1.5 nm in combination with log K_{OW} > 5 and rejected a threshold in MW 700 – 1000 g/mol. More recently, D_{max} was revised to 1.7 ± 0.2 nm [64]. It has to be noted that the effect of molecular size has to be accounted for by a smooth function, not a cut-off threshold, within their base-line model for identifying the bioaccumulation potential of chemicals together with other mitigating factors, such as ionisation and metabolism.

The advisory committee on hazardous substances proposed indicators of limited bioaccumulation potential for organic chemicals with regard to S_{oct} (< 0.002 * MW mg/l), MW (> 700-1100 g/mol) and molecular size (chain length > 4.3 nm, D_{max} > 1.74 nm) [35]. It was concluded that there would appear to be no clear cut-offs, recognizing the uncertainties in the interpretation of

experimental results. Considering that BCF-testing is particularly difficult for larger lipohilic molecules, the derived criteria should be re-evaluated and adjusted as necessary, once the CEFIC LRI 'gold standard' database on BCF is available.

Table 4. Criteria suggested as indicators of limited bioaccumulation potential for organic chemicals.

Year	Chemical class	log K_{OW}	Water solubility	Lipid solubility	Molecular weight	Molecular size	Reference
1985	polychlorinated naphthalenes	> 6	--	--	--	D_{eff} > 0.95 nm	Opperhuizen et al. [30]
1987	silicones	--	--	--	--	chain length > 5.3 nm	Opperhuizen et al. [53]
1988	organic colorants (ionic and non-ionic dyestuffs and pigments)	--	< 0.1 mg/l or > 2000 mg/l	< 10 mg/l	> 450 g/mol	D_{eff} > 1.05 nm	Anliker et al. [57,58]
1991	--	--	--	--	> 600 g/mol	--	UBA [65]
2000	--	--	--	< 2 mmol/kg	>700 g/mol	D_{eff} > 0.95 nm chain length > 4.3 nm	Environment Canada [66]
2002	diverse organics	> 5.5	--	--	--	D_{max} > 1.5 nm	Dimitrov et al. [62]
2003	diverse organics	> 5	--	--	--	D_{max} > 1.47 nm	Dimitrov et al. [63]
2003	--	--	--	--	> 700 g/mol	--	TGD [6]
2005	diverse organics	--	--	--	--	D_{max} > 1.7 ± 0.2 nm	Dimitrov et al. [64]
2006	--	--	--	< 0.002 * MW mg/l	> 700-1100 g/mol	chain length > 4.3 nm D_{max} > 1.74 nm	TC-NES sub-group on PBT [35]

Summary: With regard to study objective **'Do studies on bioconcentration support limits of uptake and accumulation potential related to size or lipid solubility of the chemicals?'** the literature review revealed:

The available information indicates that clear cut-offs in bioconcentration related to size or lipid solubility of chemicals do not exist. Apparently reduced bioconcentration frequently concerns hydrophobic chemicals that have very low aqueous solubilities. These properties make them difficult to test, and many apparently reduced BCF data may be attributed to shortcomings in the interpretation of experimental results. Major influential factors concern bioavailability (i.e. freely dissolved exposure concentrations, sorption, solubility limits in the aqueous phase and effects of solubilizers) and kinetics (i.e. attainment of steady state, lipid vs. aqueous layer diffusion control, effects of organism size and life span). Many studies that have investigated relationships between molecular dimensions and reduced uptake, i.e. based on 'lower' BCFs than expected, may actually describe experimental shortcomings or artefacts. In this context, it is interesting to note that any thresholds have been steadily increasing with time (Table 4). The increasing cut-offs with time may be explained by refined testing procedures and improved experimental design to work out the limits of model domains, i.e. generation and analysis of better-quality data for larger hydrophobic compounds.

6.3 Active Transport Mechanisms

Active transport mechanisms for transmembrane passages are, except for physiological substrates, very rare and specific for these substances [4,25]. Literature searches revealed few evidence for active uptake of environmental pollutants, e.g. (organo)metals. Unless chemicals are used as or resemble very closely physiological substrates, active uptake into organisms appears negligible.

Summary: With regard to study objective **'How relevant are active transport mechanisms for the uptake of large organic chemicals?'** the literature review revealed:

The available information indicates only minor relevance of active transport mechanisms for the uptake of large organic chemicals with regard to environmental bioaccumulation.

7. Criteria Related to Limited Bioaccumulation

The size-related criteria suggested as indicators of limited bioaccumulation potential for organic chemicals were tested on data provided by UBA.

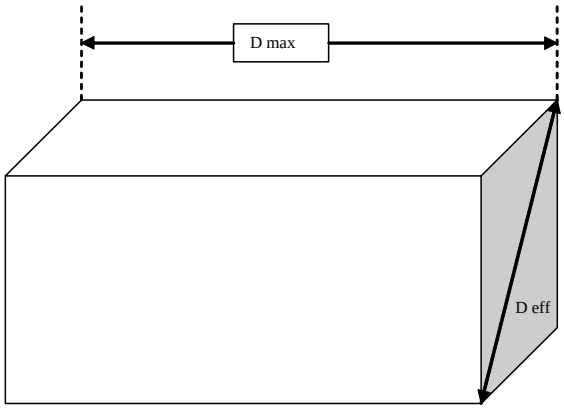
7.1 Molecular Size

Molecular size, expressed as molecular diameter, can be calculated from three-dimensional molecular structures. In a first step, the two-dimensional chemicals' structures (drawn by commercial software, e.g., ISIS/Draw) have to be converted into three-dimensional structures, which have to be optimized subsequently.

Chem3D from the Chemoffice software packet (www.cambridgesoft.com) was used for building three-dimensional structures. Optimization of the structures was performed with this software as well. However, due to the large size of molecules investigated in this study, some problems occurred during the optimization step. Therefore, a further optimization was performed using the PM3 method implemented in the MOPAC-programme (version 7.0, http://openmopac.net/).

The diameter of a molecule is not clearly defined. Starting from a mathematically optimized three-dimensional structure, the maximum diameter of a molecule can be principally calculated as the largest distance between two atoms based on their van der Waals radii. However, the structures are (a) optimized for the free molecule (ignoring any solvent effects) and (b) even for a 'free molecule' several optimized structures may be found (different conformations). This is especially important with flexible molecules, e.g. with long alkyl chains. For different conformations of the same substance, the maximum diameter may vary considerable. An

example is given below. The ‘effective maximum diameter’ is often used instead of the maximum diameter. Additionally to the effective maximum diameter the ‘effective diameter’ and the minimum diameter can be calculated. The ‘effective maximum diameter” (D_{max}) is defined such that the respective perpendicular diameter is minimal, the latter being the effective second largest diameter or simply the effective diameter D_{eff}; the effective third largest diameter D_{min} is the diameter perpendicular to both D_{max} and D_{eff}. The following figure illustrates the diameters of the "bounding box".



The molecular diameters were calculated using different software programmes:

- Programme CROSS [67] developed at Fraunhofer IME calculates the maximum, the effective and the minimum diameter as defined above.

- The algorithm developed by Cash and Nabholz [68] calculates the minimum cross-sectional diameter, which is comparable to the effective diameter from CROSS.

- The programme Mol2Mol (http://web.interware.hu/frenzy/mol2mol/index.html) calculates a ‘bounding box’, a hypothetical square box perfectly fitting the molecule. The largest, the medium and the smallest length of this square box are comparable to the maximum, effective and minimum diameter from CROSS.

Molecular diameters were calculated by the three methods for 60 compounds. Table 5 shows the correlation between the methods.

Table 5. Correlation between molecular diameters from different methods.

		R^2
D_{max} (Cross)	D_{max} (BB)	0.96
D_{eff} (Cross)	D_{eff} (Cash / Nabholz)	0.77
D_{eff} (Cross)	D_{eff} (BB)	0.91
D_{eff} (Cash / Nabholz)	D_{eff} (BB)	0.70
D_{min} (Cross)	D_{min} (BB)	0.96

While correlation between maximum diameters as well as minimum diameters (calculated by CROSS and Mol2Mol) is rather strong, the correlation between the different effective diameters is more weak, especially the correlation between the 'Cash/Nabholz' diameter and the two other methods. As a conclusion from these findings, only one maximum diameter (from CROSS) was used for subsequent analysis, but both the CROSS and the Cash/Nabholz effective diameters were evaluated.

Two examples are given for compounds with large deviations between maximum diameters from different conformations (Table 6).

Table 6. Deviations between diameters (in nm) calculated for different conformations.

Compound	Conformation	D_{max}	D_{eff}	D_{min}	heat of formation
Tridemorph	stretched alkyl chain	2.36	0.897	0.55	-120
Tridemorph	curved alkyl chain	1.69	0.933	0.85	-96
neu10	stretched alkyl chain	3.29	1.07	0.87	-261
neu10	curved alkyl chain	1.53	1.19	1.13	-264

Especially the parameter D_{max} depends strongly on the conformation, while D_{eff} is less sensitive for changes of conformation. The parameter 'heat of formation' is an indicator for the stability of a conformation: lower heat of formation stands for more stable structures. While both conformations are similarly stable for compound 'neu10', the 'curved alkyl chain'-conformation of Tridemorph is much less stable than the 'stretched alkyl chain'-conformation. However, optimization process stopped successfully for the two conformations, since local minima were found. Therefore, it is important to check the optimized structures with a molecular viewing programme.

7.2 BCF Data

BCF data were provided by the Umweltbundesamt for 31 plant protection agents and for 18 new chemicals. These chemicals were selected by two criteria: (i) molecular weight > 300 and (ii) bioaccumulation data available. Since this data set had no compounds with log BCF > 4 (range in BCF: 1.5 to 14600), additional data for compounds with very high BCF were taken from the literature. Some literature data for compounds with low BCF were also taken into account for

comparison purposes. For this additional data set, BCF data are regarded valid and range from 1.5 to 6,000,000. Data for log K_{OW} and solubility in water were provided by the Umweltbundesamt; calculated log K_{OW} (KowWin) were used, when no experimental were available.

Most BCF data from Umweltbundesamt were qualified as ‘valid data'. However, for two compounds Umweltbundesamt classified the measurements as ‘invalid’. Critical inspection of the available BCF data revealed major deficiencies in data quality of several superlipophilic compounds, as discussed in chapter 5 on bioaccumulation data quality. For nine compounds, the accumulation experiments have been conducted at concentrations above the water solubility of the test compounds. Consequently, the resulting BCF values are too low artefacts due to invalid experiments. These data, which are shown in figure 8 for demonstration purposes, were excluded from further analyses.

A compilation of the experimental data is given in the appendix. The data set from the Umweltbundesamt includes no compounds with log BCF > 4 and cannot be considered as sufficiently representative. Once the CEFIC LRI ‘gold standard’ database on BCF is available, the findings of this study should be re-examined and adjusted as necessary.

The lipophilicity range of the test chemicals covers more than ten orders of magnitude. A comparison of experimental and calculated log K_{OW} data (figure 6) shows that experimental data are often lower than the corresponding calculated values. For the very lipophilic compounds, this may be a result of experimental problems.

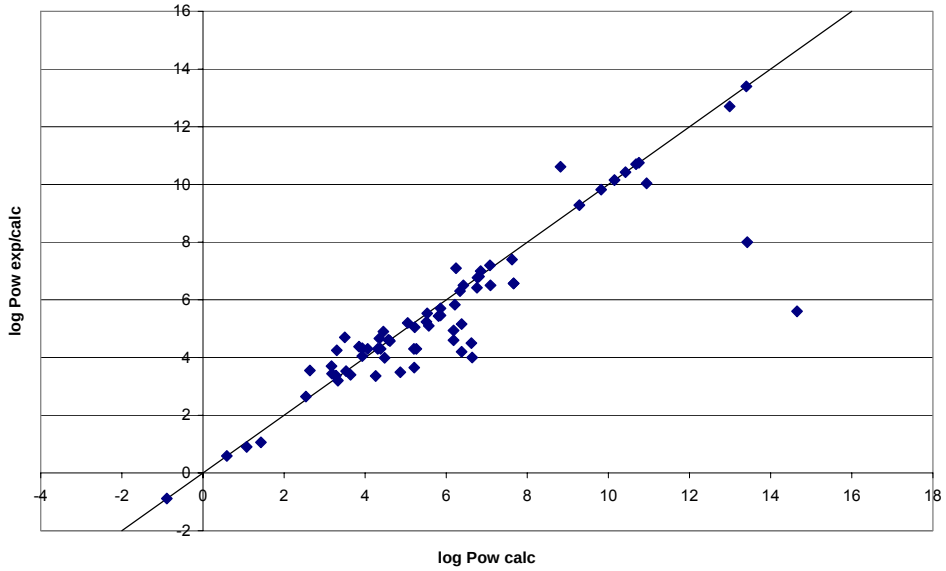


Figure 6. Plot of experimental versus calculated log K_{OW} data. Data points exactly on the diagonal are calculated values.

The relationships between bioconcentration and lipophilicity of the test chemicals were first explored graphically. No linear correlation between log BCF and log K_{OW} (R^2 = 0.0002) was found for the entire data set, as was to be expected due to inclusion of highly lipophilic compounds. However, a trend (R^2 = 0.37) can be seen for compounds with log K_{OW} < 6 (figure

7). The weak correlation is strongly influenced by the 4 values with log K_{OW} < 2. Reduction of the data set to compounds with log K_{OW} > 2 and < 6 yields R^2 = 0.24.

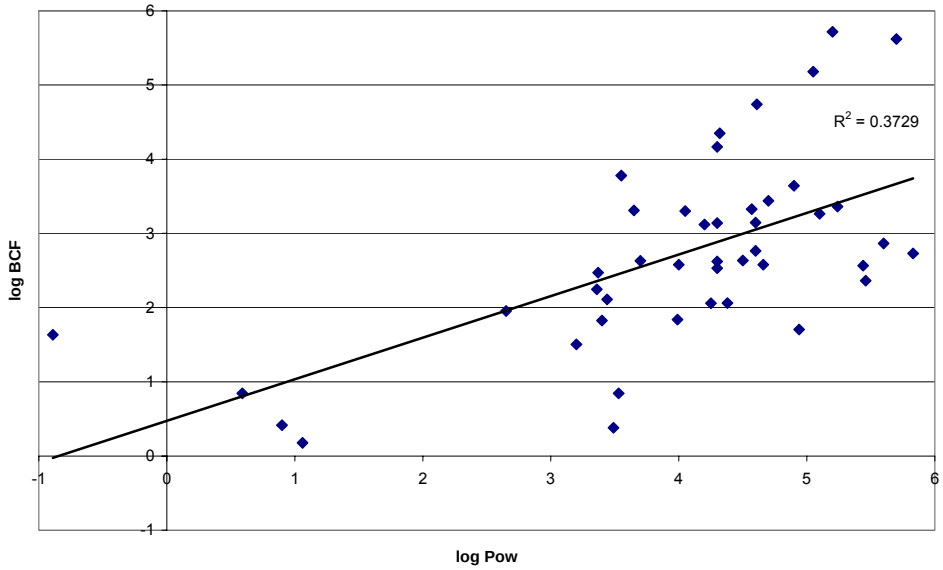


Figure 7. Correlation between log K_{OW} and log BCF for compounds with log K_{OW} < 6.

On the other hand, the 'worst case" QSAR-model (see chapter 4) leads to a nearly perfect fit in the log K_{OW} range 0 to 7 and is a clear upper limit for estimated log BCF over the entire lipophilicity range (Figure 8).

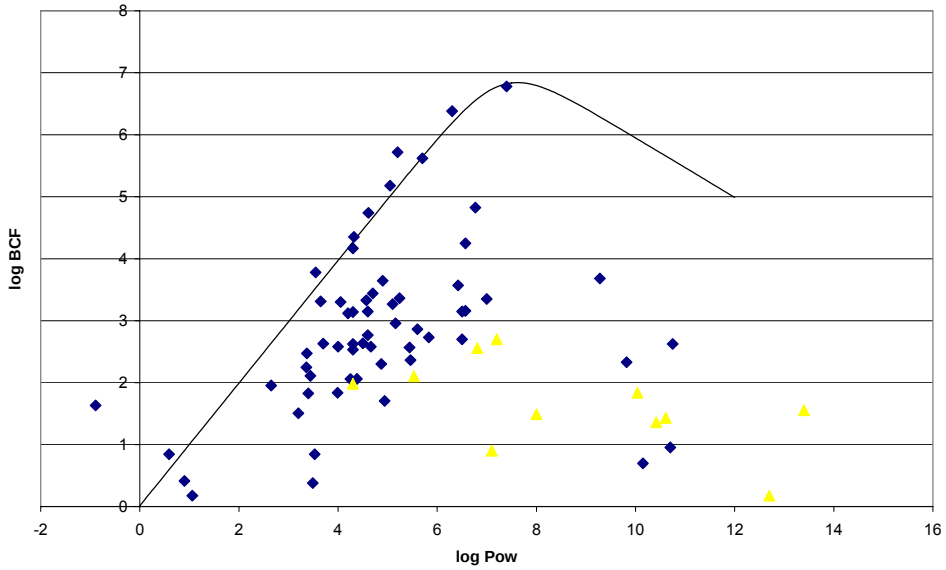


Figure 8. Plot of log BCF and log K_{OW} relative to the bilinear 'worst case' QSAR model [8], compounds with presumably invalid BCF data are marked (yellow triangles).

7.3 Application of Criteria Related to Limited Accumulation

Although the information from the literature study (chapter 2 to 6) revealed no clear cut-off triggers, some of the candidate criteria (molecular diameters, molecular weight, solubility in water) were applied to the available data set.

7.3.1 Effective and Maximum Molecular Diameter

Maximum and effective molecular diameters (calculated by CROSS and the algorithm from Cash and Nabholz [68]) were tested for their ability to identify compounds with limited accumulation. Figures 9 and 10 show the effect of the effective diameter of the test chemicals on their bioconcentration.

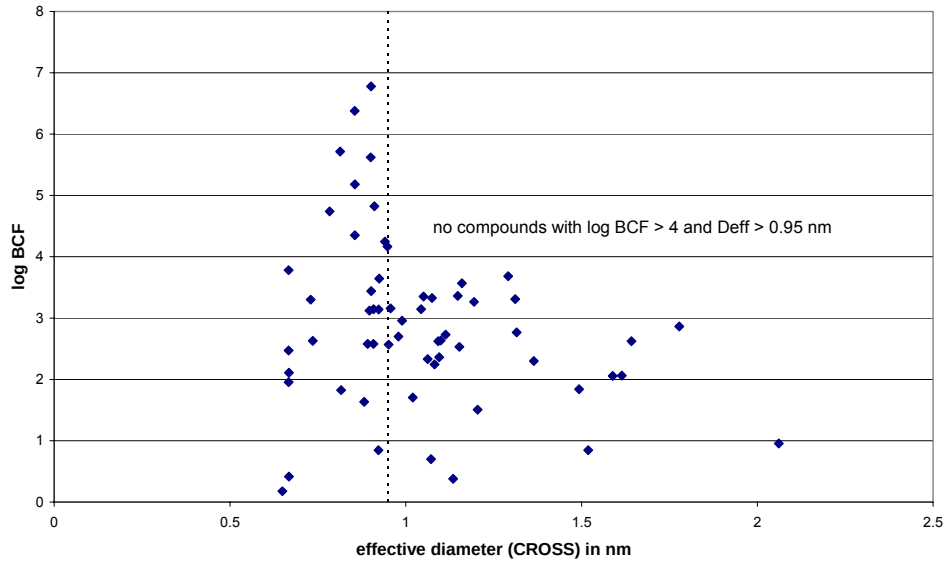


Figure 9. Plot of log BCF versus effective diameter calculated by CROSS (in nm).

A clear separation between compounds having log BCF > 4 and compounds with log BCF < 4 can be seen at an effective diameter of approx. 0.95 nm. This numerical cut-off corresponds to findings by Opperhuizen et al. [30], but no robust conclusions can be drawn. The data set is (a) too small for sound statistics and (b) not representative due to the lack of highly accumulating compounds from Umweltbundesamt. Compounds with large and small effective diameters can be found, which have rather low BCF (BCF < 100), however, low BCF values can arise either from large diameters or from other influences, such as lipophilicity. The influence of both log K_{OW} and D_{eff} can be seen in figure 10.

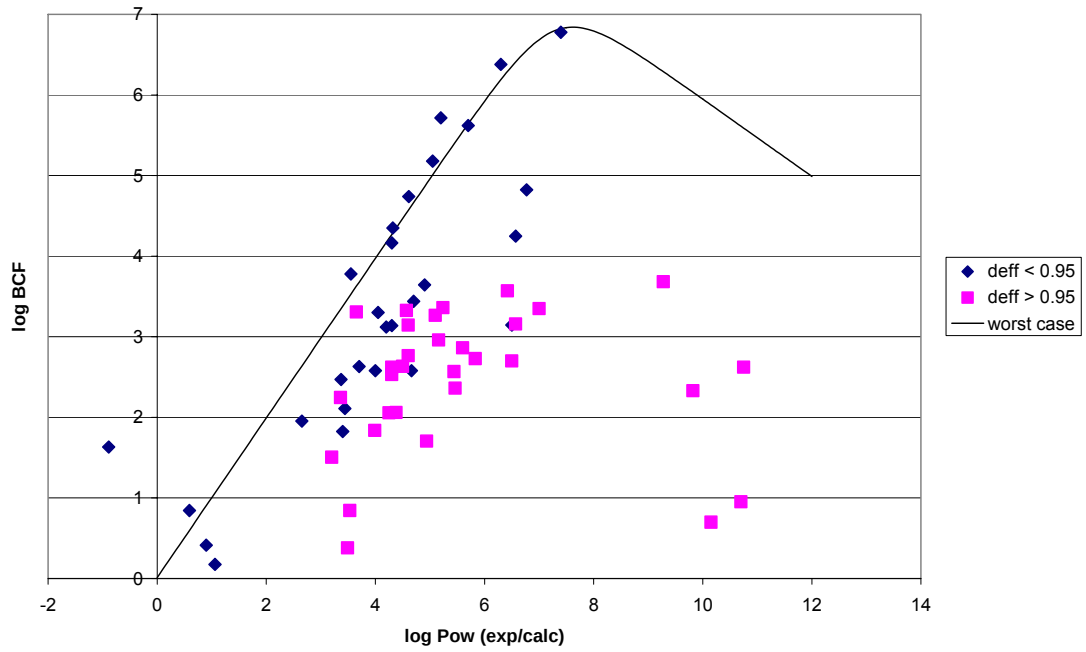


Figure 10. Plot of log BCF versus log K_{OW}. Compounds with effective diameter > 0.95 nm and compounds with lower effective diameters are distinguished.

As a general trend, it can be seen that compounds with effective diameters > 0.95 nm (a) do not show log BCFs > 4 and (b) these compounds are less bioaccumulative than estimated from the worst case function over the entire lipophilicity range. However, restrictions made above (insufficient number of data, data set not representative) have to be recognized.

In spite of the computational problems (see chapter 7.1), the maximum diameter was also taken into account. A plot of log BCF versus the maximum diameter is shown in figure 11. Again, a discrimination of compounds with log BCF > 4 can be seen at D_{max} = 1.65, but which is not as clear as for the effective diameter.

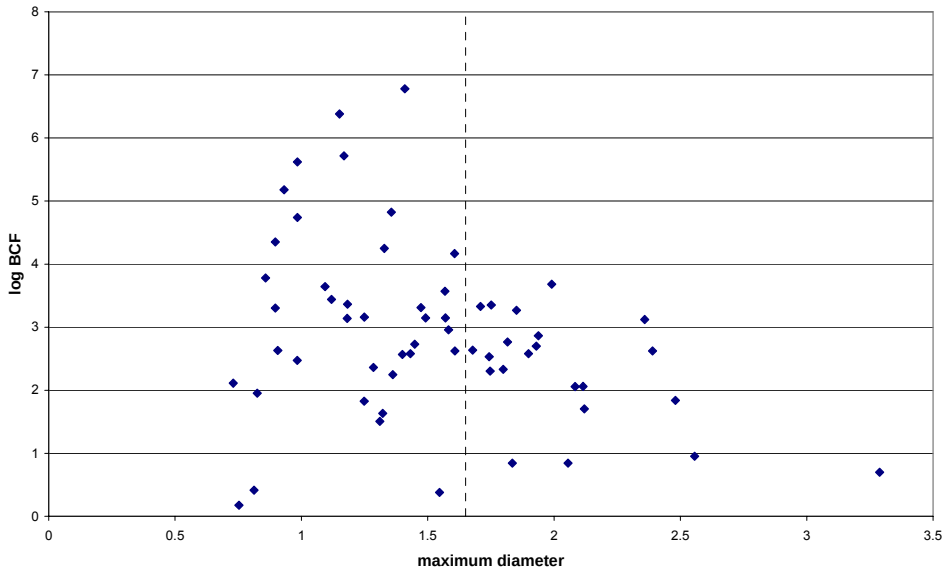


Figure 11. Plot of log BCF versus maximum diameter calculated by CROSS (in nm).

Conclusion: The above findings indicate that compounds with effective diameters > 0.95 nm (with structure optimization by MOPAC/PM3 and calculation of diameters by CROSS) may not have log BCF > 4. However, restrictions concerning the available data set (size, representativity) cause substantial uncertainties. Because current classification criteria for B (BCF = 2000; log BCF = 3.3) and vB (BCF = 5000, log BCF = 3.7) compounds are clearly lower than log BCF = 4, **the effective diameter as cut-off trigger for B/vB compounds is not sufficiently protective.**

7.3.2 'Rule of 5'

The 'rule of 5' by Lipinski et al. [50] were applied to the data set from Umweltbundesamt (see chapter 6.1). An 'alert' was assigned, when any two of the following conditions were fulfilled:

1. Molecular weight > 500 g/mol
2. Σ hydrogen bond donors (expressed as Σ OH + NH) > 5
3. Σ hydrogen bond acceptors (expressed as Σ N + O) > 10
4. log K_{OW} > 5.

Findings are shown in figure 12:

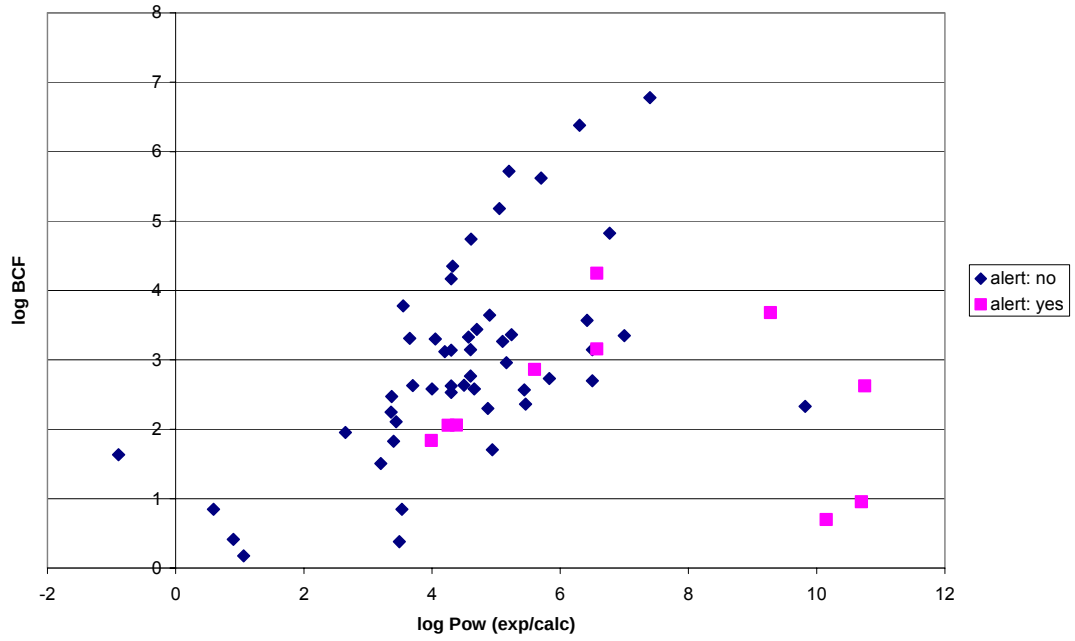


Figure 12. Plot of log BCF versus log K_{OW}. Compounds, which fulfil any two of the 'rules of 5' are marked as 'alert: yes'.

Conclusion: The above findings indicate that compounds with alerts according to the 'rule of 5' may not have log BCF > 4. However, restrictions of the available data set (size, representativity)

cause substantial uncertainties. Because current classification criteria for B (BCF = 2000; log BCF = 3.3) or vB (BCF = 5000, log BCF = 3.7) compounds are clearly lower than log BCF = 4, **'alerts' according to Lipinski's 'rule of 5' are not sufficiently protective to identify non-bioaccumulating compounds.**

7.3.3 Molecular Weight

Molecular weight has been frequently suggested as cut-off trigger. As shown in figure 13, there is a clear threshold at a molecular weight 600 g/mol. Within this dataset, no compound of molecular weight > 600 has measured log BCF > 3. If this finding could be substantiated with a robust and more representative database, e.g. the CEFIC LRI 'gold standard' database on BCF, this would mean that, independent of log K_{OW}, a compound with a molecular weight > 600 might be classified as not-B. A mechanistic interpretation of a molecular weight cut-off may not be straightforward, as the molecular weight is related to multiple properties. The aspect of limited membrane permeation due to molecular size appears of minor relevance as it is known that compounds with molecular weight > 1000 g/mol are able to permeate through membranes. Other properties interrelated with molecular weight, such as solubilities, sorption, bioavailability and absorption kinetics, are most likely to be more influential. Further investigations on valid bioacumulation data covering the entire log BCF range up to 7are needed, particularly for lipophilic chemicals with a molecular weight > 500 g/mol,.

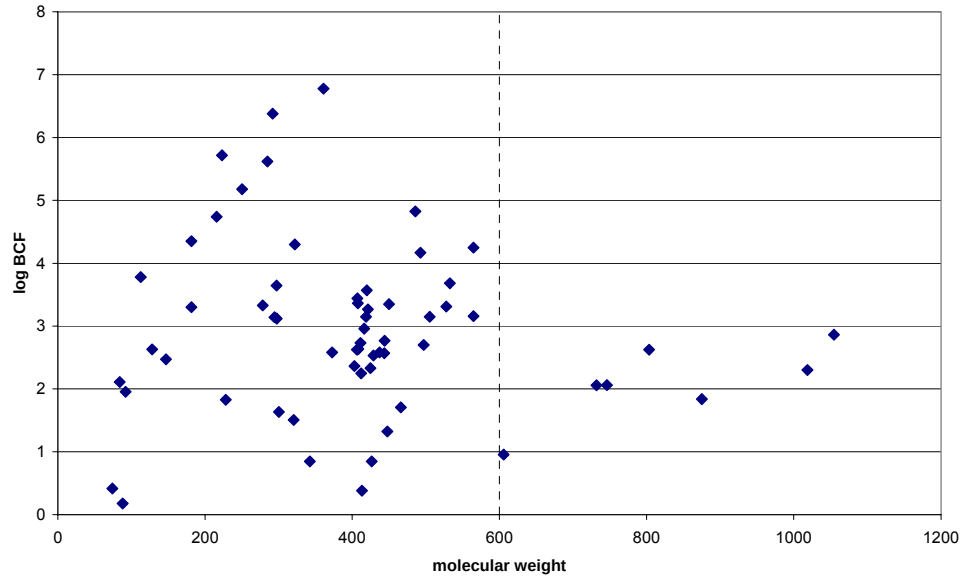


Figure 13. Plot of log BCF versus molecular weight.

Conclusion: The above findings indicate that compounds with a molecular weight > 600 g/mol may not have a log BCF > 3. However, restrictions of the available data set (size, representativity) cause substantial uncertainties. **If substantiated with valid BCF data for large lipophilic compounds, the molecular weight may be a potential candidate for use as cut-off trigger for B/vB compounds.**

7.3.4 Solubility

Data on solubility in water were available for a reduced data set of 18 compounds. No pattern could be detected, which may be used as an indication of limited bioaccumulation (Figure 14).

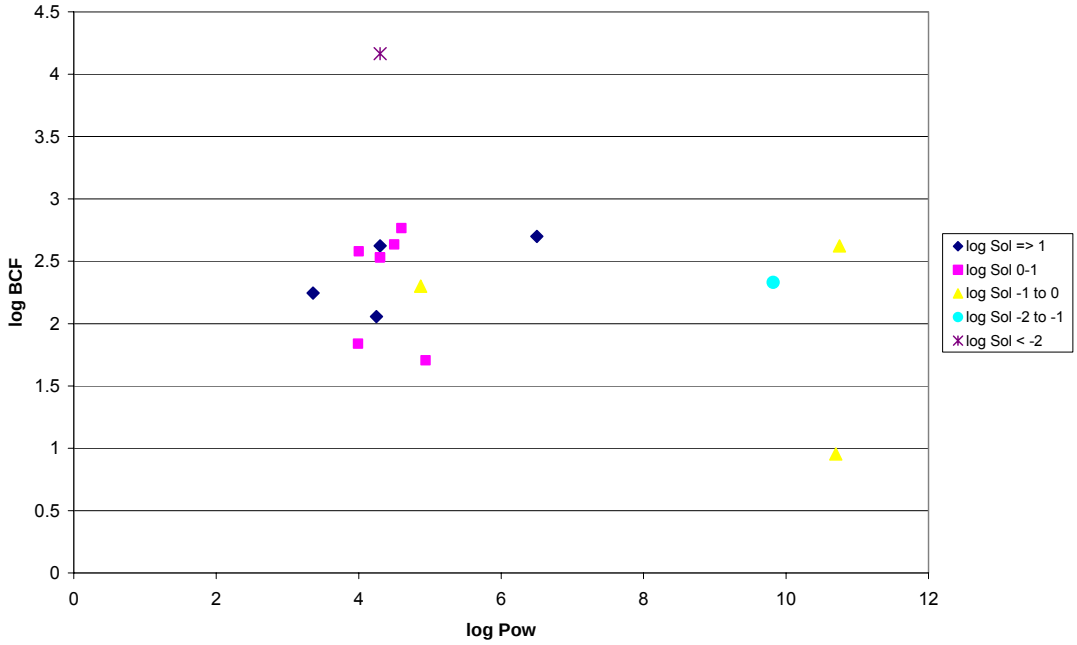


Figure 14. Plot of log BCF versus log K_{OW}. Compounds are differentiated by their water solubility.

Conclusion: The above findings indicate that **water solubility is not a useful property for cut-off trigger for B/vB compounds.**

9. Conclusions

The study has addressed five major issues to rationalize the effects of molecular size and lipid solubility on the bioaccumulation potential of environmental contaminants:

- **Do studies on biological membranes support limits of permeability related to size or lipid solubility of the chemicals?**
  The available information indicates that large high molecular weight-substances (MW > 1000 g/mol) are able to permeate through membranes and can be efficiently absorbed. No robust evidence is provided to substantiate molecular weight or size cut-offs for membrane permeation that can be applied to assess bioaccumulation. Chemicals of moderate lipophilicity and sufficiently solubility in water and membranes diffuse rapidly through aqueous and lipid phases. Large superlipophilic compounds also permeate through membranes, but at slower rates due to their low solubility in water layers. It is the slow rate of elimination that gives high log K_{OW}-chemicals their inherent bioaccumulative potential [52]. Substances that would make bad oral drugs because of minor solubility and slow absorption kinetics, can be very bioaccumulative environmental contaminants.

- **Do studies on bioconcentration support limits of uptake and accumulation potential related to size or lipid solubility of the chemicals?**
  The available information indicates that clear cut-offs in bioconcentration related to size or lipid solubility of chemicals do not exist. Apparently reduced bioconcentration frequently concerns hydrophobic chemicals that have very low aqueous solubilities. These properties make them difficult to test and many apparently reduced BCF data may be attributed to shortcomings in the interpretation of experimental results. Major influential factors concern bioavailability (i.e. freely dissolved exposure concentrations, sorption, solubility limits in the aqueous phase and effects of solubilizers) and kinetics (i.e. attainment of steady state, lipid vs. aqueous layer diffusion control, effects of organism size and life span). Many studies that have investigated relationships between molecular dimensions and reduced uptake, i.e. based on 'lower' BCFs than expected, may actually describe experimental shortcomings or artefacts. In this context, it is interesting to note that any thresholds have been steadily increasing with time (Table 4). The increasing cut-offs with time may be explained by refined testing procedures and improved experimental design to work out the limits of model domains, i.e. generation and analysis of better-quality data for larger hydrophobic compounds.

- **How relevant are active transport mechanisms for the uptake of large organic chemicals?**
  The available information indicates only minor relevance of active transport mechanisms for the uptake of large organic chemicals with regard to environmental bioaccumulation.

- **Do compound properties related to size or lipid solubility provide guidance in assessment schemes of bioaccumulative chemicals? Which parameters are**

potentially useful? Can cut-off triggers be defined?

The available information indicates that compounds with effective diameters > 0.95 nm (with structure optimization by MOPAC/PM3 and calculation of diameters by CROSS) may not have log BCF > 4. However, restrictions of the available data set (size, representativity) cause substantial uncertainties. Because current classification criteria for B (BCF = 2000; log BCF = 3.3) or vB (BCF = 5000, log BCF = 3.7) compounds are clearly lower than log BCF = 4, **the effective or maximum diameter as a cut-off trigger for B/vB compounds is not sufficiently protective.**

The findings of this study indicate that compounds with alerts according to the 'rule of 5' by Lipinski et al. [50] may not have log BCF > 4. However, restrictions of the available data set (size, representativity) cause substantial uncertainties. Because current classification criteria for B (BCF = 2000; log BCF = 3.3) or vB (BCF = 5000, log BCF = 3.7) compounds are clearly lower than log BCF = 4, **'alerts' according to Lipinski's 'rule of 5' are not sufficiently protective to identify non-bioaccumulating compounds.**

Considering the limited database available, it appears that **water solubility is not a useful property for cut-off trigger for B/vB compounds.**

Analysis of the available data indicates that compounds with molecular weight > 600 g/mol may not have log BCF > 3. However, restrictions of the available data set (size, representativity) cause substantial uncertainties. **If substantiated with valid BCF data for large lipophilic compounds, molecular weight may be a potential candidate for use as a cut-off trigger for B/vB compounds.** A mechanistic interpretation of a molecular weight cut-off may not be straightforward, as molecular weight is related to multiple properties. The aspect of limited membrane permeation due to molecular size appears of minor relevance as it is known that compounds with molecular weight > 1000 g/mol are able to permeate through membranes. Other properties interrelated with molecular weight, such as solubilities, sorption, bioavailability and absorption kinetics, are most likely more influential. Further investigations on valid bioaccumulation data covering the entire log BCF range up to 7are needed, particularly for lipophilic chemicals with a molecular weight > 500 g/mol.

- **Do high quality-data for superhydrophobic substances provide new insights to relationships between BCF and log K_{OW}?**

  QSARs relating BCF and log K_{OW} are well established and essentially valid for neutral organics of intermediate lipophilicity ($0 < \log K_{OW} < 6$). The apparent loss in linear relationships for superlipophilic compounds has been attributed – in part – to experimental artefacts. Theoretical considerations substantiate curvilinear relationships for chemicals with log $K_{OW} > 6$:

  - Aqueous phase diffusion control of water to lipid transfer
  - Differences in phase (solvent) properties of natural lipids and octanol
  - Influence of steric conformations
  - Differences in thermodynamic properties of partitioning, e.g. enthalpy changes

  To test established QSARs, BCF data were provided by Umweltbundesamt for 31 plant protection agents and for 18 new chemicals. These chemicals were selected by two criteria: molecular weight > 300 g/mol and bioaccumulation data available. Since this

data set includes no compounds with log BCF > 4 (range in BCF data: 1.5 to 14600), additional data for compounds with very high BCF were taken from the literature. Most BCF data from Umweltbundesamt were qualified as ‘valid data'. However, for two compounds Umweltbundesamt classified the measurements as ‘invalid’. Critical inspection of the available BCF data revealed major deficiencies in data quality of several superlipophilic compounds. For nine compounds, the accumulation experiments have been conducted at concentrations above the water solubility of the test compounds. Consequently, the resulting BCF values are too low artefacts due to invalid experiments. These data were excluded from further analyses.

The remaining data set from Umweltbundesamt is considered not sufficiently representative, because it includes no compounds with log BCF > 4. It is too limited with regard to activity domain as to provide new insights to relationships between BCF and log K_{OW}.

New insights to relationships between BCF and log K_{OW} cannot be provided. The currently available database is insufficient to conclusively substantiate the effects of molecular size and lipid solubility on the bioaccumulation potential of environmental contaminants. Once the CEFIC LRI ‘gold standard’ database on BCF is available, the findings of this study should be re-examined and adjusted as necessary. This study confirms again the importance to exclude experimental artefacts from analysis of bioaccumulation of superhydrophobic substances.

As size-related criteria for limited bioaccumulation appear not to be a clear cut-off trigger, a combination of multiple properties appears a viable option. Evidence-driven assessments on a case-by-case basis should also consider information on aquatic toxicities upon long-term exposure, i.e. absorption potential. The extent to which uptake in mammalian/terrestrial species may be used, still requires validation.

10. Zusammenfassung (in deutscher Sprache)

Die Studie untersucht den Einfluss von Substanzeigenschaften (Molekülgröße und Lipidlöslichkeit) auf das Biokonzentrationpotential von Umweltchemikalien:

- **Sind aus Untersuchungen an biologischen Membranen Permeabilitätsgrenzen oder hemmende Einflüsse auf die Stoffaufnahme, die der Molekülgröße oder der Löslichkeit in Oktanol zuzuordnen sind, nachgewiesen?**
  Die verfügbaren Information zeigen, dass die Aufnahme großer Substanzen mit hohem Molekulargewicht (MW > 1000 g/mol) nicht grundsätzlich behindert ist. Sie können Membranen passieren und in Organismen aufgenommen werden. Permeabilitätsgrenzen, die der Molekülgröße oder der Löslichkeit in Oktanol zuzuordnen sind, sind experimentell nicht nachgewiesen. Chemikalien geeigneter Lipophilie und Löslichkeit in Wasser und Membranen diffundieren rasch durch Wasser- und Lipidphasen. Große superlipophile Stoffe permeieren ebenso durch Membranen, aber mit verminderter Geschwindigkeit aufgrund ihrer geringen Wasserlöslichkeit. Substanzen mit hohem log K_{OW} werden nur langsam eliminiert und können deshalb ein hohes Bioakkumulationspotential aufweisen [52]. Verbindungen mit ungünstigen Eigenschaften für orale Arzneistoffe, d.h. geringer Wasserlöslichkeit und langsamer Absorption, können gleichzeitig stark bioakkumulierende Umweltchemikalien sein.

- **Lassen sich Einflüsse der Molekülgröße oder der Löslichkeit in Oktanol auf die Stoffaufnahme und das Bioakkumulationspotential aus Erkenntnissen von in Tests bestimmten Biokonzentrationsfaktoren ableiten?**
  Die verfügbaren Informationen zeigen, dass es keine eindeutigen Grenzwerte für Einflüsse der Molekülgröße oder der Löslichkeit in Oktanol auf die Stoffaufnahme und das Bioakkumulationspotential gibt. Reduzierte Biokonzentration relativ zu linearen QSAR-Modellen betrifft zumeist hydrophobe Chemikalien mit sehr geringer Wasserlöslichkeit. Diese Substanzeigenschaften verursachen experimentelle Schwierigkeiten bei der Messung von Biokonzentrationsfaktoren, sodass viele ‘zu niedrige’ BCF-Werte die fehlerhafte Durchführung und Auswertung von Testergebnissen reflektieren. Entscheidende Einflussfaktoren sind dabei die Bioverfügbarkeit (z. B. freie, gelöste Expositionskonzentrationen, Sorption, Grenzen der Wasserlöslichkeit, Effekte von Lösungsvermittlern) und die Kinetik (z. B. Erreichen des Steady-State, verminderte Diffusion in wässrigen oder Lipidphasen, Auswirkungen der Größe und Lebensdauer der Testorganismen). Viele Untersuchungen der Beziehungen zwischen molekularen Deskriptoren und scheinbar reduzierter Biokonzentration beschreiben tatsächlich experimentelle Artefakte. In diesem Zusammenhang ist es eine interessante Beobachtung, dass sämtliche Grenzwerte im Laufe der Zeit angestiegen sind (Table 4), bedingt durch verbesserte Testverfahren und experimentelles Design, um die Grenzen der Modelle zu testen, speziell für große hydrophobe Verbindungen.

- **Wie bedeutend sind aktive Transportmechanismen für die Aufnahme von organischen, umweltrelevanten Stoffen mit großen Molekülabmessungen oder hoher**

Molmasse?

Die verfügbaren Informationen deuten auf eine nur geringe Relevanz von aktiven Transportmechanismen für die Aufnahme und Bioakkumulation von organischen, umweltrelevanten Stoffen mit großen Molekülabmessungen oder hoher Molmasse.

- **Welche Schlussfolgerungen lassen sich hinsichtlich einer möglichen Einbindung von Stoffeigenschaften (Molekülgröße oder Löslichkeit in Oktanol) in die Prüf- und Bewertungsstrategien für das Bioakkumulationspotential ziehen? Welche Parameter sind dazu geeignet? Welche Werte für diese Parameter können als Grenzkriterium herangezogen werden?**

  Die Analyse der verfügbaren Daten zeigt, dass Stoffe mit einem effektiven Durchmesser > 0.95 nm (Strukturoptimierung mit MOPAC/PM3 und Berechnung des Durchmessers mit CROSS) wahrscheinlich log BCF < 4 haben. Weil die vorliegende Datenbasis hinsichtlich Größe und Repräsentativität unzureichend ist und weil die Klassifizierungskriterien für B-Stoffe (BCF = 2000; log BCF = 3.3) oder vB-Stoffe (BCF = 5000, log BCF = 3.7) deutlich niedriger liegen, ist die **Verwendung des effektiven oder maximalen Durchmessers als Cut-off Trigger für B/vB Stoffe nicht ausreichend protektiv.**

  Anwendung der 'rule of 5' von Lipinski et al. [50] auf die Daten des Umweltbundesamtes deutet an, dass Stoffe, die mindestens zwei der Regeln erfüllen, wahrscheinlich log BCF < 4 haben. Weil die vorliegende Datenbasis hinsichtlich Größe und Repräsentativität unzureichend ist und weil die Klassifizierungskriterien für B-Stoffe (BCF = 2000; log BCF = 3.3) oder vB-Stoffe (BCF = 5000, log BCF = 3.7) deutlich niedriger liegen, ist die **Verwendung von Lipinski's 'rule of 5' als Cut-off Trigger für B/vB Stoffe nicht ausreichend protektiv.**

  In Anbetracht der limitierten Datenbasis erscheint es unwahrscheinlich, dass die Wasserlöslichkeit eine geeignete Eigenschaft als Cut-off Trigger für B/vB Stoffe darstellt.

  Die Analyse der verfügbaren Daten zeigt, dass sämtliche vorliegenden Substanzen mit einem **Molekulargewicht > 600 g/mol log BCF < 3** haben. Allerdings läßt die vorliegende Datenbasis (unzureichende Größe und Repräsentativität) keine endgültigen Schlüsse zu. **Wenn es gelingt, diesen Befund anhand valider BCF Daten auch für große lipophile Stoffe und stark bioakkumulierende Substanzen abzusichern, könnte das Molekulargewicht einen potentiellen Kandidaten für einem Cut-off Trigger für B/vB Stoffe darstellen.** Die mechanistische Interpretation eines Grenzwertes auf der Basis des Molekulargewichts ist komplex, weil diese Eigenschaft mit vielen anderen korreliert. Der Aspekt der limitierten Membranpermeation aufgrund der Größe von Verbindungen erscheint ungeeignet, weil auch Chemikalien mit MW > 1000 g/mol Membranen gut passieren können. Andere Eigenschaften, die ebenfalls mit dem Molekulargewicht korreliert sind, wie Löslichkeit, Sorption, Bioverfügbarkeit und Absorptionskinetik, haben vermutlich einen größeren Einfluss. Weitere Untersuchungen sind notwendig, speziell mit lipophilen Stoffen, die ein Molekulargeicht > 500 g/mol haben, anhand valider Bioakkumulationsdaten, die das gesamte Aktivitätsspektrum mindestens bis log BCF 7 abdecken.

- **Ergeben sich aus belastbaren BCF-Daten von superhydrophoben Stoffen neue Erkenntnisse im Verhältnis BCF zu log K_{OW}?**
  QSARs zur Abschätzung des BCF aus dem log K_{OW} sind für neutrale organische Substanzen mittlerer Lipophilie (0 < log K_{OW} < 6) etabliert und grundsätzlich valide. Die beobachtete nicht-Linearität der Beziehungen für superlipophile Substanzen wird – zum Teil – Mängeln in den experimentellen BCF Daten zugeschrieben. Aus theoretischen Erwägungen sind nicht-lineare QSARs oberhalb von log K_{OW} 6 zu erwarten:
  - limitierte Diffusion in wäßrigen Phasen
  - unterschiedliches Verhalten in Lipiden oder Oktanol
  - Einfluss sterischer Konformationen
  - Unterschiedliche thermodynamische Eigenschaften, z.B. Enthalpie

  Vom Umweltbundesamt wurden BCF Daten für 31 Pflanzenschutzmittel und 18 Neustoffe zur Verfügung gestellt, um etablierte QSARs zu testen. Die Stoffe wurden anhand von zwei Kriterien ausgewählt: Sie haben alle ein Molekulargewicht > 300 und Bioakkumulationsdaten liegen vor. Weil der so erhaltene Datensatz keine Verbindungen mit log BCF > 4 (Bereich der BCF-Werte: 1.5 - 14600) enthält, wurden zusätzlich Daten aus der Literatur für stark bioakkumulierende Chemikalien verwendet.
  Die meisten der BCF-Daten des Umweltbundesamtes wurden als valide eingestuft, lediglich bei zwei Stoffen lautete die Einstufung der Datenqualität ‘nicht valide’. Eine kritische Überprüfung ergab allerdings eine Problematik bei superhydrophoben Stoffen: Bei den meisten UBA-Stoffen mit log K_{OW} > 7 wurde in den BCF-Studien mit Testkonzentrationen oberhalb der Wasserlöslichkeit gearbeitet, was zu einer Unterschätzung der BCF-Werte führt. Diese Stoffe wurden von den weiteren Auswertungen ausgeschlossen.
  Insgesamt ist der verbleibende Datensatz, der von Umweltbundesamt zur Verfügung gestellt wurde, zu klein und repräsentiert nur in unzureichender Weise die chemische Domäne und das Aktivitätsspektrum potentiell bioakkumulierender Substanzen. Daher ist es nicht möglich, neue Erkenntnisse über das Verhältnis von BCF zu log K_{OW} von superhydrophoben Stoffen zu gewinnen.

Neue Aussagen über die Beziehung zwischen BCF und log K_{OW} sind nicht möglich. Die verfügbare Datenbasis ist nicht ausreichend, um abschließend den Einfluss der Molekülgröße und der Oktanol-Löslichkeit auf das Bioakkumulationspotential festzustellen. Wenn die CEFIC LRI Goldstandard-Datenbank zur Bioakkumulation vorliegen wird, sollten sämtliche Befunde dieser Studie überprüft und, wenn notwendig, entsprechend modifiziert werden. Die Studie verdeutlicht, wie wichtig es ist, dass experimentelle Artefakte bei BCF-Studien mit superhydrophoben Stoffen vermieden werden.

Offensichtlich gibt es keine einfachen Grenzwerte bezüglich der Molekülgröße oder Oktanollöslichkeit für die Einstufung von bioakkumulierenden Umweltchemikalien. Eine Evidenzbasierte Bewertung sollte im Einzelfall alle verfügbaren Informationen zur längerfristigen aquatischen Toxizität, z. B. Absorptionspotential, berücksichtigen. Inwieweit die Aufnahme in

anderen Spezies (Säuger, andere terrestrische Organismen) verwendet werden kann, bedarf noch der Validierung.

11. Appendix: Experimental Data

Chemical/Code	logK_{OW}	BCF	log BCF	Ref.
psm1	4.66	380	2.58	UBA
psm5	4.6	583	2.77	UBA
psm6	4.7	2755	3.44	UBA
psm7	4.3	14645	4.17	UBA
psm8	4.3	420	2.62	UBA
psm9	4.3	340	2.53	UBA
psm10	4	380	2.58	UBA
psm11	5.24	2300	3.36	UBA
psm14	3.49	2	0.38	UBA
psm16	4.25	114	2.06	UBA
psm17	4.38	115	2.06	UBA
psm18	5.6	730	2.86	UBA
psm19	5.1	1842	3.27	UBA
psm20	3.2	32	1.51	UBA
psm21	4.5	431	2.63	UBA
psm22	5.83	537	2.73	UBA
psm23	5.46	230	2.36	UBA
psm24	3.65	2039	3.31	UBA
psm25	5.44	369	2.57	UBA
psm26	6.5	500	2.7	UBA
psm27	6.42	3700	3.57	UBA
psm29	4.6	1400	3.15	UBA
psm31	3.99	69	1.84	UBA
psm33	5.16	910	2.96	UBA
psm36	6.5	1400	3.15	UBA
psm39	7	2240	3.35	UBA
psm41	3.36	176	2.25	UBA
psm42	4.94	51	1.71	UBA
psm43	4.2	1318	3.12	UBA
neu1	12.7	2*	0.18	UBA
neu2	10.04	69*	1.84	UBA
neu3	7.2	500*	2.7	UBA
neu4	13.4	36*	1.56	UBA
neu5	5.53	127*	2.1	UBA
neu6	9.82	214	2.33	UBA
neu7	8	31*	1.49	UBA
neu8	4.87	200	2.3	UBA
neu9	10.7	9	0.95	UBA
neu10	10.15	5*	0.7	UBA
neu13	10.61	27*	1.43	UBA
neu14	9.28	4800	3.68	UBA
neu15	10.42	23*	1.36	UBA
neu16	10.75	420	2.62	UBA
neu17	3.53	7	0.85	UBA
neu18	-0.89	43	1.63	UBA
neu19	0.59	7	0.85	UBA
neu20	7.1	8*	0.9	UBA

Toluene	2.65	90	1.95	EU-RAR
1,2,4-trichlorobenzene	4.05	2000	3.3	EU-RAR
musk ketone	4.3	1380	3.14	EU-RAR
musk xylene	4.9	4400	3.64	EU-RAR
naphthalene	3.7	427	2.63	EU-RAR
tert.-Butylmethylether	1.06	1.5	0.18	EU-RAR
Bisphenol A	3.4	67	1.83	EU-RAR
aniline	0.9	2.6	0.41	EU-RAR
1,4-dichlorobenzene	3.37	296	2.47	EU-RAR
dibutylphthalate	4.57	2125	3.33	EU-RAR
2,2',4,4',5-pentabromodiphenylether	6.57	1440	3.16	EU-RAR
2,2',4,4',6-pentabromodiphenylether	6.57	17700	4.25	EU-RAR
tetrabromodiphenylether	6.77	66700	4.82	EU-RAR
cyclohexane	3.44	129	2.11	EU-RAR
1,3-dichlorobenzene	3.55	6025	3.78	[10]
1,3,5-trichlorobenzene	4.32	22400	4.35	[10]
1,2,3,4-tetrachlorobenzene	4.61	54950	4.74	[10]
pentachlorobenzene	5.05	151400	5.18	[10]
hexachlorobenzene	5.7	417000	5.62	[10]
2,5-dichlorobiphenyl	5.2	520000	5.72	[69]
2,2',5,5'-tetrachlorobiphenyl	6.3	2400000	6.38	[69]
2,2',4,4',5,5'-hexachlorobiphenyl	7.4	6000000	6.78	[69]
2,3,7,8-TCDD	6.8	20000	4.3	[70]

*: test concentration > solubility in water

Ref. UBA: Data provided by the Umweltbundesamt

Ref. EU-RAR: EU Risk Assessment Reports (www.ecb.jrc.it)

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