Environmental Research
of the Federal Ministry for the Environment,
Nature Conservation and Nuclear Safety

Project No. FKZ 3711 63 405/01

Contribution of non-lipid based processes to the bioaccumulation of chemicals

by

Prof. Dr. Christian Schlechtriem (FHG-IME)

Dr. Monika Nendza (AL-Luhnstedt)

Dr. Stefan Hahn and Ariane Zwintscher (FHG-ITEM)

Prof. Dr. Gerrit Schüürmann and Dr. Ralph Kühne (UFZ)

Fraunhofer-Institut für Molekularbiologie und
Angewandte Ökologie (FHG-IME),
57392 Schmallenberg
Analytisches Laboratorium für Umweltuntersuchungen
und Auftragsforschung (AL-Luhnstedt),
24816 Luhnstedt
Fraunhofer-Institut für Toxikologie und Experimentelle
Medizin (FHG-ITEM), 30625 Hannover
Helmholtz-Zentrum für Umweltforschung (UFZ),
04318 Leipzig

ON BEHALF OF THE

FEDERAL ENVIRONMENT AGENCY

Schmallenberg, 15th April 2015

Report Cover Sheet

Report No.	UBA-FB 00
Report Title	Contribution of non-lipid based processes to the bioaccumulation of chemicals
Author(s) (Family Name, First Name)	Prof. Dr. Christian Schlechtriem (FHG-IME) Dr. Monika Nendza (AL-Luhnstedt) Dr. Stefan Hahn and Ariane Zwintscher (FHG-ITEM) Prof. Dr. Gerrit Schüürmann and Dr. Ralph Kühne (UFZ)
Performing Organisation (Name, Address)	Fraunhofer-Institut für Molekularbiologie und Angewandte Ökologie (FHG-IME), 57392 Schmallenberg Analytisches Laboratorium für Umweltuntersuchungen und Auftragsforschung (AL-Luhnstedt), 24816 Luhnstedt Fraunhofer-Institut für Toxikologie und Experimentelle Medizin (FHG-ITEM), 30625 Hannover Helmholtz-Zentrum für Umweltforschung (UFZ), 04318 Leipzig
Funding Agency	Umweltbundesamt Postfach 14 06 06813 Dessau-Roßlau
Report Date (Year)	2014
Project No. (FKZ)	FKZ 3711 63 405/01
No. of Pages	92
Supplementary Notes	
Keywords	Bioaccumulation, lipid, protein, active transport, Caco-2, surfactants, polyfluorinated compounds, QSAR

Berichtskennblatt

Berichtsnummer	UBA-FB 00
Titel des Berichts	Untersuchung von nicht-lipidbasiertem Bioakkumulationsverhalten von Stoffen
Autor(en) (Name, Vorname)	Prof. Dr. Christian Schlechtriem (FHG-IME) Dr. Monika Nendza (AL-Luhnstedt) Dr. Stefan Hahn und Ariane Zwintscher (FHG-ITEM) Prof. Dr. Gerrit Schüürmann und Dr. Ralph Kühne (UFZ)
Durchführende Institution (Name, Anschrift)	Fraunhofer-Institut für Molekularbiologie und Angewandte Ökologie (FHG-IME), 57392 Schmallenberg Analytisches Laboratorium für Umweltuntersuchungen und Auftragsforschung (AL-Luhnstedt), 24816 Luhnstedt Fraunhofer-Institut für Toxikologie und Experimentelle Medizin (FHG-ITEM), 30625 Hannover Helmholtz-Zentrum für Umweltforschung (UFZ), 04318 Leipzig
Fördernde Institution	Umweltbundesamt Postfach 14 06 06813 Dessau-Roßlau
Abschlussjahr	2014
Forschungskennzahl (FKZ)	FKZ 3711 63 405/01
Seitenzahl des Berichts	92
Zusätzliche Angaben	
Schlagwörter	Bioakkumulation, Lipid, Protein, aktiver Transport, Caco-2, Tenside, polyfluorierte Stoffe, QSAR

Abstract

The aim of this study was to investigate the possible effects of specific processes on bioaccumulation in aquatic organisms: (i) the protein binding of perfluoroalkyl and polyfluoroalkyl substances, (ii) the absorption of surfactants at biological interfaces, and (iii) the specific transport processes which may not be properly addressed by log K_{ow}. Exploratory data analyses with 998 chemical substances revealed for 7% of the compounds higher bioaccumulation than estimated from log K_{ow}.

No specific classes of chemicals could be identified with a general and significant increase of bioaccumulation in aquatic organisms. A literature search was carried out for processes and substances involved in specific bioaccumulation beyond water/lipid partitioning. The results show that protein binding does not correlate with increased bioaccumulation beyond log K_{ow}. Instead, protein binding, like accumulation in storage lipids, correlates with the log K_{ow} of the substances. The bioaccumulation potential of surfactants does not correlate with log K_{ow} and is often lower than log K_{ow} based estimates. In contrast, substances with secondary active transport via carriers might show an increased absorption. Uptake of substances by transport processes can be identified *in vitro* using the Caco-2 assay, based on a human cell line, which displays both active and passive transport processes. A screening based on (estimated) P_{app} with differential thresholds for compounds of different lipophilicity was carried out.

The findings of the present study suggest three areas of future research:

- To further clarify the qualitative and quantitative role of protein binding for bioaccumulation of PFAS, systematic experimental binding studies with different protein and lipid targets would be useful
- To assess the possibility of increased dietary uptake of surfactants due to absorption to food items, quantitative information on the dietary uptake of surfactants would be desirable.
- The validity of Caco-2 based P_{app} as a screening tool for specific mechanisms of bioaccumulation has to be further evaluated by applying to a larger dataset and by comparing predicted values to measured results including supposedly difficult compounds.

Kurzbeschreibung

In diesem Forschungsvorhaben wurden die möglichen Auswirkungen von spezifischen Prozessen auf die Bioakkumulation in aquatischen Lebewesen untersucht: (i) spezifische Proteinbindung von perfluoralkyl und polyfluoralkyl Substanzen, (ii) Absorption von Tensiden in biologischen Grenzflächen, und (iii) spezifische (aktive) Aufnahmemechanismen. Eine Datenanalyse von Abweichungen von der lipidbasierten Bioakkumulation am Beispiel von 998 Chemikalien zeigte bei 7% der untersuchten Substanzen eine erhöhte Bioakkumulation verglichen zur Abschätzung durch den log K_{ow}.

Es konnten keine chemischen Substanzklassen identifiziert werden, deren Bioakkumulation in aquatischen Organismen regelmäßig signifikant gesteigert ist. Auf Basis von Daten aus der Literatur konnte gezeigt werden, dass Proteinbindungen nicht zur Erklärung von erhöhter Bioakkumulation geeignet sind. Proteinbindungen korrelieren, ebenso wie die Akkumulation in Lipiden, mit den hydrophoben Eigenschaften von Substanzen. Das Bioakkumulationspotential grenzflächenaktiver Substanzen korreliert nicht mit log K_{ow} und ist häufig geringer als aus log K_{ow} abgeschätzt. Demgegenüber können aktive Transportprozesse durchaus zu einer gesteigerten Absorption führen. Die Aufnahme von Stoffen durch Transportprozesse kann *in vitro* mit zell-basierten Caco-2 Tests, die sowohl aktive als auch passive Transportprozesse repräsentieren, identifiziert werden. Ein Screening wurde auf Basis berechneter P_{app}-Werte durchgeführt, welche zunächst nach der Lipophilie der Verbindungen differenziert wurden.

Die Ergebnisse dieser Studie weisen auf Forschungsbedarf in drei Bereichen:

- Um die qualitative und quantitative Bedeutung der Proteinbindung für die Bioakkumulation von PFAS zu klären, sollten systematische experimentelle Bindungsstudien mit verschiedenen Proteinen und Lipiden unternommen werden.
- Um die Möglichkeit der Aufnahme von Tensiden aufgrund der Absorption an Nahrung besser einschätzen zu können, sind quantitative Informationen zur Akkumulation von Tensiden via Nahrung wünschenswert.
- Die Validität des Caco-2 Models zum Bioakkumulationsscreening sollte durch Anwendung auf einen größeren Datensatz und den Vergleich von Schätzwerten mit gemessenen Ergebnissen, auch für bekannte Problemstoffe, geprüft werden.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

A = hydrogen bond donor capacity (acidity)

ABC = ATP binding cassette

ABC-transporters = ATP binding cassette – transporters

AS = alkyl sulfates

ATP = adenosine triphosphate

B = bioaccumulation

B = hydrogen bond acceptor capacity (basicity)

BAF = bioaccumulation factor

BCF = bioconcentration factor

BCRP = breast cancer resistance protein

BMF = biomagnification factor

BSA = bovine serum albumin

Caco-2 = carcinoma colon (human intestinal epithelial cells)

CMC = critical micelle concentration

CR = concentration ratio

DMAC = N,N-dimethyl-acetamide

ECHA = european chemicals agency

EU = european union

GIT = gastrointestinal tract

HIA = human intestinal absorption

HPLC = high-performance liquid chromatography

HSA = human serum albumin

IAM = immobilized artificial membrane

k_1 = uptake rate

k_2 = depuration rate

LAS = linear alkylbenzene sulfonates

L-FABP = liver fatty acid binding protein

log CHI IAM = affinity to phospholipids

log *D* = partition coefficient at a defined pH

log $K_{BSA/w}$ = bovine serum albumin/water partition coefficient

log K_{HSA} = human serum albumin/water partition coefficient

log K_{mw} = membrane/water partition coefficient

log K_{oa} = octan-1-ol/air partition coefficient

log K_{ow} = octan-1-ol/water partition coefficient

log K_{pw} = protein/water partition coefficient

log(K_{i1}) = interaction rate constant

LSER = linear solvation energy relationship

MoA = mode of action

MRP = multi-drug resistance-associated protein

n = number of data (in statistical context)

nm = nanometer

nonB = not bioaccumulating

OATP = organic anion transport polypeptide

OCTN = organic cation transport

OECD = organisation for economic co-operation and development

PAMPA = parallel artificial membrane permeation assay

P_{app} = apparent permeability coefficient (*in vitro* e.g. from Caco-2 experiments)

P_{eff} = effective permeability coefficient (*in vivo*)

PBT = persistent, bioaccumulative and toxic

PEPT1 = peptide transporter

PFAS = perfluoroalkyl and polyfluoroalkyl substances

PFBA = pentafluorobenzoic acid

PFBS = perfluorobutanesulfonic acid

PFDA = perfluorodecanoic acid

PFDoDA = perfluorododecanoic acid

PFHpA = perfluoroheptanoic acid

PFHxA = perfluorohexanoic acid

PFHxDA = perfluorohexadecanoic acid

PFHxS = perfluorohexane sulfonate

PFNA = perfluorononanoic acid

PFOA = perfluorooctanoic acid

PFODA = perfluorooctadecanoic acid

PFOS = perfluorooctanesulfonic acid

PFPA = pentafluoropentanoic acid

PFTeDA = perfluorotetradecanoic acid

PFTrDA = perfluorotridecanoic acid

PFUnDA = perfluoroundecanoic acid

P-gp = P-glycoprotein

pH = decimal logarithm of the reciprocal of the hydrogen ion activity

pk_a = logarithmic acid dissociation constant

POPs = persistent organic pollutants

PSA = polar surface area

QSAR = quantitative structure–activity relationship

r^2 = squared correlation coefficient of regression

REACH = registration, evaluation, authorisation and restriction of chemicals (European Union)

rms = root mean squared error

rp-HPLC = reverse-phase high performance liquid chromatography

S = polarity and polarizability

SGLT = sodium/glucose transporter

SHBG = sex-hormone binding globulin

SVHC = substance of very high concern

TEER = transepithelial electrical resistance

TGD = technical guidance document

TLC = thin-layer chromatography

TMAC = trimethyl ammonium chloride

TPSA = topological polar surface area

V = characteristic volume

vPvB = very persistent and very bioaccumulative

τ = biotransformation half-life

Glossary

bioaccumulation = uptake from the environment via any possible pathway

bioconcentration = uptake from the surrounding phase via adsorption, lipid diffusion etc.

biomagnification = uptake via the foodweb resulting in increased concentrations in higher trophic levels

Caco-2 = continuous cell line of heterogeneous human epithelial colorectal adenocarcinoma cells. Caco-2 is most commonly used as a confluent monolayer on a cell culture insert filter. Caco-2 is established across the pharmaceutical industry as an in vitro model of the human small intestinal mucosa to predict the absorption of orally administered drugs.

critical micelle concentration (CMC) = concentration of surfactants above which micelles form and all additional surfactants added to the system go to micelles

hydrophobicity = affinity of a molecule or a moiety for a hydrophobic environment.

k_1 = uptake rate

k_2 = elimination rate

lipophilicity = affinity of a molecule or a moiety for a lipophilic environment. It is commonly measured by its distribution behaviour in a biphasic system, either liquid-liquid (e.g., partition coefficient in octan-1-ol/water) or solid/liquid (retention on reversed-phase high performance liquid chromatography (rp-HPLC) or thin-layer chromatography (TLC) system) (http://www.chemicool.com).

log D = distribution coefficient, the partition coefficient at a defined pH value, accounting for the lower log K_{ow} of the fraction of the ionized molecules relative to the higher log K_{ow} of the fraction of the unionized molecules

log K_{ow} = thermodynamic partitioning of a chemical compound between water and an immiscible (bio)organic solvent (octan-1-ol)

LSER = linear solvation energy relationships

P_{app} = apparent permeability coefficient for compounds across membranes. P_{app} is obtained from the flow rate F of a compound into the compartment B, normalized by the surface area S through which this flow takes place and normalized again by the driving concentration A_0.

Passive diffusion = movement of biochemicals and other atomic or molecular substances across cell membranes. Unlike active transport, it does not require an input of chemical energy, being driven by the growth of entropy of the system.

Polarity (S) = the state of being electrically either positive or negative

Polarizability = the quality of being able to be polarized

TEER = transepithelial electrical resistance is used to measure the ion movement across the para-cellular pathway

1 Summary

Specific processes may increase or reduce the bioaccumulation of chemical substances in aquatic organisms related to intrinsic properties of the chemicals. Exploratory data analyses with 998 chemical substances revealed higher bioaccumulation than estimated from log K_{ow} for 7% of the compounds. However, no specific classes of chemicals could be identified with a general and significant increase of bioaccumulation in aquatic organisms.

A literature search was carried out for processes and substances involved in specific mechanisms of bioaccumulation. The following processes were considered:

- Interactions with proteins
- Distribution of surfactants
- Active/passive uptake in the gastrointestinal tract (GIT)

Potential interactions in terms of partition coefficients, rate constants and binding parameters with proteins, or CMC were analyzed for:

- Perfluoroalkyl and polyfluoroalkyl substances (PFAS)
- Surfactants
- Pharmaceuticals

Our major tools were exploratory data analyses relative to different modelling approaches. We first compared the available data for diverse processes to find trends between them. Second, we regarded possible relationships between the processes and the properties of the chemicals. Thirdly, we looked at either agreement or deviations of data for the diverse processes with/from, e.g., a log K_{ow} based reference model. Deviations from this model were taken as indicators of possible specific contributions to bioaccumulation.

The results show that protein binding is dominated by log K_{ow} and correlates with log BCF accordingly. Protein binding does not correlate with increased bioaccumulation beyond log K_{ow}. Regarding PFAS, reliable log K_{ow} are not available. The uncertainties of the estimated log D values of PFAS limit further conclusions regarding non-lipid based accumulation of PFAS. Interestingly, there is a (linear) correlation between the experimental accumulation data for PFAS and calculated log D/log K_{ow}. Furthermore, we observe an excellent agreement of experimental BCF values for PFAS with field BAF obtained with fish. There is no indication of biomagnification of PFAS in aquatic organisms. The exploratory data analyses of different parameters of protein binding of PFAS do not support protein based accumulation of PFAS.

Because the available data on PFAS are not sufficient to understand the relationships between bioaccumulation and protein binding, we analysed a set of diverse organic chemicals to gain a better picture of the relationships between bioaccumulation and protein binding. Consistent with long-standing expertise in pharmaceutical and medicinal chemistry, protein binding is correlated with log K_{ow} and log BCF. To further clarify the qualitative and quantitative role of protein binding for bioaccumulation of PFAS, systematic experimental binding studies with different protein and lipid targets would be useful.

The available bioconcentration data for surfactants are not sufficient to fully assess their bioaccumulation potential. Most of the BCF estimates are below the threshold of 2000. Only for TMAC a BCF close to this value (BCF=1960) was estimated. The BCF of surfactants other than PFAS in fish do not correlate with log Kow and are often lower than estimated based on hypothetical log Kow. Within the different chemical classes of surfactants, other properties such

as the length of the alkyl chains and the number of oxyethylene units seem to play a role with regard to the uptake and bioaccumulation of surfactants. The suitability of CMC as an alternative measure of the hydrophobicity of surfactants was tested, however, there is no indication for a general suitability of this measure. For the most part, the distribution and accumulation of surfactants in organisms depend on their absorption at biological interfaces. The metabolism of surfactants may also have an impact on the accumulation and distribution of the molecules in fish as shown for anionic and nonionic surfactants. However, information on the biotransformation kinetics as well as knowledge on the protein binding of anionic, nonionic and cationic surfactants in fish is often limited.

Surfactants may easily absorb to food items, possibly causing an increased dietary uptake. As yet, quantitative information on the dietary uptake of surfactants is limited.

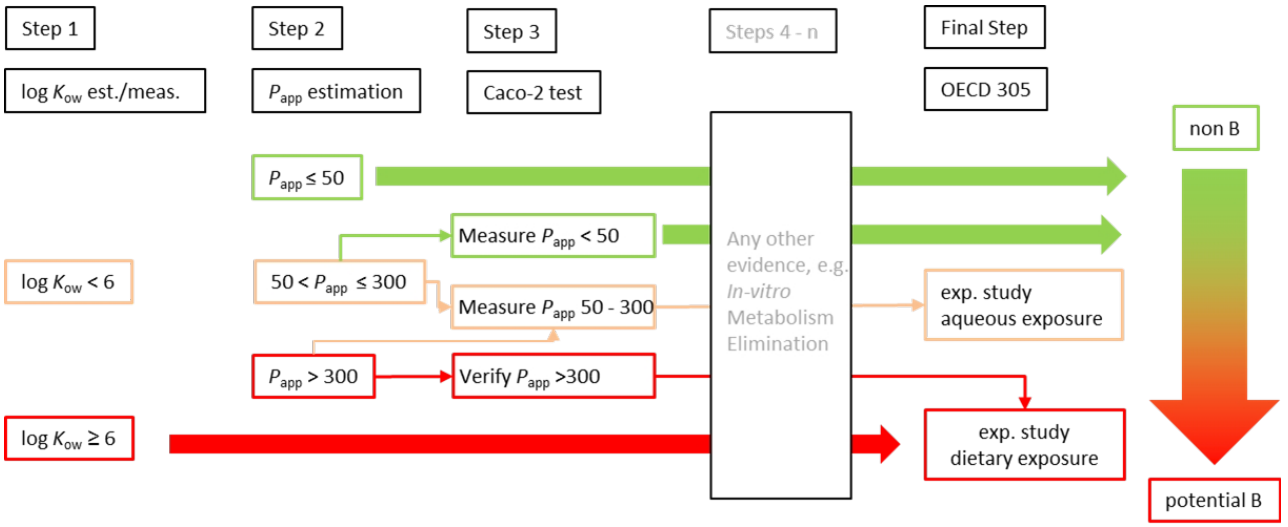
Uptake by active and passive transport processes can affect the extent of bioaccumulation of chemicals. The passive diffusion correlates well with the log K_{ow} of substances. If secondary active transport of substances via carriers occurs in addition to passive diffusion, absorption may be increased as compared to the predicted uptake based on molecular properties. Since primary active transport mostly causes efflux from cells, it rather limits the absorption of substances, and thus is an opposing mechanism with respect to bioaccumulation, i.e. reduces bioaccumulation.

The Caco-2 assay revealed a good model for the absorption potential, i.e. uptake of chemicals into organisms. The Caco-2 cell line features both active and passive absorption processes. Note, however, that the Caco-2 assay does not necessarily cover all the processes possibly involved in bioaccumulation. Caco-2 based P_{app} (apparent permeability coefficient) are, in general, positively related to the bioaccumulation potential of chemicals. High P_{app} estimates are frequently observed for bioaccumulating chemicals. Caco-2 based P_{app} depend on intrinsic properties of chemicals. P_{app} can be estimated from QSARs based on physicochemical properties and from LSERs with solvatochromic parameters. P_{app} estimates may be used to get a first impression of the absorption potential of some chemicals. However, the validity of this tool for bioaccumulation screening has to be further evaluated by applying to a larger dataset and by comparing predicted values to measured results including supposedly difficult compounds.

The classification of the bioaccumulation potential of chemicals by Caco-2 based P_{app} was adapted and verified with measured Caco-2. The assessment for possible increased bioaccumulation due to (active) uptake in the GIT is outlined in the following scheme. The screening is based on (estimated) P_{app} with differential thresholds for compounds of different lipophilicity. The next steps are *in vitro* assays. Finally, if necessary, recommendations are given for *in vivo* testing of bioaccumulation with aqueous or dietary exposure.

Metabolism in terms of biotransformation could be expected to lower bioaccumulation. Compounds with a sufficiently high rate of metabolism may be waived from BCF testing. However, estimated biotransformation rates are not suitable to further discriminate chemicals in the critical P_{app} ranges given in the above scheme.

With due regard to parent compounds and their possibly accumulating metabolites, criteria in P_{app} and log K_{ow} may be used to screen for possible effects of specific processes on the bioaccumulation of chemicals in aquatic organisms. If chemicals are already known for specific mechanisms of bioaccumulation, a specific and case-by-case assessment may be needed.



Possible candidates with specific mechanisms of bioaccumulation are organometallic compounds, chemicals reacting covalently with biomolecules under physiological conditions and irritants (H315, H320).

No specific classes of chemicals could be identified with a general and significant increase of bioaccumulation in aquatic organisms. Significant increases of the bioaccumulation (upward deviations by at least one log unit above a reference (TGD) QSAR (Veith et al. 1979)) concern about 7% of the substances. Among the chemical classes that are frequently suspected to behave in particular manners, e.g. organometallic compounds, perfluoroalkyl and polyfluoroalkyl substances (PFAS), an increased bioaccumulation is observed for 5 – 10% of the class members and thus corresponds to the respective portion of substances in the total data set. An exception are the 1,3-branched cyclohexanes with the BCF values of all four class members exceeding the reference model by at least one log unit. Further compounds with upward deviations are often polychlorinated and/or condensed aromatic compounds. Accordingly, considerable BCF underestimation (>1 log unit) is observed for unsaturated hydrocarbons including aromatic and cyclic compounds and for compounds containing Cl heteroatoms. The risk of BCF underestimation for chemicals containing other heteroatoms is lower.

The findings of the present study suggest three areas of future research:

- To further clarify the qualitative and quantitative role of protein binding for bioaccumulation of PFAS, systematic experimental binding studies with different protein and lipid targets would be useful
- To assess the possibility of increased dietary uptake of surfactants due to absorption to food items, quantitative information on the dietary uptake of surfactants would be desirable.
- The validity of Caco-2 based P_{app} as a screening tool for specific mechanisms of bioaccumulation has to be further evaluated by applying to a larger dataset and by comparing predicted values to measured results including supposedly difficult compounds.

2 Zusammenfassung

Spezifische Prozesse, welche die Bioakkumulation von chemischen Substanzen in aquatischen Organismen erhöhen oder reduzieren, können durch intrinsische Eigenschaften der Chemikalien induziert werden. Explorative Datenanalysen zeigen, dass signifikant erhöhte Bioakkumulation verglichen mit der Abschätzung aus log K_{ow} für 7% von 998 untersuchten Substanzen auftritt. Es konnten jedoch keine spezifischen Chemikalienklassen identifiziert werden, die regelmäßig eine signifikant erhöhte Bioakkumulation in aquatischen Organismen aufweisen.

Eine Literaturrecherche wurde durchgeführt, um Prozesse, die zu spezifischer Bioakkumulation von Substanzen führen können, näher zu untersuchen. Folgende Prozesse wurden berücksichtigt:

- Interaktionen mit Proteinen
- Verteilung von Tensiden
- Aktive/passive Aufnahme in den Gastrointestinaltrakt (GIT)

Potentielle Interaktionen (Verteilungskoeffizienten, Geschwindigkeitskonstanten und Bindungsparameter für Interaktionen mit Proteinen, CMC) wurden analysiert für:

- Perfluoralkyl- und polyfluoralkylsubstanzen (PFAS)
- Tenside
- Arzneimittel

Unser wichtigstes Instrument waren explorative Datenanalysen bezüglich verschiedener Modellierungsansätze. Zunächst verglichen wir die verfügbaren Daten für unterschiedliche Prozesse, um Trends zwischen ihnen festzustellen. Zweitens untersuchten wir mögliche Zusammenhänge zwischen den Prozessen und den Eigenschaften der Chemikalien. Drittens betrachteten wir Übereinstimmungen oder Abweichungen der Daten für die unterschiedlichen Prozesse mit/von, z.B., einem log K_{ow} basierten Referenzmodel. Abweichungen von diesem Model wurden als Indikator für mögliche spezifische Beiträge zur Bioakkumulation betrachtet.

Die Ergebnisse zeigen, dass Proteinbindung von log K_{ow} dominiert ist und entsprechend mit log BCF korreliert. Proteinbindung korreliert nicht mit erhöhter Bioakkumulation relativ zum log K_{ow}. Für PFAS stehen keine zuverlässigen log K_{ow} zur Verfügung. Die Unsicherheiten der berechneten log D für PFAS limitieren weitere Schlussfolgerungen hinsichtlich nicht-lipid basierter Akkumulation von PFAS. Interessanterweise zeigt sich dennoch eine (lineare) Korrelation zwischen experimentellen Daten zur Akkumulation von PFAS und berechneten log D/log K_{ow}. Auch ist eine exzellente Übereinstimmung der experimentellen BCF Werte für PFAS mit Feld BAF von Fischen festzustellen. Es gibt jedoch keine Hinweise auf Biomagnifikation von PFAS in aquatischen Organismen. Die explorativen Datenanalysen der verschiedenen Parameter zur Proteinbindung von PFAS stützen nicht die proteinbasierte Akkumulation von PFAS.

Weil die vorliegenden Daten für PFAS nicht ausreichend sind, haben wir anhand eines Datensatzes mit diversen organischen Chemikalien die Beziehungen zwischen Bioakkumulation und Proteinbindung analysiert. Gemäß der etablierten Expertise in der pharmazeutischen und medizinischen Chemie, korreliert Proteinbindung mit log K_{ow} und log BCF. Um die qualitative und quantitative Bedeutung der Proteinbindung für die Bioakkumulation von PFAS zu klären, sollten systematische experimentelle Bindungsstudien mit verschiedenen Proteinen und Lipiden unternommen werden.

Die verfügbaren Daten zur Biokonzentration von Tensiden sind nicht ausreichend um ihr Bioakkumulationspotential einzuschätzen. Die meisten BCF sind unterhalb des Grenzwerts von

2000. Nur für TMAC ist der BCF nahe dem Grenzwert (BCF=1960). Der BCF von Tensiden, außer PFAS, in Fischen korreliert nicht mit log K_{ow} und ist häufig geringer als aus hypothetischen log K_{ow} abgeschätzt. Für verschiedene chemische Klassen grenzflächenaktiver Substanzen scheinen die Länge der Alkylketten oder die Anzahl der Oxyethylen-Einheiten eine Rolle für ihre Aufnahme und Bioakkumulation zu spielen. Die Eignung der CMC als alternatives Maß der Hydrophobie von Tensiden wurde getested, aber eine generelle Eignung konnte nicht festgestellt werden. Hauptsächlich wird die Verteilung und Akkumulation von Tensiden von ihrer Absorption in biologischen Grenzflächen bestimmt. Der Metabolismus von Tensiden kann einen erheblichen Einfluss auf die Akkumulation und Verteilung der Moleküle in Fischen haben, z.B. bei anionischen und nichtionischen Tensiden. Allerdings sind die Informationen zur Biotransformationskinetik sowie der Proteinbindung von anionischen, nichtionischen und kationischen Tensiden in Fischen unzureichend.

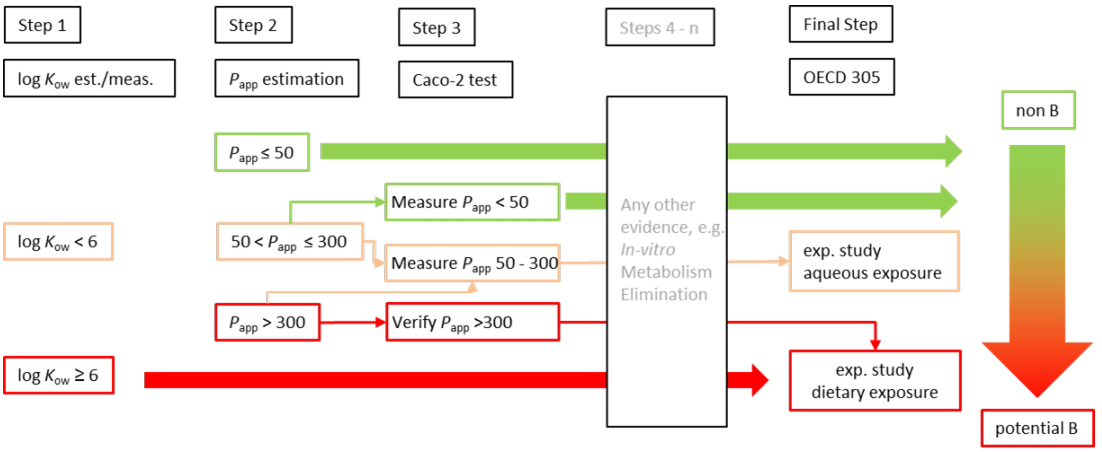
Grenzflächenaktivität von Stoffen kann zur Absorption an Nahrung führen und damit zu einem weiteren Aufnahmepfad. Allerdings sind die quantitativen Informationen zur Akkumulation von Tensiden via Nahrung begrenzt.

Die Aufnahme mittels aktiver und passiver Transportprozesse kann das Ausmaß der Bioakkumulation von Chemikalien bestimmen. Die passive Diffusion korreliert mit dem log K_{ow} der Stoffe. Wenn sekundäre aktive Prozesse (Carrier) zusätzlich zur passiven Diffusion stattfinden, kann die Aufnahme erhöht sein im Vergleich zu dem aus ihrem log K_{ow} abgeleiteten Absorptionspotential. Der primäre aktive Transport betrifft vorwiegend den Efflux von Substanzen aus den Zellen, und ist daher ein Mechanismus, der der Bioakkumulation entgegenwirken kann und so zu einer verringerten Bioakkumulation führen kann.

Caco-2 ist als Model für das Absorptionspotential etabliert, d.h. die Aufnahme von Chemikalien in Organismen. Caco-2 Zellen bieten sowohl aktive als auch passive Absorptionsprozesse. Im Prinzip sind Caco-2 basierte P_{app} (apparent permeability coefficient) positiv mit dem Bioakkumulationspotential von Chemikalien korreliert. Hohe P_{app} treten zumeist bei bioakkumulierenden Stoffen auf. Es ist dabei festzuhalten, dass Caco-2 nicht unbedingt alle Prozesse die zur Bioakkumulation beitragen können abdeckt. Caco-2 basierte P_{app} werden durch intrinsische Stoffeigenschaften bestimmt. P_{app} können mit QSARs aus physikochemischen Eigenschaften und mit LSERs anhand solvatochromischer Parameter abgeschätzt werden. P_{app} können für eine erste Einschätzung des Absorptionspotentials von Chemikalien herangezogen werden. Es ist aber notwendig die Validität dieses Model zum Bioakkumulationsscreening weiter zu prüfen durch Anwendung auf einen größeren Datensatz und den Vergleich von Schätzwerten mit gemessenen Ergebnissen, auch für bekannte Problemstoffe.

Die Klassifizierung des Bioakkumulationspotentials von Chemikalien mittels Caco-2 basierter P_{app} wurde angepasst und mit gemessenen Caco-2 überprüft. Die Einschätzung möglicherweise erhöhter Bioakkumulation aufgrund (aktiver) Aufnahme im GIT ist im folgenden Schema dargestellt. Das Screening verwendet zunächst berechnete P_{app}, differenziert nach der Lipophilie der Verbindungen. Weitere Schritte sind *in vitro* Assays. Wenn nötig, werden Empfehlungen gegeben für die *in vivo* Testung der Bioakkumulation mit Exposition aus der Wasserphase oder dem Futter.

Metabolismus und Biotransformation sollten nur eine geringe Bioakkumulation zulassen. Für Stoffe mit hohem Metabolismus wäre dann die BCF Testung nicht notwendig. Allerdings erwiesen sich berechnete Biotransformationsraten als nicht geeignet um Chemikalien mit P_{app} in den kritischen Bereichen des obigen Schemas weiter zu differenzieren.



Unter Berücksichtigung auch möglicherweise akkumulierender Metabolite, können diese Kriterien zu einem Screening auf mögliche Auswirkungen spezifischer Prozesse auf die Bioakkumulation von Chemikalien in aquatischen Organismen beitragen. Wenn für Chemikalien bereits spezifische Mechanismen der Bioakkumulation bekannt sind, ist eine geeignete Einzelfallprüfung angezeigt. Mögliche Kandidaten sind Organometalle, Stoffe, die kovalent mit Biomolekülen reagieren können oder Reizstoffe (H315, H320).

Stoffklassen, die generell eine signifikant erhöhte Bioakkumulation in aquatischen Organismen aufweisen, konnten nicht festgestellt werden. Deutlich erhöhte Bioakkumulation (Abweichungen von mindestens einer log Einheit oberhalb der Referenz (TGD) QSAR (Veith et al. 1979)) betreffen ca. 7% der Substanzen. Bei Chemikalienklassen, für die üblicherweise spezielle Interaktionen vermutet werden, z.B. Organometalle, perfluoralkyl und polyfluoralkyl Substanzen (PFAS), findet sich eine erhöhte Bioakkumulation bei 5 – 10% der Stoffe, so wie im Gesamtdatensatz. Eine Ausnahme stellen 1,3-verzweigtkettige Cyclohexane dar, die für alle identifizierten Substanzen BCF Werte zeigen, die eine oder mehrere log-Einheiten über dem log K_{ow}-basierten Referenzmodel liegen. Weitere Chemikalien, die im Vergleich zum Model häufig zu höheren BCF Werten führen, sind polychlorierte und/oder kondensierte aromatische Substanzen. Entsprechend wird erhebliche Unterschätzung von BCF (>1 log Einheit) für ungesättigte Kohlenwasserstoffe einschließlich aromatischer und zyklischer Verbindungen sowie für Stoffe mit Cl Heteroatomen beobachtet. Das Risiko der Unterschätzung von BCF ist geringer für Chemikalien mit anderen Heteroatomen.

Die Ergebnisse dieser Studie weisen auf Forschungsbedarf in drei Bereichen:

- Um die qualitative und quantitative Bedeutung der Proteinbindung für die Bioakkumulation von PFAS zu klären, sollten systematische experimentelle Bindungsstudien mit verschiedenen Proteinen und Lipiden unternommen werden.
- Um die Möglichkeit der Aufnahme von Tensiden aufgrund der Absorption an Nahrung besser einschätzen zu können, sind quantitative Informationen zur Akkumulation von Tensiden via Nahrung wünschenswert.
- Die Validität des Caco-2 Models zum Bioakkumulationsscreening sollte durch Anwendung auf einen größeren Datensatz und den Vergleich von Schätzwerten mit gemessenen Ergebnissen, auch für bekannte Problemstoffe, geprüft werden.

3 Introduction

The European chemicals' legislation REACH (European Commission 2006) aims to protect man and the environment from substances of very high concern (SVHC). Chemicals with (very) persistent, (very) bioaccumulative and toxic properties (PBT and vPvB compounds), substances that are carcinogenic, mutagenic and toxic to reproduction (CMR compounds), as well as chemicals of comparable concern (e.g. endocrine disruptors), may be subject to authorization.

Criteria to identify PBT- and vPvB-compounds are given in the REACH regulation (EU) Nr. 253/2011 as Annex XIII (European Commission 2011) and in the Guidance on information requirements and chemical safety assessment (ECHA 2012). Substances with a bioconcentration factor (BCF) >2000 are PBT candidates, and substances with BCF >5000 are vPvB candidates (provided the respective P- and T-criteria are fulfilled). The octan-1-ol/water partitioning coefficient (log K_{ow}; experimentally determined or estimated by valid QSARs) is often used to screen for bioaccumulation potential. According to the ECHA guidance (ECHA 2012 Chapter R.11: PBT Assessment) it is assumed that the substance is not B and not vB if log $K_{ow} \leq 4.5$. However, up to 30% of the B and vB chemicals have log K_{ow} between 3 and 4.5 (Nendza & Müller 2010). Furthermore, according to the guidance care must be taken in case that a substance is known to bioaccumulate by a mechanism other than passive diffusion driven by hydrophobicity. Moreover for some groups of chemicals, such as metals and surfactants, log K_{ow} is not a valid descriptor for assessing the bioaccumulation potential. If there is convincing evidence that a substance can biomagnify in the food chain (e.g. field data indicate that BMF >1) it is likely bioaccumulative (B or vB).

If the screening criteria or other evidence indicate a bioaccumulation potential, an experimental study on the aquatic bioconcentration factor according to OECD test guideline 305 is required. The standard test guideline for aquatic bioaccumulation in fish (OECD 1996) reduced the manifold uptake and elimination mechanisms in aquatic biota to the respiratory absorption via gills and the diffusion through skin. Accumulation along food webs has not been accounted for. Only the current revision of the OECD test guideline 305 (OECD 2012) extended the bioaccumulation assessments with the option to determine biomagnification factors (BMF) from feeding studies as alternatives to the determination of BCF from waterborne exposures.

The bioaccumulation of many chemicals, particularly neutral organic compounds, is directly related to their hydrophobicity expressed as the octan-1-ol/water partition coefficient log K_{ow}. Generally, bioaccumulation increases with increasing hydrophobicity of chemicals. The positive correlation implies analogous partitioning processes between biota or octan-1-ol and water, though other specific mechanisms may be relevant as well. It has been speculated that additional processes may increase the bioaccumulation of chemicals far beyond the extent to be expected from their log K_{ow}. Current screening criteria likely miss such compounds and their risks may not be recognized. Thus, the central question of this project is:

Which additional processes, beyond passive diffusion and accumulation in lipids due to thermodynamic partitioning (e.g. log K_{ow}), contribute to the bioaccumulation of which classes of chemicals and how can they be recognized?

4 Objectives

The goals of the present project were to investigate if, and to what extent, specific mechanisms of uptake, partitioning and distribution, in addition to lipid/water-partitioning, may increase the total bioaccumulation of some chemical substances. We have worked out suggestions how specific mechanisms can be identified and integrated in the bioaccumulation assessment under REACH. Our work was structured in several tasks:

1. Literature search
- Processes and symptoms of specific mechanisms of bioaccumulation
- Classes of chemicals accumulating by (non-)lipid based processes and their properties
2. Analysis of the relevance of specific mechanisms of bioaccumulation relative to the total accumulation of certain chemical substances
3. Compilation of the current scientific status for classes of chemicals with specific mechanisms of bioaccumulation based on structural or physicochemical properties
- Specific protein binding of perfluoroalkyl and polyfluoroalkyl substances (PFAS)
- Consideration of accumulation processes of surfactants
- Specific (e. g. active) mechanisms of uptake
4. Options to integrate specific mechanisms of bioaccumulation in the bioaccumulation assessment under REACH
5. Presentation of the results
- Final report
- Expert workshop
- Publications

5 Materials and methods

5.1 Literature search

We searched the literature for processes and substances involved in specific mechanisms of bioaccumulation using online databases (e.g. google scholar, ISI Web of Knowledge). We retrieved data on 33 endpoints for 892 chemicals regarding:

- Accumulation
- Uptake and absorption
- Pharmacokinetics
- Protein binding
- Active transport

The data set covers a large number of diverse chemicals including many pharmaceuticals, but often with entries for only some of the listed endpoints. The data gaps are due to specific study objectives for the different compounds. For reasons of comparison and reference scaling, we complemented these data with a data set of reliable BCF and log K_{ow} data for 998 chemicals obtained from the EU project OSIRIS (OSIRIS 2007-2011). However, it was possible for only 95

substances to retrieve reliable BCF data as well as data regarding possible specific mechanisms of bioaccumulation. The data base compiled for this project is available as an annex to the electronic version of this report in a separate Excel file.

We analyzed the data in several ways:

- Exploratory data analysis (graphic)
- Correlation analysis (statistical)
- LSER modelling

5.2 Processes and symptoms of specific bioaccumulation mechanisms

5.2.1 Protein binding

The protein binding data address multiple endpoints, covering a variety of different proteins. Most researchers use serum albumins in their experiments, bovine (BSA) more often than human (HSA). Specific proteins like rat liver fatty acid binding protein (L-FABP) are used depending on the study objectives.

The most often used endpoints for **interactions of chemicals with proteins** are protein/water partition coefficients and protein binding affinities. In the following, we give an overview of the published data sets used in the exploratory data analyses presented in section 6.2.

Binding to bovine serum albumin

Protein/water partition coefficients

- Endo & Goss 2011: experimental data (1.3: log $K_{BSA/w}$ (37 °C)) and data collected from diverse literature sources (1.3: log $K_{BSA/w}$)
- DeBruyn & Gobas 2007: literature data compilation of protein (albumin) binding data (1.3: log (protein-water partition coefficient))
- Bischel et al. 2010 ; 2011: protein/water partition coefficients (1.4: log K_{pw})

Binding affinities

- MacManus-Spencer et al. 2010: secondary association constants at lower (1.4: BSA (1 µM) log k_1) or higher BSA concentrations (1.4: BSA (10 µM) log k_1)

Binding to human serum albumin

Binding affinities

- Valko et al. 2003: affinity constants determined from HPLC retention times (1.3: log K_{HSA})

Binding to rat liver fatty acid binding protein

Binding affinities

- Woodcroft et al. 2010: interaction with recombinant rat L-FABP at pH 7.4 (1.4: log(K_{i1}) (µM))

To be able to compare the protein binding with **lipid based interactions**, we complemented the data set with:

- Bioconcentration factors: experimentally determined and validated log BCF (Nendza et al. 2015)

- Octan-1-ol/water partition coefficients: calculated consensus log K_{ow} (ChemProp, UFZ 2014)
- Affinity to phospholipids: interaction with an immobilized artificial membrane (1.3: log CHI IAM, Valko et al. 2003)

5.2.2 Absorption of Surfactants

Bioconcentration data for surfactants have been collected and critically reviewed by Tolls et al. (1994) and Tolls (1998). These studies quantified the bioconcentration of surfactants by the concentration ratio (CR) in order to refer to bioconcentration data unless there was evidence that they pertain to the definition of the BCF. The prediction of the bioaccumulation potential of surfactants by their log K_{ow} or critical micelle concentration (CMC) was investigated based on the log K_{ow} / log BCF and log CMC / log BCF relationships. The correlation of BMF estimates for PFAS with hydrophobicity measures was determined. The absorption of surfactants is described in detail in section 6.3.

5.2.3 Gastrointestinal absorption

5.2.3.1 General aspects of gastrointestinal absorption processes

Biomagnification following dietary exposure can contribute significantly to the bioaccumulation of chemicals, especially of highly lipophilic compounds. Gastrointestinal absorption processes may also influence the uptake of some chemicals and thus their bioaccumulation potential.

The general principles of gastrointestinal absorption processes were elaborated based on current literature. The literature search comprised scientific books, reviews and journal articles.

The available literature mostly focuses on known gastrointestinal absorption principles in human and rodents. Only few publications deal with these principles in fish. Nevertheless for most of the known absorption mechanisms in humans / rodents also analogues in aquatic organisms (fish) could be found. An overview of these gastrointestinal absorption processes and their occurrence in different species is presented in chapter 6.4.

Experimental data of gastrointestinal uptake are mostly available for pharmaceuticals. Since the definitions of the experimental endpoints for substances absorbed from the gastrointestinal tract differ between the cited references, they are summarized briefly in the following:

Human intestinal absorption = % HIA

The human intestinal absorption is the percentage of the orally administered molecule which is absorbed through the gastrointestinal tract and will be systemically available, i.e. in the blood system.

% Absorption

Fraction absorbed compared to the administered dose.

% oral dose absorbed

The fraction of an orally administered dose that is systemically available.

% Excretion

Fraction of an administered dose that is excreted via the urine / faeces.

(oral) bioavailability

The amount and the time in which an active ingredient is absorbed from the gastrointestinal tract and is present in the systemic circulation. Here only the metabolically unchanged drug is considered.

For intravenously administered drugs the bioavailability is defined as 100%.

The absolute bioavailability of a substance is defined as the bioavailability of a substance by any non-systemic way, compared to the intravenous administration.

Caco-2

The Caco-2 is widely used as an *in vitro* model to study intestinal absorption processes. The Caco-2 cell line was isolated in 1977 by J. Fogh from a human colon adenocarcinoma. With this *in vitro* model it is possible to address various questions regarding the gastrointestinal permeability of new drugs. It is possible to predict the absolute bioavailability, as well as to elucidate structure-transport relationships.

The Caco-2 cell line features both active and passive absorption processes. In addition, different P450 isoenzymes and phase II enzymes are expressed by the Caco-2 cells, so that the (presystemic) metabolism of compounds can be examined as well.

In a typical Caco-2 experiment the cell monolayer (A = apical site, B = basolateral site) is surrounded by 2 buffering systems. On the apical site the cell has a brush border membrane and the Tight Junctions. A brush border is the name for the microvilli covered surface of epithelium cells found in certain locations of the body. Cells that absorb substances need a large surface area in contact with the substance to be efficient. The term brush border is due to the morphology of the cells that appear very much like the bristles of a paintbrush in electron microscopy.

Caco-2 experiments measure the amount and the time that substances take to cross the cell monolayer in direction A to B or vice versa.

The measurement unit is P_{app} in cm/s.

A rough classification of Caco-2 permeability is given according to Pham-The et al. (2013)

- $P_{app} < 0.7 \times 10^{-6}$ cm/s low permeability
- 0.7×10^{-6} cm/s $< P_{app} < 16 \times 10^{-6}$ cm/s medium permeability
- $P_{app} > 16 \times 10^{-6}$ cm/s high permeability

PAMPA

PAMPA (Parallel Artificial Membrane Permeation Assay) is an assay for estimating the permeability of compounds across membranes. PAMPA is based on the simulation of membranes by mixing of lipids with organic solvents. The artificial membrane is blotted on a filter between two buffer systems (donor and acceptor). For determining the permeability of a compound, both buffer systems are analyzed for the presence of the test substance after an incubation period of about 18 hours (Vögtli 2010). Since the membrane is artificial, neither active transporter nor isoenzymes responsible for metabolism are expressed. Therefore, this assay is more likely to predict passive transport processes (van de Waterbeemd 2007).

5.2.3.2 Exploratory analysis for screening level estimation of Caco-2 based P_{app} values

Uptake by active and passive transport processes can be identified *in vitro* using the Caco-2 assay. Increased absorption of molecules may lead to higher bioaccumulation and thus systematic deviations from the dependency of the BCF on the octan-1-ol/water partition coefficient. High Caco-2 based partitioning values P_{app} may indicate an increased gastrointestinal absorption potential of compounds.

In order to evaluate Caco-2 data as an indicator of increased bioaccumulation potential, a data set with both experimental P_{app} from Caco-2 and bioaccumulation data for each compound is required. From the EU project OSIRIS a reliable BCF dataset of 998 chemicals is available (OSIRIS 2007-2011. Unfortunately the corresponding P_{app} data overlap for these 998 chemicals is rather low. Therefore, the approach followed here was to identify a few key descriptors enabling a crude approximation of Caco-2 based P_{app} values, and then to use these rough data to inspect deviations of the log K_{ow} / log BCF relationship for the validated BCF set.

For descriptor, either experimental octan-1-ol/water partition coefficients were applied when available, or predicted values were used otherwise. Furthermore, the selection of other descriptor candidates was restricted to features that can be calculated or estimated purely from the chemical structure. However, properties with existing and easily accessible data compilations were preferred. The relationship between bioaccumulation and Caco-2 based partitioning values P_{app} are described in chapter 6.4.4.

A screening level tool was developed which may be used to get a first impression of the absorption potential of some chemicals. However, the validity of this tool has to be further evaluated by applying to a larger dataset and by comparing predicted values to measured results including supposedly difficult compounds.

5.3 QSAR and LSER modelling

Different mechanisms of bioaccumulation of chemical substances are likely due to different physico-chemical properties. Knowing the relevant physicochemical properties and their relative contributions to the respective rate-limiting processes is important to understand the mechanisms. QSARs are established tools to describe the relationships between the physicochemical properties and observed effects of chemicals.

Since experimentally determined data on physicochemical properties are often lacking, we obtained estimates with ChemProp[1] (UFZ 2014), EpiWeb[2], SPARC[3], ACD/LABs and ChemAxon via ChemSpider[4] and T.E.S.T.[5]. Only if inside the applicability domain of the models, we considered the estimates to be reliable (Kühne et al. 2009). If possible, we used several independent algorithms to obtain multiple estimates of the same property and then calculated the (geometric) mean (consensus modelling) for the most reliable *in silico* results. We obtained partition coefficients for different media, e.g.,

[1] UFZ Department of Ecological Chemistry 2014. ChemProp 6.1; http://www.ufz.de/index.php?en=6738

[2] http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm

[3] http://ibmlc2.chem.uga.edu/sparc/

[4] http://www.chemspider.com/

[5] http://www.epa.gov/nrmrl/std/cppb/qsar/index.html

- log K_{ow} (octan-1-ol/water)
- log K_{oa} (octan-1-ol/air)
- log K_{mw} (membrane/water)
- log K_{pw} (protein/water)

(Sub-)structures, (extra)thermodynamic and solvatochromic descriptors were used to assess the effects of the size and dimensionality, polarity, solubility and volatility of chemicals in different (bio)phases. We used LSER models to analyze in detail the relevant contributions of hydrogen bonding acceptors and donors as well as the influence of molecular size on the partitioning of substances between phases with different properties, e.g. lipid/water, membrane/water, protein/water. Simplified LSER models take the general form:

Log BCF = $a\ A + b\ B + v\ V + c$ (Equation 1)

with A = hydrogen bonding donor capacity, B = hydrogen bonding acceptor capacity, V = intrinsic (characteristic) molecular volume, and a, b, v = regression coefficients, c = intercept (e.g. Taft et al. 1985, Kamlet et al. 1988a,b, Abraham et al. 1989, Hickey & Passino-Reader 1991, Schüürmann et al. 2006).

5.3.1 Reference (TGD) model for log BCF/log K_{ow} relationships

To test the hypothesis of non-lipid based bioaccumulation of chemicals, we looked for deviations of their bioaccumulation from lipid based models in terms of log K_{ow} based QSARs. This approach required to obtain a reference model for log BCF/log K_{ow} relationships.

A previous project (Müller & Nendza 2011) has reviewed available methods and tools for estimating the BCF of pesticides and biocides with a focus on the applicability for regulatory purposes. The report compiled, compared and discussed 15 log K_{ow} based QSARs that are well established and essentially valid for neutral organics of intermediate hydrophobicity ($0 < \log K_{ow} < 6$). The study included the QSAR from Veith et al. (1979) that was recommended according to the EU Technical Guidance Document on Risk Assessment (European Commission 2003).

Most of the log K_{ow} based QSARs are very similar in terms of slope and intercept, though the models have been developed with different datasets (different compound classes), for different ranges of log K_{ow}, for different species and according to different test protocols. The recent ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7c: Endpoint specific guidance (2014a) recommends 4 commonly used QSAR models for predicting fish BCFs. The first is the linear model from Veith et al. (1979):

$\log BCF = 0.85 \log K_{ow} - 0.70$ (Equation 2)

(n = 55, r = 0.95, species: *Pimephales promelas*, chemicals: heterogeneous dataset, range of log K_{ow}: 1 – 7.05)

Equation 2 has been derived more than thirty years ago, well before QSAR quality criteria (OECD 2007) have been established. Formal analysis (Müller & Nendza 2011) revealed that the model violates at least three of the five agreed OECD principles, namely *1. a defined endpoint* [different test species, different exposure times and regimes, different methods of quantification of test substances (specific analysis of parent compound vs. total radioactivity) and arbitrary correction factors applied to some data], *2. an unambiguous algorithm* (the model is not completely reproducible), *3. a defined domain of applicability* (the training set is not

clearly defined). Still, Equation 2 can be considered laterally validated based on its good agreement with other QSARs, see Müller & Nendza 2011. This and its regulatory acceptance (ECHA 2014, 2014a), prompted to select Equation 2 as reference (TGD) model for log BCF/log K_{ow} relationships for the purposes of this study.

6 Results and Discussion

6.1 Chemicals / chemical classes and their properties

6.1.1 Quantitative relevance of increased bioaccumulation

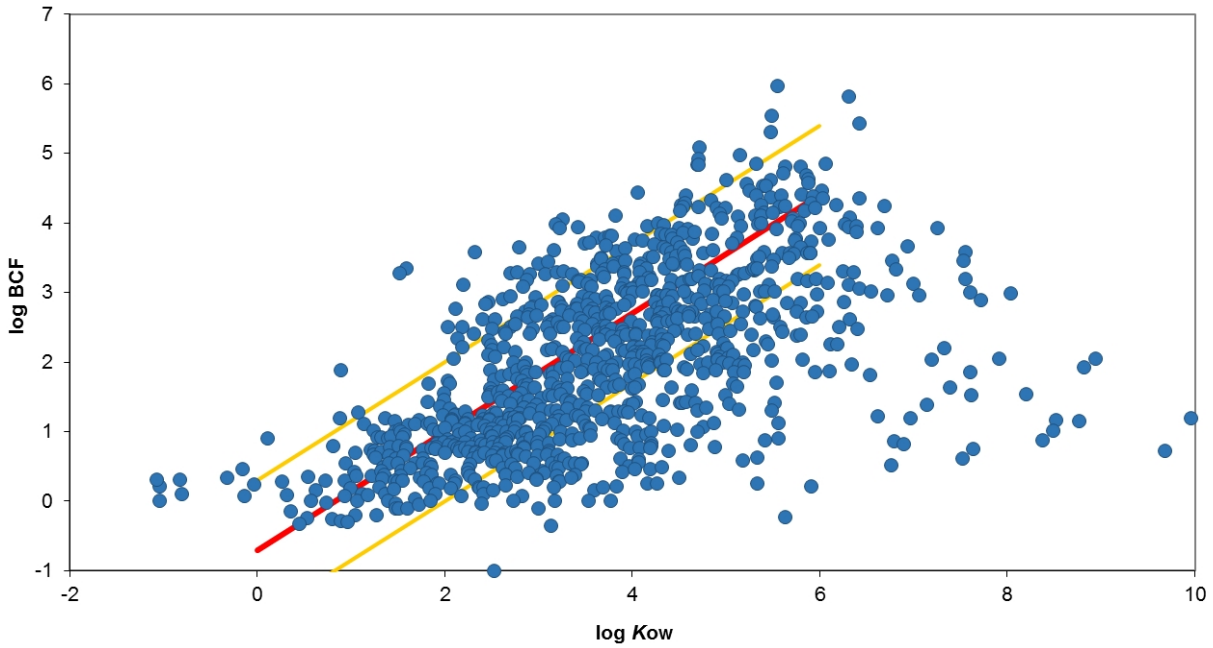
Intrinsic properties of chemicals may induce specific processes that may increase or reduce the bioaccumulation of these substances in aquatic organisms:

- Protein binding
- Active uptake
- Ionisation
- Metabolism

How large is the portion of substances with specific intrinsic properties which lead to significantly increased bioaccumulation in aquatic organisms?

Hydrophobicity has emerged as the key parameter for assessing the potential environmental impact of contaminants and constitutes a measure of the preference of a substance for either aqueous or non-aqueous phases. The partitioning between compartments of different polarity determines the rate and the direction of the transport of chemicals in the environment and thus their accumulation in some of its components. The octan-1-ol/water partition coefficient (log K_{ow}) quantifies the thermodynamic partitioning of a compound between an aqueous and an organic phase (octan-1-ol), which is considered to simulate natural hydrophobic phases (e.g. membranes) (Nendza 1998). However, log K_{ow} can describe multiple processes. On the one hand it accounts for the passive diffusion and, on the other hand, may as well contribute size and solubility related aspects of active transport. Deviations of BCF values of chemicals higher than estimates from their log K_{ow} values may quantify the possible extent of specific mechanisms of bioaccumulation in addition to water/lipid partitioning. Systematic upward deviations are to be expected if additional interactions increase the total bioaccumulation beyond the common log BCF/log K_{ow} relationships (reference model). We explored the general relationship with the example of 998 substances with validated experimental BCF data for a broad range of organic compound classes obtained from the EU project OSIRIS (OSIRIS 2007-2011) (Nendza et al. 2015). In Figure 1, we observe substantial scatter in both directions relative to the log K_{ow} based QSAR model by Veith et al. 1979 (reference (TGD) model (European Commission 2003)). However, downward scatter, i.e. BCF values are lower than expected from the log K_{ow} of the chemicals, is much more frequent than upward scatter, i.e. BCF values are higher than expected from the log K_{ow} of the chemicals. We observe 82 compounds with BCF values exceeding the log K_{ow} estimates by one order of magnitude or more (NOTE: deviations less than one log unit are likely due to experimental variability (Nendza et al. 2010)). Among the 82 compounds are 8 substances with measured and predicted BCF <10 and another 3 substances with measured and predicted BCF <100 and thus without regulatory relevance. **In conclusion, the** log K_{ow} **based QSARs may underestimate the BCF for about 7% of the substances.**

Figure 1: Exploratory data analysis of 998 substances with validated experimental BCF data (OSIRIS 2007-2011) relative to a log K_{ow} based QSAR model (Equation 2) by Veith et al. (1979).

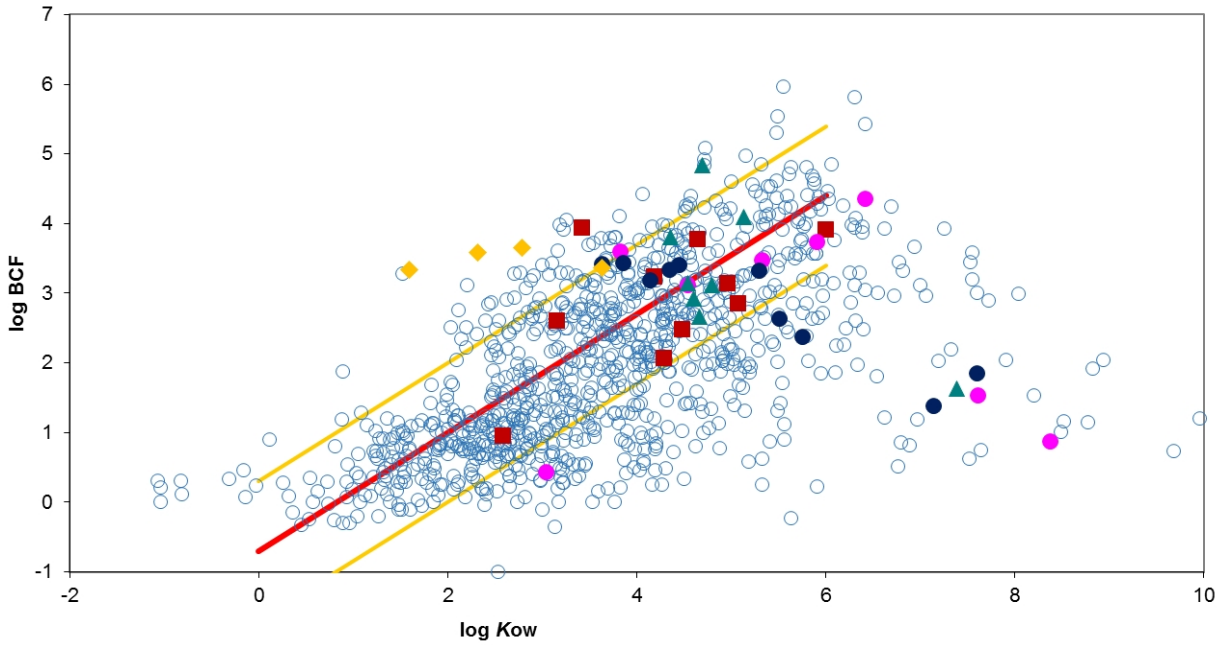


Red line: reference (TGD) model (Veith et al. 1979, European Commission 2003). Scatter of data points within the yellow lines (margins of one log unit) are likely due to experimental variability. Deviations above the upper yellow line indicate the possibility for additional specific mechanisms of bioaccumulation. Deviations below the lower yellow line indicate processes reducing the bioaccumulation of substances, e.g. biotransformations.

6.1.2 Which classes of chemicals are likely to exhibit significant increase of their bioaccumulation in aquatic organisms?

The exploratory data analysis presented in Figure 1 was detailed with regard to chemical classes that are frequently suspected to behave in particular manners, e.g. organometallic compounds, perfluoroalkyl and polyfluoroalkyl substances (PFAS), triphenyl phosphates, compounds with alkyl chains >6 carbon atoms, 1,3-branched cyclohexanes. Figure 2 shows that the BCF values of these compounds are generally high, mostly above 1000, but only one chemical of each of these classes reveals significantly increased BCF above the reference model. The other members of these classes have bioaccumulation about or below the reference model. Thus the portions of outliers with increased BCF above the reference model are the same within the suspicious classes of chemicals as in the total data set. The only observed exception are the **1,3-branched cyclohexanes** with all four class members having BCF values being one or more log-units above the estimates by the reference model. Other compounds with upward deviations are often **polychlorinated and/or condensed aromatic compounds**.

Figure 2: Exploratory data analysis of 998 substances with validated experimental BCF and log K_{ow} data relative to a log K_{ow} based QSAR model (Equation 2) by Veith et al. (1979) by chemical classes.



Red line: reference (TGD) model (Veith et al. 1979, European Commission 2003). Chemical classes that are frequently suspected to behave in particular manners are indicated, e.g. organometallic compounds (red squares), perfluoroalkyl and polyfluoroalkyl substances (PFAS) (pink circles), triphenyl phosphates (green triangles), compounds with alkyl chains >6 carbon atoms (d'blue circles), 1,3-branched cyclohexanes (yellow diamonds). The yellow lines indicate one log unit deviations above or below the reference model.

The most important findings of our exploratory data analyses on chemicals with possible increase of their bioaccumulation in aquatic organisms can be summarized as follows:

- Significant increases of their bioaccumulation (upward deviations by at least one log unit above a reference (TGD) QSAR (Veith et al. 1979, Equation 2)) concern about 7% of the substances.
- Among the chemical classes that are frequently suspected to behave in particular manners, e.g. organometallic compounds, perfluoroalkyl and polyfluoroalkyl substances (PFAS), an increased bioaccumulation is observed for 5 – 10% of the class members and thus corresponds to the respective portion of substances in the total data set.
- An exception are the 1,3-branched cyclohexanes with the BCF values of all four class members exceeding the reference model by at least one log unit.
- Further compounds with upward deviations are often polychlorinated and/or condensed aromatic compounds.

6.1.3 Chemical classes

For the data set of 998 compounds with validated experimental BCF values, the deviations from the Veith et al. (1979) QSAR (Equation 2) were categorized into three classes. Most concern relates to compounds with an underestimation of log BCF by more than 1 log unit. The class with deviations less than 1 log unit covers the well predicted compounds including data variability.

The class with downward deviations below -1 log unit relates to the compounds with an overestimation of log BCF by Equation 2.

After exclusion of compounds with experimental BCF below 200 corresponding to one order of magnitude below the B threshold, this classification has been separately performed by compound classes. The results are listed in Table 6- 1.

Unsaturated hydrocarbons including aromatic and cyclic compounds turned out to be the most problematic with regard to BCF underestimation. Also predictions for compounds containing Cl heteroatoms should be used with caution.

Chemicals containing other heteroatoms tend to be more polar and typically bioaccumulate even less than predicted from log K_{ow}.

Table 6- 1: Classification of the deviations of log BCF predictions by Veith et al. (1979) in terms of log $BCF_{experimental}$ – log $BCF_{predicted}$.

Chemical class	>1	-1...1	<-1
1 Hydrocarbon	35 (34%)	65 (64%)	2 (2%)
1.1 Nonaromatic	19 (54%)	14 (40%)	2 (8%)
1.1.1 Saturated, alkyl chain	-	6 (75%)	2 (25%)
1.1.2 Saturated, alkyl ring	10 (83%)	2 (17%)	-
1.1.3 Double bonds	9 (60%)	6 (40%)	-
1.2 Aromatic	16 (24%)	51 (76%)	-
1.2.1 Single aromatic ring	5 (22%)	18 (88%)	-
1.2.2 Biphenyl type	2 (29%)	5 (71%)	-
1.2.3 Fused aromatic rings	9 (24%)	28 (76%)	-
2 Halogen hydrocarbon	23 (23%)	78 (78%)	3 (<1%)
2.1 Chlorine	19 (24%)	59 (76%)	2 (<1%)
2.1.1 Nonaromatic	6 (55%)	5 (45%)	-
2.1.1.1 Saturated, alkyl ring	1 (50%)	1 (50%)	-
2.1.1.2 Double bonds	5 (55%)	4 (45%)	-
2.1.2 Aromatic	13 (19%)	54 (79%)	1 (2%)
2.1.2.1 Single aromatic ring	3 (15%)	17 (85%)	-
2.1.2.2 Biphenyl type	7 (18%)	33 (82%)	-
2.1.2.3 Fused aromatic rings	3 (38%)	4 (50%)	1 (12%)

Chemical class	>1	-1...1	<-1
2.2 Bromine	2 (11%)	15 (83%)	1 (6%)
2.1.1.1 Saturated, alkyl chain	-	1 (100%)	-
2.1.1.2 Saturated, alkyl ring	1 (100%)	-	-
2.2.2 Aromatic	1 (6%)	14 (88%)	1 (6%)
2.2.2.1 Single aromatic ring	-	11 (100%)	-
2.2.2.2 Biphenyl type	1 (7%)	13 (86%)	1 (7%)
2.3 Iodine	-	1 (100%)	-
2.4 Mixed halogen	2 (33%)	4 (67%)	-
3 Oxygen	4 (4%)	87 (76%)	23 (20%)
3.1. Without halogen	2 (5%)	33 (87%)	3 (8%)
3.1.1 OH group	-	17 (94%)	1 (6%)
3.1.2 Ether/furane	1 (11%)	8 (89%)	-
3.1.3 Peroxid	-	2 (100%)	-
3.1.4 Ketone/quinone	-	4 (100%)	-
3.1.5 Acid	-	1 (50%)	1 (50%)
3.1.6 Ester	1 (33%)	1 (33%)	1 (33%)
3.2 With halogen	2 (2%)	56 (72%)	20 (26%)
3.2.1 OH group	-	4 (67%)	2 (33%)
3.2.2 Ether/furane	2 (3%)	47 (71%)	17 (26%)
3.2.3 Acid	-	1 (100%)	-
3.2.4 Ester	-	2 (100%)	-
3.2.5 Mixed O groups	-	2 (67%)	1 (33%)
4 Nitrogen	-	21 (95%)	1 (5%)
4.1.1 Amine (incl. halogen)	-	11 (92%)	1 (8%)
4.1.2 Nitrile	-	2 (100%)	-
4.1.3 Hydrazine	-	2 (100%)	-
4.1.4 Aromatic azine	-	4 (100%)	-

Chemical class	>1	-1...1	<-1
4.1.5 Azol	-	3 (100%)	-
4.1.6 Other	-	2 (100%)	-
4.1.7 Mixed	-	1 (100%)	-
4.2 With O and N	1 (3%)	21 (72%)	7 (24%)
4.2.1 Separate O and N groups	-	14 (74%)	5 (26%)
4.2.2 N-C=O groups	1 (25%)	3 (75%)	-
4.2.3 NO groups	-	20 (91%)	2 (9%)
5 Sulfurus	-	8 (80%)	2 (20%)
5.1 No other heteroatom	-	4 (67%)	2 (33%)
5.2 With N	-	4 (100%)	-
5.3 SO groups	2 (25%)	6 (75%)	-
5.4 With O and N	1 (20%)	4 (80%)	-
6 Phosphorus	3 (10%)	21 (72%)	5 (17%)
6.1 With O	2 (14%)	9 (64%)	3 (21%)
6.3 With S	-	6 (75%)	2 (25%)
6.4 SO groups	1 (100%)	-	-
6.5 With other atom types	1 (9%)	8 (73%)	2 (18%)
6.5.1 No O, N, S, P	-	4 (100%)	-
6.5.2 With O	1 (20%)	2 (40%)	2 (40%)
6.5.3 With N	-	1 (100%)	-

The most important findings of our exploratory data analyses on chemical structures can be summarized as follows:

- Considerable BCF underestimation (Column “>1”) is likely for unsaturated hydrocarbons including aromatic and cyclic compounds and for compounds containing Cl heteroatoms.
- The risk of BCF underestimation for chemicals containing other heteroatoms is lower.

6.2 Protein binding

6.2.1 Perfluoroalkyl and polyfluoroalkyl substances (PFAS)

Interactions of chemicals with proteins have been hypothesized to be an important mechanism of bioaccumulation causing higher levels of contaminants in organisms than estimated from their log K_{ow}. Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are fluorosurfactants (Buck et al. 2011, Stahl et al. 2011) and have been the frequently named examples for high bioaccumulation due to protein binding. For comparative analyses, we collected experimental BCF and field BAF for PFAS from the literature (Martin et al. 2003b; Arnot & Gobas 2006; de Voogt et al. 2006; Jeon et al. 2010) as well as various parameters of protein binding (Valko et al. 2003; DeBruyn & Gobas 2007; MacManus-Spencer et al. 2010; Woodcroft et al. 2010; Bischel et al. 2011; Endo & Goss 2011).

To test the hypothesis of non-lipid based bioaccumulation of PFAS, we first looked for deviations of the bioaccumulation of PFAS from lipid based models in terms of log K_{ow} based QSARs. This approach required to obtain log K_{ow} data for PFAS at biologically relevant conditions. However, it is difficult to obtain good log K_{ow} data and other partitioning descriptors of PFAS for several reasons.

- PFAS are strong acids with low pK_a values. They are completely ionized at pH values above 5. In bioaccumulation studies with fish, the pH values in different compartments are mostly above 7. For example, the pH value of the test medium (water) of bioaccumulation studies with fish is generally about 8. The pH value in the blood of, e.g., rainbow trout is also about 8 at 12-14°C (Paul 2001). The pH values in muscle tissues of brown trout are approx. 7.2 (Butler & Day 1993). The pH values at the outside of gill membranes can be about 0.4 pH units lower than the surrounding water, i.e. about 7.6. In case of weak organic acids, the lower pH may cause a local increase of the fraction of the unionised form and thus slightly increase their uptake (Fent 2003). In the case of strong acids like PFAS, only the ionized forms are expected in the aqueous phases at the outside and within the fish.
- Only few experimental log K_{ow} data are available for PFAS and these are highly variable due to ionization and surfactant properties of PFAS. Reliable experimental log K_{ow} data or other partitioning descriptors of PFAS as points of reference at biologically relevant conditions ('Gold Standard') are not available.
- PFAS are not covered by the applicability domains of the established computational models to estimate log K_{ow}. The reason is the lack of reliable experimental data for training sets of models. Therefore, fragment approaches must extrapolate from structures with only few fluorine atoms and lack the interaction terms for poly- and perfluorinated compounds. Likewise, LSER models are only based on calculated descriptors since the respective experimental data have not been obtained. To this end, the log K_{ow} estimates from fragment approaches and LSER models are both extrapolations outside their applicability domains and uncertainties (much) larger than ± 0.5 log units have to be expected. Still, both methods result in similar estimates, see Table 6- 2.
- The currently best approach to estimate log K_{ow} of PFAS is consensus modelling, i.e. the mean of independent estimates by, e.g., vcclab (Webster & Ellis 2011), ChemProp (UFZ 2014) and ACD (2015), to compensate for conflicting results of individual algorithms.
- The established approach to quantify the partitioning of ionisable substances is with the Henderson-Hasselbalch equation. It calculates log D, the partition coefficient at a

defined pH value, accounting for the lower log K_{ow} of the fraction of the ionized molecules relative to the higher log K_{ow} of the fraction of the unionized molecules. The log D is also termed distribution coefficient. However, due to the complete dissociation (i.e. dissociation rate = 100%) of PFAS at biologically relevant pH values, the Henderson-Hasselbalch equation is not meaningful to calculate log D of PFAS at pH >5. Note that log D values of PFAS do not differ for pH 7.2 (fish muscle tissue), pH 7.6 (outside of fish gills) and pH 8 (fish blood and surrounding test medium).

- The currently best approximation of the log D values of the ionized forms of PFAS at pH >5 is to subtract a correction constant of -3.75 (ACD 2015) from the log K_{ow} values of the unionized forms of PFAS. This procedure is a gross but educated guess of the log D values of the ionized forms of PFAS based on expert judgment, see Table 6- 2.

Table 6- 2: Physicochemical properties, bioaccumulation and protein binding data of PFAS.

CAS	Name	log K_{ow} (cons.)[1]	log K_{ow} (LSER)[2]	log D (pH >5)[3]	pK_a[4]	log BCF[5]	log BAF[6]	BMF[7]	log K_{PW}[8]	log k_1 BSA (1 µM)[9]	log k_1 BSA (10 µM)[9]	log Ki_1 L-FABP (µM)[10]	SHBG displ. log EC10 (mM)[11]
375-22-4	PFBA	2.18	1.94	-1.57	0.37								3.68
2706-90-3	PFPA	2.83	2.69	-0.93	0.40				3.40			2.78	
307-24-4	PFHxA	3.44	3.42	-0.31	0.42			0.16	4.05			2.42	
375-85-9	PFHpA	4.20	4.19	0.45	0.47				4.23			1.79	
335-67-1	PFOA	4.90	4.88	1.15	0.50	2.04	1.64	0.04	4.14	5.18	4.52	1.12	3.63
375-95-1	PFNA	5.72	5.64	1.97	0.52				4.05	5.78	4.69	0.78	
335-76-2	PFDA	6.45	6.40	2.70	0.52	3.28	3.36	0.23	3.86	5.48	5.26		3.40
2058-94-8	PFUnDA	7.12	7.13	3.37	0.52	3.85	3.70	0.28	3.70	4.30	5.48		
307-55-1	PFDoDA	7.74	7.89	3.99	0.52	4.40		0.43	3.30				
72629-94-8	PFTrDA	8.37	8.61	4.62	0.52								
376-06-7	PFTeDA	9.13	9.35	5.38	0.52	4.44		1.00					
67905-19-5	PFHxDA	9.34	10.84	5.59	0.52								
16517-11-6	PFODA	7.30	9.85	3.55	4.78								
375-73-5	PFBS	2.01	0.98	-1.75	-3.57				3.86				>3.9
355-46-4	PFHxS	3.10	2.43	-0.66	-3.34	1.79			4.30				>3.9
1763-23-1	PFOS	4.28	3.92	0.53	-3.27	3.55	3.72	0.37	4.10				3.55

[1]: Mean of the estimates by vcclab (Webster & Ellis 2011), ChemProp (UFZ 2014) and ACD (2015)
[2]: LSER-Modell from Abraham et al. 1994
[3]: log D = log $K_{ow(cons.)}$ - 3.75 (ACD 2015)

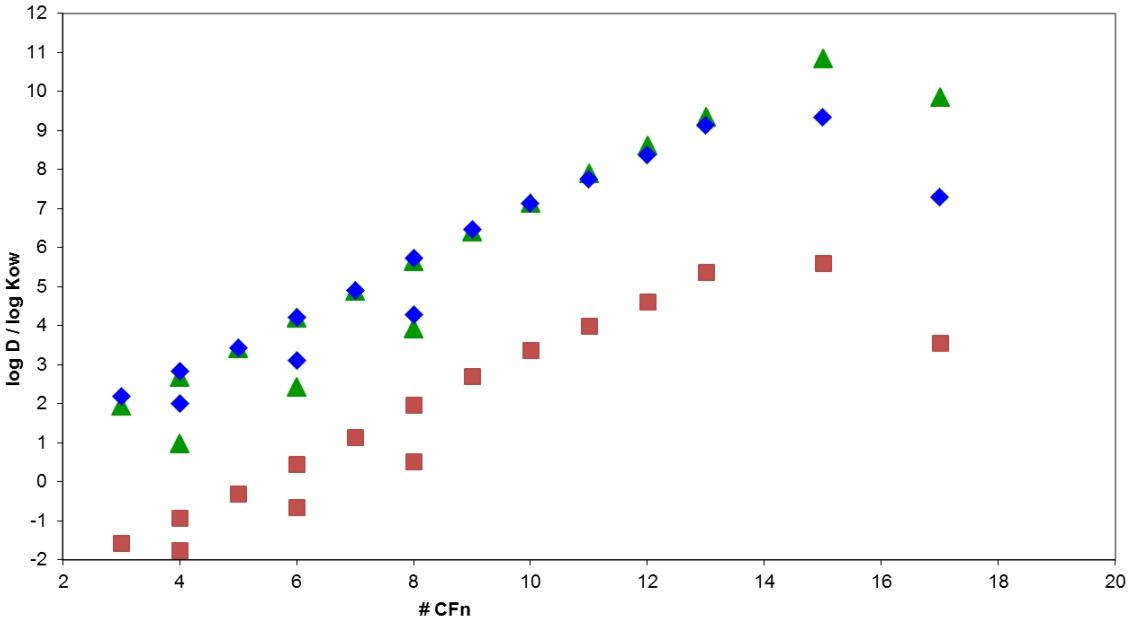
[4]: pKa classic from ACD 2015
[5]: Average experimental BCF from Martin et al. 2003b and Jeon et al. 2010
[6]: Field BAF for fish from de Voogt et al. 2006
[7]: Average experimental BMF from Martin et al. 2003a and Goeritz et al. 2013
[8]: Protein (BSA)/Water partition coefficient from Bischel et al. 2011
[9]: Secondary association constants at lower (1 µM) or higher (10 µM) BSA concentrations from MacManus-Spencer et al. 2010
[10]: Interaction with recombinant rat L-FABP at pH 7.4 (log(K_{i1}) (µM)) from Woodcroft et al. 2010
[11]: Estimated *in vivo* carp (*Cyprinus carpio*) serum estradiol displacement from sex-hormone binding globulins (SHBGs) EC10 (mM) from Jones et al. 2003

The data used for the analysis of the bioaccumulation of PFAS and the possible contributions of protein binding are listed in the following Table 6- 2. Results of the exploratory data analyses are presented in Figure 3 to Figure 10.

The exploratory data analyses addressed several issues:

- We compared the log K_{ow} (unionized) and the log D (ionized) of PFAS (Figure 3) to look for differences of the hydrophobicities of unionized and ionized PFAS and observed:
  (1.) The log K_{ow} estimates from fragment approaches and LSER models for the unionized forms of PFAS are in excellent agreement with a tendency of the LSER-derived data to smaller values at the lower end and higher data at the upper end of the scale. Overall, both methods appear to capture the partitioning of PFAS equally well and may thus be used interchangeably.
  (2.) The log D estimates are in the same rank order as the log K_{ow} estimates because a constant correction term was used to calculate log D from log K_{ow}. The PFAS are completely ionized at pH values above 5 and the degree of ionization of the compounds under conditions relevant to bioaccumulation in fish is 100%.
  (3.) The constant correction term of -3.75 (ACD 2015) to convert the log K_{ow} values of the unionized forms of PFAS to the log D of the respective ionized forms results in an even downward shift of the data points.
  (4.) The analysis of the log D (ionized) of PFAS, as compared to log K_{ow} (unionized), does not allow to differentiate non-lipid based accumulation.

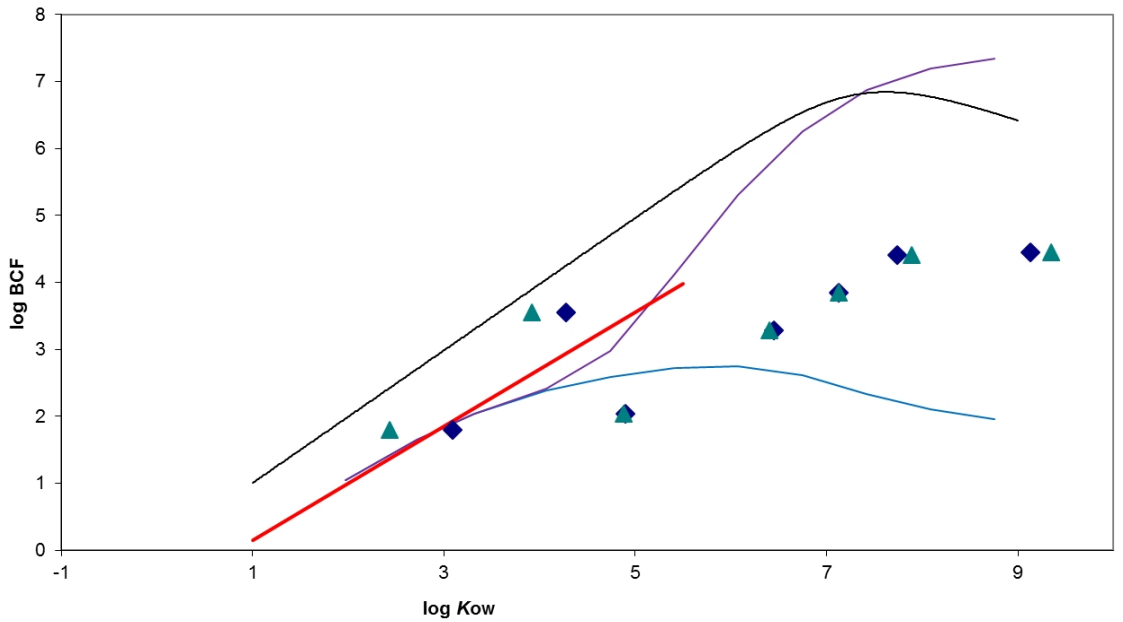
Figure 3: Comparison of log K_{ow} (unionized) and log D (ionized) of PFAS.



Exploratory data analysis of the hydrophobicity parameters of PFAS: mean log K_{ow} (blue diamonds) by vcclab (Webster & Ellis 2011), ChemProp (UFZ 2014) and ACD (2015); LSER based log K_{ow} (green triangles) from Abraham et al. 1994; log D (brown squares) calculated from log K_{ow}; # CFn: number of fluorinated carbons in PFAS molecules.

- We compared the log BCF of PFAS relative to log K_{ow} based QSAR models based on log K_{ow} of the unionized forms of PFAS (Figure 4) to look for deviations of unionized PFAS from lipid based models and observed:
  (1.) There is a positive correlation between the experimental accumulation data and predicted log K_{ow}. This is in accordance with Ng and Hungerbühler (2014). The bioaccumulation of PFAS is close to or even less than estimated with the log K_{ow} based reference QSAR model (Veith et al. 1979) and always much less than the lipid based 'worst case' QSAR. Note that these QSARs were derived for unionized compounds only.
  (2.) The correlation between log BCF and log K_{ow} of PFAS, particularly the same slope as the established QSARs, supports similar processes, i.e. lipid based accumulation.

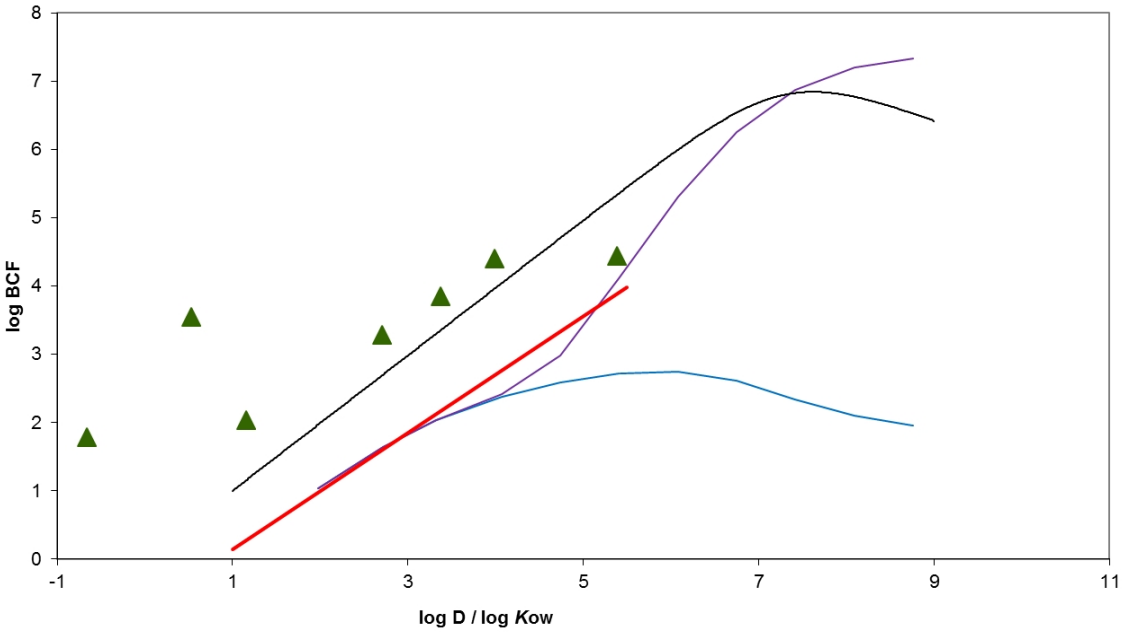
Figure 4: Exploratory data analysis of the bioaccumulation of PFAS relative to log K_{ow} based QSAR models.



Exploratory data analysis of the bioaccumulation of PFAS relative to log K_{ow} based QSAR models: log K_{ow} based QSAR model by Veith et al. 1979 (red line, reference (TGD) model); worst case QSAR by Nendza 1991 (black line); log BCF (blue line) and log BAF (purple line) model by Arnot and Gobas 2006 and US EPA 2011; average experimental log BCF from Martin et al. 2003b and Jeon et al. 2010; mean log K_{ow} (d'blue diamonds) by vcclab (Webster & Ellis 2011), ChemProp (UFZ 2014) and ACD (2015); LSER based log K_{ow} (green triangles) from Abraham et al. 1994.

- We compared the log BCF of PFAS relative to log K_{ow} based QSAR models based on log D of the ionized forms of PFAS (Figure 5) to look for deviations of ionized PFAS from lipid based models and observed:
  (1.) The data pattern is the same as with log K_{ow}, since the log D estimates are in the same rank order as the log K_{ow} estimates because a constant correction term was used to calculate log D from log K_{ow}.
  (2.) The constant correction term of -3.75 (ACD 2015) to convert the log K_{ow} of the unionized forms of PFAS to the log D of the respective ionized forms results in a left-ward shift of the data points, moving them mostly beyond the log K_{ow} based QSARs.
  (3.) The log BCF data points of the perfluoroalkylcarboxylates are still close to the worst case QSAR, but those of the perfluoroalkylsulfonates are beyond the models. However, due to the uncertainty of the correction constant (at least ± 1 log unit), no deviations should be quantified. Furthermore, the perfluoroalkylsulfonates with log D values below 1 are clearly outside the range of the models.
  (4.) The uncertainties of the estimated log D values of PFAS limit further conclusions regarding non-lipid based accumulation of PFAS.

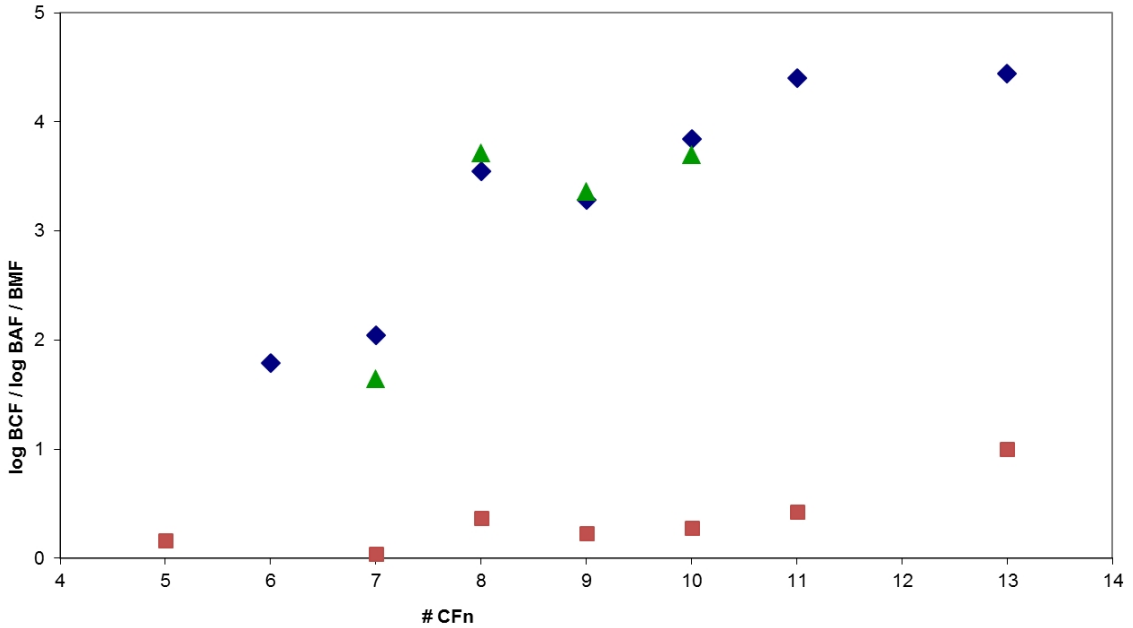
Figure 5: Exploratory data analysis of the bioaccumulation of PFAS relative to log K_{ow} based QSAR models.



Exploratory data analysis of the bioaccumulation of PFAS relative to log K_{ow} based QSAR models: log K_{ow} based QSAR model by Veith et al. 1979 (red line, reference (TGD) model); worst case QSAR by Nendza 1991 (black line); log BCF (blue line) and log BAF (purple line) model by Arnot and Gobas 2006 and US EPA 2011; average experimental log BCF from Martin et al. 2003b and Jeon et al. 2010; log D (green triangles) calculated from log K_{ow} (mean from vcclab (Webster & Ellis 2011), ChemProp (UFZ 2014) and ACD (2015)) – 3.75.

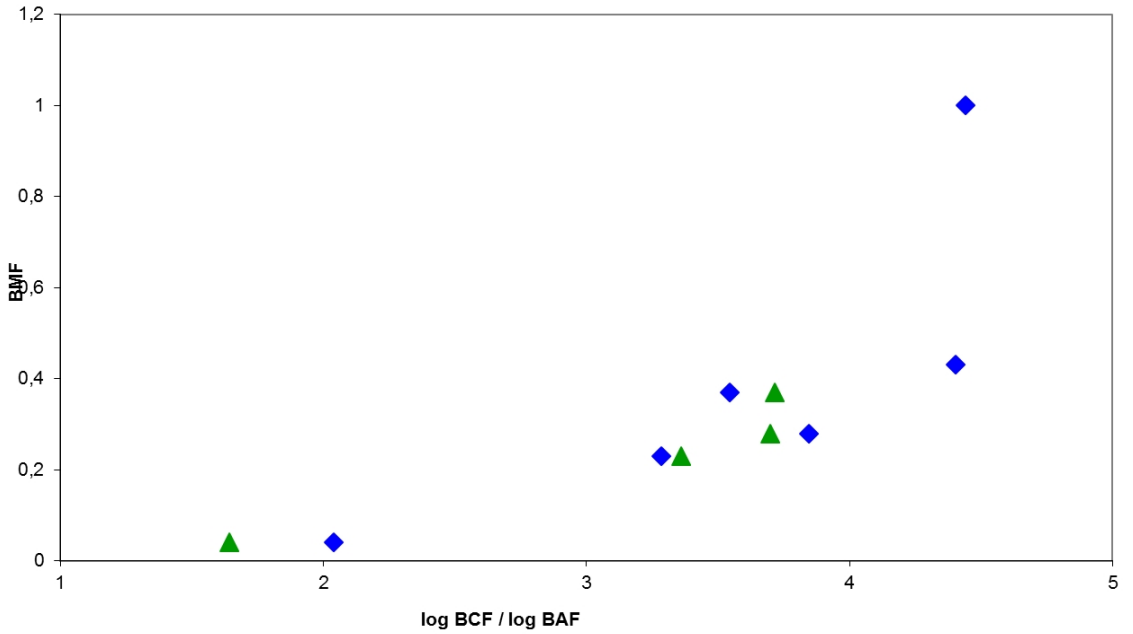
- We compared different metrics of bioaccumulation, log BCF, log BAF and BMF, of PFAS (Figure 6, Figure 7) to look for effects of uptake routes on mechanisms and magnitudes of bioaccumulation of PFAS and observed:
  (1.) The BCF, BAF and BMF of PFAS increase with increasing number of fluorinated carbons in the PFAS molecules.
  (2.) There is excellent agreement of experimental BCF values for PFAS with field BAF obtained with fish. The deviations are mostly less than 0.2 log units, which is less than the experimental variability of BCF data of most organic chemicals. Because the BCF and BAF data are from different sources, it is unlikely that they are biased by the same errors. The excellent agreement of experimental BCF for PFAS with field BAF indicates that BCF may be acceptable predictors of field BAF for PFAS. It furthermore suggests that water-borne exposure is the relevant route of uptake of PFAS also in the field.
  (3.) The available BMF are also below 1, except PFTeDA with a BMF of 1.00. There is no indication of biomagnification of PFAS.
  (4.) The collinearity of BCF, BAF and BMF of PFAS indicate equivalent mechanisms of bioaccumulation.

Figure 6: Relationships between the number of fluorinated carbons in PFAS molecules and different metrics of bioaccumulation, log BCF, log BAF and BMF .



Comparison of the different metrics of bioaccumulation, log BCF, log BAF and BMF, of PFAS: average experimental log BCF (blue diamonds) from Martin et al. 2003b and Jeon et al. 2010; log BAF (green triangles) from de Voogt et al. 2006; BMF (brown squares) from Martin et al. 2003a and Goeritz et al. 2013; # CFn: number of fluorinated carbons in PFAS molecules.

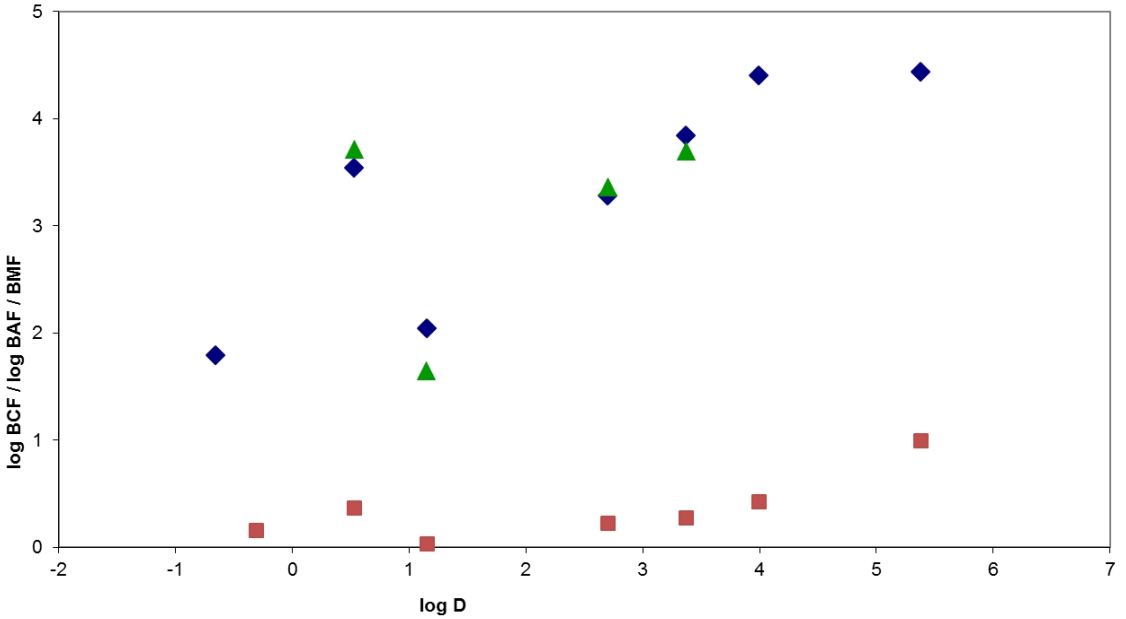
Figure 7: Comparison of different metrics of bioaccumulation, log BCF, log BAF and BMF, of PFAS.



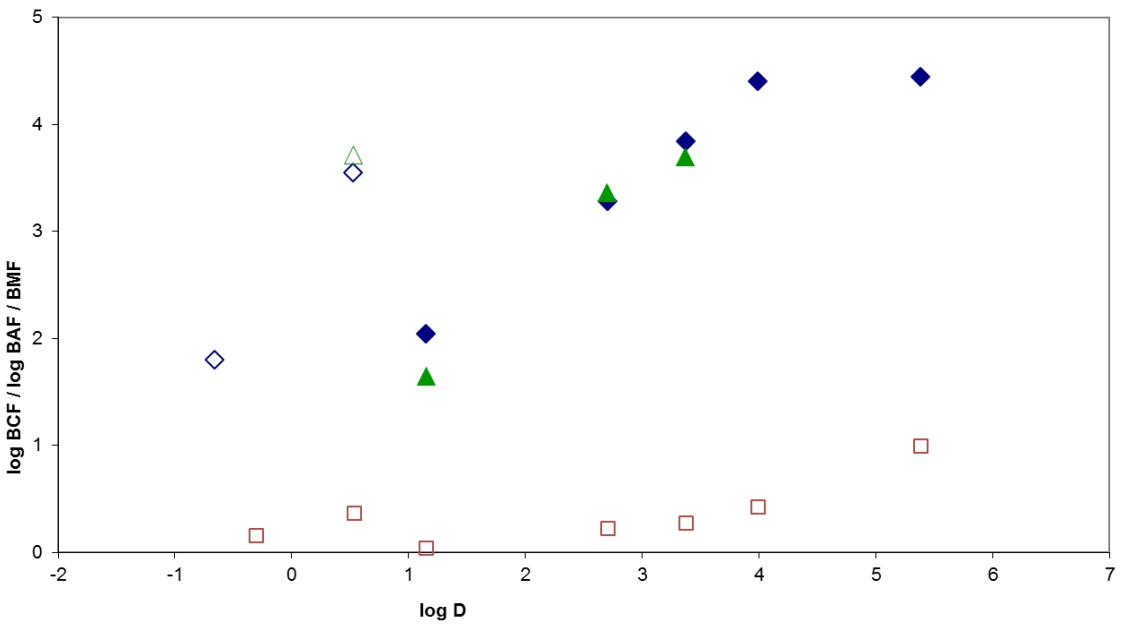
Comparison of the different metrics of bioaccumulation, log BCF, log BAF and BMF, of PFAS: average experimental log BCF (blue diamonds) from Martin et al. 2003b and Jeon et al. 2010; log BAF (green triangles) from de Voogt et al. 2006; BMF from Martin et al. 2003a and Goeritz et al. 2013.

- We compared different metrics of bioaccumulation, log BCF, log BAF and BMF, of PFAS (Figure 8) based on log D to look for relationships with the hydrophobicity of the ionized PFAS and observed:
  (1.) There are correlations between the experimental accumulation data, log BAF, log BCF and BMF, and log D. Three groups can be distinguished, see Figure 8a-c.
  (2.) Figure 8a shows that log BCF and log BAF of the perfluoroalkylcarboxylates among the PFAS are linearily related to log D. The slope is similar to the established lipid based QSARs, supporting similar processes, i.e. lipid based accumulation.
  (3.) Figure 8b shows that log BCF and log BAF of the perfluoroalkylsulfonates among the PFAS are also linearily related to log D. Again, the slope is similar to the established lipid based QSARs, supporting similar processes, i.e. lipid based accumulation. However, the intercept is about 1 log unit higher. The explanations are most likely systematic errors in log K_{ow} of the perfluoroalkylsulfonates as compared to the perfluoroalkylcarboxylates. Further reasons could be either different bioaccumulation capacities of the perfluoroalkylsulfonates as compared to the perfluoroalkylcarboxylates or experimental artefacts due to the two perfluoroalkylsulfonates being investigated in different laboratories. The differences in the accumulation of the perfluoroalkylcarboxylates and the perfluoroalkylsulfonates are, however, within the range of experimental variability and may be resolved if more data, particularly on the accumulation of perfluoroalkylsulfonates, become available.
  (4.) Figure 8c shows a correlation between the experimental BMF data and log D. It is directed by the data point of PFTeDA with a BMF of 1.00. A distinction between perfluoroalkylcarboxylates and perfluoroalkylsulfonates is not possible because PFOS is the only perfluoroalkylsulfonate with a measured BMF. There is no indication of biomagnification of PFAS.
  (5.) The same relationships as with log D also exist with log K_{ow}, due to the constant correction term of -3.75 (ACD 2015) to convert the log K_{ow} values of the unionized forms of PFAS to the log D of the respective ionized forms, resulting in an even horizontal shift of the data points.

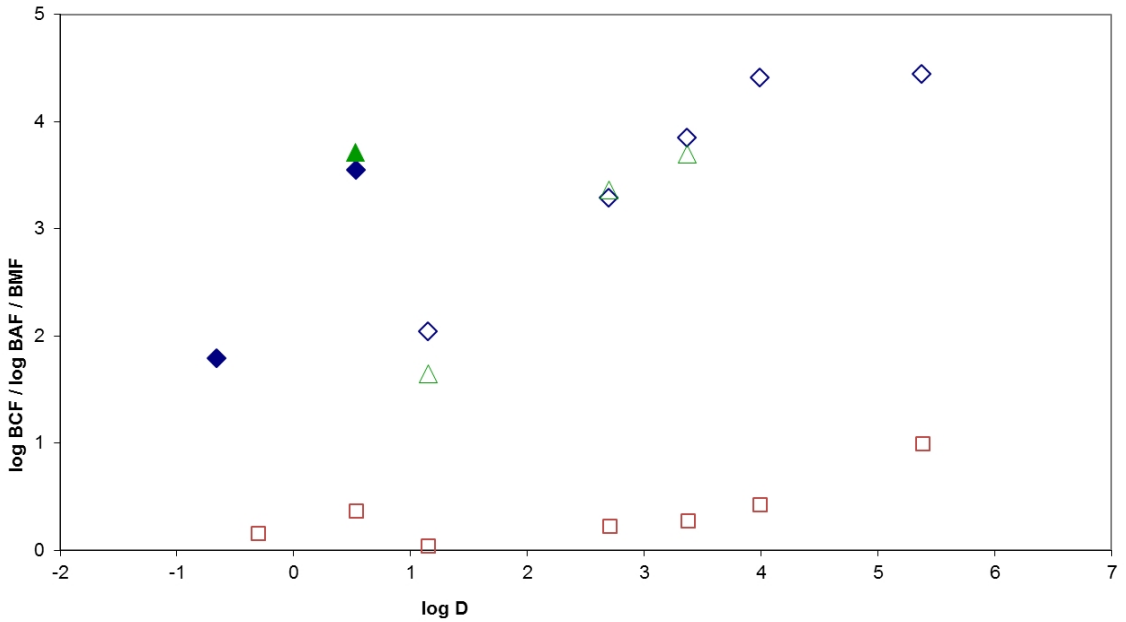
Figure 8: Relationships between log D and different metrics of bioaccumulation, log BCF, log BAF and BMF, of PFAS.
a: log BAF and log BCF of perfluoroalkylcarboxylates
b: log BAF and log BCF of perfluoroalkylsulfonates
c: BMF of perfluoroalkylcarboxylates and perfluoroalkylsulfonates



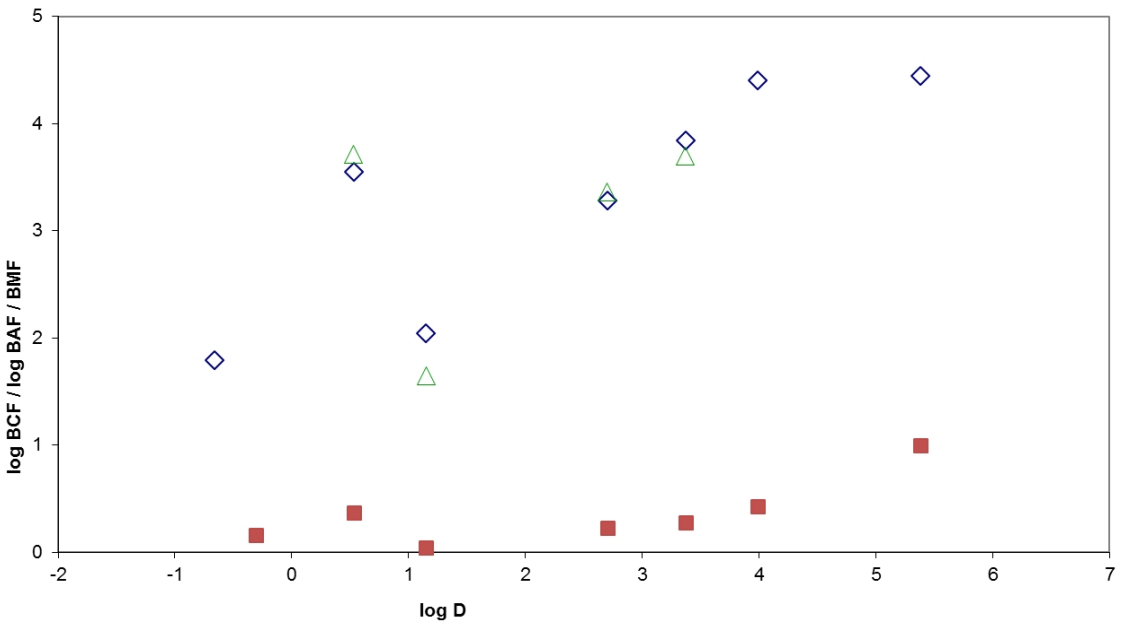
a: log BAF and log BCF of perfluoroalkylcarboxylates:



b: log BAF and log BCF of perfluoroalkylsulfonates:



c: BMF of perfluoroalkylcarboxylates and perfluoroalkylsulfonates:



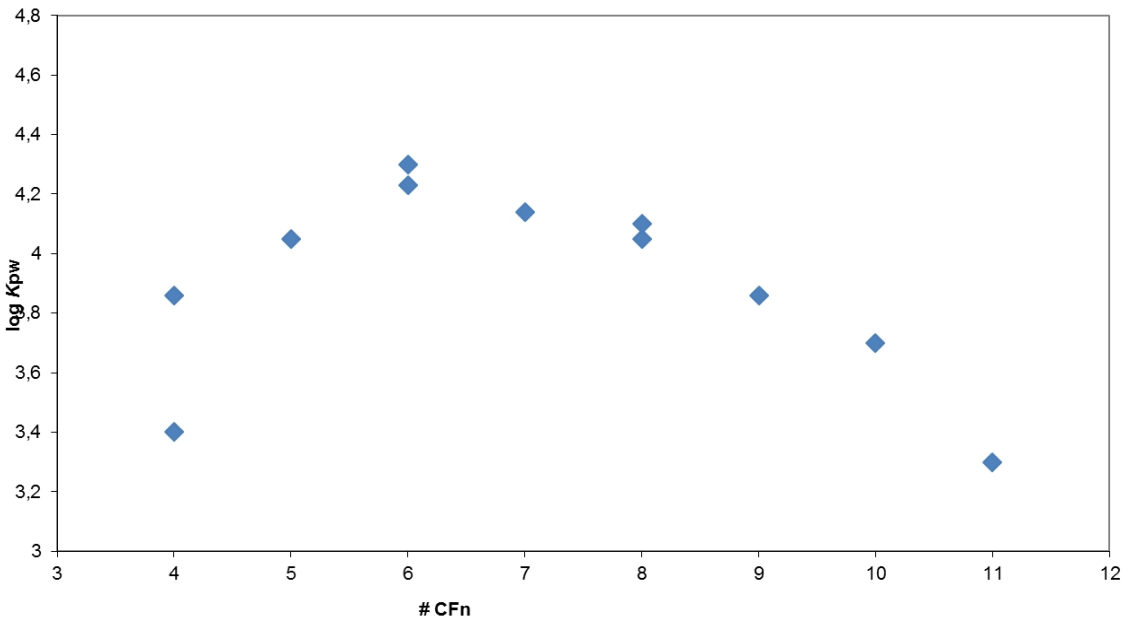
Relationship between log D and the different metrics of bioaccumulation, log BCF, log BAF and BMF, of PFAS: log BAF (green triangles) from de Voogt et al. 2006; BMF (brown squares) from Martin et al. 2003a and Goeritz et al. 2013; average experimental log BCF (blue diamonds) from Martin et al. 2003b and Jeon et al. 2010; log D calculated from log K_{ow} (mean from vcclab (Webster & Ellis 2011), ChemProp (UFZ 2014) and ACD (2015).

We compared protein/water partition coefficients (log K_{pw}) with log K_{ow} and the different metrics of bioaccumulation, log BCF, log BAF and BMF, of PFAS (Figure 9a-c) to look for effects of protein binding on the bioaccumulation of PFAS and observed:
(1.) Figure 9a shows that the protein/water partition coefficients (log K_{pw}) of PFAS are within a narrow range (about 1 log unit) and increase with number of fluorinated carbons

in PFAS molecules up to 6, then gradually decrease with further increasing chain length. PFOS and PFOA have log K_{pw} values of ~4.1, see Table 6- 2.
(2.) Figure 9b shows that log K_{pw} and log K_{ow} are matching for short- and intermediate-chain PFAS, with up to 7 fluorinated carbons in the molecules, incl., e.g., PFOA and PFOS. Because log K_{pw} decreases for PFAS with #CFn >7, while log K_{ow} increases for PFAS with #CFn >7, long-chain PFAS appear to partition more into lipids than into proteins.
(3.) Figure 9c shows that log BCF and log BAF decrease with increasing log K_{pw} of PFAS, indicating an opposite trend of association with proteins and bioaccumulation. The anti-correlation between log BCF and log BAF and log K_{pw} does not support similar processes and provides evidence for non-protein based accumulation of PFAS.

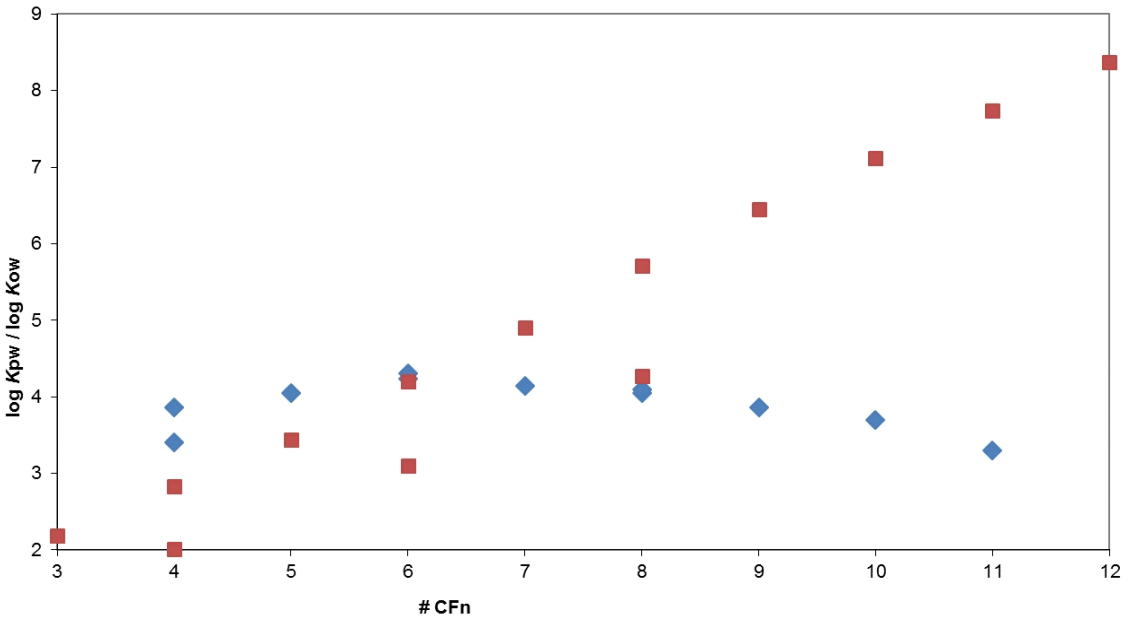
Figure 9: **Relationships between the number of fluorinated carbons in PFAS molecules, log K_{pw}, log K_{ow} and different metrics of bioaccumulation, log BCF, log BAF and BMF.**
a: log K_{pw} of PFAS
b: log K_{pw} and log K_{ow} of PFAS
c: log K_{pw} and log BCF, log BAF and BMF of PFAS

a: log K_{pw} of PFAS:



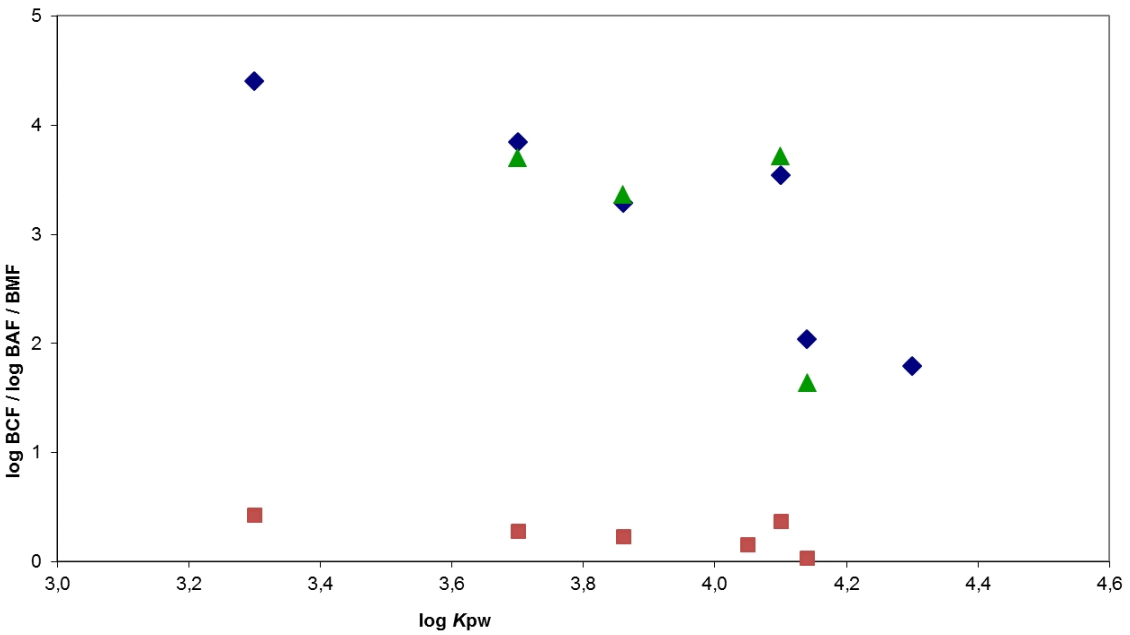
Protein/water partition coefficients (log K_{pw}) from Bischel et al. 2011; # CFn: number of fluorinated carbons in PFAS molecules.

b: log K_{pw} and log K_{ow} of PFAS:



Protein/water partition coefficients log K_{pw} (blue diamonds) from Bischel et al. 2011; mean log K_{ow} (brown squares) by vcclab (Webster & Ellis 2011), ChemProp (UFZ 2014) and ACD (2015); # CFn: number of fluorinated carbons in PFAS molecules.

c: log K_{pw} and log BCF, log BAF and BMF of PFAS:

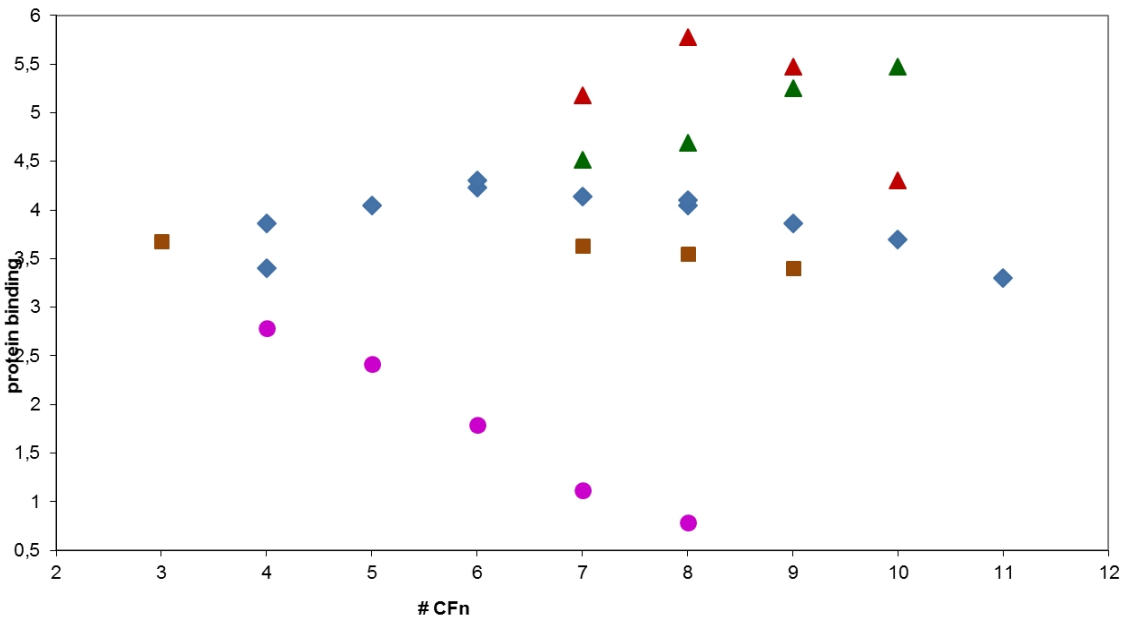


Average experimental log BCF (blue diamonds) from Martin et al. 2003b and Jeon et al. 2010; log BAF (green triangles) from de Voogt et al. 2006; BMF (brown squares) from Martin et al. 2003a and Goeritz et al. 2013; protein/water partition coefficients (log K_{pw}) from Bischel et al. 2011.

- We compared protein/water partition coefficients (log K_{pw}) with different metrics of protein binding of PFAS (Figure 10) to look for different aspects of protein binding of PFAS and observed:

(1.) The protein/water partition coefficients (log K_{pw}) of PFAS are not directly correlated with the other metrics of protein binding of PFAS.
(2.) The secondary association constants are dependent on BSA concentrations and show a decreasing trend at lower (1 µM) BSA concentrations but an increasing trend at higher BSA concentrations (10 µM).
(3.) Interactions with recombinant rat L-FABP at pH 7.4 (log(K_{i1}) linearily decrease with increasing number of fluorinated carbons in the PFAS molecules.
(4.) The estimated *in vivo* carp (*Cyprinus carpio*) serum estradiol displacement from sex-hormone binding globulins (SHBGs) is invariant for different PFAS.
(5.) The different parameters of protein binding of PFAS represent a multitude of mechanisms and do not support protein based accumulation of PFAS.

Figure 10: Relationships between the number of fluorinated carbons in PFAS molecules and different metrics of protein binding.



Protein/water partition coefficients log K_{pw} (blue diamonds) from Bischel et al. 2011; secondary association constants at lower BSA concentrations (1 µM, red triangles) or higher BSA concentrations (10 µM, green triangles) from MacManus-Spencer et al. 2010; interaction with recombinant rat L-FABP (pink circles) at pH 7.4 (log(K_{i1}) (µM)) from Woodcroft et al. 2010; estimated *in vivo* carp (*Cyprinus carpio*) serum estradiol displacement from sex-hormone binding globulins (SHBGs) EC10 (brown squares) from Jones et al. 2003; # CFn: number of fluorinated carbons in PFAS molecules.

We compared 2 published LSER models, one for log K_{ow} (Abraham et al. 1994) and one for binding to human serum albumin (HSA) (Valko et al. 2003) to look for different contributions of molecular interactions (Equation 3, Equation 4):

$$\log K_{ow} = 0.562\ E - 1.054\ S + 0.032\ A - 3.46\ B + 3.814\ V + 0.088 \quad \text{(Equation 3)}$$

$$\log \text{HSA} = 0.82\ E - 0.36\ S + 0.18\ A - 1.97\ B + 1.62\ V - 1.28 \quad \text{(Equation 4)}$$

Calculation of the differences of the coefficients of Equation 3 and Equation 4 results in a hypothetical model (Equation 5) of the relationship between HSA and K_{ow} (ΔAO).

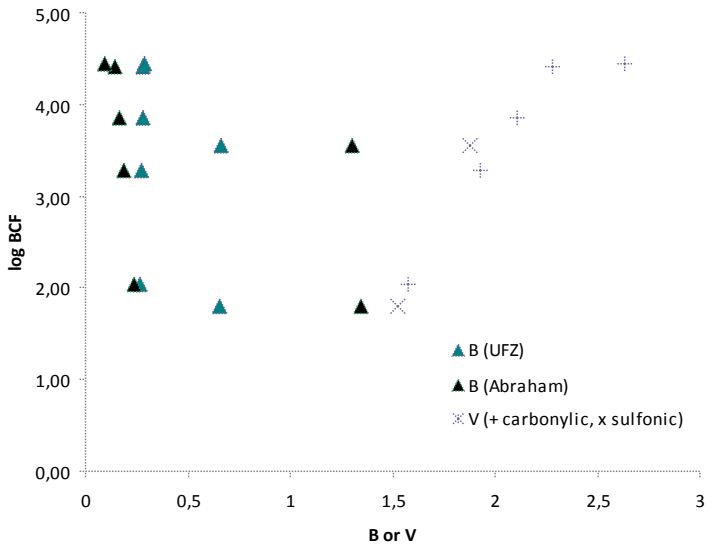
$$\log \Delta AO = 0.258\ E + 0.694\ S + 0.148\ A + 1.49\ B - 2.194\ V - 1.368 \quad \text{(Equation 5)}$$

The differences are most evident for the parameters *B* (hydrogen bonding acceptor capacity) and *V* (intrinsic (characteristic) molecular volume). Both coefficients are large. The large

positive value of *B* means that high ability of a molecule to accept hydrogen bonding (=basicity) indicates preference for proteins as compared to lipids. The parameter *V* represents the size of the molecules. The large negative value of *V* means that increasing size of molecules indicates preference for lipids as compared to proteins, i.e large molecules tend to partition into lipids but not into proteins.

Figure 11 illustrates the trend, modelled by *V*, that PFAS with higher BCF appear to partition more into lipids and less into proteins. We observe a marked increase of *V* with increasing BCF, indicating that high accumulation is most likely due to uptake into lipid phases. In contrast to *V*, the effect of *B* on BCF is almost constant and only discriminates perfluoroalkylcarboxylates and perfluoroalkylsulfonates. The *B* values for the perfluoroalkylsulfonates are a little larger and may indicate a slight preference for protein binding as compared to perfluoroalkylcarboxylates. The constant *B* values within the groups, however, suggests that the BCF of PFAS is independent of the extent of protein binding.

Figure 11: Relationships between experimental log BCF and *B* (hydrogen bonding acceptor capacity) and *V* (intrinsic (characteristic) molecular volume) of PFAS.



The theoretical analysis of LSER models for log K_{ow} (Abraham et al. 1994) and binding to human serum albumin (HSA) (Valko et al. 2003) indicates that the effect of protein binding on the bioaccumulation of PFAS may be less than generally assumed.

Figure 12 illustrates the relationship between log BCF and the molecular weight of PFAS. The link is the collinearity of chain length, molecular weight and molecular volume of PFAS. The longer the chain (= number of fluorinated carbons in PFAS molecules), the higher is the molecular weight and the larger is the volume of the molecules. Note that, regarding the influence of the molecular weight on the bioaccumulation of PFAS, we observe no discrimination of perfluoroalkylcarboxylates and perfluoroalkylsulfonates.

Figure 12: Relationships between experimental log BCF and molecular weight (MW) of PFAS.

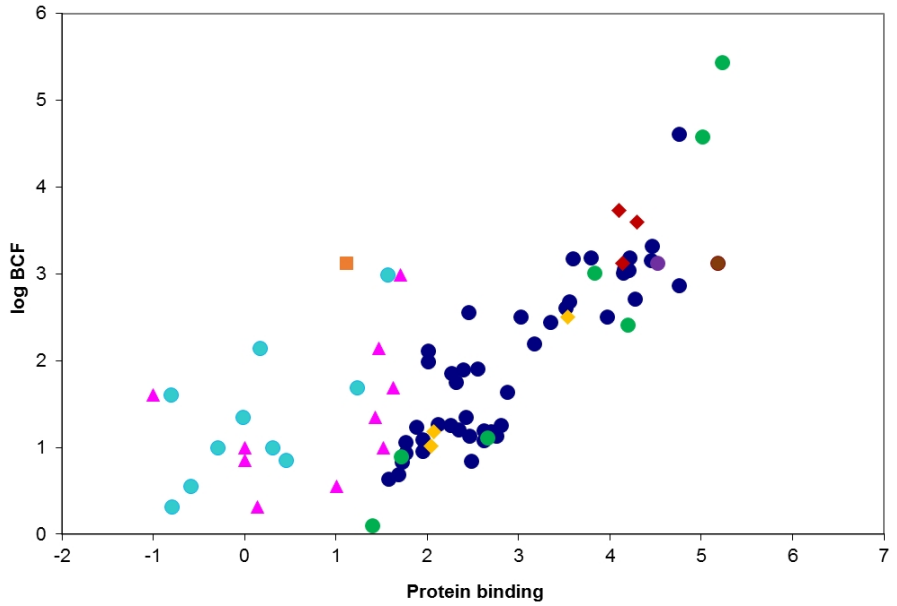
The most important findings of our exploratory data analyses on PFAS can be summarized as follows:

- Reliable log K_{ow} of PFAS are not available, thus PFAS are not covered by the applicability domains of the established computational models like fragment approaches or LSER.
- The uncertainties of the estimated log D values of PFAS limit further conclusions regarding non-lipid based accumulation of PFAS.
- There is a (linear) correlation between the experimental accumulation data for PFAS and calculated log D/log K_{ow}.
- There is excellent agreement of experimental BCF values for PFAS with field BAF obtained with fish.
- There is no indication of biomagnification of PFAS.
- The different parameters of protein binding of PFAS do not support protein based accumulation of PFAS.
- To further clarify the mode of bioaccumulation of PFAS, systematic experimental binding studies with different protein and lipid targets would be useful.

6.2.2 Relationships between bioaccumulation and protein binding

Because the available data of PFAS are not sufficient to understand the relationships between bioaccumulation and protein binding, we analysed a set of diverse organic chemicals to gain a better picture of the relationships between bioaccumulation and protein binding. We applied exploratory data analyses to the data detailed in section 5.2. We first plotted experimental BCF data against data on protein binding. The overall picture (Figure 13) indicates some trends of increasing BCF with increasing protein binding, however, with substantial scatter.

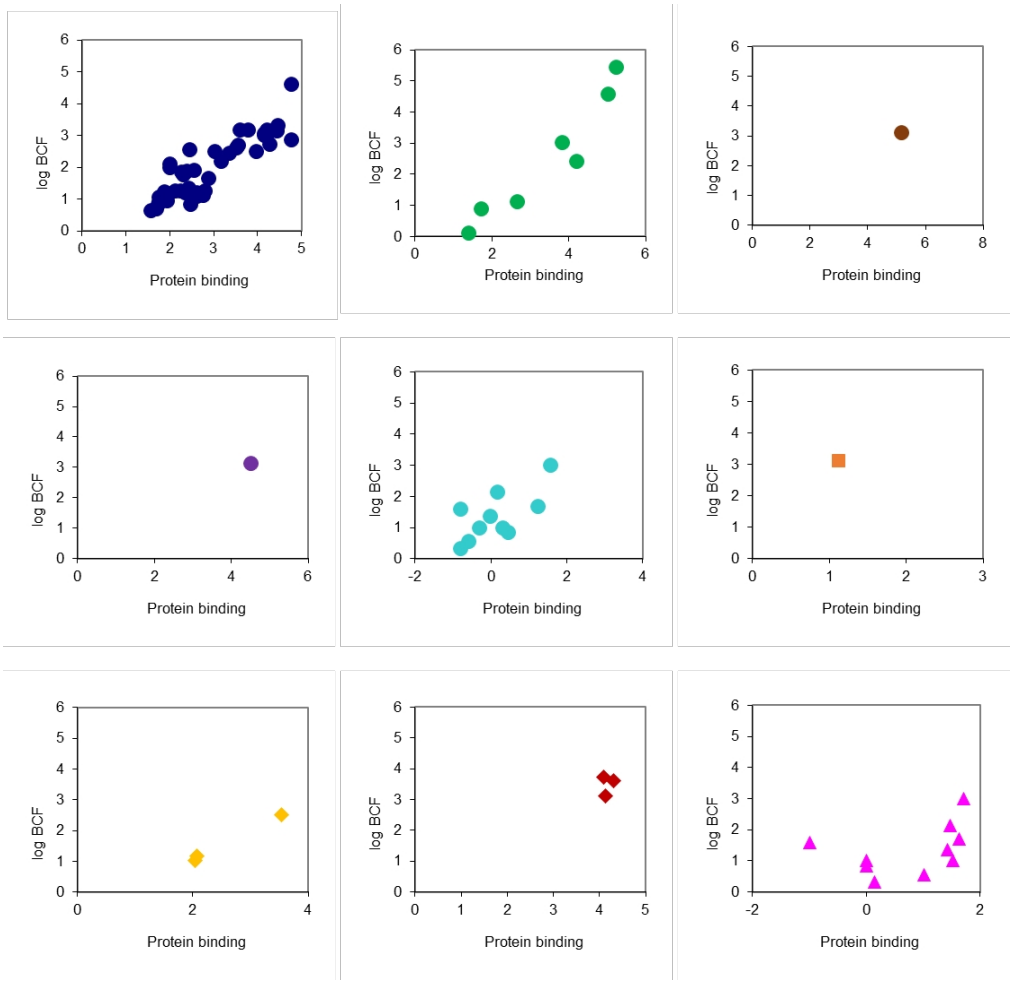
Figure 13: Relationships between bioaccumulation and multiple parameters of protein binding.



Relationships between bioaccumulation and multiple parameters of protein binding: log $K_{BSA/w}$ (37 °C) (d'blue circles) and log $K_{BSA/w}$ (green circles) from Endo & Goss 2011; BSA (1 µM) log k_1 (brown circles) and BSA (10 µM) log k_1 (purple circles) from MacManus-Spencer et al. 2010; log K_{HSA} (l'blue circles) from Valko et al. 2003; log(K_{i1}) L-FABP ((l'brown squares) from Woodcroft et al. 2010; log protein/water partition coefficients from DeBruyn & Gobas 2007 (orange diamonds) and Bischel et al. 2011 (d'red diamonds); log CHI IAM from Valko et al. 2003; log BCF from Nendza et al. 2015.

Detailed inspections of the relationships between bioaccumulation and individual endpoints of protein binding indicate correlations between BCF and binding to bovine serum albumin (Figure 14). The other endpoints of protein binding do not support specific conclusions, partly due to the lack of data.

Figure 14: Relationships between bioaccumulation and individual parameters of protein binding.



Relationships between bioaccumulation and individual parameters of protein binding: log $K_{BSA/w}$ (37 °C) (d'blue circles) and log $K_{BSA/w}$ (green circles) from Endo & Goss 2011; BSA (1 µM) log k_1 (brown circles) and BSA (10 µM) log k_1 (purple circles) from MacManus-Spencer et al. 2010; log K_{HSA} (l'blue circles) from Valko et al. 2003; log(K_{i1}) L-FABP ((l'brown squares) from Woodcroft et al. 2010; log protein/water partition coefficients from DeBruyn & Gobas 2007 (orange diamonds) and Bischel et al. 2011 (d'red diamonds); log CHI IAM from Valko et al. 2003; log BCF from Nendza et al. 2015.

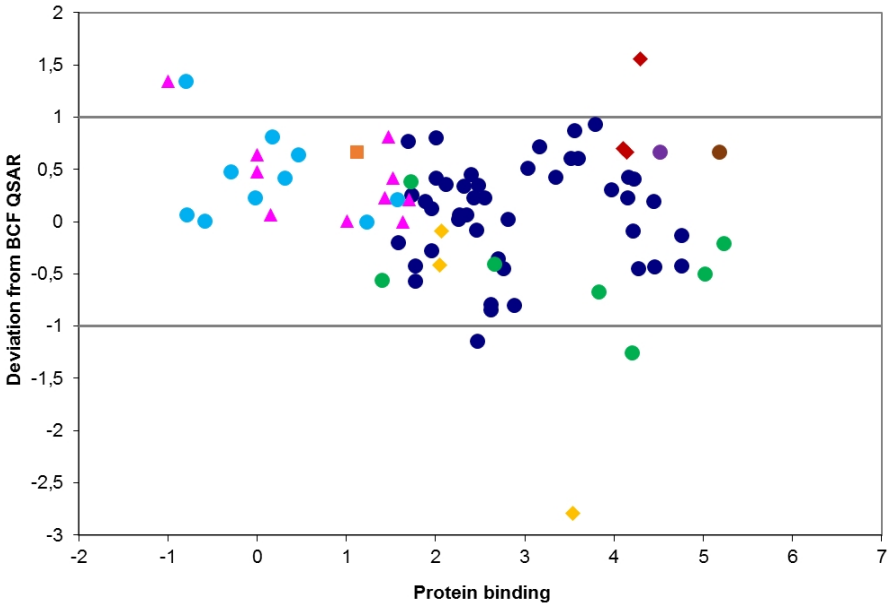
6.2.3 Relationships between the deviations from log K_{ow} based bioaccumulation models and protein binding

Because the direct relationships between bioaccumulation and protein binding were not conclusive regarding the contribution of protein binding to increased bioaccumulation, we continued the exploratory data analyses with a different metric for the bioaccumulation potential of the chemicals. We used the difference between the experimental BCF value and the BCF estimate according to the log K_{ow} based QSAR model by Veith et al. 1979 (Equation 2, reference (TGD) model). If this difference is >1, it is assumed to reflect the possibility for additional specific mechanisms of bioaccumulation. If this difference is <-1, it may indicate processes reducing the bioaccumulation of substances, e.g. biotransformations. Deviations between -1 and +1 are not significant since they are within the experimental variability of BCF values.

Figure 15 shows that three compounds each deviate by more than 1 log unit above or below the log K_{ow} based QSAR model by Veith et al. 1979 (Equation 2, reference (TGD) model), respectively. This number is clearly too low to draw any reasonable conclusions except that the

deviations from the log K_{ow} based reference QSAR are NOT related to the protein binding potential of chemicals.

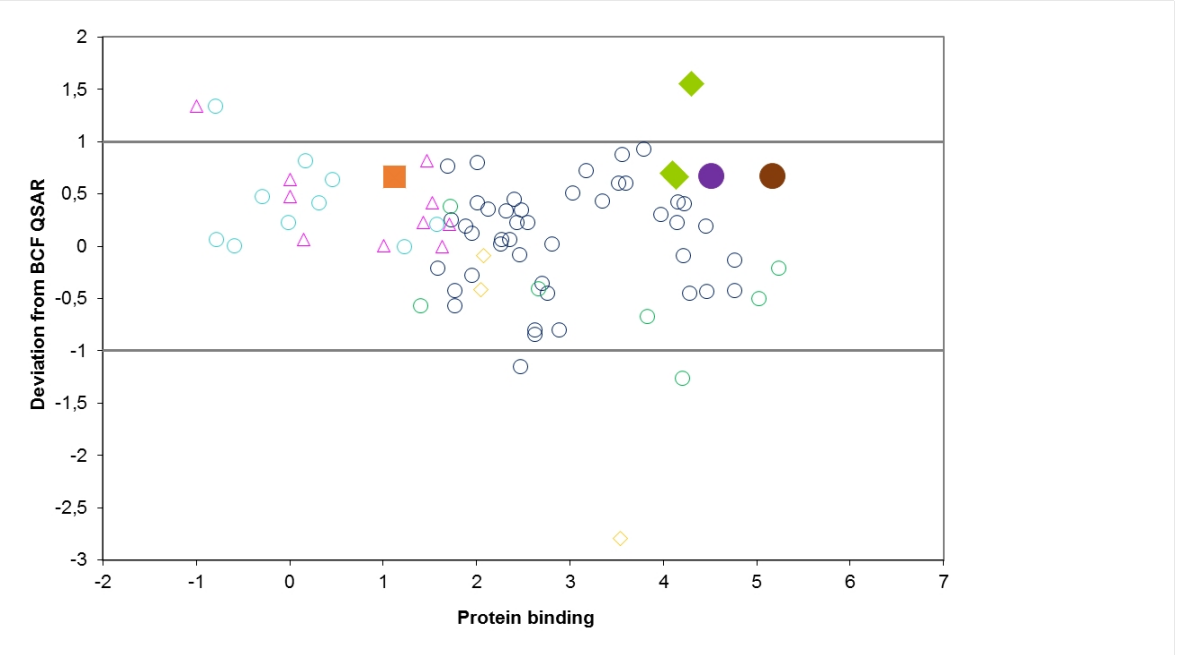
Figure 15: Relationships between deviations from a log K_{ow} based bioaccumulation QSAR (Veith et al. 1979) and multiple parameters of protein binding.



Relationships between deviations from a log K_{ow} based bioaccumulation QSAR (Veith et al. 1979) and multiple parameters of protein binding: log $K_{BSA/w}$ (37 °C) (d'blue circles) and log $K_{BSA/w}$ (green circles) from Endo & Goss 2011; BSA (1 µM) log k_1 (brown circles) and BSA (10 µM) log k_1 (purple circles) from MacManus-Spencer et al. 2010; log K_{HSA} (l'blue circles) from Valko et al. 2003; log(K_{f1}) L-FABP ((l'brown squares) from Woodcroft et al. 2010; log protein/water partition coefficients from DeBruyn & Gobas 2007 (orange diamonds) and Bischel et al. 2011 (d'red diamonds); log CHI IAM from Valko et al. 2003.

Highlighting the PFAS in the data display (Figure 16) confirms the previous observations (section 6.2.1) that specific protein binding contributions to the bioaccumulation of PFAS do not result in BCF higher than estimated from log K_{ow}.

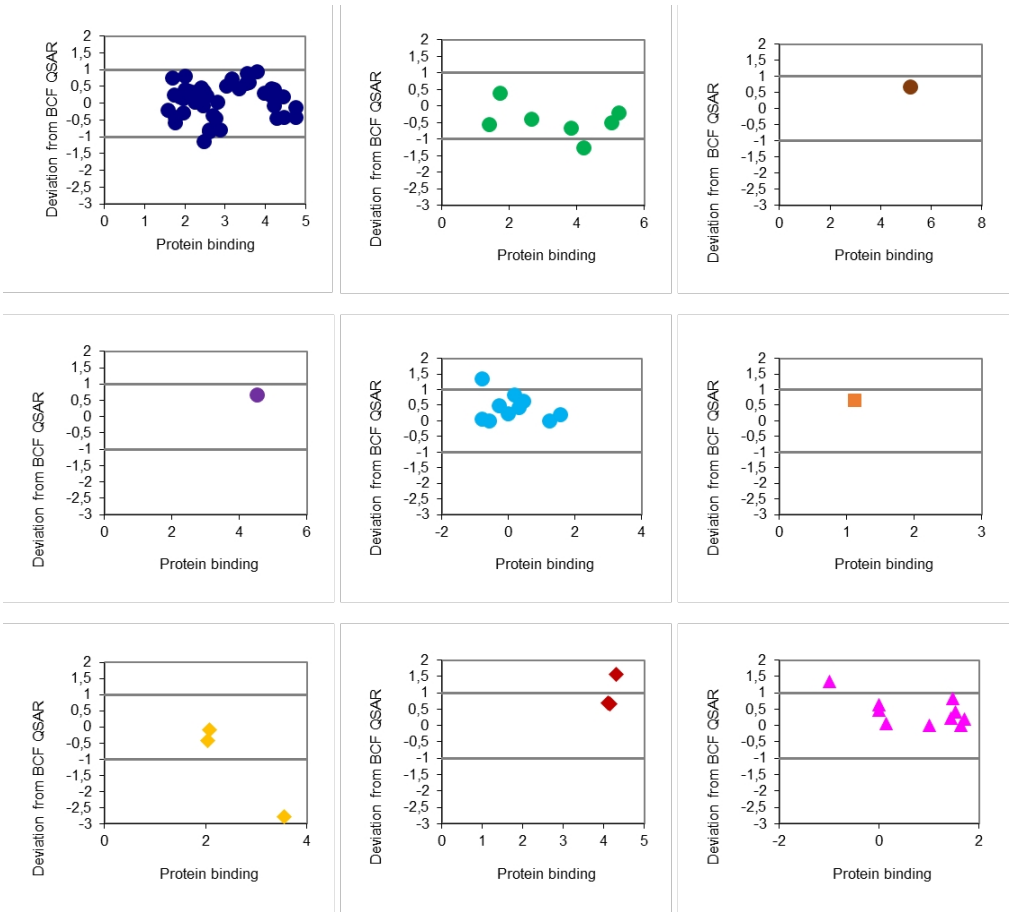
Figure 16: Relationships between deviations from a log K_{ow} based bioaccumulation QSAR (Veith et al. 1979) and multiple parameters of protein binding, highlighting PFAS



Relationships between deviations from a log K_{ow} based bioaccumulation QSAR (Veith et al. 1979) and multiple parameters of protein binding: log $K_{BSA/w}$ (37 °C) (d'blue circles) and log $K_{BSA/w}$ (green circles) from Endo & Goss 2011; BSA (1 µM) log k_1 (brown circles) and BSA (10 µM) log k_1 (purple circles) from MacManus-Spencer et al. 2010; log K_{HSA} (l'blue circles) from Valko et al. 2003; log(K_{i1}) L-FABP ((l'brown squares) from Woodcroft et al. 2010; log protein/water partition coefficients from DeBruyn & Gobas 2007 (orange diamonds) and Bischel et al. 2011 (d'red diamonds); log CHI IAM from Valko et al. 2003.

Detailed inspections of the relationships between the deviations from log K_{ow} based bioaccumulation and individual endpoints of protein binding confirm the above findings (Figure 17). Random scatter of deviations are observed for the binding to bovine serum albumin. The other endpoints of protein binding do not support specific conclusions due to the lack of data.

Figure 17: Relationships between deviations from a log K_{ow} based bioaccumulation QSAR (Veith et al. 1979) and individual parameters of protein binding.



Relationships between deviations from a log K_{ow} based bioaccumulation QSAR (Veith et al. 1979) and individual parameters of protein binding: log $K_{BSA/w}$ (37 °C) (d'blue circles) and log $K_{BSA/w}$ (green circles) from Endo & Goss 2011; BSA (1 µM) log k_1 (brown circles) and BSA (10 µM) log k_1 (purple circles) from MacManus-Spencer et al. 2010; log K_{HSA} (l'blue circles) from Valko et al. 2003; log(K_{i1}) L-FABP ((l'brown squares) from Woodcroft et al. 2010; log protein/water partition coefficients from DeBruyn & Gobas 2007 (orange diamonds) and Bischel et al. 2011 (d'red diamonds); log CHI IAM from Valko et al. 2003.

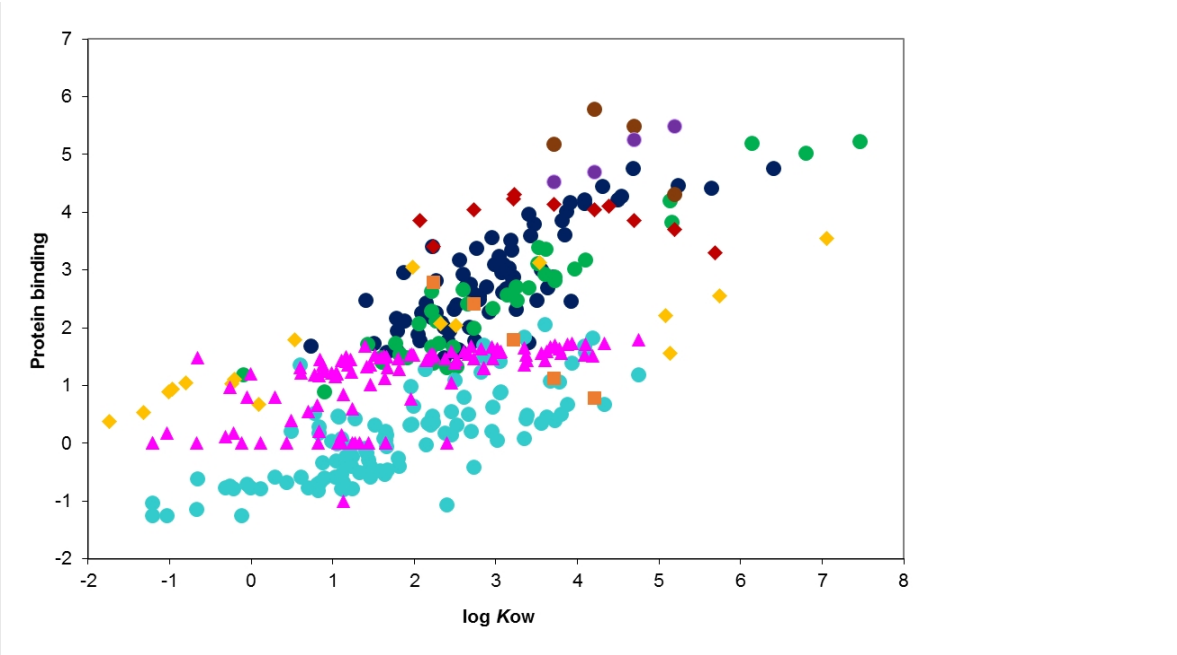
6.2.4 Relationships between protein binding and log K_{ow}

The exploratory data analyses were completed with graphical displays of the direct relationships between protein binding and log K_{ow}. We see in Figure 18 a clear trend of protein binding increasing with log K_{ow}. This trend is consistent with long-standing expertise in pharmaceutical and medicinal chemistry.

Highlighting the PFAS in the data display (Figure 19) indicates contradictory observations with some increase, some decrease or even invariability of protein binding relative to log K_{ow}. Consistent correlations between protein binding and log K_{ow} of the PFAS are not evident.

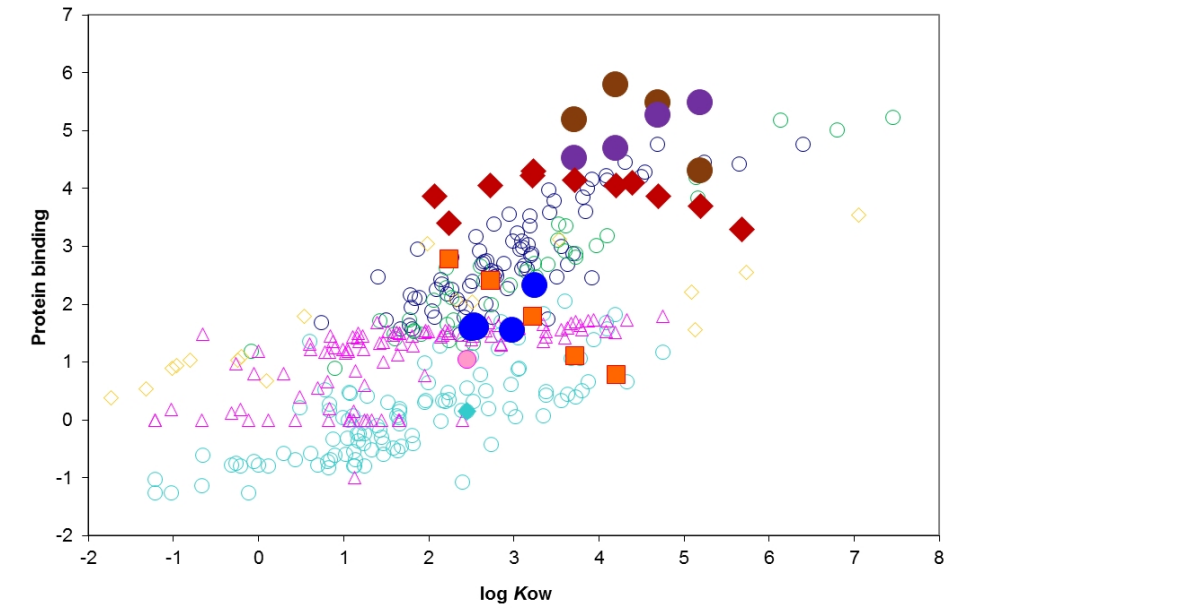
Detailed inspections of the relationships between the individual endpoints of protein binding and log K_{ow} confirm the above findings (Figure 20). Trends of increase of protein binding with log K_{ow} are observed for the binding to bovine and human serum albumin. The other endpoints of protein binding do not support specific conclusions.

Figure 18: Relationships between multiple parameters of protein binding and log K_{ow}.



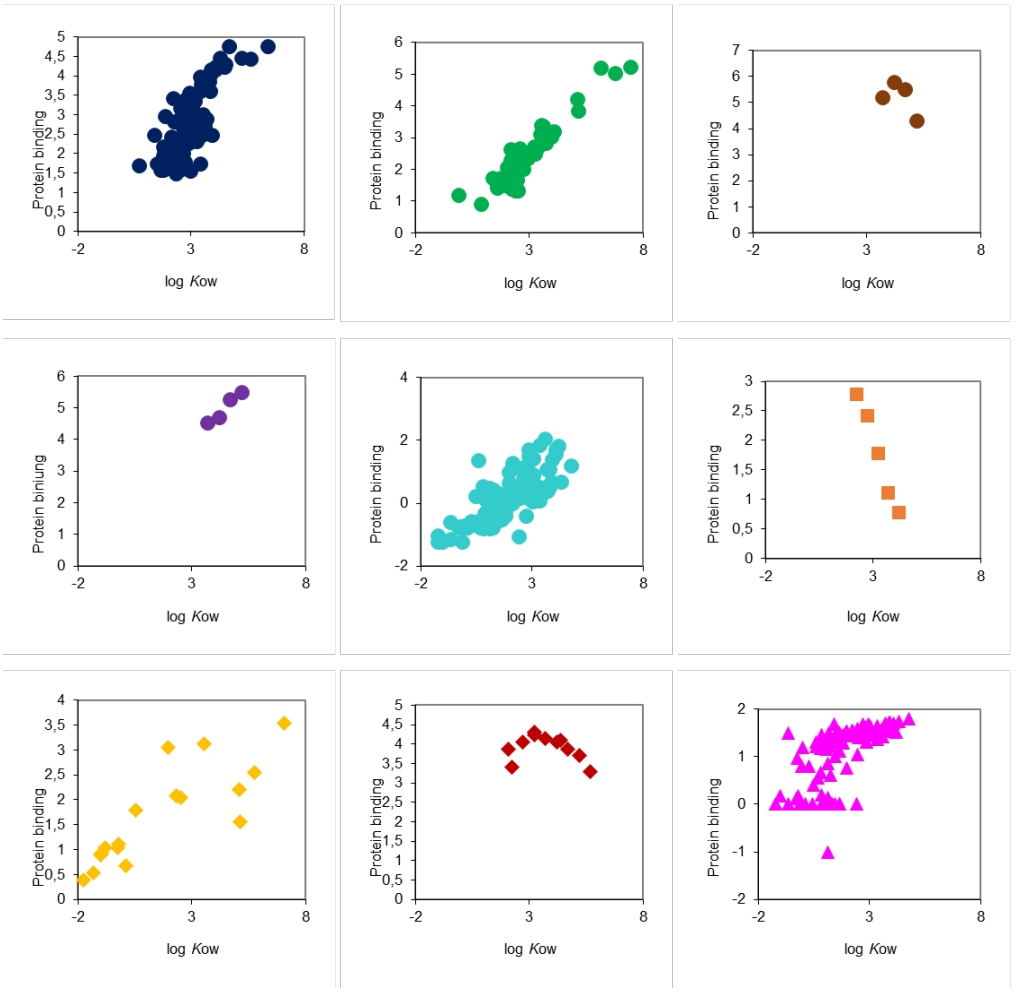
Relationships between multiple parameters of protein binding and log K_{ow}: log $K_{BSA/w}$ (37 °C) (d'blue circles) and log $K_{BSA/w}$ (green circles) from Endo & Goss 2011; BSA (1 µM) log k_1 (brown circles) and BSA (10 µM) log k_1 (purple circles) from MacManus-Spencer et al. 2010; log K_{HSA} (l'blue circles) from Valko et al. 2003; log(K_{i1}) L-FABP ((l'brown squares) from Woodcroft et al. 2010; log protein/water partition coefficients from DeBruyn & Gobas 2007 (orange diamonds) and Bischel et al. 2011 (d'red diamonds); log CHI IAM from Valko et al. 2003.; log K_{ow} from ChemProp (UFZ 2014).

Figure 19: Relationships between multiple parameters of protein binding and log K_{ow}, highlighting PFAS.



Relationships between multiple parameters of protein binding and log K_{ow}, highlighting PFAS: log $K_{BSA/w}$ (37 °C) (d'blue circles) and log $K_{BSA/w}$ (green circles) from Endo & Goss 2011; BSA (1 µM) log k_1 (brown circles) and BSA (10 µM) log k_1 (purple circles) from MacManus-Spencer et al. 2010; log K_{HSA} (l'blue circles) from Valko et al. 2003; log(K_{i1}) L-FABP ((l'brown squares) from Woodcroft et al. 2010; log protein/water partition coefficients from DeBruyn & Gobas 2007 (orange diamonds) and Bischel et al. 2011 (d'red diamonds); log CHI IAM from Valko et al. 2003.; log K_{ow} from ChemProp (UFZ 2014).

Figure 20: Relationships between individual parameters of protein binding and log K_{ow}.



Relationships between individual parameters of protein binding and log K_{ow}: log $K_{BSA/w}$ (37 °C) (d'blue circles) and log $K_{BSA/w}$ (green circles) from Endo & Goss 2011; BSA (1 µM) log k_1 (brown circles) and BSA (10 µM) log k_1 (purple circles) from MacManus-Spencer et al. 2010; log K_{HSA} (l'blue circles) from Valko et al. 2003; log(K_{fi}) L-FABP ((l'brown squares) from Woodcroft et al. 2010; log protein/water partition coefficients from DeBruyn & Gobas 2007 (orange diamonds) and Bischel et al. 2011 (d'red diamonds); log CHI IAM from Valko et al. 2003.; log K_{ow} from ChemProp (UFZ 2014).

The most important findings of our exploratory data analyses on protein binding can be summarized as follows:

- Protein binding is correlated with log K_{ow} and log BCF.
- Protein binding does not correlate with significantly increased bioaccumulation beyond log K_{ow}.
- Protein binding does not provide additional information about the quantitative bioaccumulation of substances beyond log K_{ow}.
- To further clarify the qualitative and quantitative role of protein binding for bioaccumulation, systematic experimental binding studies with different protein and lipid targets would be useful.

6.3 Absorption of Surfactants

Surfactants are surface active agents which lower the interfacial tension between two liquids. Due to their amphiphilic nature (i.e. they contain both a hydrophilic and a hydrophobic part)

they tend to absorb at biological interfaces such as membrane/(interstitial) water interfaces as well as air/water interfaces, food/water interfaces or glass walls. The latter may hamper the determination of their aqueous concentrations. Therefore, it should be critically examined according to TGD OECD 305 (OECD 2012) whether the aqueous bioconcentration test is feasible for a particular surfactant. Otherwise, the dietary study should be considered as more appropriate. Data on the biomagnification of surfactants other than PFAS are not yet available, and therefore, information on the bioaccumulation of surfactants described in this report are mainly derived from bioconcentration studies.

6.3.1 Anionic surfactants

Anionic surfactants contain anionic functional groups at their head, such as sulfate, sulfonate, phosphate and carboxylate. Anionic surfactants show a wide range of BCF values (Table 6- 3). However, the high variation of BCF obtained in different studies with the same compounds indicates that the comparison of the results should be carried out with caution. Variation could be explained by several factors such as exposure time, test species and feeding conditions (Tolls et al. 1997). In addition, environmental factors like water hardness may also influence the bioconcentration of surfactants (Tovell et al. 1974).

Table 6- 3: Bioconcentration data of anionic surfactants (three significant digits displayed)

Acronym	CAS	Aqu.Conc. (µM)	k_1	k_2	BCFkin	BCF	Species	Reference
Alkylbenzene								
C12-A					6300	35	*Lepomis macrochirus*	Werner & Kimerle (1982)
Dodecyl						130	*Leuciscus idus*	Freitag et al. 1985
Linear alkylbenzene sulfonates (LAS)								
C12-LAS	25155-30-0	0.23	159	0.57	279	108	*Lepomis macrochirus*	Bishop & Maki (1980)
C12-LAS	25155-30-0	0.23	159	0.57	279	145	*Lepomis macrochirus*	Bishop & Maki (1980)
C12-LAS	25155-30-0	2.3	76	0.54	141	227	*Lepomis macrochirus*	Bishop & Maki (1980)
C12-LAS	25155-30-0	2.3	76	0.54	141	280	*Lepomis macrochirus*	Bishop & Maki (1980)
C12-LAS	25155-30-0	0.29	499	2.2	227	231	*Brachidanio rerio*	Coenen (1988)

C12-LAS	25155-30-0	0.3				173	*Pimephales promelas*	Kimerle et al. (1975)
C12-LAS	25155-30-0	0.4				245	*Pimephales promelas*	Kimerle et al. (1975)
C12-LAS	25155-30-0					551*	*Pimephales promelas*	Comotto et al. (1979)
C13.1-LAS						472*	*Pimephales promelas*	Comotto et al. (1979)
C13-LAS	26248-24-8	0.28				385	*Pimephales promelas*	Kimerle et al. (1975)
C13-LAS	26248-24-8	0.31				293	*Pimephales promelas*	Kimerle et al. (1975)
C13-LAS	26248-24-8					1220*	*Pimephales promelas*	Comotto et al. (1979)
C11.6-LAS		2.63				50	*Pimephales promelas*	Kimerle et al. (1975)
C11.6-LAS						269*	*Pimephales promelas*	Comotto et al. (1979)
C11.7-LAS		1.45	25	0.24	104	104	*Lepomis macrochirus*	Kimerle et al. (1981)
Alkyl Sulfates (AS)								
C12-AS	151-21-3	13.9	3.4	0.48	7.1	7.15	*Proterorhinus marmoratus*	Topcuoglu & Birol (1982)
C12-AS	151-21-3	0.093				2.7	*Cyprinus carpio*	Wakabayashi et al. (1987)
C12-AS	151-21-3	1.39				4.6	*Cyprinus carpio*	Wakabayashi et al. (1987)
C12-AS	151-21-3	13.9				2.6	*Cyprinus carpio*	Wakabayashi et al. (1987)
Alkyloxyethylene sulfate surfactants								
C12-EO3S	13150-00-0	0.8	11.1				*Cyprinus carpio*	Kikuchi et al. (1980)
C12-EO5S	15826-20-7	0.7	2.2				*Cyprinus carpio*	Kikuchi et al. (1980)
Perfluorinated alkyl substances								

PFOA	335-67-1		0.53	130	4.0		*Oncorhynchus mykiss*	Martin et al. (2003b)
PFDA	335-76-2		29	62	450		*Oncorhynchus mykiss*	Martin et al. (2003b)
PFUnA	2058-94-8		120	46	2700		*Oncorhynchus mykiss*	Martin et al. (2003b)
PFDoA	307-55-1		700	38	18000		*Oncorhynchus mykiss*	Martin et al. (2003b)
PFTA	376-06-7		580	24	23000		*Oncorhynchus mykiss*	Martin et al. (2003b)
PFOS	1763-23-1		53	48	1100		*Oncorhynchus mykiss*	Martin et al. (2003b)
PFHxS	307-24-4		0.62	65	9.6		*Oncorhynchus mykiss*	Martin et al. (2003b)

*based on dry weight; k_1 = uptake rate; k_2 = depuration rate

Surfactants have a hydrophilic head group and are thus not expected to have a fast transport through biological membranes (Tolls & Sijm 1995). However, tissue analyses of blood and gills as part of fish bioconcentration studies showed that uptake of surfactants through the gills is a rapid process (Tolls & Sijm 1995). Surfactants are distributed in the body via the blood system.

Kikuchi et al. (1978) investigated the bioconcentration of C_{12}-LAS and C_{12}-AS in common carp (*Cyprinus carpio*). Based on autoradiograms it was shown that these surfactants are rapidly absorbed and easily distributed through tissues and organs. The highest surfactant concentrations were found in gills, blood, hepatopancreas, kidney and gall bladder.

Also other tissue distribution studies on fish found highest concentrations of anionic surfactants in the gall bladder (Granmo & Kollberg 1976; Neufarth et al. 1978; Kikuchi et al. 1978, 1980; Goodrich et al. 1991; Tovell et al. 1975; Comotto et al. 1979; Kimerle et al. 1975, 1981; Toshima et al. 1992). An explanation for the high concentrations in the gall bladder is that the surfactants are metabolized in the liver and the metabolites are subsequently excreted into the gall bladder (Tolls & Sijm 1995). The content of the gall bladder is not emptied into the gut when fish are fasting showing that the feeding regime may have a significant effect on the BCF of anionic surfactants (Tolls & Sijm 1995). This was shown by Tovell et al. (1975) and Comotto et al. (1979) who compared the elimination of dodecyl sulfate and LAS in starved and fed fish (Tolls & Sijm 1995).

Data from experiments with chromatographic analyses showed the biotransformation of anionic surfactants (Kimerle et al. 1975; Comotto et al. 1979; Kikuchi et al. 1980; Wakabayashi et al. 1987). However, most data are based on Liquid Scintillation Counting analysis which does not allow for differentiated quantification of metabolites and parent substance. Only a few studies investigated more closely the metabolism of this group of surfactants giving clear indications for the metabolism of LAS in fish. Data from Burke et al. (1991) showed that LAS are rapidly metabolized in rainbow trout to phenyldodecane which is the non-sulfonated analog of C_{12}-LAS. In another study (Tovell et al. 1975) a metabolite (4-butyrolactone) of C_{12}-AS was identified after hydrolysis of the sulfate ester. Studies with radiolabelled C_{12}-LAS by Tolls et al. (1997) resulted in a higher BCF for the metabolite than for the parent compound with a BCF of 173-245

and 62 for metabolite and parent compound, respectively. A similar result was observed for C_{13}-LAS.

In contrast to anionic surfactants such as LAS or AS, PFAS are metabolically inert, have a long biological half-life and thus tend to bioaccumulate along the food chain leading to increased PFAS concentrations in upper-trophic level organisms (Giesy & Kannan 2001; Kannan et al. 2005). The potential of PFAS to bioaccumulate seems to increase with the number of fluorinated carbon atoms and is obviously higher for perfluoroalkyl sulfonates compared with perfluoroalkyl carboxylates with similar chain lengths (Martin et al. 2003a, b; Goeritz et al. 2013). Dietary exposure bioaccumulation fish tests on rainbow trout with PFAS have been carried out (Martin et al. 2003 b; Goeritz et al. 2013). Both studies showed that biomagnification of PFAS was below the threshold of 1 and that bioaccumulation in the aquatic food web can be mainly attributed to bioconcentration processes in fish. It was shown that the size of the animals has no impact on the outcome of the studies (Table 6- 4).

Table 6- 4: Biomagnification of PFAS in juvenile and market-size rainbow trout *Oncorhynchus mykiss*

Acronym	CAS	α	I	k_2	BMFkin	Animal size (g)	Reference
PFAS							
PFBS	375-73-5	5.98	2.53	64.2	0.02	400	Goeritz et al. (2013)
PFDA	335-76-2	110	1.5	70	0.23	2	Martin et al. (2003a)
PFDoA	307-55-1	130	1.5	47	0.43	2	Martin et al. (2003a)
PFHxA	307-24-4	55.8	2.53	79.2	0.18	400	Goeritz et al. (2013)
PFHxA	307-24-4	70	1.5	76	0.14	2	Martin et al. (2003a)
PFNA	375-95-1	52.2	2.53	57.5	0.23	400	Goeritz et al. (2013)
PFOA	335-67-1	13.8	2.53	97.3	0.04	400	Goeritz et al. (2013)
PFOA	335-67-1	59	1.5	230	0.038	2	Martin et al. (2003a)
PFOS	1763-23-1	72.1	2.53	43.0	0.42	400	Goeritz et al. (2013)
PFOS	1763-23-1	120	1.5	54	0.32	2	Martin et al. (2003a)
PFTA	376-06-7	130	1.5	20	1.0	2	Martin et al. (2003a)
PFUnA	2058-94-8	110	1.5	61	0.28	2	Martin et al. (2003a)

α = assimilation efficiency; k_1 = uptake rate; k_2 = depuration rate; I = Ingestion rate

BMF = $\alpha * I / k_2$

6.3.2 Cationic surfactants

For this category of surfactants, the hydrophilic part is positively charged, e.g. with a quaternary ammonium ion.

Table 6- 5: Bioconcentration data of cationic surfactants (three significant digits displayed).

Acronym	CAS	Aqu.Conc.	k_1	k_2	BCFkin	BCF	Species	Reference
Dialkyldimethyl ammonium compounds (DMAC)								
(C16/18)2-DMAC		0.034				32	*Lepomis macrochirus*	Lewis & Wee (1983)
(C18)2-DMAC	107-64-2					104	*Pimephales promelas*	Versteeg & Shorter (1992)
(C18)2-DMAC	107-64-2		32	0.31	103		*Pimephales promelas*	Versteeg & Shorter (1992)
(C18)2-DMAC	107-64-2		18	0.48	37.5		*Pimephales promelas*	Versteeg & Shorter (1992)
(C18)2-DMAC	107-64-2		1.6	0.58	2.8		*Pimephales promelas*	Versteeg & Shorter (1992)
Monoalkyltrimethyl ammonium compounds (TMAC)								
C16/18-TMAC	112-02-7 / 112-03-8					1960	*Pimephales promelas*	Versteeg & Shorter (1992)
C16/18-TMAC	112-02-7 / 112-03-8		235	0.12	1960		*Pimephales promelas*	Versteeg & Shorter (1992)
C12-TMAC	112-00-5					35	*Pimephales promelas*	Versteeg & Shorter (1992)
C12-TMAC	112-00-5		20	0.58	34.5		*Pimephales promelas*	Versteeg & Shorter (1992)
C8-TMAC	10108-86-8					2.4	*Pimephales promelas*	Versteeg & Shorter (1992)
C8-TMAC	10108-86-8		1.1	0.48	2.3		*Pimephales promelas*	Versteeg & Shorter (1992)

With regard to TMAC it could be clearly shown that the chain length of surfactants correlates with the bioconcentration potential (BCF value) of the molecules. A BCF increase with increasing length of the alkyl chain was observed (Versteeg & Shorter 1992). The uptake of cationic surfactants occurs via the gills (Tolls & Sijm 1995). Differences in the absorption of cationic surfactants – TMAC is better absorbed by the gills as DMAC – might be explained by the different head groups of the molecules (Tolls & Sijm 1995). While the monoalkyl cationic surfactants such as TMAC form micelles, the cationic surfactants with two alkyl chains such as DMAC have a tendency to form bilayers (Tanford 1980; Scherer & Seelig 1989). DMAC compounds may thus be stabilized in the lipid bilayer of the membranes leading to a slower transport through the membrane comparing to TMAC (Tolls & Sijm 1995). In contrast to anionic and nonionic surfactants information on the biotransformation of cationic surfactants is not available (Tolls & Sijm 1995).

6.3.3 Nonionic surfactants

Nonionic surfactants are surfactants with a non-charged hydrophilic part, e.g. ethoxylate. BCF data (Table 6- 6) for nonionic surfactants were described by Bishop and Maki (1980) and Wakabayashi et al. (1987). They investigated the influence of the number of oxyethylene units on the bioconcentration behavior of dodecyl oxyethylene ethers (C_{12}-EO_n). The presumption was that an increased number of oxyethylene units leads to a higher BCF. However, in comparison with other studies, this could not be confirmed (Tolls et al. 1995). A comparison from Tolls et al. (2000) showed that the BCF of alcohol ethoxylate (C_nEO_8) increase with an increase of the length of the alkyl chain (

Table 6- 7).

Table 6- 6: Bioconcentration data of nonionic surfactants (three significant digits displayed).

Acronym	CAS	Aqu.Conc.	k_1	k_2	BCFkin	BCF	Species	Reference
Alkyloxyethylene surfactants								
C14-EO7	27306-79-2	0.41				721	*Lepomis macrochirus*	Bishop & Maki (1980)
C14-EO7	27306-79-2	0.41	903	1.23	734	731	*Lepomis macrochirus*	Bishop & Maki (1980)
C14-EO7	27306-79-2	4.1				684	*Lepomis macrochirus*	Bishop & Maki (1980)
C14-EO7	27306-79-2	4.1	959	1.2	799	799	*Lepomis macrochirus*	Bishop & Maki (1980)
C12-EO4	9002-92-0	0.69	189	0.61	310	309	*Cyprinus carpio*	Wakabayashi et al. (1987)
C12-EO8	9002-92-0	0.45	53.1	0.24	221	222	*Cyprinus carpio*	Wakabayashi et al. (1987)
C12-EO16	9002-92-0	0.28	0.95	0.22	4.3	4.3	*Cyprinus carpio*	Wakabayashi et al. (1987)

Table 6- 7: BCF data for alcohol ethoxylates (C_nEO_8) with different alkyl chain lengths (three significant digits displayed).

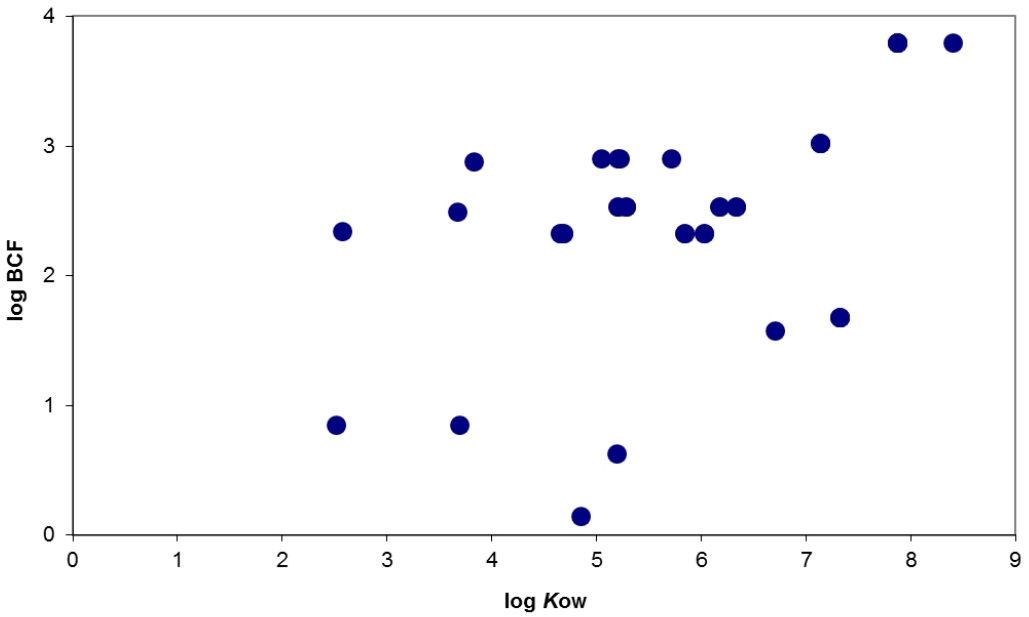
C_n	BCF
12	12.7
13	49.9
14	56.7
15	388

Like anionic surfactants, nonionic surfactants are metabolized in fish (Tolls & Sijm 1995). Tolls and Sjim (1999) showed in a BCF study with radiolabelled $C_{14}EO_8$ a higher accumulation of a metabolite compared to the parent compound.

6.3.4 Correlation between log K_{ow} and BCF estimates

A range of log K_{ow} data are presented for surfactants in the literature (Tolls 1998). It was shown in Chapter 6.2 that the log K_{ow} of PFAS correlates with their log BCF and that protein binding does not provide additional information about the quantitative bioaccumulation of such compounds. However, when log K_{ow} and BCF estimates of other, non-perfluorinated, surfactants are compared no correlation was found which might be explained by the amphiphilic nature of those substances (Figure 21). Experimental BCF of surfactants seem to be independent of log K_{ow}. However, due to the tendency to aggregate at the interface of liquid-liquid systems the log K_{ow} should be used with caution. Formation of emulsions is a serious experimental problem in the determination of octan-1-ol/water partition coefficients for surfactants (Tolls 1998). Therefore, estimates of this parameter are very difficult or even impossible to measure and most probably incorrect. In addition to that, measurements of log K_{ow} for ionic surfactants will yield distribution ratios rather than partition coefficients (Schwarzenbach et al. 1993). As an alternative measure of hydrophobicity, the critical micelle concentration (CMC) was suggested (Tolls & Sijm 1995).

Figure 21: Relationship between calculated log Kow and log BCF (experimental data) of surfactants.

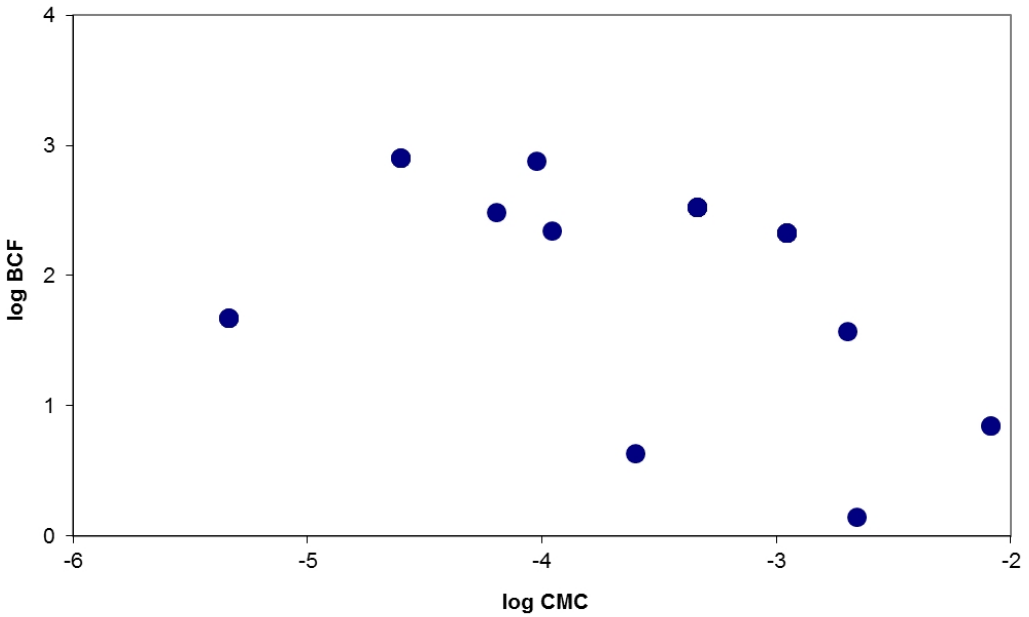


6.3.5 Correlation between CMC and BCF estimates

The CMC is the concentration at which half of the molecules in solution are associated as micelles and thus represents the maximum concentration of freely dissolved surfactants. The more hydrophobic a surfactant is, the less favorable it is for those molecules to remain dissolved

in water (Tolls 1998). There is, however, no simple linear relationship between BCF and CMC of surfactants (Figure 22). Instead, high variability leads to low r^2 of the correlation. Tolls and Sijm (1995) concluded that CMC might not be a good measure of the hydrophobicity of surfactants from different classes.

Figure 22: Relationship between log CMC and log BCF (experimental data) of surfactants.



The most important findings of our exploratory data analyses on surfactants can be summarized as follows:

- The available bioconcentration data for surfactants are not sufficient to fully assess their bioaccumulation potential. Most of the BCF estimates are below the threshold of 2000. Only for TMAC a BCF close to this value (BCF=1960) was estimated.
- The bioconcentration of surfactants other than PFAS in fish does not correlate with log K_{ow}, however other properties such as the length of the alkyl chains and number of oxyethylene units seem to play a role with regard to the uptake and bioaccumulation of surfactants.
- The suitability of CMC as an alternative measure of the hydrophobicity of surfactants was tested; however, there is no indication for a general suitability of this measure.
- For the most part, the distribution and accumulation of surfactants in organisms depend on their absorption at biological interfaces. The metabolism of surfactants may also have an impact on the accumulation and distribution of the molecules in fish as shown for anionic and nonionic surfactants. However, information on the biotransformation kinetics as well as knowledge on the protein binding of anionic, nonionic and cationic surfactants in fish is often limited.
- Surfactants may easily absorb to food items, possibly causing an increased dietary uptake. As yet, quantitative information on the dietary uptake of surfactants is limited.

6.4 Gastrointestinal absorption

6.4.1 Relevant (passive/active) gastrointestinal absorption mechanisms

After oral uptake a substance will enter the gastrointestinal tract. The epithelium of the gastrointestinal tract of various species maintains the exchange barrier for solids and fluids. After oral administration a molecule can penetrate gastrointestinal membranes in different ways due to several transport mechanisms.

The respective transport mechanisms are divided basically into two groups: passive uptake and active transport (Figure 23).

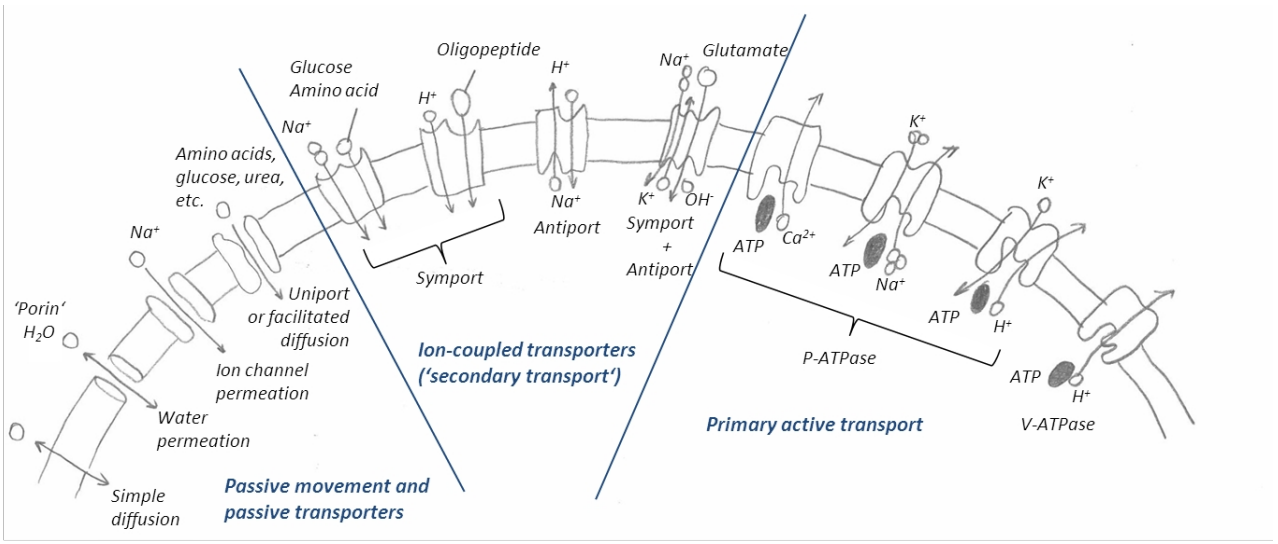
The passive uptake pathway is divided into two different mechanisms:

- Paracellular permeability is driven by transepithelial gradients which allow a molecule to cross the tight junctions between intestinal enterocytes through pores (prerequisite: non-ionized, hydrophilic molecules) (Hurst et al. 2007).
- Transcellular transport, in which the molecule has to pass the lipid membrane (prerequisite: lipophilicity).

For the active uptake various pathways from the gastrointestinal tract are known. The active uptake is differentiated in the so-called influx and efflux transporters that are either responsible for the selective uptake (influx) of a molecule or its release (efflux) from the interior of the cell (Hurst et al. 2007). It is important to emphasize that the relevance of each of the two routes is dependent on the respective substance-specific molecular properties (Thomas et al. 2006).

Which transport mechanism is existent for an individual molecule is depending on its physicochemical properties. Physiological factors such as the intestinal pH, blood circulation, lymph flow, the pathological state, drug interactions, nutrition, and the dissolution behavior in the mucus are some factors that are species specific and might differ between animals.

Figure 23: General Scheme of membrane transport.



Adapted from: „Environmental Physiology of Animals“, Willmer, Stone, Johnson; Blackwell Science Ltd.; 2000

As an *in vitro* model for determining the intestinal absorption processes in humans the Caco-2 monolayers are often used in pharmaceutical industry. One advantage of this model is the

possibility to investigate the membrane absorption processes including both - the passive transport (paracellular and transcellular) and active transport processes (Fujikawa et al. 2005).

6.4.2 Passive transport in the GIT

Passive diffusion requires no energy. Rather the molecule diffuses through the cell membrane due to a concentration gradient. Thereby, the rate of diffusion is dependent on the area and thickness of the membrane as well as on the substance-specific diffusion coefficients. In the intestine the permeability of the tight junctions decreases significantly from the proximal to the distal end. This pore size is equal to the size of uncharged molecules (jejunum approximately 0.8 nm; ileum approximately 0.4 nm; Colon approximately 0.2 nm) and the ion selectivity (Thews & Vaupel 2005). The diffusion rate is quantitatively described by Fick's law of diffusion (Equation 6). The diffusion coefficient is depending on the diffusion medium (for example, viscosity and absolute temperature) and the nature of the diffusing molecule (such as molecular size, structure, lipophilicity, pK_a and charge) according to Ficks law of diffusion

(Equation 6)

with D = diffusion coefficient in m^2s^{-1}.

As already mentioned, the passive permeability of biological membranes can be divided into 2 groups: the paracellular diffusion and the transcellular transport.

a) Paracellular diffusion in the GIT

In the paracellular diffusion a molecule passes biological membranes using the tight junctions between the intestinal enterocytes via pores. As a prerequisite for the paracellular diffusion, the molecular size should not exceed 250 Da and the molecule should be of hydrophilic nature (log K_{ow} <0) (Hurst et al. 2007). Since the total membrane complex adduced a negative charge, positively charged molecules can pass the tight junctions faster than negatively charged molecules (Karlson et al. 1999). Overall there are only minor species-related differences in the paracellular diffusion between rats and humans (He et al. 1998). Therefore, the rat is a good model for predicting human bioavailability of compounds that are absorbed through the paracellular route.

b) Transcellular membrane transport

The passive transcellular transport of substances is directed from the apical site of the membrane, through the cytoplasm to the basolateral site of the membrane. A distribution equilibrium is established at the membrane surface according to the partition coefficient; the transport within the membrane is then according to the diffusion law. Generally hydrophobic organic substances, as well as monoglycerides and fatty acids are capable to diffuse through biological membranes due to their lipophilicity (Daughterty 1999). However, for an unrestricted permeation of membranes a substance should preferably be both hydrophilic and lipophilic (Artursson et al. 2001).

A measure for permeability is often the logarithm of the octan-1-ol/water partition coefficient (log K_{ow}) of a substance in the non-ionized state. For substances with log K_{ow} values near 2 a complete absorption is generally predicted (Artursson et al. 2001; Lipinski 2000). For substances with log K_{ow} values >4 the permeability gradually begins to decline since the substance is then sufficiently hydrophobic that it easily penetrates into the lipid bilayer, but it cannot leave the

aqueous interior of the membrane though the basolateral site of the membrane. In the case of ionizable substances the log *D* is commonly used. The log *D* is the logarithm of the octan-1-ol/water partition coefficient at a particular pH value. This value is often used to determine a more meaningful database for interpretation of processes in the intestine (Artursson et al. 2001, Hou et al. 2004, van de Waterbeemd & Testa 2008).

6.4.3 Active transport

The active membrane transport is divided into three main mechanisms: the secondary active transport using carrier, the primary active transport using ATP and the vesicle transport.

a) Secondary active transport

Carrier mediated or secondary active mechanisms allow facilitated transcellular absorption of certain substances. This process consists of two parallel mechanisms. First an active transport part, which transports a molecule out of the cell and a second simultaneous bidirectional passive transport mechanism utilizing the existing electronic gradient. In summary the secondary active transport uses a specific gradient that originates from primary active transport (ATP dependent) but the secondary active transport itself does not use ATP (Boudker & Verdon 2010).

Especially for hydrophilic substances the transport through membranes might be facilitated via secondary active transport mechanism by coupling the substance temporarily to the carrier and therefore allowing it to have sufficient lipophilic properties to cross membranes (Artursson et al. 2001; Lennernäs 2007).

An example of such a secondary active system is the sodium-dependent glucose transport in the intestine. The ATP dependent sodium pump causes a directed electrochemical gradient for sodium, which is the driving force for uptake of glucose cotransport from the intestinal lumen into the cell. Finally the increased glucose concentration in the cell leads to a facilitated diffusion of glucose through the basolateral membrane out of the cell.

b) Primary active transport

The primary active transport along the gastrointestinal tract is catalyzed by several membrane bound enzymes under ATP consumption. Several substrates can be carried against an electrochemical potential. In summary the intestinal transport mechanisms can be divided into two classes: the influx transporter and efflux transporters (Kunta & Sinko 2004).

Influx Transporter

In the following some influx transporter are listed exemplarily, which play an important role for the absorption of substances in the gastrointestinal tract (Dobson et al. 2008).

- Peptide transporter (PEPT 1)
- Organic anion-transporting polypeptides (OATPs)
- Nucleosid transporter
- Amino acid transporter
- Monosaccharid transporter
- Bile acid transporter

Molecules that are substrates of those influx transporters may have a higher intestinal absorption than predicted by their physicochemical properties.

Efflux Transporter

Compared to the influx transporters the efflux transporter solve as an absorption barrier that limits the oral bioavailability of selected substances. Here, substances are released back into the intestinal lumen directly after their uptake by secretion (Anderle et al. 2004).

Most efflux transporters belong to the family of ATP binding cassette (ABC) and are formed at the apical surface of intestinal enterocytes (Fagerholm 2007).

The following transporters belong to the ABC transporters:

- P-glycoprotein (P-gp)
- Breast cancer resistance protein (BCRP)
- Multi-drug resistance associated protein (MRP)

These transporters play an important role in limiting intestinal absorption processes.

c) Vesicle transport (endocytosis / exocytosis)

In addition, there are other active processes on cell surfaces by which a substrate can be transported across cell membranes, such as endo-and exocytosis.

Endocytosis is the inclusion of materials from outside of the plasma membrane to inside the cell. Finally the compounds are released by lysis of the vesicle. In the case that liquids are transported the mechanism is called pinocytosis, if solids are transported the mechanism is called phagocytosis.

The exocytosis is a mechanism where substances are transported from the interior of the cell to the extracellular space. In the cytosol located vesicles fuse with the cell membrane and the transported substance is released.

Membrane transport / absorption mechanisms

The principal routes of absorption in the gastrointestinal tract (GIT) were studied for different species (fish, rodents, humans). Relevant passive and active uptake mechanisms are summarized in Table 6- 8. The potential uptake mechanisms are listed and distinguished according to the particular molecules transported and in which species this mechanism is present. Additionally suitable example substances are listed.

Table 6- 8: Relevant passive and active absorption mechanisms in the gastrointestinal tract (GIT).

Uptake	Mechanisms	Molecules transported	Species	Examples
Passive	Paracellular (Filtration, diffusion, osmosis)	Small, uncharged, polar	Ubiquitous	H_2O, Glycerol, N_2
	Transcellular	Lipophilic		Neutral organics
	Facilitated diffusion Uniporter	Polar, charged		Amino acids, urea, glucose, Na^+

Secondary active	Coupled transport / co-transport	Peptides (PEPT1) Monosaccharides (SGLT1) Organic Cations (OCTN) Organic Anions (OATP)	Rodents / humans PEPT1 expression in fish[1a,b]	PEPT1:[3] Cephalosporin antibiotics OATPs:[4] Azithromycin
Primary active	ATP dependent transport	e.g. Pharmaceuticals Influx / Efflux (ABC)	Rodents / humans / fish[2]	ABC:[5a,b] Methotrexate Digoxine Phytosterole
Other (active)	Endocytosis	Fluids (pinocytosis) Solids (phagocytosis)	Ubiquitous (eukaryotic cells)	Uptake of e.g. food components
	Exocytosis	Cellular components (in vesicles)		Secretion of macromolecules

PEPT1 = PEPtide Transporter, ABC = ATP Binding Cassette; OCTN = organic cation Transport, OATP = organic anion transport polypeptide, SGLT = sodium/glucose transporter

1a) Rønnestad et al. 2010 / 1b) Bakke et al. 2010 / 2) Popovic et al. 2010/ 3) Lee & Yamamoto 1990 / 4) Garver et al. 2008 / 5a) van de Waterbeemd & Testa 2008 / 5b) Izar et al. 2011.

The most important findings of our exploratory data analyses on passive and active gastrointestinal absorption can be summarized as follows:

- The passive diffusion correlates well with the log K_{ow} of substances.
- Substances which use secondary active transport via carriers might have an increased absorption compared to their predicted absorption using their molecular properties. In these cases an underestimation of BCF values might be possible.
- The primary active transport mostly limits the absorption of substances, and thus it is an opposing mechanism with respect to bioaccumulation.

6.4.4 Relationships between bioaccumulation and uptake in the GIT

6.4.4.1 Approach and data set

To develop a screening level tool for the prediction of the absorption potential of chemicals, BCF values from the OSIRIS dataset and corresponding uptake information were taken from different data sources (Zhao et al. 2004; Linnakoski et al. 2006; Veber et al. 2002; Broccatelli 2012; Leung et al. 2012; Pham-The et al. 2013). The most important criteria for selection were a sufficient number of datapoints, available information on chemical structures and potential relevance of the endpoints with respect to the uptake. The chemical structures were preferably taken from the specified code or structure formulas. In other cases the ChemProp database (UFZ 2014) and additional sources were used to determine the specific structures of the substances.

From the available database 78 compounds with experimental log P_{app} values were analyzed for their correspondence to some physicochemical properties. This approach is based on mechanistic knowledge, data availability considerations and feasibility estimations. Candidate properties with known impact on permeability were selected and tested.

The polar surface area (PSA), the log K_{ow} and the molar weight have been identified to be suitable for that purpose. Alternatively, a set of solvatochromic parameters turned out to be useful.

Notably, the error distribution is not uniform over all data. There are some substantial outliers with logarithmic P_{app} differences of up to 6 corresponding to deviations of six orders of magnitude for P_{app}. Several other data fit much better. Outliers obscure the regression line in an undesired manner. This effect is particularly large in small data sets. In order to obtain a realistic trend, removal of the largest outliers is justified for that reason here. So, we omitted 6 items from the initial set. With the final set of 72 compounds, the analyses were carried out again.

The resulting correlations of BCF and P_{app} values from Caco-2 assays with physicochemical descriptors do not meet the quality standards for quantitative models. This is mainly due to the limited number of suitable data points. Thus, the result is not a fully qualified QSAR, but a screening tool.

6.4.4.2 Estimations of Caco-2 based P_{app}

The octan-1-ol/water partition coefficient is a surrogate for the partitioning coefficient between lipid and water and it is expected to play a significant but not exclusive role in permeation. Estimation models for log K_{ow} are generally available as well as a quite large number of experimental data. For the current study the ChemProp (UFZ 2014) consensus model has been used in cases when no experimental data were known, but any other reliable model would also be suitable instead.

Since permeation through pores is important, the size of molecules is relevant. The molecular weight is applied here as a rough descriptor of the molecule size. The molecular weight can always be calculated as long as the exact chemical structure is available.

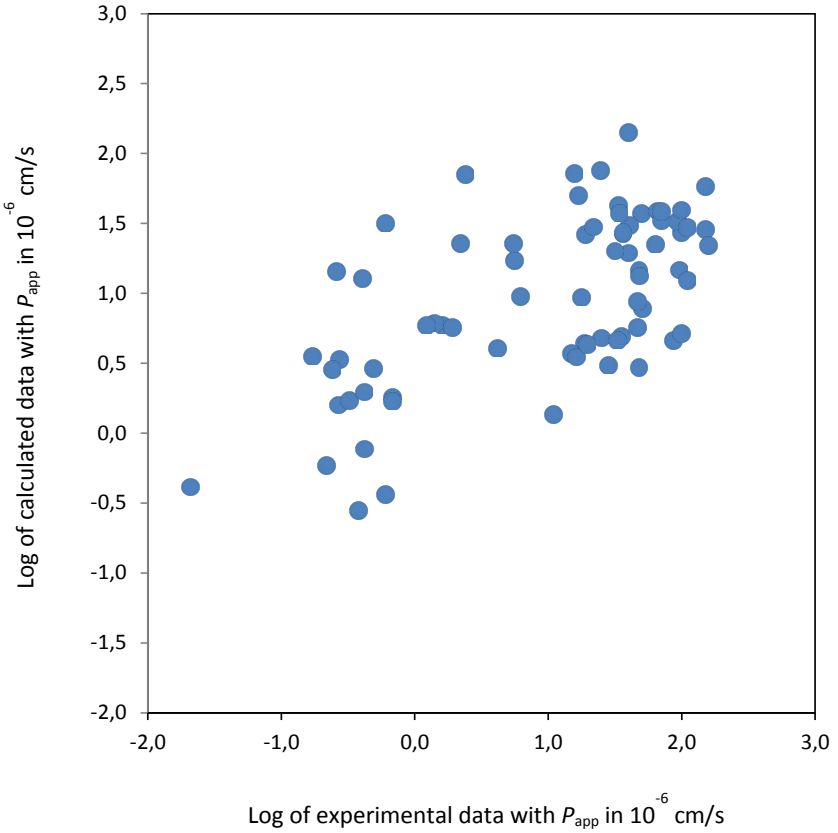
The polar surface area (*PSA*) is known to have an important influence on permeability, thus it is included here also. *PSA* can be predicted in terms of the "topological PSA" (*TPSA*) from the chemical structure by a fragment model (Ertl et al. 2000). This model is implemented in ChemProp (UFZ 2014) for automated runs.

In order to obtain similar orders of magnitude for the model parameters, *TPSA* (Å^2) and molar weight (D) have been scaled through dividing by 100. Multiple linear regression then yields

$$\log P_{app} = 0.17 \log K_{ow} - 0.3 \frac{MW}{100} - 0.7 \frac{TPSA}{100} \qquad \text{(Equation 7)}$$

with P_{app} in 10^{-6} cm/s, *TPSA* in Å^2, and *MW* in D. The statistics for n = 72 are a squared regression coefficient r^2 = 0.39 and root mean squared error *rms* = 0.76. Obviously, and as expected, affinity to lipids as expressed by log K_{ow} increases the permeability, while molecule size and uneven charge distribution at the molecular surface decrease it. The plot of the approximately estimated log P_{app} values vs. the experimental data is shown in Figure 24.

Figure 24: P_{app} estimated from physicochemical properties by Equation 7 vs. experimental data (x) with P_{app} in 10^{-6} cm/s.



Interestingly, Equation 7 shows some noticeable accordance with the work of Hou et al. 2004. They published a series of different equations, with several descriptors including the descriptors used in this work, even though not in this combination. Correlating to *TPSA* only they obtain a coefficient of -0.010 for P_{eff} that corresponds to P_{app} (Equation 8):

$$\log P_{eff} = -4.36 - 0.010\ TPSA \qquad \text{(Equation 8)}$$

This is quite similar to $-\frac{0.7}{100} = -0.007$ in Equation 7. Their statistics of r^2 = 0.41 and *rms* = 0.61 are comparable to our regression, however their data set was limited to a range of ca. 2.5 orders of magnitude in comparison to the more than 4 orders of magnitude of our data. Furthermore, their log K_{ow} regression yields a coefficient of 0.236, similar to 0.17 in Equation 7:

$$\log P_{eff} = -5.469 + 0.236 \log K_{ow} \qquad \text{(Equation 9)}$$

with an r^2 of 0.22 and *rms* = 0.66. Both models of Hou et al. (2004) provide a rather larger intercept of -4.36 and -5.469, respectively. Our molar weight term on average contributes to roughly -1 and thus covers ca. ¼ of this constant. The remaining offset of above -3 obviously is a matter of the unit applied. Unfortunately, Hou et al. (2004) do not give the units of the data in their paper but taking into account the observed agreement we suppose their unit of P_{app} (or P_{eff} in their nomenclature) is 10^{-3} cm/s.

Alternatively, LSERs with solvatochromic parameters were applied. This approach basically represents the major potentially important factors determining partitioning. These are hydrogen bonding, polarity and polarisability, electronic interactions and molecular size. The parameters are scaled in a balanced manner and the coefficients can easily be compared and mechanistically interpreted with regard to the underlying interactions. When a certain

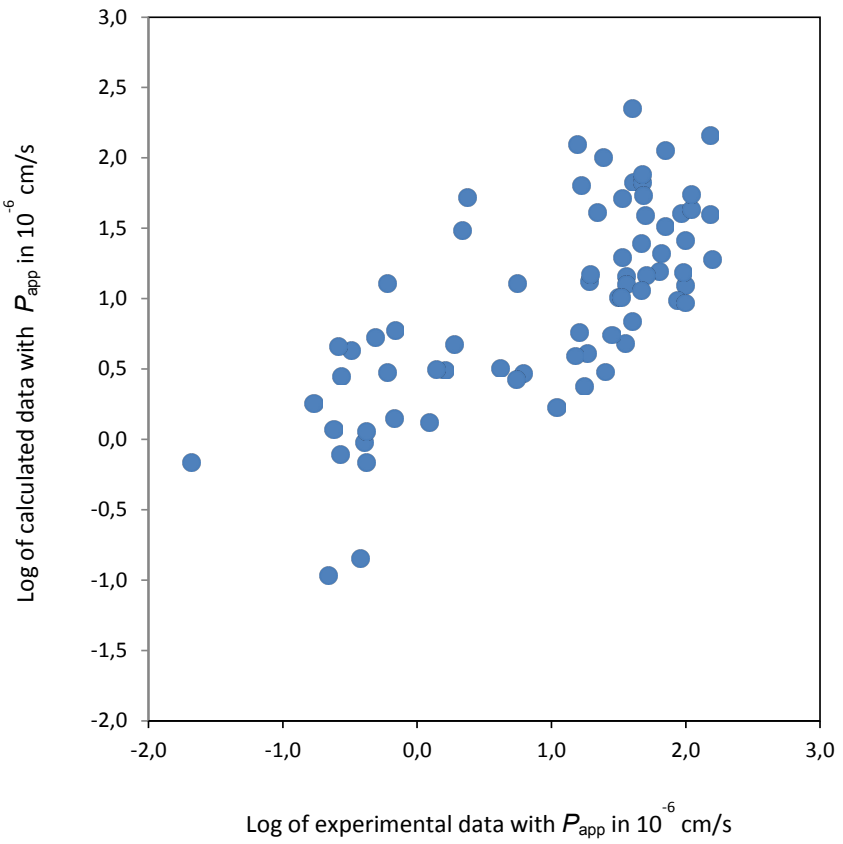
interaction is not important for a particular partitioning process, the respective term can be omitted. Omitting terms and thus reducing the number of parameters to be fitted is especially desirable here with respect to the small size of the data set. The resulting LSER equation is

$$\text{Log } P_{app} = -0.88\ A - 0.80\ B + 0.11\ S \qquad \text{(Equation 10)}$$

again with P_{app} in 10^{-6} cm/s. *A* is the hydrogen bond donor capacity (acidity) and *B* the hydrogen bond acceptor capacity (basicity) of the solute. There is a similar strong negative effect of both features. The opportunity to participate in intermolecular hydrogen bonds, no matter whether as a donor or as an acceptor, retains compounds in the aqueous phase because of the energy gain by building hydrogen bonds with water. The parameter *S* denotes polarity and polarisability. The slightly positive effect of this factor may be surprising at first glance particularly when comparing to the strong negative coefficient of *TPSA* in Equation 7. A possible but rather hypothetical explanation is that this slightly positive value numerically compensates a moderate overestimation through *A* and *B*.

The statistics for the fitting of Equation with again $n = 72$ are $r^2 = 0.52$ and $rms = 0.67$. The plot of the estimated vs. the experimental values is shown in Figure 25.

Figure 25: P_{app} estimated from solvatochromic parameters by Equation 10 vs. experimental data (x) with P_{app} in 10^{-6} cm/s.



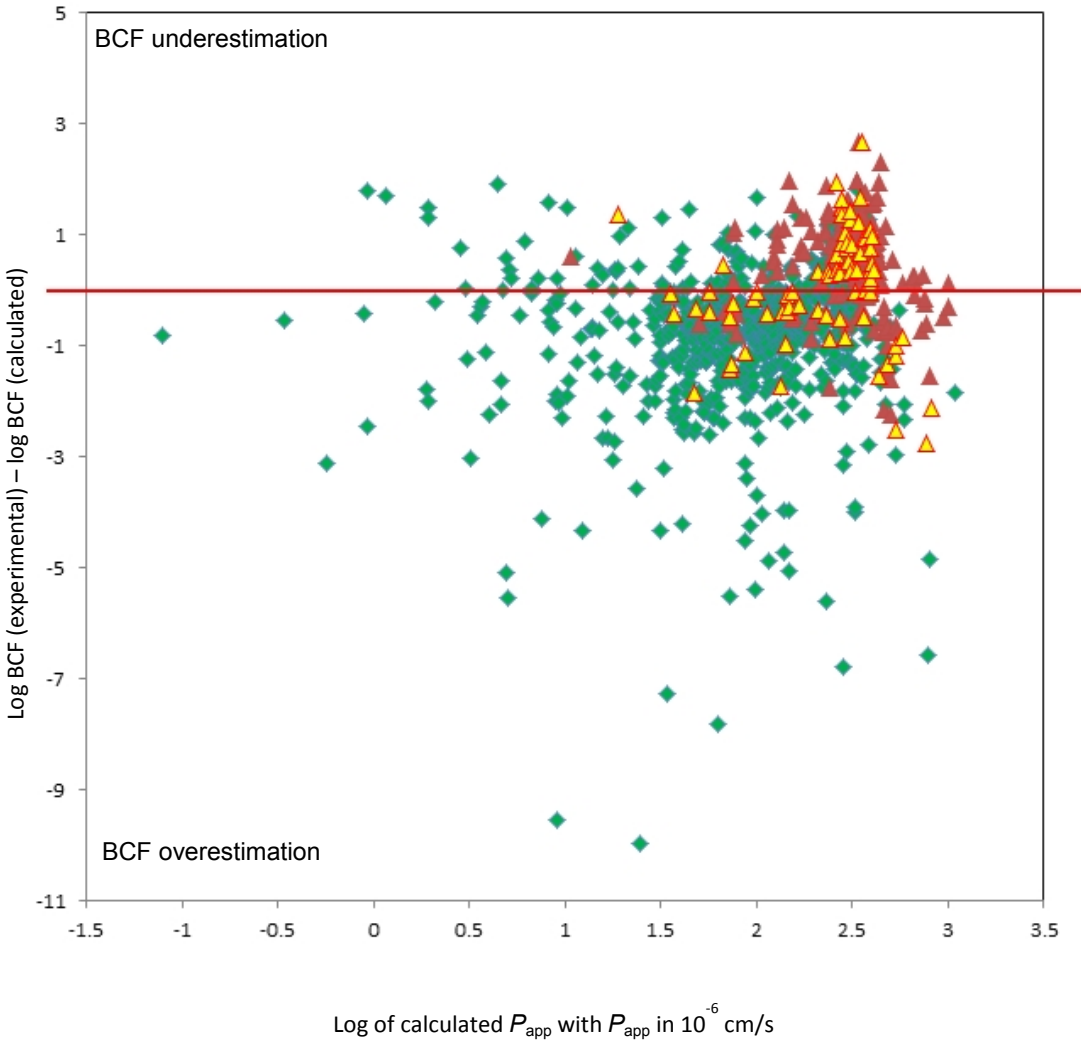
In result, the performance of both approaches is comparable. Since the LSER with solvatochromic parameters (Equation 10) yields slightly superior statistics, it was selected for the bioaccumulation study.

6.4.4.3 Relationships between Caco-2 based P_{app} and BCF deviations

As explained, reliable experimental log BCF data were available for 998 chemicals (OSIRIS 2007-2011). To estimate the lipid based bioaccumulation, a reference (TGD) model (Equation 2) by Veith et al. (1979) for log BCF as used in TGD/EUSES (European Commission 2003) was applied. Experimental or calculated log K_{ow} values as explained above were used.

First, the deviations from the log K_{ow} based log BCF model expressed as (log $BCF_{experimental}$ – log $BCF_{predicted}$) have been compared directly to the log P_{app} estimations from Equation 10. Using this expression, underestimation of the BCF yields positive values and overestimation yields negative values.

Figure 26: Log $BCF_{experimental}$ – log $BCF_{predicted}$ vs. logarithmic P_{app} estimated from solvatochromic parameters.



True BCF classes based on experimental data are coded by colors, green denotes BCF <1000 (clearly nonB), yellow denotes 1000 ≤ BCF <2000 (nonB, but close to B), and red denotes BCF ≥2000 (B). The horizontal red line marks the border between overestimation (bottom) and underestimation (top) of the BCF by a reference model (Equation 2).

The comparison is shown in Figure 26. The deviations from the log K_{ow} reference model are plotted against the modeled logarithmic permeability log P_{app}. Values below the red line result from BCF overestimation and thus are generally less critical than values above the red line

originating from an underestimation of the BCF. The estimated permeability increases from left to right in the graph.

BCF underestimation is very important with high BCF, but less relevant for actually low BCF values. To visualize this, the true BCF classes based on the experimental data are coded by colors in the figure. Compounds with BCF <1000 (i.e. safely nonB) are shown in green, chemicals with BCF ≥ 1000 but below 2000 (i.e. BCF close to but still below the nonB/B threshold) in yellow, and compounds truly to be classified as B (i.e. BCF at least 2000) in red.

The data points shown in yellow (medium BCF, but nonB) or red (high BCF, B) triangles concentrate on the upper right side of the plot. This indicates that rather high P_{app} estimations are generally observed for bioaccumulating chemicals. Furthermore, a large fraction of the true B compounds (red triangles) are located above the red line, corresponding to underestimation of the BCF by the octan-1-ol/water partition coefficient. For some of them the deviation is larger than one order of magnitude, as they are located above +1 on the y axis of Figure 26.

On the other hand, large predicted P_{app} values do not automatically mean BCF underestimation or even large BCF values. Even rightmost of the plot, both underestimation and overestimation occurs, and the respective chemicals belong to all of the three classes.

Due to the latter, a fully discriminating model based on estimated P_{app} is not possible, and, by the way, was never expected. However, the findings can be exploited to derive some useful reliable criteria based on estimated P_{app} that considerably go beyond rules of thumb, as shown below.

6.4.4.4 Relationships between Caco-2 based P_{app} and BCF

For appropriate screening criteria of increased bioaccumulation relative to estimations based on the Veith et al. (1979) model (Equation 2), Caco-2 based classifications have been verified with respect to underestimation of log BCF in the critical range. For this purpose, experimental log BCF are plotted against log K_{ow} and the derived P_{app} classes are marked by colors.

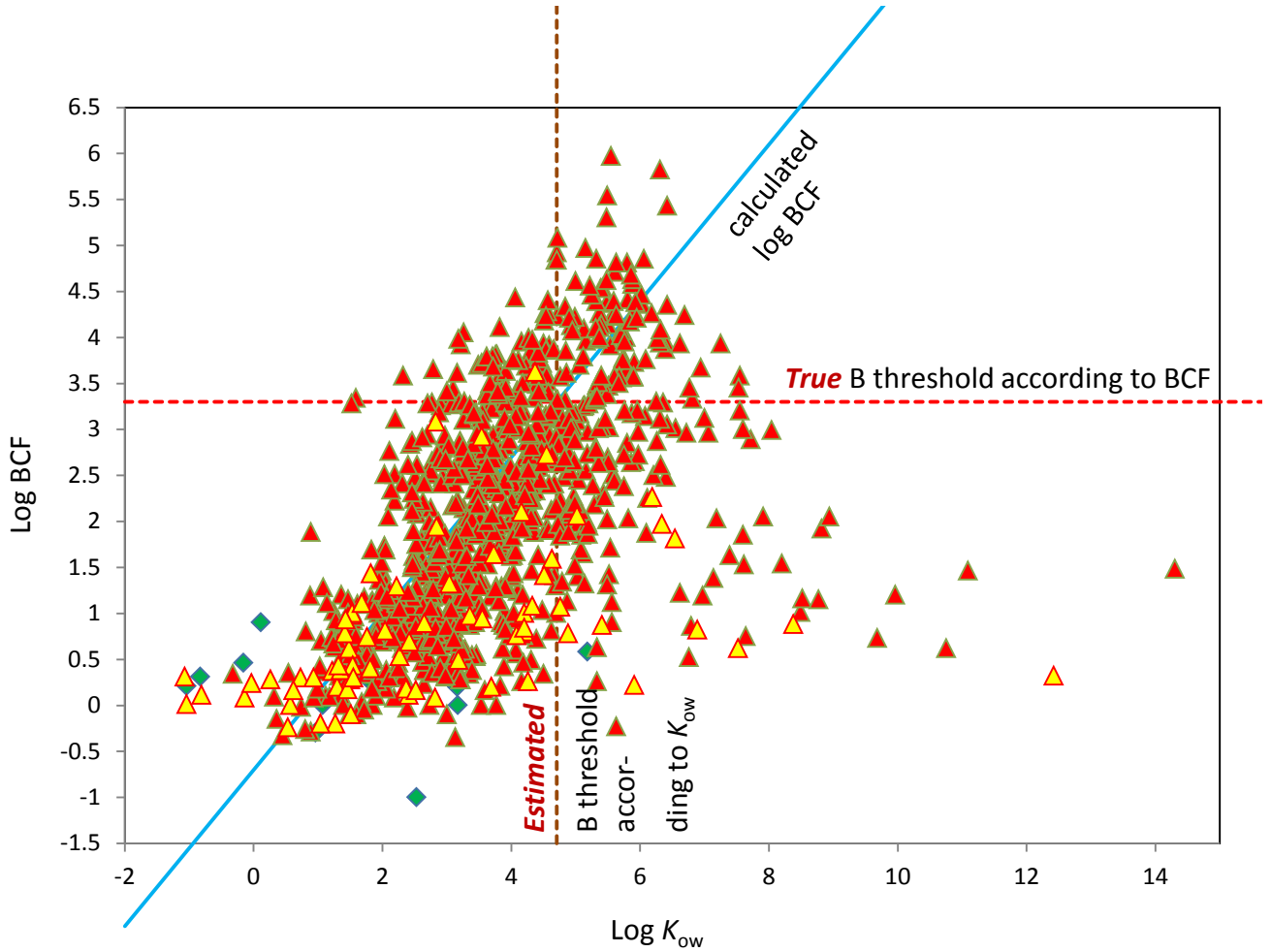
Figure 27 shows the respective plot, applying Caco-2 based P_{app} classifications by Pham-The et al. (2013) into low transport (green: below $0.7x10^{-6}$ cm/s), medium transport (yellow: from $0.7x10^{-6}$ cm/s to $16x10^{-6}$ cm/s) and high transport (red: above $16x10^{-6}$ cm/s). BCF underestimation through log K_{ow} occurs when the value is located left of the blue line. If additionally located above the red horizontal line, the compound will be classified wrongly as nonB if its location in the plot is left of the vertical brown line. Right of it, Equation 2 already predicts the compounds to be B.

It can be seen that the application of the Caco-2 classification by Pham-The et al. (2013) leads to a high amount of compounds with high permeability prediction. Therefore, the classifications might need to be adapted for the prediction of increased bioaccumulation in fish. There are several explanations possible to interpret these findings. First of all the Caco-2 cell line is a human derived intestinal cell line and it is most frequently used to test the absorption potential of pharmaceuticals in the GIT. Several influencing factors have to be considered to assign these values for fish intestines such as the transepithelial electrical resistance (TEER). TEER values of about 10 – 50 Ω cm^2 indicate high permeability, whereas values of >1000 Ω cm^2 indicate low permeability (Arnot et al. 2009). The TEER of Caco-2 cells (230 Ω cm^2) resembles that of both mammalian intestines (20 – 100 Ω cm^2) and fish intestines (25-50 Ω cm^2) (Nichols et al. 2009; de Wolf et. al 2007). However, the transepithelial resistance in fish is about factor 10 lower as compared to Caco-2 cells. As a lower TEER value indicates a greater absorption potential via the paracellular routes (i.e. tight junctions), fish intestines are considered to be more permeable

than indicated by Caco-2. As a consequence, the classification scheme for the Caco-2 values has to be adapted (increased).

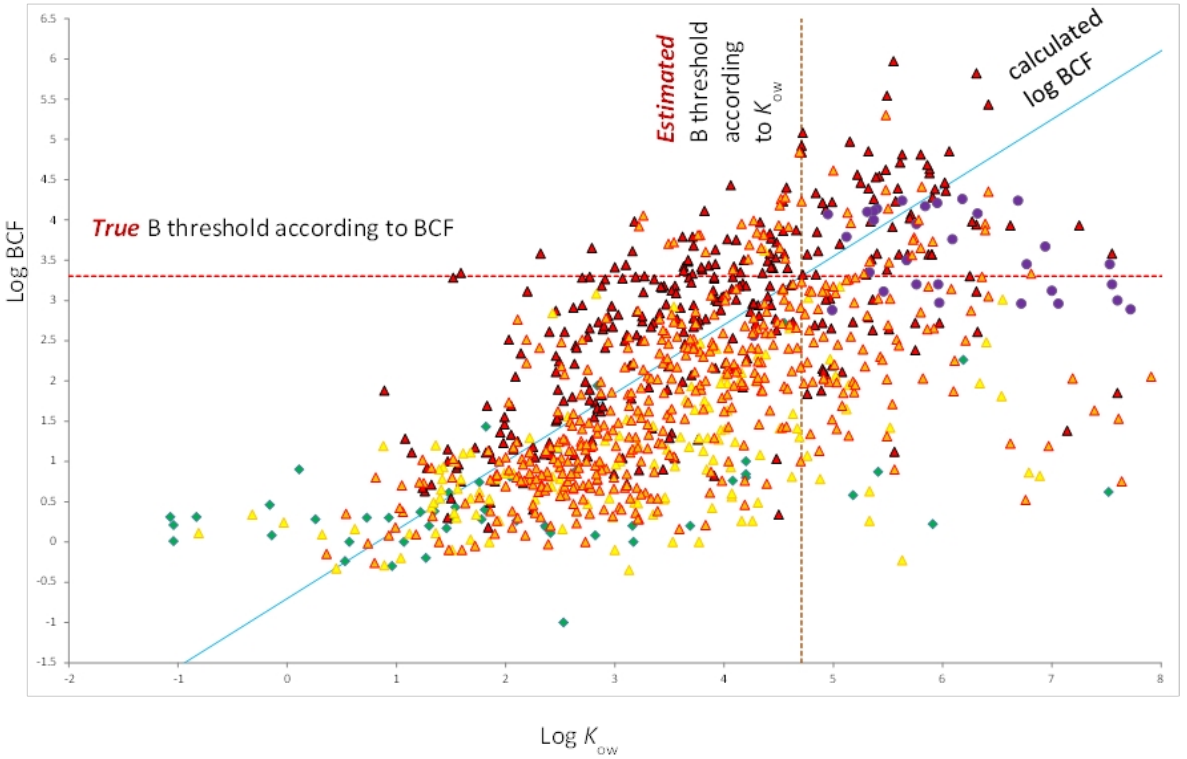
Therefore, P_{app} data are separated into 5 new adapted classes: (1) below $10x10^{-6}$ cm/s, (2) from $10x10^{-6}$ cm/s to below $50x10^{-6}$ cm/s, (3) from $50x10^{-6}$ cm/s to below $250x10^{-6}$ cm/s, (4) from $250x10^{-6}$ cm/s to below $500x10^{-6}$ cm/s, (5) above $500x10^{-6}$ cm/s. The plot in Figure 28 is similar to Figure 27, but this new classification is applied. The new classes are marked in green, yellow, orange, red, and violet, in that order.

Figure 27: Experimental log BCF vs. log K_{ow} with color-coded P_{app} ranges according to Pham-The et al. (2013).



Green denotes low transport (below $0.7x10^{-6}$ cm/s), yellow medium transport (from $0.7x10^{-6}$ cm/s to $16x10^{-6}$ cm/s) and red high transport (above $16x10^{-6}$ cm/s). The blue diagonal line represents the calculation results for log BCF from Equation 2. The horizontal red line marks the B threshold (below nonB, above B), and the vertical brown line marks the classification into nonB (left) and B (right) resulting from the estimated log BCF. BCF underestimation from log K_{ow} occurs when the value is located left of the blue line. If additionally located above the red horizontal line, the compound will be classified wrongly as nonB if its location in the plot is left of the vertical brown line. Right of it, Equation 2 already predicts the compounds to be B.

Figure 28: Experimental log BCF vs. log K_{ow} with respect to adopted classes of modelled P_{app} coded by colors.



Green denotes class 1 (<10x10^{-6} cm/s), yellow class 2 (10x10^{-6} cm/s to <50x10^{-6} cm/s), orange class 3 (50x10^{-6} cm/s to <250x10^{-6} cm/s) , red class 4 (250x10^{-6} cm/s to <500x10^{-6} cm/s) and violet class 5 (500x10^{-6} cm/s and higher). The irrelevant section of log K_{ow} >8 is omitted for clarity.

For class 1 (P_{app} below 10x10^{-6} cm/s), the BCF may be either underestimated or overestimated when using the log K_{ow} approach (Equation 2) by Veith et al. (1979). However, despite of the fact that this class includes some compounds with rather high log K_{ow}, all of these chemicals have experimental BCF below the B threshold (BCF <2000). Therefore, the bioaccumulation potential of these substances is of no concern.

For class 2 (P_{app} 10x10^{-6} cm/s to <50x10^{-6} cm/s), BCF overestimation by the log K_{ow} approach is typical (94%), but there are also a few (6) cases of underestimation. However, underestimation yielding a wrong classification to be nonB only appears in one case together with log K_{ow} above 4 (diethylstilbestrol ($C_{18}H_{20}O_2$) with an estimated log K_{ow} of 4.34).

For class 3 (P_{app} 50x10^{-6} cm/s to <250x10^{-6} cm/s), even if in most cases there is an overestimation of the BCF by the log K_{ow} approach as well (98%), there are still a rather notable number (11) of underestimations. However, wrong classifications as nonB instead of B only occur in 2 cases with predicted log K_{ow} above 3.

For class 4 (P_{app} 250x10^{-6} cm/s to <500x10^{-6} cm/s), there are a significant number of underestimations (34, i.e. 10%) as well as overestimations by the log K_{ow} approach. Wrong classification as nonB instead of B occurs with predicted log K_{ow} above 1 in 12 cases. The 54 compounds with very high P_{app} (class 5) also exhibit rather high log K_{ow}. Here, the well-known decline of BCF takes place. Some compounds predicted as B may have a BCF slightly below the B threshold, but there is no critical underestimation at all.

The most important findings of our exploratory data analyses on Caco-2 based P_{app} can be summarized as follows:

- P_{app} can be estimated from physicochemical properties: log K_{ow}, molecular size and polar surface area. Affinity to lipids as expressed by log K_{ow} increases the permeability, while molecule size and uneven charge distribution at the molecular surface decrease it.
- P_{app} can be estimated from LSERs with solvatochromic parameters: hydrogen bond donor capacity (acidity) and hydrogen bond acceptor capacity (basicity) of the solute. There is a similar strong negative effect of both features. The opportunity to participate in intermolecular hydrogen bonds, no matter whether as a donor or as an acceptor, retains compounds in the aqueous phase because of the energy gain by building hydrogen bonds with water.
- High P_{app} estimates are generally observed for bioaccumulating chemicals. The compounds (1.5%) which are actually B based on BCF studies but wrongly assigned to be nonB by the K_{ow} approach are mostly found in class 3 and 4, i.e. P_{app} values between 50 and $500x10^{-6}$ cm/s, with one exception in class 2.
- P_{app} estimates may be used to get a first impression of the absorption potential of some chemicals. However, the validity of this tool has to be further evaluated by applying to a larger dataset and by comparing predicted values to measured results including supposedly difficult compounds.

6.4.5 Metabolism

Metabolism was initially considered as an additional criterion to exclude compounds from being classified as bioaccumulative. This was tested with predicted half-lives of biotransformation τ (Arnot et al. 2009).

Figure 29 corresponds to Figure 25, with compounds predicted to be biotransformed rapidly marked by circles. Obviously, biotransformation prevents from transport in terms of P_{app}. However, this takes effect only for compounds that are not very bioaccumulative already as shown in Figure 30 as a repetition of Figure 29. Here, compounds with half-lives below 1 h^{-1} (upper graph) and even 10 h^{-1} (lower graph) are marked by circles.

It can be seen that metabolism may be a mitigating factor for bioaccumulation. However, it is obvious that it cannot be exploited for any safe exclusion of non-lipid based bioaccumulation.

Figure 29: P_{app} estimated from solvatochromic parameters by Equation vs. experimental data. Compounds with biotransformation half-lives τ below 1 h^{-1} are indicated by circles.

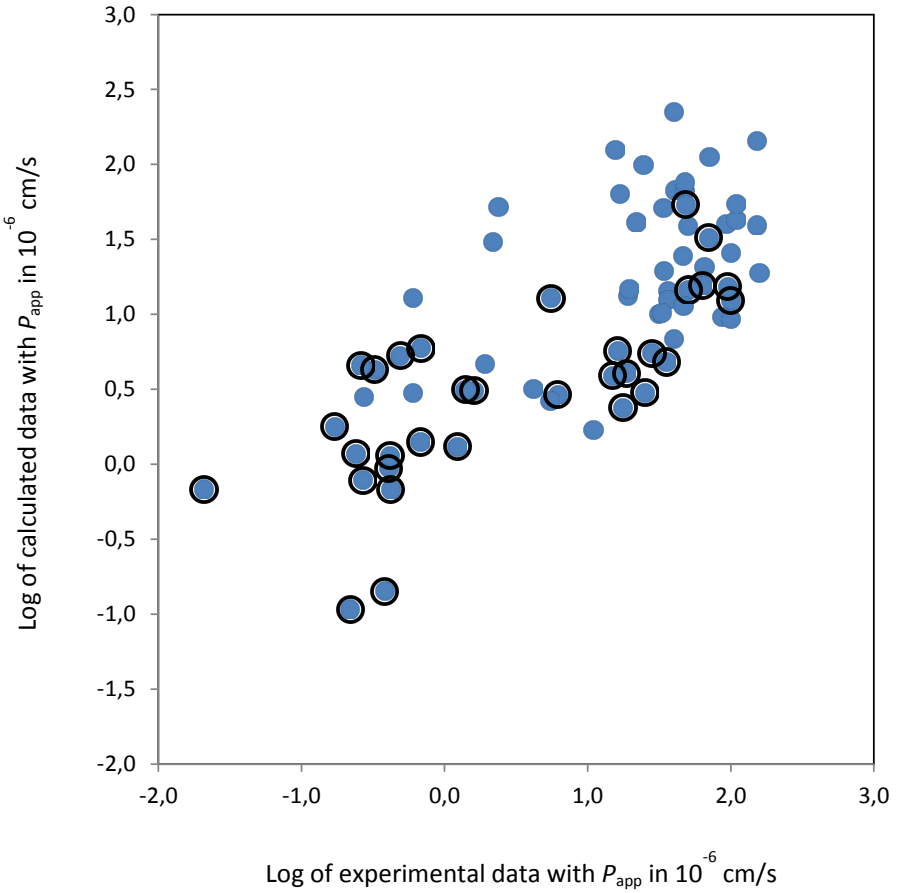
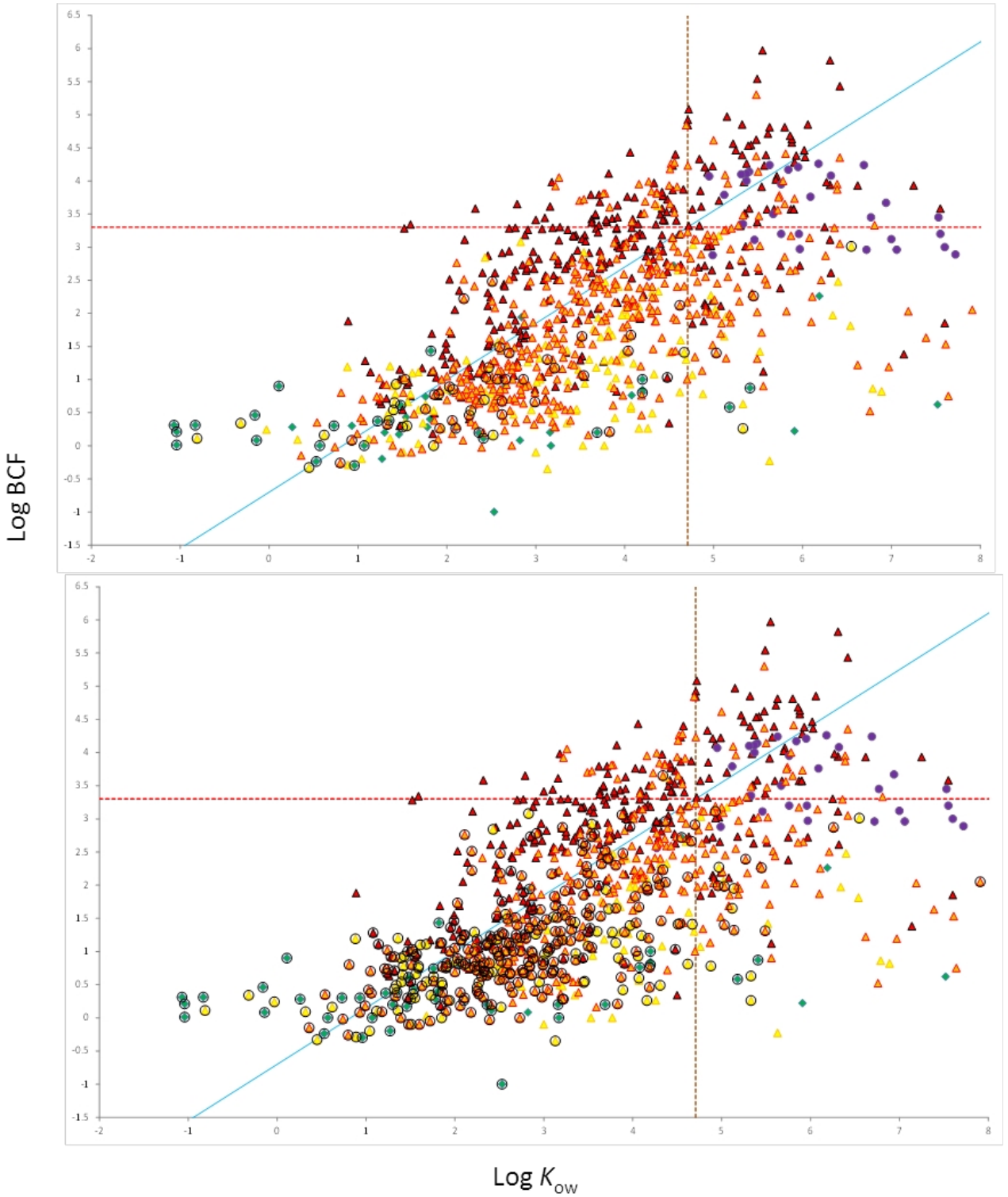


Figure 30: Experimental log BCF vs. log K_{ow} with respect to degradation (relevant log K_{ow} range). The color codes and the additional lines are the same as explained in Figure 29. Compounds with predicted biotransformation half-lives below 1 h^{-1} (upper part) or 10 h^{-1} (lower part) are additionally indicated by circles.



7 Screening criteria for increased bioaccumulation

With due regard to parent compounds and their possibly accumulating metabolites, criteria may be used to screen for possible effects of specific processes on the bioaccumulation of chemicals in aquatic organisms. If chemicals have already known specific mechanisms of bioaccumulation, a specific and case-by-case assessment may be needed. Possible candidates are organometals, chemicals reacting covalently with biomolecules under physiological conditions or irritants (H315, H320).

7.1 Protein binding

Protein binding is dominated by log K_{ow} and correlates with log BCF accordingly. Protein binding does not correlate with increased bioaccumulation beyond log K_{ow}. Regarding the bioaccumulation and related intrinsic properties of PFAS (section 6.2.1), the most important findings of our exploratory data analyses can be summarized as follows:

- Reliable log K_{ow} of PFAS are not available, thus PFAS are not covered by the applicability domains of the established computational models like fragment approaches or LSER.
- The uncertainties of the estimated log D values of PFAS limit further conclusions regarding non-lipid based accumulation of PFAS.
- There is a (linear) correlation between the experimental accumulation data for PFAS and calculated log D/log K_{ow}.
- There is excellent agreement of experimental BCF values for PFAS with field BAF obtained with fish.
- There is no indication of biomagnification of PFAS.
- The different parameters of protein binding of PFAS do not support protein based accumulation of PFAS.
- To further clarify the mode of bioaccumulation of PFAS, systematic experimental binding studies with different protein and lipid targets would be useful.

Regarding the protein binding of diverse organic chemicals, the most important findings of our exploratory data analyses (section 6.2.2 - 6.2.4) can be summarized as follows:

- Protein binding is correlated with log K_{ow} and log BCF.
- Protein binding does not correlate with significantly increased bioaccumulation beyond log K_{ow}.
- Protein binding does not provide additional information about the quantitative bioaccumulation of substances beyond log K_{ow}.
- To further clarify the qualitative and quantitative role of protein binding for bioaccumulation, systematic experimental binding studies with different protein and lipid targets would be useful.

7.2 Absorption of Surfactants

The current status on bioaccumulation of surfactants (section 6.3) can be summarized as follows:

The available bioconcentration data for surfactants are not sufficient to fully assess their bioaccumulation potential. Most of the BCF estimates are below the threshold of 2000. Only for TMAC a BCF close to this value (BCF=1960) was estimated. The bioconcentration of surfactants other than PFAS in fish does not correlate with log K_{ow}, however other properties such as the length of the alkyl chains and number of oxyethylene units seem to play a role with regard to the uptake and bioaccumulation of surfactants. The suitability of CMC as an alternative measure of a surfactant's hydrophobicity was tested, however, there is no indication for a general suitability of this measure. For the most part, the distribution and accumulation of surfactants in organisms depend on their absorption at biological interfaces. The metabolism of surfactants may also have an impact on the accumulation and distribution of the molecules in fish as shown for anionic and nonionic surfactants. However, information on the biotransformation kinetics as well as knowledge on the protein binding of anionic, nonionic and cationic surfactants in fish is often limited. The surface activity may cause absorption to food items and contribute to an increased dietary uptake. As yet, quantitative information on the dietary uptake of surfactants is limited.

7.3 Caco-2

The Caco-2 assay revealed a good model for the absorption potential, i.e. uptake of chemicals into organisms. The Caco-2 assay does not cover other processes involved in bioaccumulation. The most important findings of our exploratory data analyses on passive and active gastrointestinal absorption (section 6.4) can be summarized as follows:

- The passive diffusion correlates well with the log K_{ow} of substances.
- Substances which use secondary active transport via carriers might have an increased absorption compared to their predicted absorption using their molecular properties. In these cases an underestimation of BCF values might be possible.
- The primary active transport mostly limits the absorption of substances, and thus it is an opposing mechanism with respect to bioaccumulation.

Estimation of Caco-2 based P_{app} is possible based on intrinsic properties of chemicals. Caco-2 based P_{app} is, in general, positively related with the bioaccumulation potential of chemicals. The most important findings of our exploratory data analyses can be summarized as follows:

- P_{app} can be estimated from physicochemical properties: log K_{ow}, molecular size and polar surface area. Affinity to lipids as expressed by log K_{ow} increases the permeability, while molecule size and uneven charge distribution at the molecular surface decrease it.
- P_{app} can be estimated from LSERs with solvatochromic parameters: hydrogen bond donor capacity (acidity) and hydrogen bond acceptor capacity (basicity) of the solute. There is a similar strong negative effect of both features. The opportunity to participate in intermolecular hydrogen bonds, no matter whether as a donor or as an acceptor, retains compounds in the aqueous phase because of the energy gain by building hydrogen bonds with water.
- High P_{app} estimates are generally observed for bioaccumulating chemicals. The compounds (1.5%) which are actually B based on BCF studies but wrongly assigned to be nonB by the K_{ow} approach are mostly found in class 3 and 4, i.e. P_{app} values between 50 and $500x10^{-6}$ cm/s, with one exception in class 2.

- P_{app} estimates may be used to get a first impression of the absorption potential of some chemicals. However, the validity of this tool has to be further evaluated by applying to a larger dataset and by comparing predicted values to measured results including supposedly difficult compounds.

The classification of the bioaccumulation potential of chemicals by Caco-2 based P_{app} was adapted and verified with measured Caco-2 data (Table 7- 1). The following rules are suggested regarding Caco-2 based P_{app}, estimated with Equation:

- For estimated values below $10x10^{-6}$ cm/s or above $500x10^{-6}$ cm/s, abnormal bioaccumulation due to transport processes is not relevant for B classification.
- For estimated values above $10x10^{-6}$ cm/s together with log K_{ow} above 4, or above $50x10^{-6}$ cm/s with log K_{ow} above 3, or above $250x10^{-6}$ cm/s with log K_{ow} above 1 but in any case not exceeding $500x10^{-6}$ cm/s and log K_{ow} of 7, the estimated P_{app} should be confirmed by a Caco-2 measurement. This is still less expensive and ethically preferred in comparison to a possible BCF measurement. However, if the experiment does confirm the P_{app} range, experimental BCF determination may be considered, or the compound may precautionarily be classified as B without experimental BCF.
- With estimated values in the P_{app} range from $250x10^{-6}$ cm/s to $500x10^{-6}$ cm/s and log K_{ow} above 3, the compound is very likely to be B.
- With estimated values in the P_{app} range from $10x10^{-6}$ cm/s to $500x10^{-6}$ cm/s but below the respective log K_{ow} thresholds, increased bioaccumulation due to transport processes is not relevant with regard to B classification.

Table 7- 1: Classification of the bioaccumulation potential of chemicals by Caco-2 based P_{app}.

Ranges of estimated properties		Transport contribution		Bioaccumulation potential
P_{app} [10^{-6} cm/s]	Log K_{ow}	Probability	Relevance for B	
Class 1 <10	any	unlikely	not relevant	nonB
Class 2 10 – 50	<4	possible	not relevant	potential nonB
	4 – 4.5		to be confirmed	potential nonB
	>4.5		not relevant	possible B
Class 3 50 – 250	<3	possible	not relevant	potential nonB
	3 – 4.5		to be confirmed	potential B
	>4.5		not relevant	possible B
Class 4 250 – 500	<1	possible	not relevant	potential nonB
	1 – 3	possible	to be confirmed	potential B
	3 – 4.5	likely	relevant	very likely B
	>4.5	possible	not relevant	possible B

Ranges of estimated properties		Transport contribution		Bioaccumulation potential
P_{app} [10^{-6} cm/s]	Log K_{ow}	Probability	Relevance for B	
Class 5 >500	any	not relevant		potential B

The assessment for possible increased bioaccumulation due to (active) uptake is outlined in Figure 32. The screening is based on (estimated) Caco-2 with differential thresholds in P_{app} for compounds of different lipophilicity. The next steps are *in vitro* assays. Finally, if necessary, recommendations are given for *in vivo* testing of bioaccumulation with aqueous or dietary exposure.

7.4 Other criteria

7.4.1 Chemical classes

No specific classes of chemicals could be identified with a general and significant increase of bioaccumulation in aquatic organisms. Based on the prediction error of the Veith et al. (1979) equation (Figure 1, Table 6- 1) most concern relates to compounds with an underestimation of log BCF by more than one log unit. The most important findings of our exploratory data analyses on chemicals with possible increase of their bioaccumulation in aquatic organisms (section 6.1 - 6.2) can be summarized as follows:

- Significant increases of their bioaccumulation (upward deviations by at least one log unit above a reference (TGD) QSAR (Veith et al. 1979, Equation 2) concern about 7% of the substances.
- Among the chemical classes that are frequently suspected to behave in particular manners, e.g. organometallic compounds, perfluoroalkyl and polyfluoroalkyl substances (PFAS), an increased bioaccumulation is observed for 5 – 10% of the class members and thus corresponds to the respective portion of substances in the total data set.
- An exception are the 1,3-branched cyclohexanes with the BCF values of all four class members exceeding the reference model by at least one log unit.
- Further compounds with upward deviations are often polychlorinated and/or condensed aromatic compounds.

The most important findings of our exploratory data analyses on effects of chemical structures on under or overestimation of BCF (section 6.3) can be summarized as follows:

- Considerable BCF underestimation (>1 log unit) is likely for unsaturated hydrocarbons including aromatic and cyclic compounds and for compounds containing Cl heteroatoms.
- The risk of BCF underestimation for chemicals containing other heteroatoms is lower.

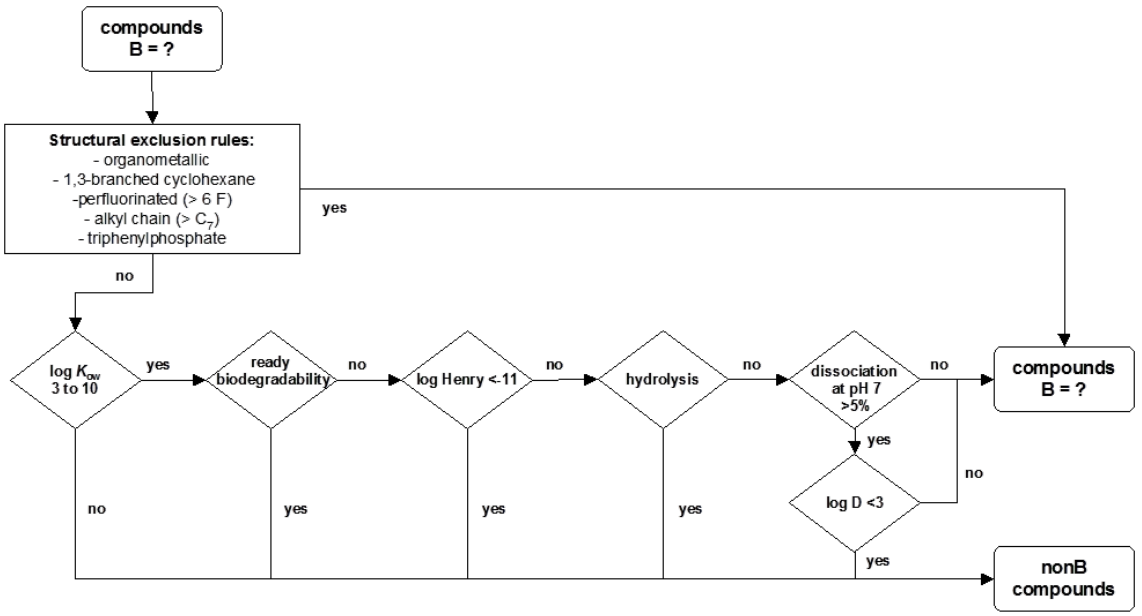
7.4.2 Physicochemical properties

Different physicochemical alerts have been suggested to screen for specific mechanisms of bioaccumulation. Examples of efficient screening criteria are provided with the BCF waiving scheme (Nendza & Müller 2010, Nendza & Herbst 2011, Nendza et al. 2015). The BCF waiving scheme allows to identify substances with low aquatic bioaccumulation (nonB, BCF <2000) based on physicochemical properties related to media-specific exposures and bioavailability

(hydrophobicity, air/water partitioning, biodegradability, hydrolysis, ionization). Figure 31 shows the workflow of the BCF waiving scheme. Output are classifications of chemicals as either nonB (non-bioaccumulative, BCF definitively <2000) or ”B = ?“ (BCF may be >2000). The bioaccumulation of compounds classified as nonB may be evaluated based on available data without new *in vivo* testing. In contrast, the assessment of the bioaccumulation of compounds classified as ”B = ?“ requires comprehensive studies because their BCF may be >2000.

Within its applicability domain, excluding specific classes of bioaccumulative chemicals like organometallics, 1,3-branched cyclohexanes, perfluoroalkyl and polyfluoroalkyl substances (PFAS), substances with an acyclic alkyl moiety (chain length ≥ C_7), aromatic triphenylphosphates, the BCF waiving scheme performs with 100% sensitivity (no false negatives) and 60% efficacy (waiving potential). Prediction confidence of the BCF waiving scheme is determined from (i) the number of physicochemical property criteria triggered by query compounds, (ii) the distance of property estimates from thresholds and (iii) the structural similarity with known nonB and B substances.

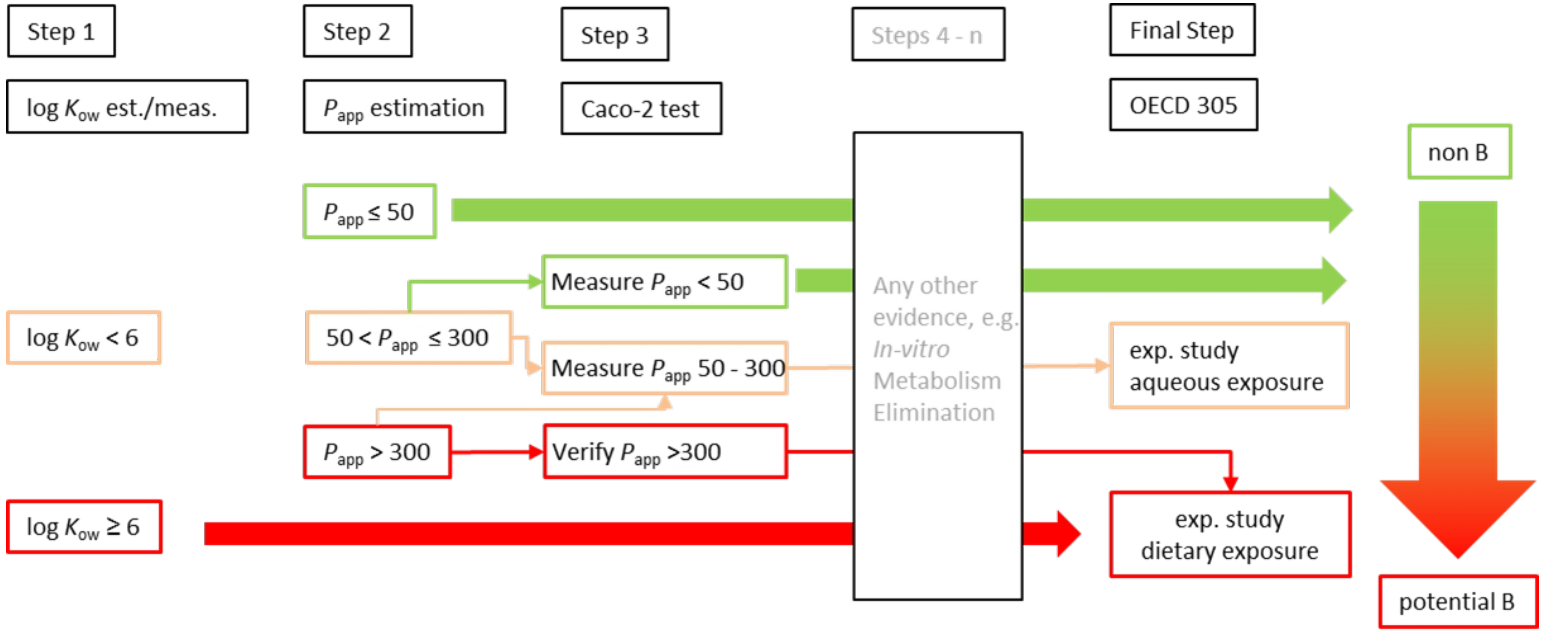
Figure 31: BCF Waiving Scheme based on screening criteria to identify nonB compounds (Nendza et al. 2015).



7.4.3 Metabolism

Theoretically, metabolism in terms of biotransformation could be expected to lower bioaccumulation. Thus, compounds with a sufficiently high rate of metabolism may be waived from BCF testing. However, estimated biotransformation rates are not suitable to further discriminate chemicals in the critical P_{app} range given in section 7.3.

Figure 32: Identification of chemicals with a potential for increased bioaccumulation due to (active) uptake.



8 References

Abraham MH, Chadha HS, Whiting GS, Mitchell RC. 1994. Hydrogen bonding. 32. An analysis of water-octanol and water-alkane partitioning and the Δ log P parameter of Seiler. J Pharm Sci 83: 1084-1100.

Abraham MH, Grellier PL, Prior DV, Duce PP, Morris JJ, Taylor PJ. 1989. Hydrogen bonding. Part 7. A scale of solute hydrogen-bond acidity based of values for complexation in tetrachloromethane. J Chem Soc Perkin Trans II: 699-711.

ACD 2015. ACD/Percepta ACD/Labs 2015 Release (Build 2726, 27 Nov 2014) Advanced Chemistry Development, Inc. www.acdlabs.com.

Anderle P. et al. 2004. Intestinal membrane transport of drugs and nutrients: genomics of membrane transporters using expression microarrays. European Journal of Pharmaceutical Sciences 21: 17–24.

Arnot JA, Meylan W, Tunkel J, Howard PH, Mackay D, Bonnell M, Boethling RS 2009. A quantitative structure-activity relationship for predicting metabolic biotransformation rates for organic chemicals in fish. Environ Toxicol Chem 28: 1168-1177.

Arnot JA, Gobas FAPC. 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environ Rev 14: 257-297.

Artursson P, Palm K. et al. 2001. Caco-2 monolayers in experimental and theoretical predictions of drug transport. Adv Drug Deliv Rev 46(1-3): 27-43.

Bakke S, Olderbakk Jordal AE, Gómez-Requeni P, Verri T, Kousoulaki K, Aksnes A, Rønnestad I. 2010. Dietary protein hydrolysates and free amino acids affect the spatial expression of peptide transporter PepT1 in the digestive tract of Atlantic cod (*Gadus morhua*). Comp Biochem Physiol Part B 156: 48–55.

Bischel HN, MacManus-Spencer L, Luthy RG. 2010. Noncovalent interactions of long-chain perfluoroalkyl acids with serum albumin. Environ Sci Technol 44: 5263–5269.

Bischel HN, MacManus-Spencer LA, Zhang C, Luthy RG. 2011. Strong associations of short-chain perfluoroalkyl acids with serum albumin and investigation of binding mechanisms. Environ Toxicol Chem 30: 2423–2430.

Boudker O, Verdon G. 2010. Structural perspectives on secondary active transporters. Trends in Pharmacol Sci 31: 418–426.

Bishop WE, Maki AW. 1980. A critical comparison of bioconcentration test methods. In: Aquatic Toxicology, Eaton, J.G., P.R. Parrish and A.C. Hendricks (Eds), American Society for Testing and Materials, ASTM STP 707, pp. 116-129.

Broccatelli F. 2012. QSAR models for P-glycoprotein transport based on a highly consistent data set. J Chem Inf Model 52: 2462-2470.

Buck RC, Franklin J, Berger U, Conder JM, Cousins IT, de Voogt P, Jensen AA, Kannan K, Mabury SA, van Leeuwen SPJ. 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integr Environ Assess Manag 7:513–541.

Burke AB, Millbum P, Huckie KR, Hutson DH. 1991. Uptake and elimination of radioactivity from rainbow trout (*Oncorhynchus mykiss*) following exposure to [14C-ring]-2-pbenyldodecane, Environ Toxicol Chem 10: 1467-1477.

Butler and Day 1993. The relationship between intracellular pH and swimming performance of brown trout exposed to neutral and sublethal pH. J: exp. Biol. 176, 271-284.

Coenen W. 1988. Einfluss des linearen Alkylbenzosulfonates (LAS) auf die Kinetik von Lindane, 4-Nitrophenol und DDT biem Zebrabãrbling. Diploma Thesis, University of Mainz

Comotto RM, Kimerle RA, Swisher RD. 1979. Bioconcentration and metabolism of linear alkylbenze- ne sulfonate by daphnids and fatbead minnows. In: Aquatic Toxicology, Marking, L.L. and R.A. Kimerle (Eds.), American Society for Testing and Materials, ASTM STP 667, pp. 232-250.

Daugherty AL, Mrsny JR. 1999. Transcellular uptake mechanisms of the intestinal epithelial barrier, Part one. Pharmaceutical Science and Technology Today, April 1999: 144–151.

deBruyn AMH, Gobas FAPC. 2007. The sorptive capacity of animal protein. Environ Toxicol Chem 26(9): 1803–1808.

de Voogt P, Berger U, de Coen W, de Wolf W, Heimstad E, McLachlan M, van Leeuwen S, van Roon A. 2006. Perfluorinated organic compounds in the European environment. Perforce, FP6-NEST-508967.

De Wolf W, Comber M, Douben P, Gimeno S, Holt M, Leonard M, Lillicrap D, Sijm D, van Egmond R, Weisbrod A, Whale G. 2007. Animal use replacement, reduction and refinement: Development of an integrated testing strategy for Bioconcentration of chemicals in fish. Integrat Environ Assess Manag 3(1): 3-17.

Dobson PD, Kell DB. 2008. Carrier-mediated cellular uptake of pharmaceutical drugs: an exception or the rule? Nat Rev Drug Discov 7(3): 205-220.

ECHA. 2012. Guidance on information requirements and chemical safety assessment Part C: Endpoint specific guidance. Guidance for the implementation of REACH (version 1.1) November 2012.

ECHA. 2014. Guidance on information requirements and chemical safety assessment. Chapter R.7a: Endpoint specific guidance. Version 2.4.

ECHA. 2014a. Guidance on information requirements and chemical safety assessment. Chapter R.7c: Endpoint specific guidance. Version 2.0.

Endo S, Goss KU. 2011. Serum albumin binding of structurally diverse neutral organic compounds: data and models. Chem Res Toxicol 24: 2293–2301. dx.doi.org/10.1021/tx200431b

Ertl P, Rohde B, Selzer P. 2000. Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. J Med Chem 43: 3714-3717.

European Commission. 2003. Technical guidance document on risk assessment in support of the Commission directive 93/67/EEC on risk assessment for new notified substances and in support of the commission regulation (EC) 1488/94 on risk assessment for existing substances and to support directive 98/8/EC of the European parliament and of the council concerning the placing of biocidal products on the market, Part III.

European Commission. 2006. REGULATION (EC) No 1907/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93

and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. European Commission, Brussels, Belgium.

European Commission. 2011. COMMISSION REGULATION (EU) No 253/2011 of 15 March 2011 amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards Annex XIII

Fagerholm U. 2007. Prediction of human pharmacokinetics – gastrointestinal absorption. J Pharm Pharmacol 59(7): 905–916.

Fent, K., 2003. Ökotoxikologie. 2nd Edition. Thieme Verlag, Stuttgart.

Freitag, D, Ballhorn, L, Geyer, H, Korte, F. 1985. An experimental method for the assessment of the behaviour of organic chemicals in the ecosphere by means of simple laboratory tests with C14 labelled chemicals. Chemosphere 14:1589-1616.

Fujikawa M, Ano R, et al. 2005. Relationships between structure and high-throughput screening permeability of diverse drugs with artificial membranes: application to prediction of Caco-2 cell permeability. Bioorg Med Chem 13(15): 4721-4732.

Garver E, Hugger ED, Shearn SP, Rao A, Dawson PA, Davis CB, Han C. 2008. Involvement of intestinal uptake transporters in the absorption of azithromycin and clarithromycin in the rat. Drug Metab Dispos 36(12): 2492-2498.

Giesy JP, Kannan K. 2001. Global distribution of perfluorooctane sulfonate in wildlife. Env Sci Technol 35:1339-1342.

Goeritz I, Falk S, Stahl T, Schäfers C, Schlechtriem C. 2013. Biomagnification and tissue distribution of perfluoroalkyl substances (PFASs) in market-size rainbow trout (*Oncorhynchus mykiss)*. Environ Toxicol Chem 32 (9): 2078-2088.

Goodrich MS, Melancon MJ, Davis RS, Lech LL. 1991. The toxicity, bioaccumulation, metabolism and elimination of dioctyl sodium sulfosuccinate DSS in rainbow trout (*Oncorhynchus mykiss*). Water Res 25: 119-124.

Granmo A, Kollberg S. 1976. Uptake pathways and elimination of a nonionic surfactants in cod (*Gadus morrhua* L.). Water Res 10 (3): 189-194.

He YL, Murby S, Warhurst G. et al. 1998. Species differences in size discrimination in the paracellular pathway refl ected by oral bioavailability of poly(ethylene glycol) and D-peptides. J Pharm Sci 87(5): 626-633.

Hickey JP, Passino-Reader DR. 1991. Linear solvation energy relationships: "rules of thumb" for estimation of variable values. Environ Sci Technol 25: 1753-1760.

Hou TJ, Zhang W, Xia K, Qiao XB, Xu XJ. 2004. ADME evaluation in drug discovery 5. Correlation of Caco-2 permeation with simple molecular properties. J Chem Inf Comput Sci 44: 1585-1600.

Hurst S, Loi CM, et al. 2007. Impact of physiological, physicochemical and biopharmaceutical factors in absorption and metabolism mechanisms on the drug oral bioavailability of rats and humans. Expert Opin Drug Metab Toxicol 3(4): 469-489.

Izar MC, Tegani DM, Kasmas SH, Fonseca FA. 2011. Phytosterols and phytosterolemia: gene–diet interactions. Genes Nutr 6:17–26.

Jeon J, Kannan K, Lim HK, Moon HB, Kim SD. 2010. Bioconcentration of perfluorinated compounds in blackrock fish, *Sebastes schlegeli*, at different salinity levels. Environ Toxicol Chem 29(11): 2529–2535.

Jones PD, Hu W, De Coen W, Newsted JL, Giesy JP. 2003. Binding of perfluorinated fatty acids to serum proteins. Environ Toxicol Chem 22(11): 2639–2649.

Kamlet MJ, Doherty RM, Abraham MH, Marcus Y, Taft RW. 1988a. Linear Solvation Energy Relationships. 46. An improved equation for correlation and prediction of octanol/water partition coefficients of organic nonelectrolytes (including strong hydrogen bond donor solutes). J Phys Chem 92: 5244-5255.

Kamlet MJ, Doherty RM, Abraham MH, Taft RW. 1988b. Solubility properties in biological media. 12. Regarding the mechanism of nonspecific toxicity or narcosis by organic nonelectrolytes. Quant Struct-Act Relat 7: 71-78.

Kannan K, Tao L, Sinclair E, Pastava SD, Jude DJ, Giesy JP. 2005. Perfluorinated compounds in aquatic organisms at various trophic levels in a Great Lakes food chain. Arch Environ Contam Toxicol 48:559-566.

Karlsson J, Ungell A, Grasjo J, Artursson P. 1999. Paracellular drug transport across intestinal epithelia: influence of charge and induced water flux. Eur. J Pharm Sci 9(1): 47-56.

Kikuchi M, Wakabayashi M, Kojima H, Yoshida T. 1978. Uptake, distribution, and elimination of sodium linear alkylbenzenesulfonate and sodium alkyl sulfate in carp. Ecotoxicol Environ Saf 2: 115-127.

Kikuchi M, Wakabayashi M, Kojima H, Yoshida T. 1980. Bioaccumulation profiles of 35S-labelled sodium alkylpoly(oxyethylene) sulfates in carp (*Cyprinus carpio*). Water Res 14(10): 1541–1548. doi:10.1016/0043-1354(80)90022-6.

Kimerle RA, Swisher RD, Schroeder-Comotto RM. 1975. Surfactant structure and aquatic toxicity. Proc. IJC Symposium of Structure Activity Correlations in Studies on Toxicity and Bioconcentration with Aquatic Organisms, pp. 22-35.

Kimerle RA, Macek KJ, Sleight III BH, Burrows ME. 1981. Bioconcentration of linear alkylbenzene sulfonates (LAS) in bluegill (Lepomis macrochirus). Water Res 15: 251-256.

Kühne R, Ebert RU, Schüürmann G. 2009. Chemical domain of QSAR models from atom-centered fragments. J Chem Inf Model 49: 2660-2669.

Kunta JR, Sinko PJ. 2004. Intestinal drug transporters: *in vivo* function and clinical importance. Curr Drug Metab 5(1): 109-124.

Lee VHL, Yamamoto A. 1990.Penetration and enzymatic barriers to peptide and protein absorption. Adv Drug Delivery Rev 4: 171-207.

Lennernäs H. 2007. Intestinal permeability and its relevance for absorption and elimination. Xenobiotica 37(10-11): 1015-1051.

Leung SSF, Mijalkovic J, Borrelli K, Jacobson MP. 2012. Testing physical models of passive membrane permeation. J Chem Inf Model 52: 1621-1636.

Lewis, MA and Wee, VT. 1983. Aquatic safety assessment for cationic surfactants. Environmental Toxicology and Chemistry 2: 105 118.

Linnankoski J, Mäkelä JM, Ranta VP, Urtti A, Yliperttula M. 2006. Computational prediction of oral drug absorption based on absorption rate constants in humans. J Med Chem 49: 3674-3681.

Lipinski CA. 2000. Drug-like properties and the causes of poor solubility and poor permeability. J Pharmacol Toxicol Methods 44(1): 235-249.

MacManus-Spencer LA, Tse ML, Hebert PC, Bischel HN, Luthy RG. 2010. Binding of perfluorocarboxylates to serum albumin: a comparison of analytical methods. Anal Chem 82: 974–981.

Martin JW, Mabury SA, Solomon KR, Muir DCG. 2003a. Dietary accumulation of perfluorinated acids in juvenile rainbow trout (*Oncorhyncus mykiss*). Environ Toxicol Chem 22, 189-195.

Martin JW, Mabury SA, Solomon KR, Muir DCG (2003b): Bioconcentration and tissue distribution of perfluorinated acids in rainbow trouts (*Oncorhyncus mykiss*). Environ Toxicol Chem 22, 196-204.

Müller M, Nendza M. 2011. Comparative analysis of estimated and measured BCF data (OECD 305) – Literature study with a special focus on differential accumulation of (mixtures of) stereoisomers. UBA-Texte 15/2011, http://www.uba.de/uba-info-medien-e/4088.html, Federal Environment Agency, Dessau-Roßlau, Germany.

Ng CA, Hungerbühler K. 2014. Bioaccumulation of Perfluorinated Alkyl Acids: Observations and Models. Environ. Sci. Technol. 2014, 48, 4637−4648

Nendza M. 1991. QSARs of bioconcentration: validity assessment of log Pow/log BCF correlations, in: Nagel R, Loskill R. (Eds.), Bioaccumulation in aquatic systems. VCH, Weinheim.

Nendza M. 1998. Structure-activity relationships in environmental sciences. Ecotoxicology Series 6. Chapman & Hall, London.

Nendza M, Müller M. 2010. Screening for low aquatic bioaccumulation (1): Lipinski's 'Rule of 5' and molecular size. SAR QSAR Environ Res 21: 495-512.

Nendza M, Herbst T. 2011. Screening for low aquatic bioaccumulation (2): Physico-chemical constraints. SAR QSAR Environ Res 22: 351-364.

Nendza M, Müller M, Wenzel A. 2014. Discriminating toxicant classes by mode of action: 4. Baseline and excess toxicity. SAR QSAR Environ Res (in press).

Neufarth A, Eckert HG, Kellner H, Loetzsch K. 1978. Aspects of environmental safety of distearyldi- methylammonium chloride, 6th report presented at the CED congress, March 1978, Madrid, Spain, p 205-216.

Nichols JW, Bonnell M, Dimitrov S, Escher B, Han X, Kramer N. 2009. Bioaccumulation assessment using predictive approaches. Integr Environ Assess Manag 5(4): 577–597.

OECD. 1996. Guidelines for testing of chemicals. 305. Bioconcentration: Flow-through fish test. OECD, Paris.

OECD. 2007. Guidance document on the validation of (quantitative) structure-activity relationships [(Q)SAR] models. Series on testing and assessment No. 69, Paris.

OECD. 2012. Organisation for Economic Cooperation and Development, 2012: Draft TG 305: Bioaccumulation in fish: Aqueous and dietary exposure, Paris.

OSIRIS. Optimized Strategies for Risk Assessment of Industrial Chemicals through Integration of Non-Test and Test Information. EU Project, contract no. GOCE-CT-2007-037017, 2007-2011.

OSIRIS project website: http://www.osiris-reach.eu/. OSIRIS webtool website: http://osiris.simpple.com/OSIRIS-ITS/welcome.do.

Paul, R.J. 2001. Physiologie der Tiere. Systeme und Stoffwechsel. Thieme Verlag, Stuttgart.

Pham-The H, González-Álvarez I, Bermejo M, Garrigues T, Le-Thi-Thu H, Cabrera-Pérez MÁ. 2013. The use of rule-based and QSPR approaches in ADME profiling: A case study on Caco-2 permeability. Mol Inf 32: 459-479.

Popovic M, Zaja R, Loncar J, Smital T. 2010. A novel ABC transporter: The first insight into zebrafish (Danio rerio) ABCH. Marine Environ Res 69: 11–13.

Rønnestad I, Murashita K, Kottra G, Jordal AE, Narawane S, Jolly C, Daniel H, Verri T. 2010. Molecular cloning and functional expression of Atlantic salmon peptide transporter 1 in Xenopus oocytes reveals efficient intestinal uptake of lysine-containing and other bioactive di- and tripeptides in teleost fish. J Nutr 140: 893–900.

Scherer PG, Seelig J. 1989. Electric charge effect on phospolipid headgroups. Phospatidylcholine in mixtures with cationic and anionic amphiphiles, Biochemistry 28: 7720-7728.

Schüürmann G, Ebert RU, Kühne R. 2006. Prediction of physicochemical properties of organic compounds from 2D molecular structure – fragment methods vs. LFER models. Chimia 60: 691-698.

Schwarzenbach RP, Gschwend PM, Imboden DM. 1993. Environmental Organic Chemistry, Wiley, New York.

Stahl T, Mattern D, Brunn H. 2011. Toxicology of perfluorinated compounds. Environmental Sciences Europe 23:38. http://www.enveurope.com/content/23/1/38.

Taft RW, Abraham MH, Doherty RM, Kamlet MJ. 1985. The molecular properties governing solubilities of organic nonelectrolytes in water. Nature 313: 384-386.

Tanford C. 1980. The hydrophobic effect: Formation of micelles and biological membranes. Wiley Interscience, New York, pp 42-59.

Thews G, Vaupel P. 2005. Vegetative Physiologie, 5. Auflage, Springer, Heidelberg.

Thomas VH, Bhattachar S. et al. 2006. The road map to oral bioavailability: an industrial perspective. Expert Opin Drug Metab Toxicol 2(4): 591-608.

Tolls J. 1998. Bioconcentration of surfactants. Thesis University Utrecht.

Tolls J, Haller M, De Graaf I, Thijssen MATC, Sijm DTHM. 1997. Bioconcentration of LAS: experimental determination and extrapolation to environmental mixtures. Environ Sci Technol 31: 3426-3431.

Tolls J, Haller M, Labee E, Verweij M, Sijm DTHM. 2000. Experimental determination of bioconcentration of the nonionic surfactant alcohol ethoxylate. Environ Toxicol Chem 19(3): 646–653.

Tolls J, Sijm DTHM. 1995. A preliminary evaluation of the relationship between bioconcentration and hydrophobicity for surfactants. Environ Toxicol Chem 14(10): 1675–1685.

Tolls J, Sijm DTHM. 1999. Bioconcentration and biotransformation of the nonionic surfactant octaethylene glycol monotridecyl ether 14C-C13EO8. Environ Toxicol Chem 18(12): 2689–2695.

Tolls J, Sijm DTHM, Kloepper-Sams P.1994. Surfactant bioconcentration - a critical review.

Chemosphere 29:693-717

Topcuoglu, S., and E. Birol, 1982. Bioaccumulation of sodium alkyl Sulphate, zinc chloride and their mixture in young goby, *Proteorhinus marmoratus* Pall. Turk J. Nucl. Sci. 9(3):100-107. (as cited in Painter and Zabel, 1988).

Toshima S, Moriya T, Yoshimura K. 1992. Effects of polyoxyethylene (20) sorbitan monooleate on the acute toxicity of linear alkylbenzenesulfonate (C12-LAS) to fish. Ecotox Environ Saf 24: 26-36.

Tovell PWA, Howes D, Newsome CS. 1975. Absorption, metabolism and excretion by goldfish of the anionic detergent sodium lauryl sulphate. Toxicology 4: 17-29.

Tovell PWA, Newsome CS, Howes D. 1974. Effect of water hardness on the toxicity of an anionic detergent to fish. Water Res 8: 291-296.

UFZ Department of Ecolocical Chemistry. 2014. ChemProp 6.1. http://www.ufz.de/index.php?en=6738. Visited 2014/04/28.

US EPA. 2011. EPI Suite v4.1. Washington, DC, U.S.Environmental Protection Agency.

Valko K, Nunhuck S, Bevan C, Abraham MH, Reynolds GP. 2003. Binding to human serum albumin. Relationships with octanol/water and immobilized artificial membrane lipophilicity. J Pharm Sci 92(11): 2236-2248.

van de Waterbeemd H, Testa, B; Drug bioavailability: estimation of solubility, permeability, absorption and bioavailability. Wiley-VCH, 16.12.2008.

van de Waterbeemd, H. 2007. Property-based lead optimization. In Pharmacokinetic Profiling in Drug Research: Biological, Physicochemical, and Computational Strategies. Testa B, Krämer SD, Wunderli-Allenspach H, Folkers G (Eds.). Weinheim, Germany, Wiley-VCH Verlag GmbH & Co. KGaA: 25-45.

Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. 2002. Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem 45: 2615-2623.

Veith GD, Defoe DL, Bergstedt BV. 1979. Measuring and estimating the bioconcentration factor of chemicals in fish. J Fish Board Can 36: 1040-1048.

Versteeg DJ, Shorter SJ. 1992. Effect of organic carbon on the uptake and toxicity of quartemary ammonium compounds to the fathead minnow, Pimephales promelas. Environ Toxicol Chem 11: 571-580.

Vögtli E. 2010. Moderne Pharmakokinetik; Transport durch Membranen, 1. Auflage Juli 2010 Wiley-VCH, Weinheim.

Wakabayashi M, Kikuchi M, Sato A, Yoshida T. 1987. Bioconcentration of alcohol ethoxylates in carp (*Cyprinus carpio*), Ecotoxicol Environ Saf 13: 148-163.

Webster EM, Ellis DA. 2011. Equilibrium modelling: a pathway to understanding observed perfluorocarboxylic and perfluorosulfonic acid behavior. Environ Toxicol Chem 30: 2229-2236.

Werner, AF and RA, Kimerle. 1982. Uptake and distribution of C12 alkylbenzene in bluegill (*Lepomis macrochirus*). Environ. Toxicol. Chem. 1:143-146.

Willmer P, Stone G, Johnson I. 2000. Environmental Physiology of Animals. Hoboken, NJ, USA, Blackwell Science Ltd.

Woodcroft MW, Ellis DA, Rafferty SP, Burns DC, March RE, Stock NL, Trumpour KS, Yee J, Munro K. 2010. Experimental characterization of the mechanism of perfluorocarboxylic acids' Liver

protein bioaccumulation: the key role of the neutral species. Environ Toxicol Chem 29(8): 1669–1677.

Zhao YH, Le J, Abraham MH, Hersey A, Eddershaw PJ, Luscombe CN, Boutina D, Beck G, Sherborne B, Cooper I, Platts JA. 2004. Evaluation of human intestinal absorption data and subsequent derivation of a quantitative structure-activity relationship (QSAR) with the Abraham descriptors. J Pharm Sci 90: 749-784.