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Comparative analysis of estimate and measured BCF data (OECD 305)

Literature study with a special focus on differential
accumulation of (mixtures of) stereoisomers

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Comparative analysis of estimated and measured BCF data (OECD 305)

Literature study with a special focus on differential accumulation of (mixtures of) stereoisomers

by

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16. Abstract The present study addresses the bioaccumulation of biocides and pesticides for regulatory purposes. The major findings are: • Calculated values of the Bioconcentration Factor (BCF) according to equation 74 of the TGD do not necessarily represent the ‘worst case’. • The inherent uncertainty in calculated BCF values requires careful consideration of potential mitigating factors to conclude on the applicability of TGD equation 74 on a case-by-case basis. • The acceptable uncertainty in either measured or calculated BCF values is context-dependent and varies with the relevant regulatory threshold (100 (if not readily degradable), 1000 (if readily degradable), 2000 (B compounds) or 5000 (vB compounds)). Transferability (read-across) of BCF-results between individual stereoisomers and different mixtures of stereoisomers is limited to those with similar metabolic rates (to be supported by (in vitro) experimental evidence). Significant differences in bioaccumulation between stereoisomers occur if selective reactions in chiral biophases take place. Among the possible processes, stereoselective biotransformation (metabolism in fish) is by far the most relevant. Rates and extents of biotransformation in fish can greatly differ between stereoisomers and it is recommended to include isomer-specific information about biotransformation in bioaccumulation assessments. If read-across is requested, it should be supported by (experimental) evidence for similar metabolic rates in fish.		
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15. Zusätzliche Angaben		
16. Kurzfassung Die vorliegende Studie befasst sich mit der Bioakkumulation von Bioziden und Pestiziden unter regulatorischen Gesichtspunkten. Die wesentlichen Ergebnisse waren: • Die berechneten Werte des Biokonzentrationsfaktors (Bioconcentration factor: BCF) auf der Basis von Gleichung 74 des TGD stellen nicht notwendigerweise einen “worst case” dar. • Die inhärente Unsicherheit in berechneten BCF-Werten erfordert die sorgfältige Erwägung potentiell abschwächender Faktoren, und die Anwendbarkeit der Gleichung 74 des TGD muss in jedem Einzelfall geprüft werden. • Die akzeptable Unsicherheit gemessener oder berechneter BCF-Werten ist kontextabhängig und ändert sich mit den relevanten regulatorischen Grenzwerten (100 (wenn nicht leicht biologisch abbaubar), 1000 (wenn leicht biologisch abbaubar), 2000 (B-Stoffe) oder 5000 (vB-Stoffe)). Die Übertragbarkeit (“read-across”) von BCF-Daten zwischen individuellen Stereoisomeren und unterschiedlichen Mischungen von Stereoisomeren ist limitiert auf solche Fälle mit ähnlichen Metabolismusraten (gestützt auf experimentelle (in vitro) Hinweise). Signifikante Unterschiede in der Bioakkumulation unterschiedlicher Stereoisomere treten auf, wenn selektive Reaktionen in chiralen Biophasen stattfinden. Unter den möglichen Prozessen ist die stereoselektive Biotransformation (Metabolismus im Fisch) bei weitem der wichtigste. Ausmaß und Geschwindigkeit der Biotransformation in Fischen können sich zwischen Stereoisomeren deutlich unterscheiden und es wird empfohlen, isomer-spezifische Informationen über die Biotransformation in Bioakkumulationsbeurteilungen zu berücksichtigen. Wenn “read-across“ gefordert wird, sollte dies durch (experimentelle) Hinweise auf ähnliche Biotransformationsraten gestützt werden.		
17. Schlagwörter Biokonzentration, BCF, Akkumulation, QSAR, Stereoisomere		
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Disclaimer: The present study addresses the bioaccumulation of biocides and pesticides for regulatory purposes. The objective is to improve general decision support, but not to evaluate individual substances.

Data and model analyses started with large datasets of diverse chemicals in the public domain and were tested with confidential data for few pesticides and new chemicals provided by Umweltbundesamt (UBA). The available data basis leads to several implications:

- The data on Bioconcentration Factors (BCF) collected in the different **datasets** have been quality-checked, but still there is considerable **inhomogeneity** with regard to:
 - analytical method (whole body, total radioactivity, parent compound)
 - exposure concentration (nominal, measured, constant)
 - attainment of steady state

Absent documentation does not allow to extract focussed datasets within the present study.

- The different datasets in the public domain feature **diverse chemicals**:
 - industrial chemicals
 - pesticides
 - biocides
 - (veterinary) pharmaceuticals
- The data distribution between the different datasets is similar:
 - log BCF mean ~ 2.9
 - BCF values > 2000 ~10-15%
 - BCF values < 2000 ~85-95%
- The inherent **variability** of measured BCF data is ~1 order of magnitude and often may be larger. Even more uncertainties may apply to QSAR estimates.
- The regulatory assessment of biocides and pesticides requires BCF data from comparable **laboratory test methods**.
- Regression-based models cannot describe the **worst case**, but represent an average with downward and upward deviations.
- **QSAR models** cannot work well with BCF values obtained from total radioactivity because activity (for mixture of parent compound and metabolites) and descriptor data (parent compound) address different substances.
- A true worst case model must deliver 100 % sensitivity (**no false negatives**), though at the cost of false positives.

Three major consequences of these implications are:

- Calculated BCF values according to equation 74 of the TGD do not necessarily represent the 'worst case'.
- The inherent uncertainty in calculated BCF values requires careful consideration of potential mitigating factors to conclude on the applicability of TGD equation 74 on a **case-by-case basis**.
- The **acceptable uncertainty** in either measured or calculated BCF values is context-dependent and varies with the relevant regulatory threshold (100 (if not readily degradable), 1000 (if readily degradable), 2000 (B compounds) or 5000 (vB compounds)).

1 Introduction

Bioaccumulation potential of chemicals is frequently assessed from bioconcentration, conventionally measured according to OECD 305 (Bioaccumulation: Flow-through Fish Test) (OECD, 1996) and expressed as Bioconcentration Factor (BCF). These studies deliberately reduce the manifold uptake and elimination mechanisms in aquatic organisms to respiratory absorption via gills and diffusion through the skin (e.g. Arnot and Gobas, 2003; Sijm et al. 2007). Despite the simplifications, testing costs are high and a minimum of 108 fish are consumed in each OECD 305 bioconcentration guideline study (ILSI HESI, 2006).

The environmental assessment of biocides according to the directive 98/8/EG (European Communities, 1998) principally requires estimation of the aquatic bioaccumulation potential from $\log K_{OW}$. For substances with a $\log K_{OW} > 3$ and predicted entry in the environment, additionally a study on bioconcentration according to OECD 305 is necessary to evaluate the potential of bioaccumulation on basis of experimental data. The BCF calculations shall use equation 74 of the EU Technical Guidance Document on Risk Assessment (TGD), Part II (European Communities, 2003).

$$\log BCF = 0.85 \log K_{OW} - 0.70 \quad (\text{Veith et al. 1979})$$

In the framework of biocide registration, it has been argued that this QSAR always represents the worst case as compared to experimental data, because it is solely based on $\log K_{OW}$. The present study tests the worst-case hypothesis by:

- Addressing statistical arguments related to regression-based models (why they always deliver average values);
- Comparing experimental data on biocides and pesticides with calculated values according to equation 74 of the TGD (why many chemicals accumulate less but some compounds accumulate much more regarding to calculation results);
- Evaluating reliability of predictions with consideration of the (structural) applicability domain of QSAR models (why many biocides and pesticides are outside the applicability domain of equation 74 of the TGD);
- Recommending alternative worst-case models for BCF prediction (why considerations beyond statistical fit are necessary).

The applicability of QSARs and read-across becomes even more complex when 3-D differences of chemical isomers have to be considered. To address the possibilities and limitations of transferability of BCF-results between individual stereoisomers and different mixtures of isomers, this study reviews:

- Characteristics of stereoisomeric chemicals (why 2-D similarity may be 3-D dissimilarity);
- Functional similarity of (stereoisomeric) chemicals (why structure and physico-chemical properties (lipophilicity, metabolic capacity, molecular size, dissociation) are relevant in BCF QSARs and read-across);
- Stereoselective processes involved in bioaccumulation (why stereoselective biotransformations and bioisomerism may affect the bioaccumulation of stereoisomers);

and illustrates the findings with a worked example of differential bioaccumulation of indoxacarb isomer(mixture)s.

2 Background

2.1 Processes affecting bioaccumulation

Accumulation of dissolved contaminants in biota occurs mostly by direct absorption, but also along the trophic web. With regard to organisms, the accumulation processes are defined according to the mode of uptake of contaminants:

- Bioaccumulation: uptake from the environment via any possible pathway;
- Biomagnification: uptake via the foodweb resulting in increased concentrations at higher trophic levels;
- Bioconcentration: uptake from the surrounding phase via absorption, lipid diffusion, etc.

Bioaccumulation is governed by four major processes (Hodgeson and Levi, 1994; Pfeifer et al. 1984) :

- Absorption: Uptake of chemical substances from food, water, air, sediment, or soil, by transport across biological membranes into the systemic circulation, e.g. across fish gills, intestine, skin.
- Distribution: Circulation of chemical substances throughout the body, binding to plasma proteins or tissue components like fat or bone. The chemical may be distributed to a tissue and elicit a toxic response; other tissues may serve as sink or as temporary depot allowing for slow release into circulation.
- Metabolism: Enzymatic transformation of chemical substances: During phase I, a polar group is introduced into the molecule, which increases its water solubility and renders it a suitable substrate for phase II reactions. In phase II, the altered molecule combines with an endogenous substrate and is excreted. Metabolism is often a detoxification mechanism, but in some cases, metabolism may activate the parent compound. Intermediates or final products may cause toxicity (toxification).
- Excretion: Elimination of chemical substances: Soluble molecules are removed through renal filtration and passed into urine. Fat soluble chemicals may be conjugated and excreted in bile (faeces). Chemicals with nutritional benefit may be

broken down and ultimately exhaled as CO₂. Volatile substances may also be exhaled directly through the lungs.

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

Absorption processes

The uptake of xenobiotics from the point of exposure to the site of action or storage is the key to worst-case assessment of bioaccumulation and involves passages through a number of tissues with transfer of the chemical across multiple membranes (e.g. mucosa, capillary wall, cell membrane). Because the other three principal processes either do not affect (distribution) or even reduce (metabolism, excretion) the total body burden, hence bioaccumulation, they are not detailed here.

The principal architecture of membranes according to the fluid mosaic model is universal, though differentiated by distinct lipid types and structural and functional proteins (Seydel and Wiese, 2002). Several mechanisms operate to absorb compounds into the body (Pfeifer et al. 1984):

- Passive diffusion (lipid diffusion, membrane permeation): Molecules diffuse across cell membranes into cells, and they can pass between cells, along their concentration gradient. Factors that may affect passive transport of substances across cell membranes concern properties of the diffusible substances as well as the cell membrane (Abraham et al. 1994). Fick's first law of diffusion and Nernst distributions rationalize that absorption of diffusible compounds relates to their molecular size and the respective lipid/water partition coefficients. The chemical factors that influence interphase partitioning are solute charge, dipolarity/polarizability, hydrogen bonding capacity and molecular size (Jensen, 1976; Seydel and Wiese, 2002). A recent literature review (Nendza and Müller, 2007a) of studies on uptake via biological membranes indicates that also large high molecular weight-substances (MW > 1000 g/mol) are able to permeate through membranes and can be efficiently absorbed. No robust evidence is provided to substantiate molecular weight or size cut-offs for membrane permeation that can be applied to assess bioaccumulation. Chemicals of moderate lipophilicity and sufficient solubility in water and membranes diffuse rapidly through aqueous and lipid phases. Large superlipophilic compounds also permeate through membranes, but at slower rates, due to their low solubility in water layers. The slow rate of elimination gives high log K_{OW} chemicals their inherent bioaccumulative potential (Arnot and Gobas, 2003).

- Ion pair transport: Compensation by ion pair formation enables passive diffusion of charged molecules across membranes.
- Filtration: Small molecules with molecular weight (MW) < 100 g/mol can pass through pores within membranes (diameter ~ 0.4 – 0.8 nm), but this process is considered more important for elimination than absorption.
- Active transport: Molecules are transported, usually against their concentration gradient, by specific carrier proteins. This route is important for gastrointestinal absorption of essential nutrients. Efflux proteins, such as P-glycoprotein, can shunt molecules out of cells. Active transport mechanisms for transmembrane passages are, except for physiological substrates, very rare and specific for these substances (Pfeifer et al. 1984; Abraham et al. 1994). Literature searches revealed few evidence for active uptake of environmental pollutants, e.g. (organo)metals. Unless chemicals are used as or resemble very closely physiological substrates, active uptake into organisms appears negligible.
- Endocytosis: Uptake of dissolved or particulate material into cells can occur by invagination of the plasma membrane and its internalisation in a membrane-bound vesicle. Endocytosis can be segregated into unspecific uptake of extracellular fluids including solutes via mechanisms which are independent of ligand-binding (pinocytosis), and receptor-mediated endocytosis for selective uptake of, e.g., hormones, growth factors, enzymes, plasma proteins (Clathrin-mediated endocytosis, caveolar endocytosis).

2.2 QSAR-modelling of bioconcentration

Quantitative structure-activity relationships (QSAR) make use of the fact that bioaccumulation of stable organic compounds is governed by partitioning between aqueous and lipid phases. The predominant process of passive diffusion is frequently formalized in log K_{OW} -dependent QSAR models. It is often assumed, that the log K_{OW} -based BCF estimates represent a 'worst case' reference point. Reduced bioaccumulation may be due to mitigating factors:

- Substance-specific properties, e.g. dissociation, solubility, volatility;
- Substance/biota interactions, e.g. degradation, metabolism, uptake and elimination kinetics.

Estimating bioconcentration factors (BCF) from octanol/water partition coefficients (log K_{OW}) is well established and essentially valid for neutral organics of intermediate lipophilicity ($0 < \log K_{OW} < 6$) (European Communities, 2003; Nendza, 1991; Nendza, 1998; Dearden, 2004).

QSAR models on the basis of descriptors other than $\log K_{OW}$ (e.g. solubility in water, topological indices, geometric properties like surface area) have also been reported, however, they are often not useful for predictions since either experimental data are required (solubility) or the equations are restricted to very narrow datasets. Problems occur, if the applicability domains of the QSARs are exceeded.

BCFBAF (formerly BCFWIN) as a part of the EPI Suite Package (<http://www.epa.gov/oppt/exposure/pubs/episuite.htm>) is a popular computer program for estimating BCF. This program estimates

- a) $\log$ BCF (BioConcentration Factor) using two different regression equations, one for compounds with $\log K_{OW}$ between 1 and 7 and one for compounds with $\log K_{OW} > 7$. The models are based $\log K_{OW}$ and combined with correction factors for some compound classes;
- b) $\log$ BAF (BioAccumulation Factor): *“This model includes mechanistic processes for bioconcentration and bioaccumulation such as chemical uptake from the water at the gill surface (BCFs and BAFs) and the diet (BAFs only), and chemical elimination at the gill surface, fecal egestion, growth dilution and metabolic biotransformation (Arnot and Gobas, 2003). Other processes included in the calculations are bioavailability in the water column (only the freely dissolved fraction can bioconcentrate) and absorption efficiencies at the gill and in the gastrointestinal tract. The model requires the octanol-water partition coefficient (K_{OW}) of the chemical and the normalized whole-body metabolic biotransformation rate constant ($k_{M, N}$; /day) as input parameters to predict BCF and BAF values.”* (Explanation from the help file of the software).

2.3 Data quality considerations

Because this study concerns the extrapolation of properties of chemicals in the regulatory context, it is of utmost importance to be aware of the (lack of) accuracy of the respective data and the uncertainties innate in calculation procedures. In addition to the validity of the methods and the reliability of the results obtained by these methods, relevance (whether the data are meaningful and useful for a particular purpose) and adequacy (whether the available information allows clear decision making for risk assessment purposes) need to be considered. In the present context, three issues are particularly liable to major error rates:

2.3.1. Experimental BCF data depend on multiple factors, like, for example, fish species, age, life stage, gender, size and physiological conditions (e.g. lipid content, test organism health), water conditions (e.g. temperature, particle/total or dissolved organic carbon contents, pH) and purity of the test chemical. Even high quality BCF data (with regard to validity and reliability) have variabilities of > 0.5 log units for at least 35% of all chemicals and

> 1 log unit for at least 10% of all chemicals and may exceed 2 log units in a few cases (Nendza et al. (2010)).

A detailed analysis of BCF data for lindane based on data from AQUIRE demonstrates the large variety in BCF data: *"The AQUIRE data base is a part of the United States Environmental Protection Agency's ECOTOX Database (US-EPA ECOTOX Database). In 2005 more than 480,000 test records, covering 6,000 aquatic and terrestrial species and 10,000 chemicals, were included. The primary source of ECOTOX data is the peer-reviewed literature, with test results identified through comprehensive searches of the open literature. The bioconcentration factor sub-file includes 13,356 aquatic chemical records and 19 terrestrial chemical records, collected from over 1,100 publications, and encompassing approximately 700 distinct chemicals. The use of the on-line database is free and can be accessed through the Internet at www.epa.gov/ecotox."* (taken from ECHA: "Guidance on information requirements and chemical safety assessment Chapter R.7c: Endpoint specific guidance").

ECOTOX: *"It is recommended that users consult the original scientific paper to ensure an understanding of the context of the data retrieved from the ECOTOX data base."*

The ECOTOX database contains 224 entries on bioaccumulation of lindane, 93 of them for different fish species. The lowest BCF reported is 23 (Macek et al. 1976), but this value is from accumulation in muscle tissue only. The lowest BCF reported for "whole organism" is 297 (La Rocca, 1991). In total there are 24 values flagged "whole organism" which are listed in table 1.

Table 1: Experimental BCF values for lindane (CAS 58-89-9) from ECOTOX (whole fish if not otherwise specified)

Species	BCF	Effect Meas*	Exposure Duration (d)	Source	Additional Remarks
Oncorhynchus mykiss	297	GACC	0.125 - 5	La Rocca et al. 1991	-
Lepomis macrochirus	360	GACC	0.125 - 5	La Rocca et al. 1992	
Danio rerio	560	GACC	0.125 - 5	La Rocca et al. 1993	
Poecilia reticulata	730	GACC	0.125 - 5	La Rocca et al. 1994	
Poecilia reticulata	12800	GACC	0.125 - 5	La Rocca et al. 1991	Lipid (original reference, although mentioned as „whole organism” in ECOTOX !)
Danio rerio	13400	GACC	0.125 - 5	La Rocca et al. 1991	
Oncorhynchus mykiss	14600	GACC	0.125 - 5	La Rocca et al. 1991	
Lepomis macrochirus	15400	GACC	0.125 - 5	La Rocca et al. 1991	
Oncorhynchus mykiss	770	RSDE	7	Oliver and Niimi 1985	-
Oncorhynchus mykiss	770	RSDE	75	Oliver and Niimi 1985	-
Oncorhynchus mykiss	880	RSDE	7	Oliver and Niimi 1985	-
Oncorhynchus mykiss	1100	RSDE	21	Oliver and Niimi 1985	-
Oncorhynchus mykiss	1200	RSDE	96	Oliver and Niimi 1985	-
Pseudorasbora parva	1246	GACC	1 - 14	Kanazawa 1981	-
Pseudorasbora parva	1246	RSDE	14	Kanazawa 1980	-
Pseudorasbora parva	1246	RSDE	14	Kanazawa 1981	-
Oncorhynchus mykiss	1400	RSDE	50	Oliver and Niimi 1991	-
Oncorhynchus mykiss	1700	RSDE	75	Oliver and Niimi 1991	-
Oncorhynchus mykiss	1900	RSDE	35	Oliver and Niimi 1991	-
Oncorhynchus mykiss	2100	RSDE	35	Oliver and Niimi 1991	-
Oncorhynchus mykiss	2100	RSDE	50	Oliver and Niimi 1991	-
Oncorhynchus mykiss	2100	RSDE	96	Oliver and Niimi 1991	-
Oncorhynchus mykiss	2100	RSDE	96	Oliver and Niimi 1991	-
Oncorhynchus mykiss	291000	RSDE	21	Oliver and Niimi 1985	Seems to be typing error, should read: 2100

* Effect measurement according to ECOTOX documentation: GACC = global accumulation, RSDE: residues

The BCF data for lindane range from 297 (whole fish) to 15400 (lipid content). The very high value of 291000 was recognised to be a typographical error after consultation of the original publication. The next largest value (15400) is based on lipid content and does not compare to the whole fish QSAR results. The largest value for whole fish is 2100 (i.e. B-compound), whereas based on a calculated log K_{OW} of 4.26 the TGD equation delivers a BCF value of 834 (i.e. non-B-classification).

For two datasets (CAESAR, BCFBAF, see sections 3.3 and 3.4 for further details) the experimental BCF values for diverse chemicals have been compared (Figure 1). Though there is very good or good agreement for most substances (70% deviate < 0.2 log units (due to many identical entries in both databases), 85% deviate < 0.5 log units), also some major discrepancies are easily evident (4% deviate > 1 log unit). Unfortunately, the reasons for the differences in the experimental BCF data for the same compounds are not available from the database documentations and can only be resolved by inspection of the original study reports.

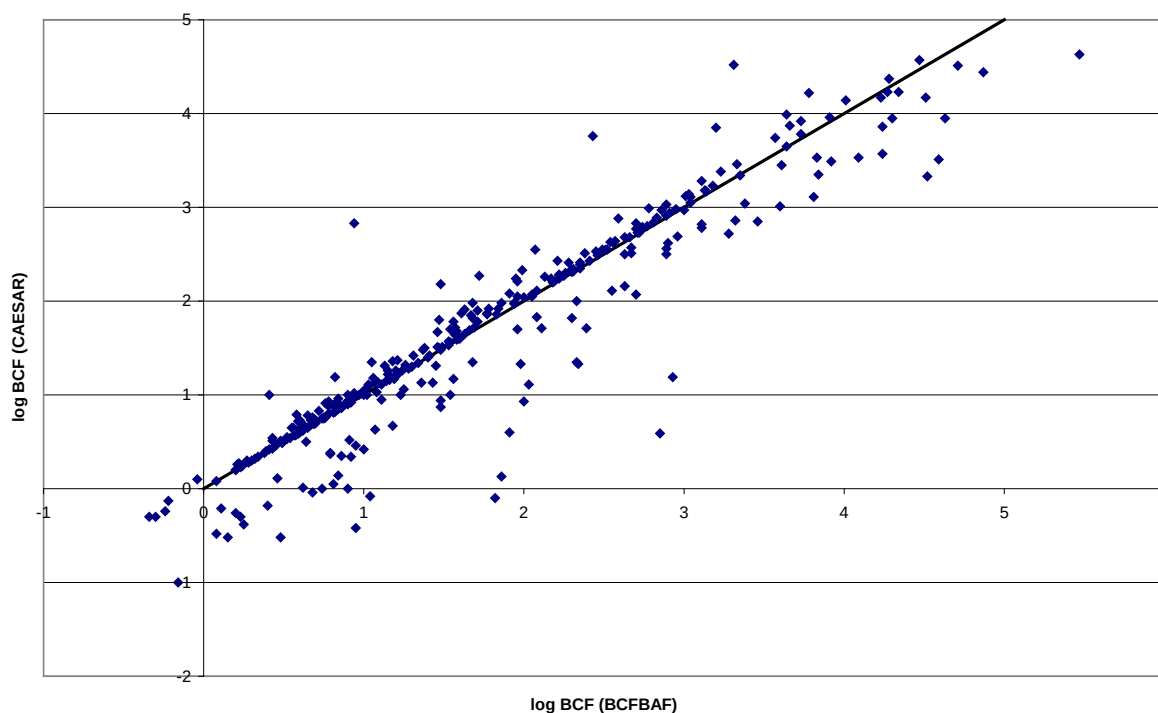


Figure 1: Comparison of experimental BCF values from the CAESAR and BCFBAF databases.

2.3.2. Regression-based QSARs must bear at least the error carried by the input data that were used to build the models. In the case of biological effect data, variability can be due to either technical (e.g., identity of test substance, deviations of test protocols, differences in exposure conditions) or inherent biological (e.g., species, strain, age and sex of test animals, seasonal influence) factors. However, experience has revealed minimum error rates in QSAR models of about ± 0.3 log units. Expectations of lesser variability are not realistic.

2.3.3a. Experimental log K_{OW} data may be affected by several factors, like, for example, concentration dependency and non-attainment of equilibrium, glass/surface adsorption effects, compound and solvent impurities. Variability in measured log K_{OW} values greatly increases towards the extremes of the log K_{OW} scale, i.e. below 0 and above 5, and may exceed 2 orders of magnitude.

2.3.3b. Calculated log K_{OW} data may be obtained from many commercial and freely available software packages, for a review see, e.g., Cronin & Livingstone (2004). The software packages differ in their computational method, e.g., group contribution, fragmental, atomic values, LSER (linear solvation energy relationships), topological descriptors. Most computational methods for log K_{OW} are so-called global models for inert organic compounds, and they frequently fail for inorganics, surface active and chelating compounds,

organometallic compounds, partly or fully dissociated compounds, compounds of extremely high or low lipophilicity, mixtures and impure compounds.

The currently available log K_{OW} calculation programmes have been found to provide inaccurate estimates for a variety of compounds to different extents. This applies particularly to the very high ($\log K_{OW} > 5$) and very low ($\log K_{OW} < 0$) ranges of lipophilicity (due to the variability of the measured values in this range) and/or if intramolecular interactions are not adequately accounted for by the respective algorithms. There are examples of variability by more than 2 log units relative to experimental data and none of the methods perform in a superior manner throughout. A detailed analysis of many estimation models and a comparison of the estimated data for > 90000 compounds is given in Mannhold et al. (2009). These authors confirm again, that consensus log K_{OW} values, i.e. the average of multiple predictions, is most reliable.

For the pesticide dataset from Umweltbundesamt (see 3.1), the correlation between

(a) experimental and estimated log K_{OW} (KOWWIN, <http://www.epa.gov/oppt/exposure/pubs/episuite.htm>) (Figure 2) and

(b) experimental log K_{OW} data from different sources (Figure 4) has been examined.

Comparison of the experimental pesticide dataset from Umweltbundesamt with calculated values using the model KOWWIN (Figure 2) reveals deviations between measured and estimated log K_{OW} values of one order of magnitude (and more), especially in the high lipophilicity range.

Experimental values for pesticides (data from KOWWIN) compared with calculated values using the model KOWWIN (Figure 3) shows better agreement between experimental and estimated log K_{OW} values. However, it has to be noted, that the experimental data from the KOWWIN database have been used to optimize the estimation software.

Comparing experimental log K_{OW} values from Umweltbundesamt with experimental log K_{OW} values from KOWWIN (Figure 4), it becomes apparent that also experimental data from different sources may differ by more than one order of magnitude.

Recommendation: The geometric mean of multiple log K_{OW} values, calculated independently by different methods, bears the best approximation to the true value.

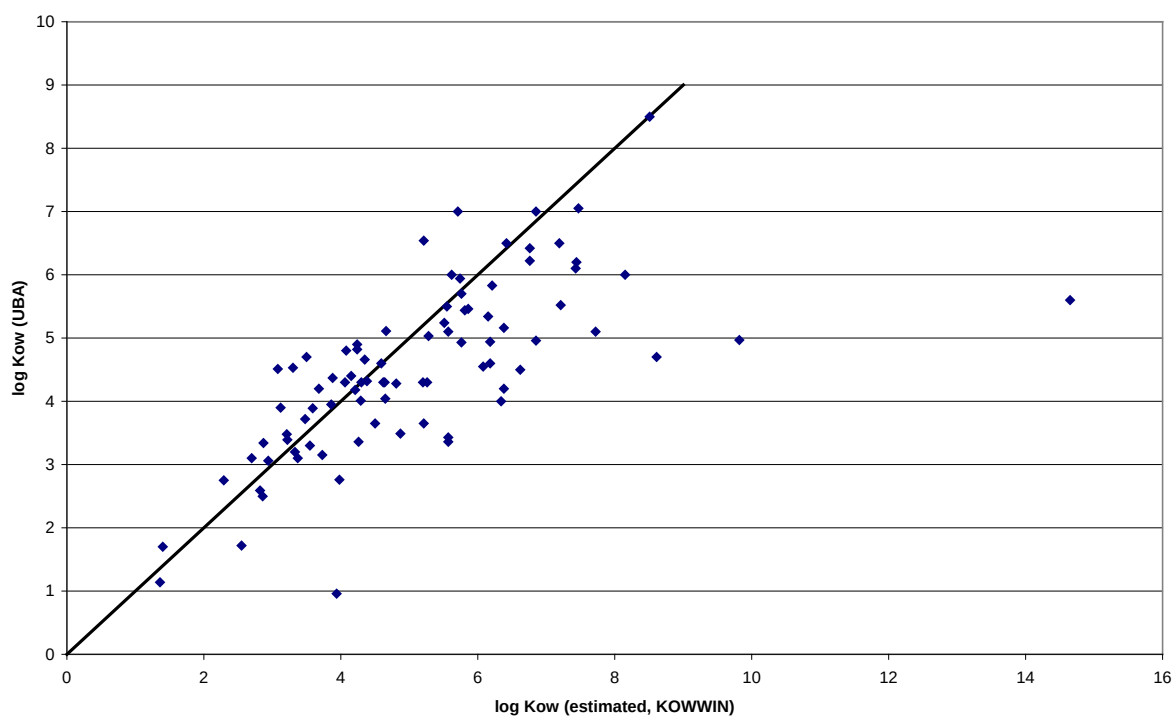


Figure 2: Comparison of experimental and estimated log K_{OW} values for pesticides (experimental data from Umweltbundesamt, estimation model: KOWWIN).

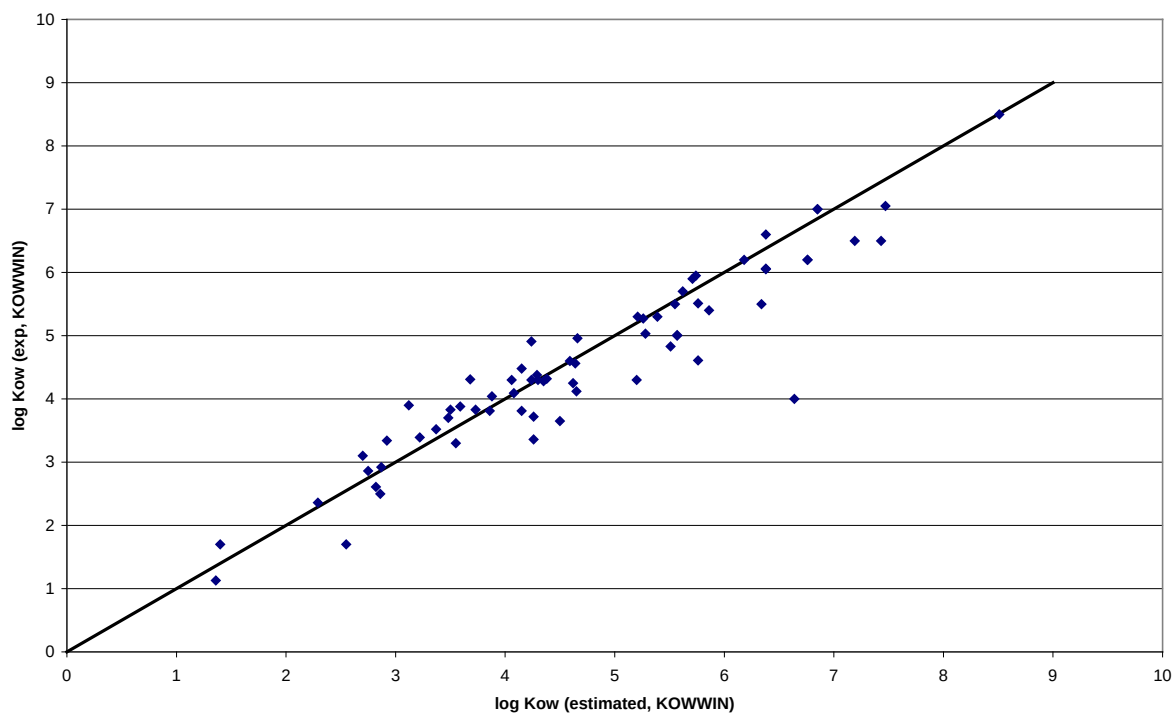


Figure 3: Comparison of experimental and estimated log K_{OW} values for pesticides (experimental data from KOWWIN, estimation model: KOWWIN).

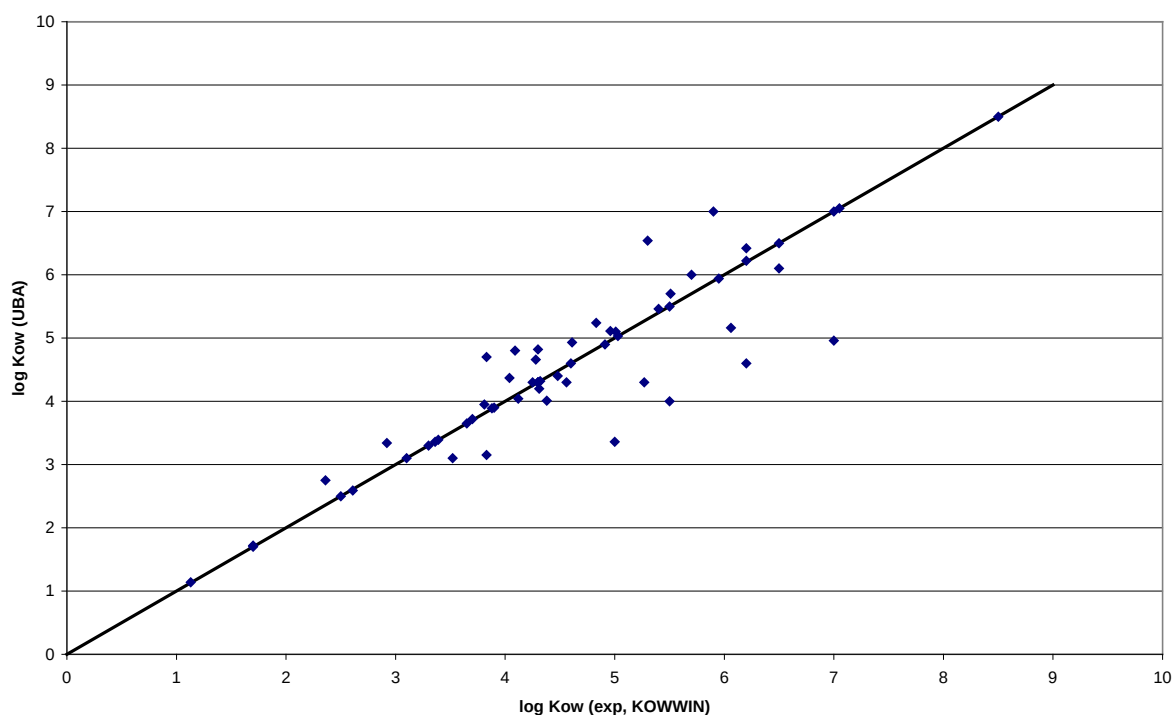


Figure 4: Comparison of experimental log K_{OW} values for pesticides from Umweltbundesamt and experimental log K_{OW} values from KOWWIN

2.4 Overview of existing QSAR-models

Numerous QSAR models for bioconcentration, mostly based on log K_{OW} , have been reported in the past three decades. A recent review of existing QSAR-models for BCF estimation compiled 11 QSAR equations for different datasets (Pavan et al. 2008). *PropertEst*, the QSAR software used at the Umweltbundesamt, features a total of 12 QSAR models for BCF (fish, daphnids, algae), many of them also discussed by Pavan et al. (2008). 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, most of them are very similar in terms of slope and intercept (Figure 5).

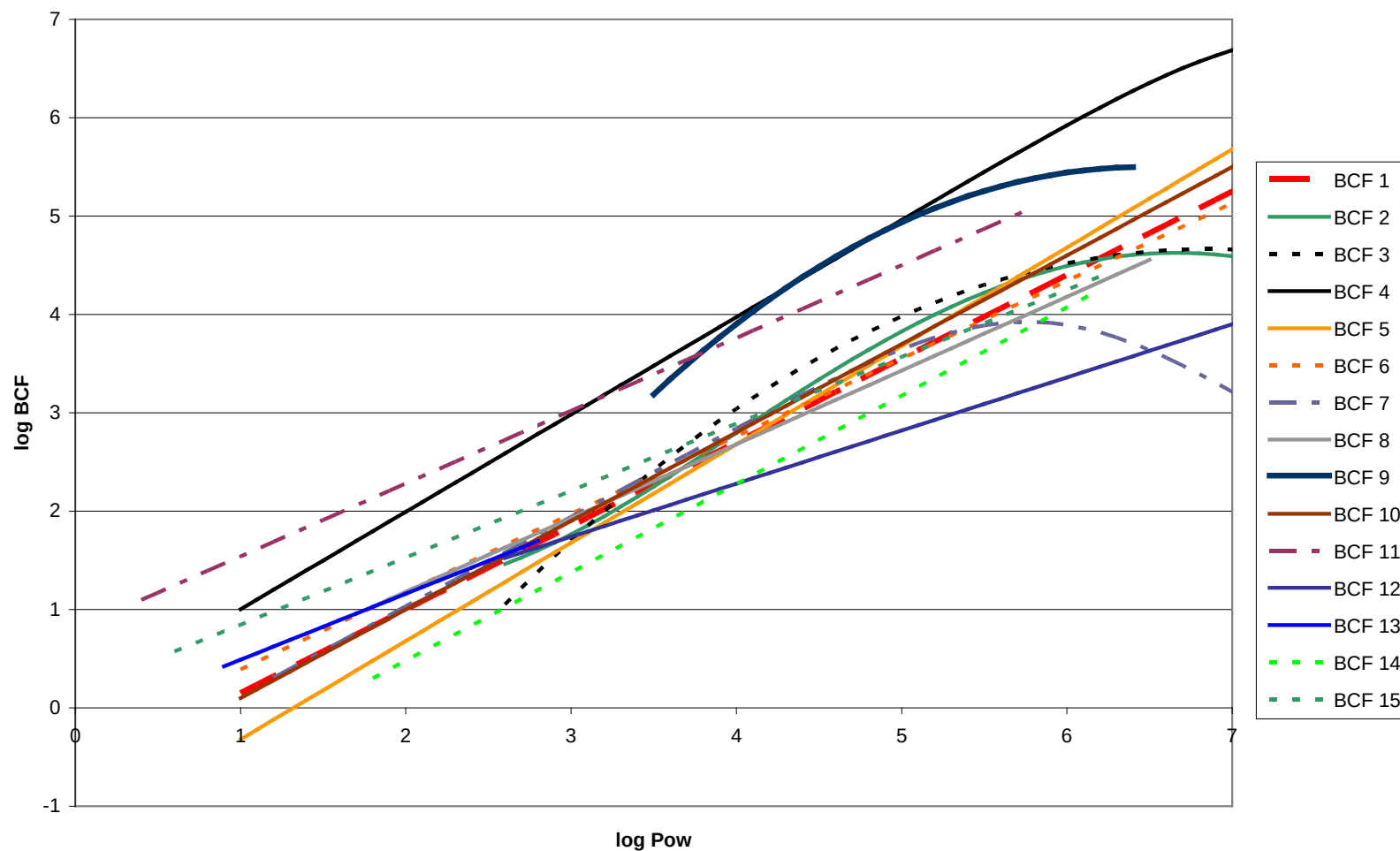


Figure 5: Comparison of QSAR models for BCF: Models no.1 to 13 are based on data for various fish species, model 14 is for Daphnia and model 15 for Chlorella (algae).

1) Veith et al. 1979

$$\log \text{BCF} = 0.85 \log K_{\text{OW}} - 0.70$$

n = 55, r = 0.95, species: *Pimephales promelas*, chemicals: heterogeneous dataset

range of log K_{OW} : 1 – 7.05

This model is recommended in the Technical Guidance Documents on Risk Assessment, part II, (TGD equation 74) for substances with log K_{OW} between 0 and 6 (for a detailed discussion of this QSAR, see 2.2.1.1.).

2) Connell and Hawker, 1988

$$\log \text{BCF} = (6.9 \cdot 10^{-3}) \log K_{\text{OW}}^4 - 0.185 \log K_{\text{OW}}^3 + 1.55 \log K_{\text{OW}}^2 - 4.18 \log K_{\text{OW}} + 4.79$$

n=45, species: fish (various), chemicals: heterogeneous dataset

range of log K_{OW} : 2.6 – 9.8

3) European Communities, 2003

$$\log \text{BCF} = -0.20 \log K_{\text{OW}}^2 + 2.74 \log K_{\text{OW}} - 4.72$$

n = 43, r = 0.883, species: fish (various), chemicals: heterogeneous dataset

This model is recommended in the Technical Guidance Documents on Risk Assessment, part II, (TGD) for substances with log $K_{\text{OW}} > 6$. The model (TGD equation 75) is based on data from: Connell, D.W., Hawker, D.W. "Use of Polynomial Expressions to describe the Bioconcentration of Hydrophobic Chemicals by Fish", Ecotox. Environ. Saf. 16, 242 - 257, 1988.

range of log K_{OW} : 2.6 – 9.8

4) Nendza, 1991

$$\log \text{BCF} = 0.99 \log K_{\text{OW}} - 1.47 \log(4.97 \cdot 10^{-8} K_{\text{OW}} + 1) + 0.0135$$

n = 132, species: fish (various), chemicals: heterogeneous dataset

range of log K_{OW} : 1 – 11

The "worst case"-bilinear model calculates the maximum bioaccumulation potential to be expected for compounds. The model has not been derived by regression, therefore, neither statistical parameters nor confidence intervals are available.

5) Mackay, 1982

$$\log \text{BCF} = \log K_{\text{OW}} - 1.32$$

$n = 44$, $r = 0.95$, $s = 0.25$, species: fish (various), chemicals: heterogeneous dataset, mainly chlorinated hydrocarbons

range of $\log K_{\text{OW}}$: 1 – 7.1

6) Veith et al. 1983

$$\log \text{BCF} = 0.79 \log K_{\text{OW}} - 0.40$$

$n=122$, $r=0.927$, $s=0.49$, species: fish (various), chemicals: heterogeneous dataset, mainly halogenated compounds

range of $\log K_{\text{OW}}$: 1 – 6.9

7) Bintein et al. 1993

$$\log \text{BCF} = 0.91 \log K_{\text{OW}} - 1.975 \cdot \log(6.8 \cdot 10^{-7} \cdot K_{\text{OW}} + 1) - 0.786$$

$n = 154$, $r = 0.95$, $s = 0.347$, species: fish (various), chemicals: heterogeneous dataset

This model is recommended by the authors for compounds with $\log K_{\text{OW}} > 6$.

range of $\log K_{\text{OW}}$: 1.2 – 8.5

8) Schüürmann and Klein, 1988

$$\log \text{BCF} = 0.75 \log K_{\text{OW}} - 0.32$$

$n = 32$, $r = 0.87$, $s = 0.54$, species: fish (various), chemicals: heterogeneous dataset, mainly chlorinated and polycyclic hydrocarbons

range of $\log K_{\text{OW}}$: 1.8 – 6.5

9) Köneman and van Leeuwen, 1980

$$\log \text{BCF} = 3.41 \log K_{\text{OW}} - 0.264 \log K_{\text{OW}}^2 - 5.513$$

$n = 6$, $r = 0.999$, $s = 0.039$, species: *Poecilia reticulata*, chemicals: chlorobenzenes

range of $\log K_{\text{OW}}$: 3.5 – 6.4

This model is based on 6 compounds from the same compound class. However, the model should be applicable for similar organic compounds (small, inert molecules, not degrading) within the range of applicability ($\log K_{\text{OW}}$ between 3.5 and 6.4).

10) Lu et al. 1999

$$\log \text{BCF} = 0.9 \log K_{\text{OW}} - 0.8$$

n = 80, r = 0.944, species: various fish, chemicals: diverse non-polar chemicals

range of log K_{OW} : 1 – 7.1

11) Escuder-Gilabert et al. 2001

$$\log \text{BCF} = 0.74 \log K_{\text{OW}} + 0.8$$

n = 66, r = 0.917, species: various fish, chemicals: diverse

range of log K_{OW} : 0.3 – 5.8

12) Neely et al. 1974

$$\log \text{BCF} = 0.54 \log K_{\text{OW}} + 0.12$$

n = 8, r = 0.949, species: *Salmo gairdneri*, chemicals: halogenated aromatics

range of log K_{OW} : 2.6 – 7.6

13) Zok et al. 1991

$$\log \text{BCF} = 0.67 \log K_{\text{OW}} - 0.18$$

n = 9, r = 0.934, species: *Brachydanio rerio*, chemicals: substituted anilines

range of log K_{OW} : 0.9 – 2.8

14) Hawker and Connell, 1986

$$\log \text{BCF} = 0.898 \log K_{\text{OW}} - 1.315$$

n = 22, r = 0.962, s = 0.293, species: *Daphnia pulex*, chemicals: heterogeneous dataset

range of log K_{OW} : 1.8 – 6.2

15) Geyer et al. 1984

$$\log \text{BCF} = 0.681 \log K_{\text{OW}} + 0.164$$

n = 41, r = 0.902, s = 0.466, species: *Chlorella fusca*, chemicals: heterogeneous dataset

range of log K_{OW} : 0.6 – 6.2

The following table 2 is intended to assist in pre-selecting an appropriate model for a given chemical. However, applicability has to be checked in more detail on the basis of the training set of the model (see chapter 2.6).

Table 2: Compilation of QSAR-models for bioconcentration factor

Dataset	Range of log K_{ow}	Species	Reference	No
anilines, substituted	0.9 – 2.8	<i>Brachydanio rerio</i>	Zok et al. 1991	13)
chlorobenzenes	3.5 – 6.4	<i>Poecilia reticulata</i>	Köneman et al. 1980	9)
mainly chlorinated and polycyclic hydrocarbons	1.8 – 6.5	fish (various)	Schüürmann and Klein, 1988	8)
mainly chlorinated hydrocarbons	1 – 7.1	fish (various)	Mackay, 1982	5)
halogenated aromatics	2.6 – 7.6	<i>Salmo gairdneri</i>	Neely et al. 1974	12)
mainly halogenated compounds	1 – 6.9	fish (various)	Veith et al. 1983	6)
diverse non-polar chemicals	1 – 7.1	fish (various)	Lu et al. 1999	10)
heterogeneous dataset	1 – 7.05	<i>Pimephales promelas</i>	Veith et al. 1979	1)
heterogeneous dataset	2.6 – 9.8	fish (various)	Connell and Hawker, 1988	2)
heterogeneous dataset	2.6 – 9.8	fish (various)	European Communities, 2003	3)
heterogeneous dataset	1 - 11	fish (various)	Nendza, 1991	4)
heterogeneous dataset	1.2 – 8.5	fish (various)	Bintein et al. 1993	7)
heterogeneous dataset	0.3 – 5.8	fish (various)	Escuder-Gilabert et al. 2001	11)
heterogeneous dataset	1.8 – 6.2	<i>Daphnia pulex</i>	Hawker and Connell, 1986	14)
heterogeneous dataset	0.6 – 6.2	<i>Chlorella fusca</i>	Geyer et al. 1984	15)

2.5 Closer examination of eq. 74 of the TGD

The “EU Technical Guidance Document on Risk Assessment, Part II (TGD 2003)” recommends its eq. 74 (= eq. 1 in Chapter 2.3 of this report) for chemicals with a log K_{ow} between 2 and 6 for estimating BCF. Therefore, this equation and the underlying dataset have been investigated in more detail:

The dataset, on which eq. 74 of the TGD is based, is heterogeneous with mostly non-polar (highly chlorinated) compounds, but also some polar compounds and some detergents. The chemical diversity may cause substantial variability of the model. Furthermore, several inconsistencies have been observed with this model:

1. According to the original reference (Veith et al. 1979) the model is based on either 55 compounds (as given in the text) or 59 compounds (as given in figure 3 of the original reference), while their table 4 lists 62 compounds with associated BCF data. The authors have excluded three compounds, resulting in a training set of 59 compounds. However, the equation could not be reproduced unless all compounds with censored BCF data were eliminated as well, resulting in a dataset of 52 compounds:

$$\log \text{BCF} = 0.847 \log K_{\text{OW}} - 0.691 \text{ (reported: } \log \text{BCF} = 0.85 \log K_{\text{OW}} - 0.70)$$

2. For this reduced dataset, the correlation coefficient r (given as 0.947) could not be reproduced. The correlation coefficient for the dataset with 52 compounds is 0.921. The correlation coefficient of 0.947 could be reproduced when using the upper limit for compounds with censored data as the actual $\log \text{BCF}$. In this case, however, the reported regression equation could not be reproduced.
3. Some compounds were included twice in the dataset with different BCF data and in some cases with different $\log K_{\text{OW}}$ data, hence the size of the dataset is further reduced to $n = 48$:
 - Hexachlorobenzene: same $\log K_{\text{OW}}$, different $\log \text{BCF}$ (different species);
 - FWA-2-A same as NTS-1: different $\log K_{\text{OW}}$ and different $\log \text{BCF}$ (same species);
 - FWA-3-A same as DASC-3: same $\log \text{BCF}$, different $\log K_{\text{OW}}$;
 - FWA-4-A same as DASC-4: same $\log \text{BCF}$, different $\log K_{\text{OW}}$.
4. For some compounds, censored $\log \text{BCF}$ have been given (example: atrazine with $\log \text{BCF} < 0.9$). It is assumed, that the upper limit value has been used for the QSAR model. For one compound the $\log \text{BCF}$ is given as a range (0.32 – 1.0), recalculation of the model was possible when using the mean value of 0.32 and 1, i.e. 0.66.
5. More than half of the BCF data were produced by one laboratory (using the same fish species and same exposure time (32 days), while the remaining data were collected from the literature. These used different fish species and the exposure times ranged from 4 days to 304 days. The biological input data therefore lack consistency.
6. The $\log \text{BCF}$ data for rainbow trout have been adapted to the other data by adding an arbitrary value of 0.5.

The TGD eq. 74 model has been derived thirty years ago, well before QSAR quality criteria (OECD, 2007) have been established. While 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), it can be considered laterally validated (good agreement with other QSARs, see Fig. 5).

2.6 Applicability of QSAR models / Optimum prediction space

In general, use of QSAR models for prediction of data for chemicals dissimilar to the training set may lead to unreliable results. To reduce the risk of “wrong” estimates, some limitations need to be considered:

- 1) Extrapolation has to be avoided: all input parameter values should be within the range of the respective input parameters of the training set. Example: the range of $\log K_{OW}$ for a training set is 2.5 to 6. In this case, usage of the QSAR model for a compound with $\log K_{OW}$ of 8 is not recommended.
- 2) Compounds for which an estimate is requested, should be sufficiently similar to the compounds included in the training set. However, similarity is not easily defined and it is dependent on the endpoint to be estimated. Example: alkanes and alkenes may be “similar” for a model estimating BCF from $\log K_{OW}$, but they are not similar with regard to reactivity.

One way to consider chemical similarity is based on compound classes: a QSAR model should be used for predictions only for compounds within the same compound class(es). However, definition of compound classes may not be unique and this method is not applicable for datasets with multifunctional compounds.

Ideally, similarity should relate to the rate-limiting interactions that cause the observed response, i.e. in case of bioaccumulation the similarity metrics should relate to partitioning, transformation, dissociation, volatilisation, etc., hence cover aspects of chemical (structural and physico-chemical) similarity and functional (toxicological) similarity (see Section 5.1).

A rather simplistic approach to define molecular similarity uses *atom centred fragments* (ACFs) (Dimitrov et al. 2005). To define the “structural domain” of a QSAR model the molecular structure is divided into fragments:

- Each non-hydrogen atom is used as the centre of a fragment, see example below;

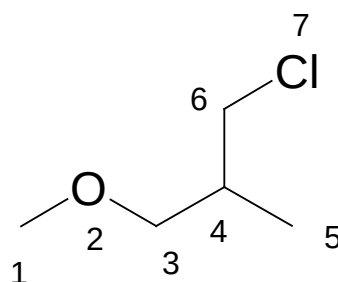
- For 1st order ACFs, all non-hydrogen atoms adjacent to the current “centre atom” are included in the fragment;
- For 2nd order ACFs, all atoms adjacent to the “first order neighbours” are additionally included in the fragments;
- For higher order ACFs, further neighbour atoms are included in the fragments;
- Each compound from the training set is divided into the related ACFs. All ACFs for all training set compounds are collected in a database;
- The compound for which an estimate is required is divided into ACFs as well and the compound is considered to be within the structural domain only if all ACFs can be found in the database from training set.

In this study only 1st order ACFs have been used. Higher order ACFs lead to a more strict definition of the structural domain, i.e. less compounds may be found within the domain.

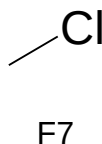
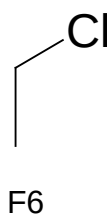
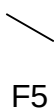
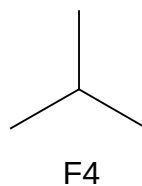
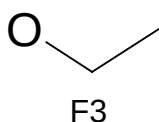
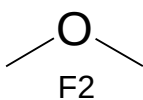
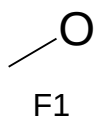
Example for building ACFs:

Consider 1-chloro-2-methylpropane methyl ether as a compound to be divided into 1st order ACFs. Atom no. 1 is the starting point to build fragment F1, there is only one non-hydrogen neighbour atom (the oxygen atom) and the ACF consist of two atoms. Atom no. 2 is the starting point to build fragment F2. There are two non-hydrogen neighbors (both are carbon atoms) and the ACF consists of three atoms. The same procedure is applied to any further atoms.

Compound:
1-chloro-2-methylpropane methyl ether



Atom Centered Fragments:



3 Comparison of experimental and calculated BCF data

To identify the possibilities and limitations of QSARs for estimating the BCF of pesticides and biocides in the regulatory framework for Umweltbundesamt, experimental and calculated BCF data have been comparatively analysed for several different datasets.

The applicability domain of eq. 74 of the TGD has been assessed using log K_{OW} descriptor range and 1st order atom centred fragments. All compounds of the datasets 3.1 – 3.4 have been checked, whether they are inside the applicability domain or not. It should be noted that other metrics of similarity to define the optimum prediction space may come up with different results.

3.1 Pesticides and new chemicals

Data for 88 pesticides as well as some data for new chemicals were retrieved at the Umweltbundesamt, Dessau. These data are confidential and are not explicated in this report, Only “whole body” BCF data, mainly based on total radioactivity and partly (~10%) based on parent compound, were used. In the case of more than one result, always the maximum BCF has been taken for further examination. Experimental log K_{OW} values were used for calculating the log BCF when available (85 compounds), otherwise the log K_{OW} values were calculated by KOWWIN (3 compounds).

Figure 6 illustrates that both TGD models 74 and 75 overestimate the BCF for 62 of 88 compounds (70%) compounds investigated in this study, i.e. for these substances estimated BCF values are larger than the measured values. Underestimations by TGD model 74 occur for 26 of 88 of compounds (30%) of this study and generally remain within one order of magnitude. Deviations within one order of magnitude compare with experimental uncertainties but uncertainties of calculated BCF values may not be sufficient for a worst-case assessment in the regulatory context. Moreover, it should be noted that no safety factors are used for the evaluation of bioaccumulation potential in the risk assessment of chemical substances.

For two compounds (lindane and novaluron), even larger upward deviations were found. For lindane, the deviation is caused by only this BCF value (15400) being measured in the lipid fraction of the fish (cf. ECOTOX database (<http://cfpub.epa.gov/ecotox/>)). Other BCF data from ECOTOX database for lindane are with BCF values between 297 and 2100 much lower and thus are closer to the calculated value of 834 using the TGD model 74 (see 2.3).

For novaluron (BCF: 14645), a much lower value of 2091 (“Verified data used for regulatory purposes”) is reported for example in the Pesticide Properties Data Base (PPDB) (contains reviewed data only) (<http://sitem.herts.ac.uk/aeru/iupac/Reports/487.htm>). On the basis of a calculated $\log K_{OW}$ of 5.26 the BCF is calculated (TGD eq. 74) as 5900.

As a general observation from comparison of experimental and calculated BCF data, it was found that compounds which are inside the applicability domain show lower deviation from eq. 74 than compounds which are outside the applicability domain of the model. However, there are only eight compounds of the dataset inside the domain, thus no final conclusion on the usability of this approach can be drawn. The rationale for the small number of compounds inside the domain is that pesticides in the UBA dataset have structural elements like e.g. heteroaromatic rings, which are not included in the training set of TGD eq. 74.

Except for lindane and novaluron, where the experimental data are questionable, there is no underestimation by more than one order of magnitude. Estimates by the worst case equation represent in all cases the upper limit for wet-weight-based BCF values.

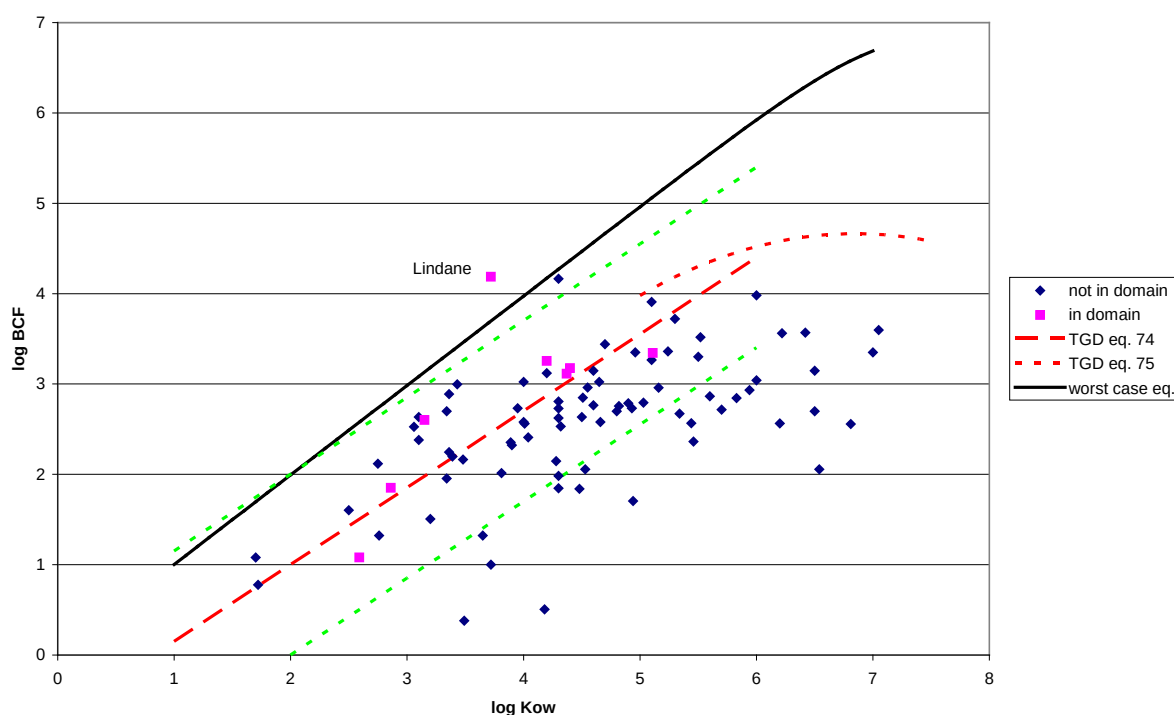


Figure 6: Comparison of experimental BCF data for pesticides (n=88) with TGD models and the worst case QSAR model (Eq. 4 from chapter 2.4) (green line: deviation interval of one order of magnitude relating to TGD eq. 74).

Additionally to the TGD equations 74 and 75, the BCFBAF (formerly BCFWIN) program was used to estimate log BCF (see Figure 7). Most of the log BCF values are estimated with deviations less than one order of magnitude, however, there are more underestimations of

bioconcentration (i.e. experimental log BCF is higher than the estimated value) than overestimations while the TGD model shows much less underestimations (see Figure 6). The same behaviour is found for the log BAF value (Figure 8).

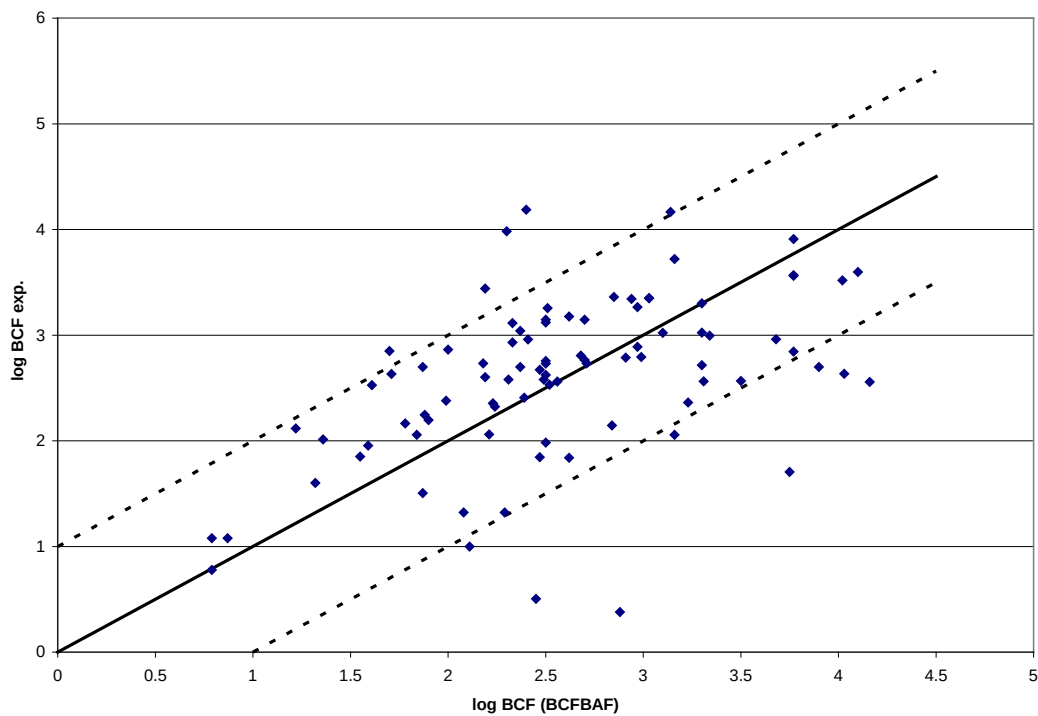


Figure 7: Comparison of experimental BCF data for pesticides (n=88) with BCFBAF estimates (log BCF) for pesticides (dashed lines: deviation interval of one order of magnitude).

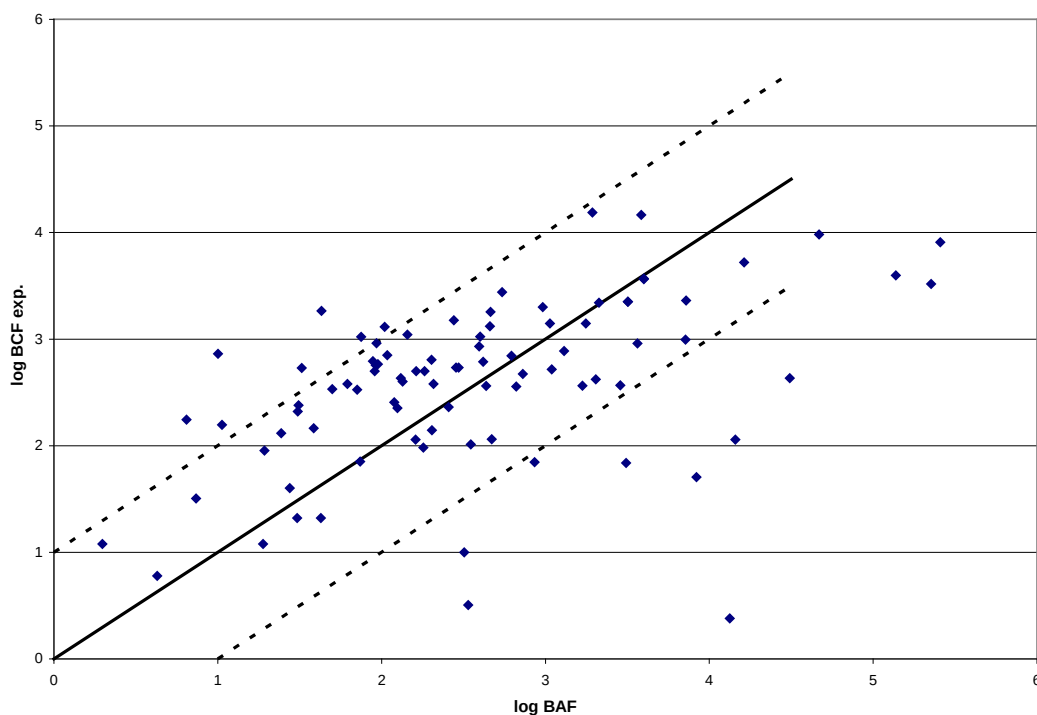


Figure 8: Comparison of experimental BCF data for pesticides (n=88) with BCFBAF estimates (log BAF) for pesticides (dashed lines: deviation interval of one order of magnitude).

In detail:

- Eq. TGD 74 shows 30% (26 of 88 compounds) underestimations (problematic for regulatory purposes) and 70% (62 of 88 compounds) overestimations (worst case, therefore no problem for regulatory purposes); 17 estimated BCF values (19%) deviate less than 0.2 log units.
- BCFWIN (log BCF) shows 63% (55 of 88 compounds) underestimations (problematic) and 37% (33 of 88 compounds) overestimations (not problematic); 18 estimated BCF values (21%) deviate less than 0.2 log units.
- BCFBAF (log BAF) shows 56% (49 of 88 compounds) underestimations (problematic) and 44% (39 of 88 compounds) overestimations (not problematic); 19 estimated BCF values (22%) deviate less than 0.2 log units.

As a consequence, both parameters from BCFBAF (log BCF and log BAF) are less suitable for estimation of BCF values than the TGD models for the regulatory assessment of pesticides.

For new chemicals only few data (n=13) were available. Some fit well to the TGD eq. 74, but most of them are much less bioaccumulating than predicted (Figure 9). Since chemical structures were not available, the test on applicability domain could not be performed. Due to the small dataset no recommendation can be given for these chemicals.

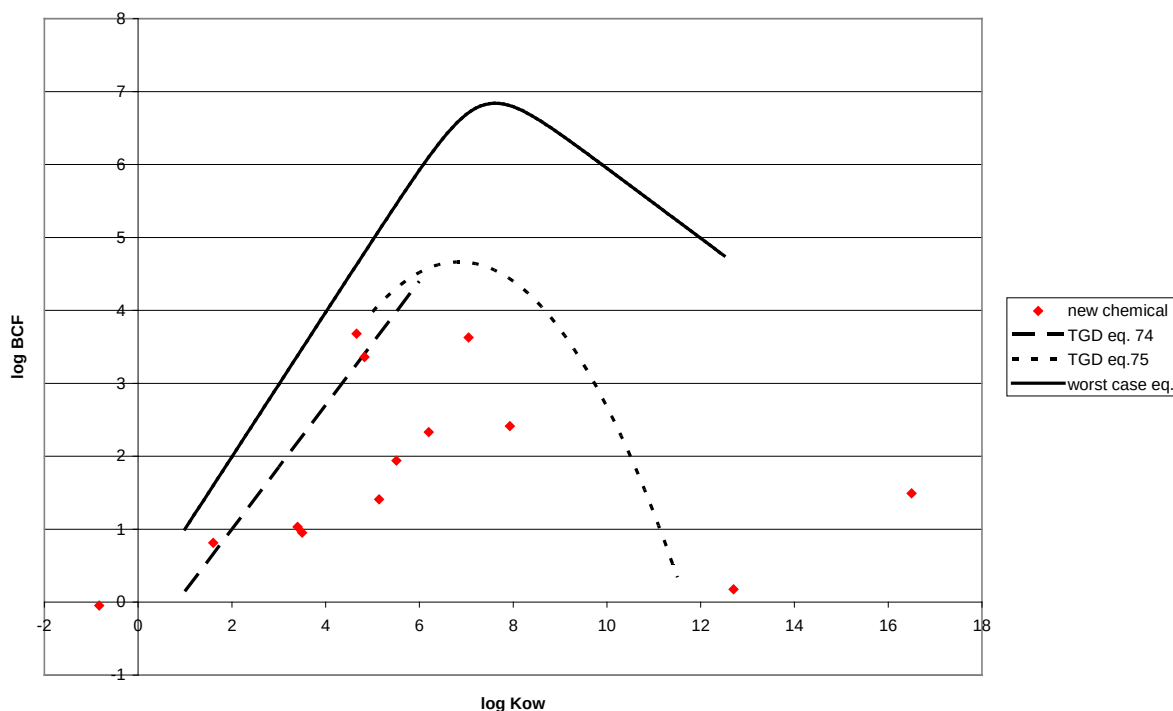


Figure 9: Comparison of experimental BCF data for new chemicals (n=13) with TGD models and the worst case QSAR model.

3.2 BCF Gold standard database

453 BCF data from different compound classes (industrial chemicals, pesticides, biocides) have been extracted from the “BCF Goldstandard Database” (EURAS (2007): CEFIC LRI Goldstandard Database: <http://ambit.acad.bg/ambit/php/euras.php>). Most of the compounds of the database have been obtained from MITI where they have been measured for regulatory purposes. The corresponding log K_{OW} data were calculated using KOWWIN (see section 2.3.3 and recommendation page 13)

210 of the 453 compounds are inside the applicability domain (as defined by log K_{OW} range and ACF 1st order), the remaining 243 compounds are not inside the applicability domain defined this way.

As can be seen from figures 10 and 11, most of the log BCF values estimated by TGD eq. 74 are within one order of magnitude to the experimental data. However, the ratio of “well predicted” compounds is considerably higher for the compounds inside the applicability domain (185 of 210 compounds, 88%) compared to the compounds outside the applicability domain (138 of 243 compounds, 57%). The BCF is underestimated with a calculation according to TGD eq. 74 for 104 of 210 compounds (50%) inside, and for 111 of 243 (46%) outside the applicability domain. However, for only 2 of 210 (1%) compounds inside the applicability domain the BCF is underestimated by more than one order of magnitude, but for 41 of 243 (17%) compounds outside the applicability domain. Especially for the compounds inside the domain only few hydrophilic compounds are considerably more accumulating than predicted. Still, for these hydrophilic compounds, experimental and calculated BCF values are < 100 .

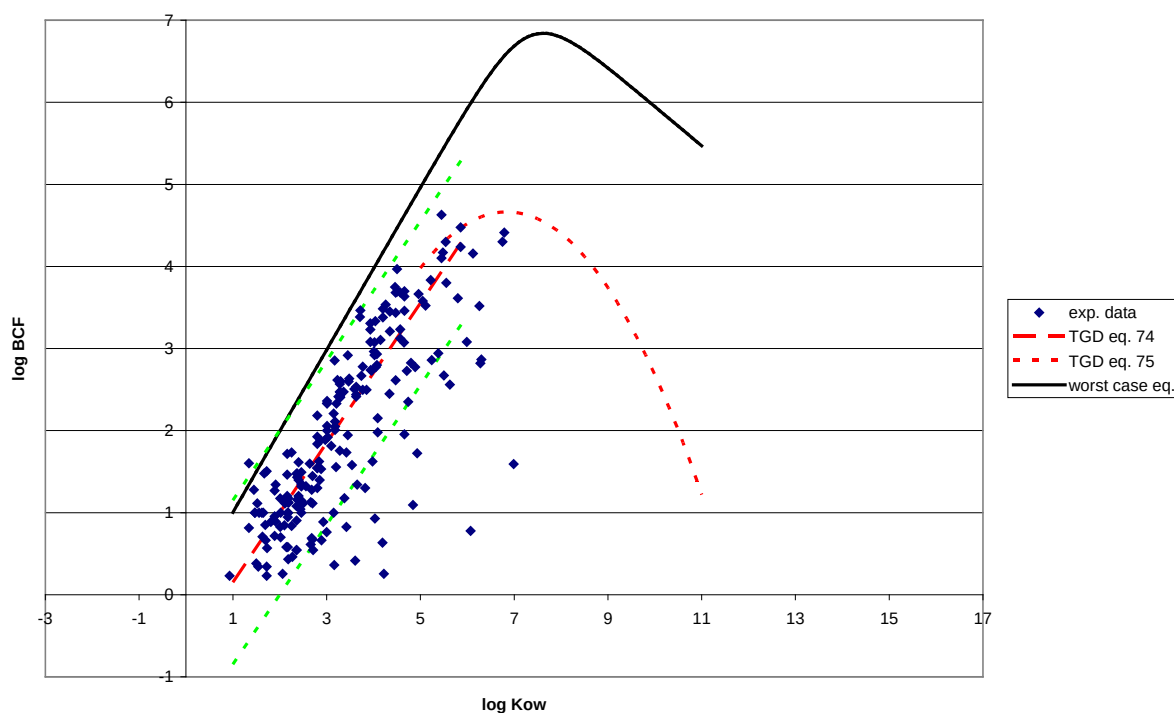


Figure 10: Comparison of experimental BCF data from the “gold standard” dataset with TGD models and the worst case QSAR model for the 210 compounds that are inside the applicability domain (dashed lines: deviation interval of one order of magnitude).

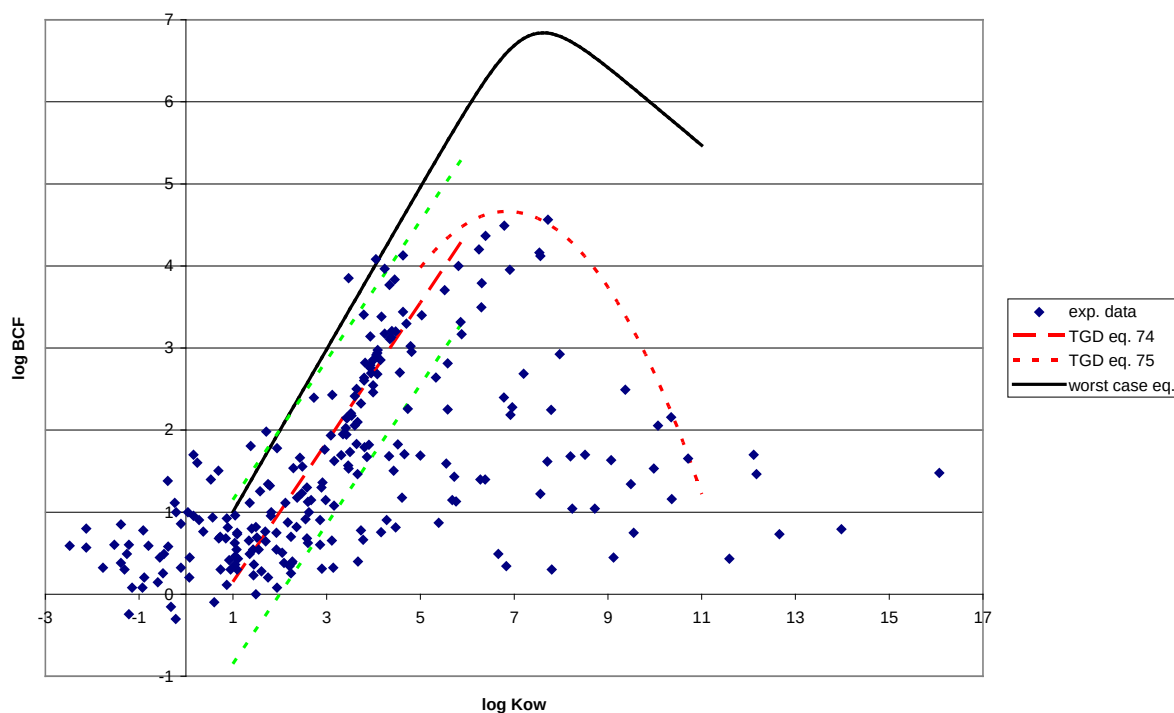


Figure 11: Comparison of experimental BCF data from the “gold standard” dataset with TGD models and the worst case QSAR model for the 243 compounds that are outside the applicability domain (dashed lines: deviation interval of one order of magnitude).

3.3 CAESAR dataset

BCF data for 461 compounds from different chemical classes have been taken from Zhao et al. (2008) which were collected and validated in the course of the EU-project CAESAR (<http://www.caesar-project.eu/index.php?page=results§ion=endpoint&ne=1>). The corresponding log K_{OW} data were calculated using KOWWIN.

Figures 12 and 13 show very similar results compared to figures 10 and 11 since most of the data are identical to the data from the gold standard dataset: approximately 300 compounds from the CAESAR dataset are included in the gold standard dataset as well.

The BCF is underestimated with a calculation according to TGD eq. 74 for 53 of 245 (22%) compounds inside the applicability domain and for 77 of 216 (37%) compounds outside the applicability domain. A deviation less than one order of magnitude is found for 198 of 245 (89%) compounds inside, and 121 of 216 (56%) compounds outside the applicability domain.

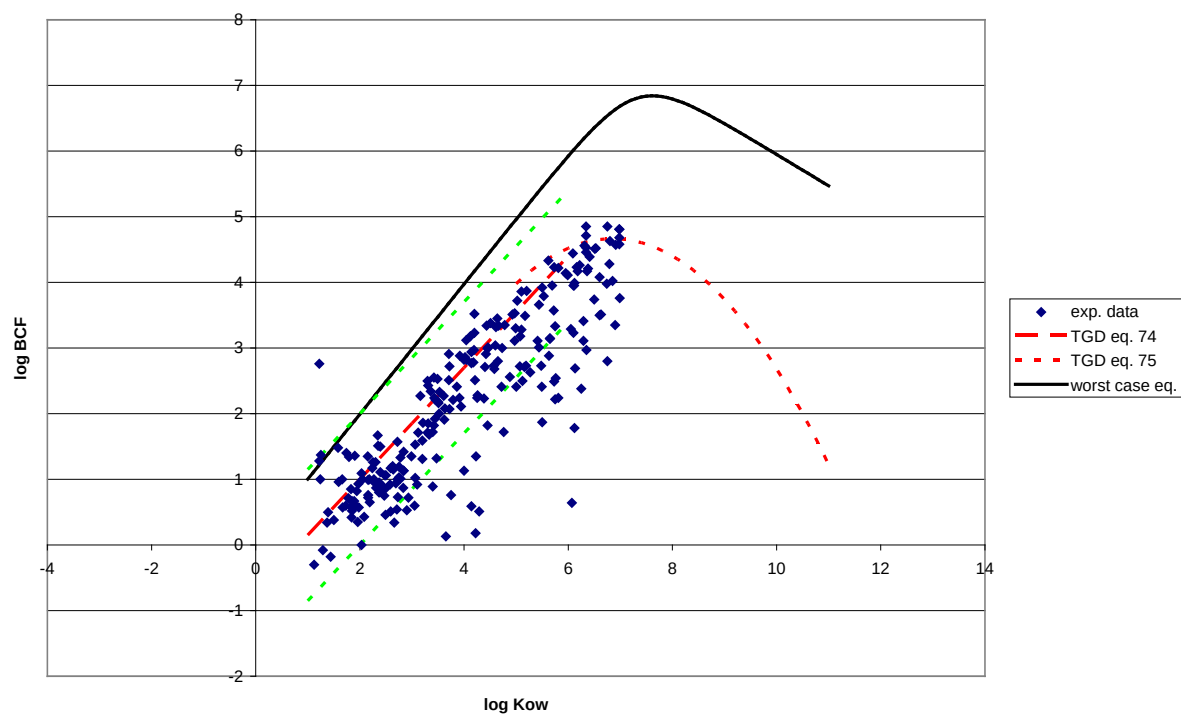


Figure 12: Comparison of experimental BCF data for the 245 compounds inside the applicability domain from the “CAESAR” dataset with TGD models and the worst case QSAR model (dashed lines: deviation interval of one order of magnitude).

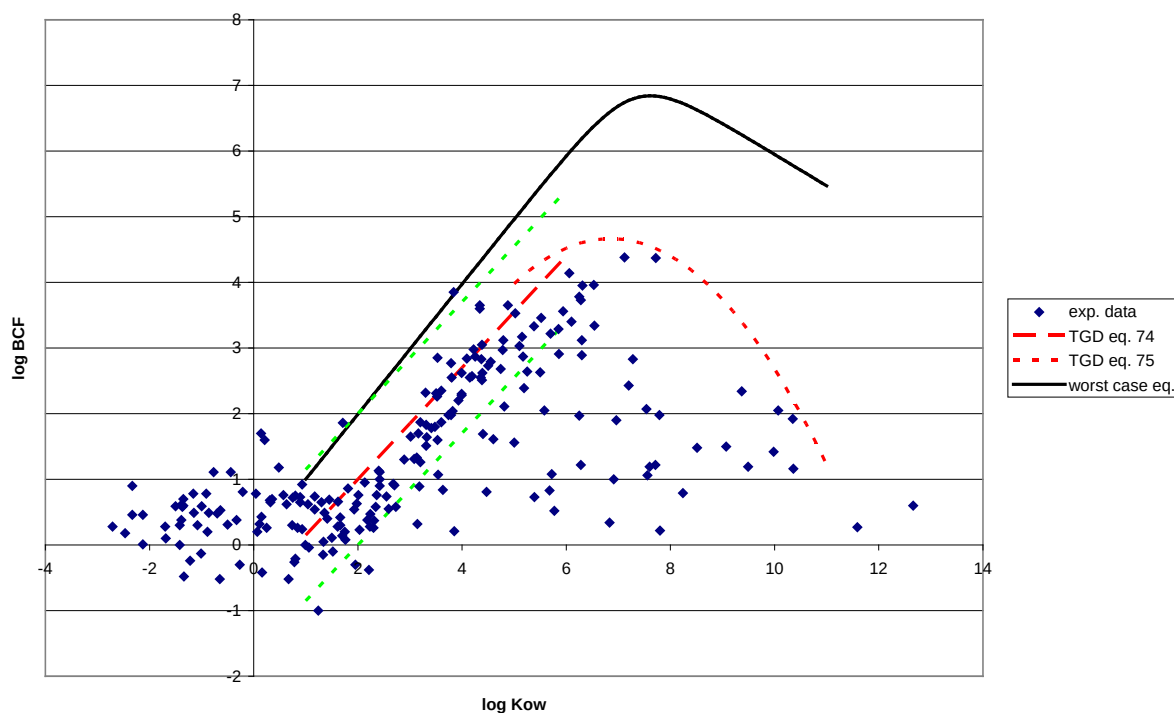


Figure 13: Comparison of experimental BCF data for the 216 compounds outside the applicability domain from the “CAESAR” dataset with TGD models and the worst case QSAR model (dashed lines: deviation interval of one order of magnitude).

3.4 BCFBAF (formerly BCFWIN) dataset

BCFBAF (formerly BCFWIN) includes a database with experimental BCF data for 610 compounds, which were used to develop and refine the BCFWIN- and BCFBAF-models. Most of the compounds are included in the gold standard dataset (see Section 3.2), therefore the following evaluation is restricted to the 218 compounds not included in the gold standard dataset.

118 of the 218 compounds are inside the applicability domain, for 95 (81%) of these 118 compounds the estimated log BCF (TGD eq. 74) is within a range of one order of magnitude. For the remaining 100 compounds (outside the applicability domain) 56% are estimated with deviations > 1 log unit.

The BCF is underestimated with a calculation according to TGD eq. 74 for 45 of 118 (38%) compounds inside the applicability domain and for 26 of 100 (26%) compounds outside the applicability domain.

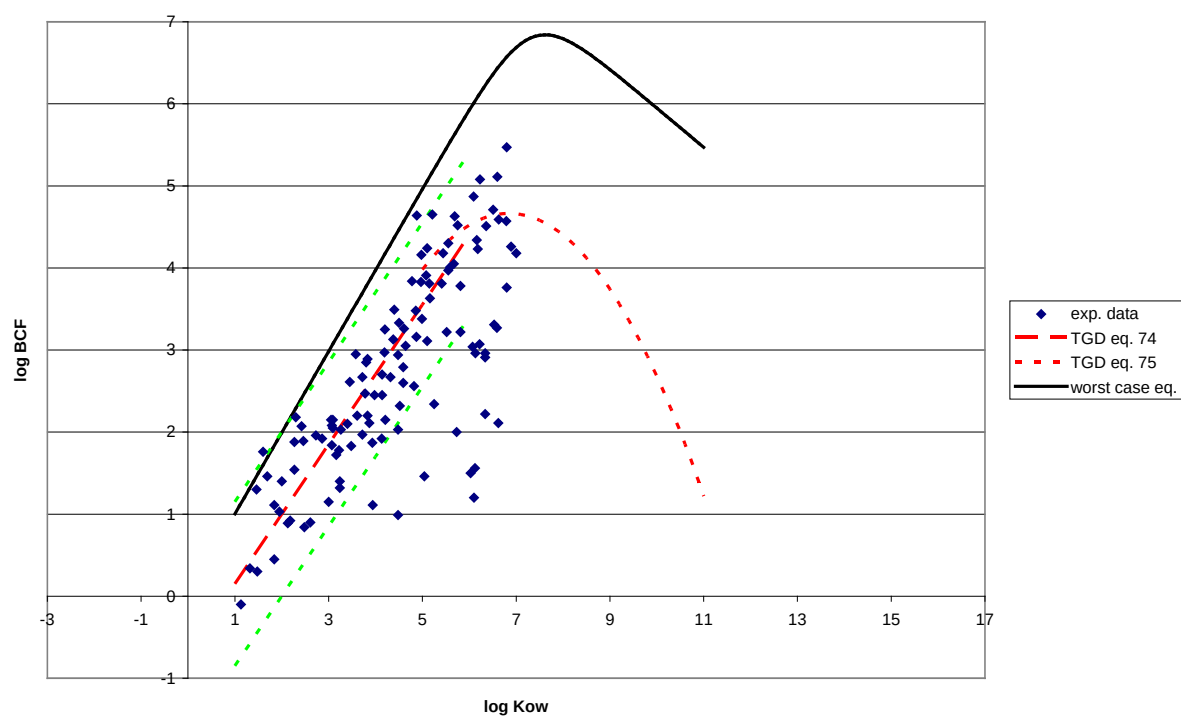


Figure 14: Comparison of experimental BCF data for the 118 compounds inside the applicability domain from the BCFBAF dataset with TGD models and the worst case QSAR model (dashed lines: deviation interval of one order of magnitude).

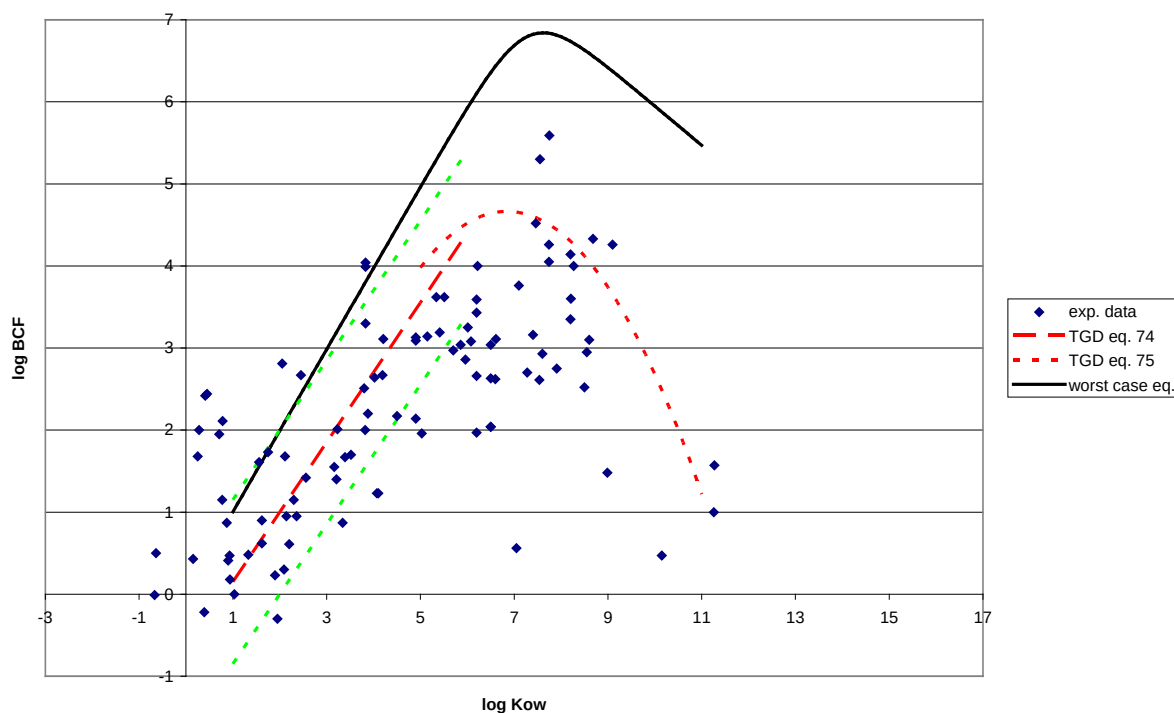


Figure 15: Comparison of experimental BCF data for the 100 compounds outside the applicability domain from the BCFBAF dataset with TGD models and the worst case QSAR model (dashed lines: deviation interval of one order of magnitude).

Summary

1. Most linear regression models for the estimation of $\log BCF$ from $\log K_{OW}$ show similar intercepts and slopes compared to equation 74 (TGD) (see Fig. 5), therefore the results of the estimates are often close. However, the magnitude of the deviations between measured and calculated BCF varies for different QSARs. There is no single QSAR model that constantly delivers minimum deviations. Models using additional correction factors (like BCFBAF) do not necessarily improve the reliability of the BCF estimations.
2. For the pesticide dataset from Umweltbundesamt, equation 74 of the TGD shows (very) large deviations for some compounds (more than one order of magnitude: 28 of 88 compounds, more than two orders of magnitude: 6 of 88). Bioconcentration is overestimated in all these cases (i.e. the experimental value is (much) lower than the calculated one). For regulatory purposes the overestimation of BCF is unproblematic.
3. For 26 of 88 compounds (30%) of the pesticide dataset from Umweltbundesamt, the BCF is underestimated by equation 74 of the TGD, but not more than one order of magnitude (assuming that the two very high values for lindane and novaluron are not realistic). For regulatory purposes the underestimation of BCF by one order of

magnitude may be problematic. As no safety factor is used for BCF data, protective values shall be preferred.

4. Comparison of experimental BCF values from different databases (e.g. CAESAR and BCFBAF (Fig. 1)) reveals that experimental values show deviations by more than one order of magnitude in some cases. As information about data generation is often scarce, regarding e.g. fish species, attainment of steady state, total radioactivity vs. parent compound analytics, etc., the degree of variability of measured data is unknown. Inhomogeneity of experimental data causes major ambiguity in QSARs based on these data. Also, QSAR models cannot work well with BCF values obtained from total radioactivity because activity (for mixtures of parent compound and metabolites) and descriptor data (parent compound) address different substances.
5. A protective upper limit of bioconcentration can be calculated using the worst case model (equation 4 of this report) where $\log \text{BCF}$ corresponds to $\log K_{\text{OW}}$ for compounds with $\log K_{\text{OW}} < 6$. This worst case model is highly sensitive (no false negatives), but reveals low specificity (many false positives). This finding is confirmed by the other datasets examined in this study.
6. For the datasets examined in this study, the estimation error is lower for compounds within the applicability domain of eq. 74 (TGD) (applicability domain as defined by structural similarity and range of $\log K_{\text{OW}}$). It is therefore recommended to verify the applicability domain for every QSAR estimation.
7. TGD eq. 74 seems to be suitable to estimate bioaccumulation, provided that the compounds are inside the applicability domain and deviations of one order of magnitude between calculated and experimental BCF values can be accepted. However, it needs to be realised that there are cases, where the measured BCF is considerably higher than the calculated one, e.g. for carbosulfan a measured BCF of 990 is reported and the calculated BCF ($\log K_{\text{OW}} = 3.43$) is only 164.
8. Even for calculated data which lie within one order of magnitude of the measured data, the deviation may not be acceptable for regulatory purposes. It has to be concluded that equation 74 of the TGD does not necessarily represent the worst case. The inherent variability of measured BCF data is ~ 1 order of magnitude and often may be larger. Even more uncertainties may apply to QSAR estimates. The acceptable uncertainty in either measured or calculated BCF values is context-dependent and varies with the relevant regulatory threshold (100 (if not readily degradable), 1000 (if readily degradable), 2000 (B compounds) or 5000 (vB compounds)).

4 Stereoselective Bioaccumulation

The following sections present evidence from a literature review about possibilities and limitations of transfer (read-across) of BCF-results between individual stereoisomers as well as between different mixtures of stereoisomers. Starting points to understanding enantioselective bioaccumulation are two aspects:

- Characteristics of stereoisomeric chemicals
- Processes involved in bioaccumulation of stereoisomers
 - o passive uptake of (non)racemic chemicals from food and water
 - o enantioselective processing in the animal:
 - selective transport across membranes
 - selective metabolism

Consideration of stereochemistry in combination with process-based biology suggests that functional similarity of stereoisomers with regard to complex bioaccumulation pathways is the key to assess the reliability and relevance of read-across between stereoisomers. Only if 3-D similarity between stereoisomers with regard to rate-limiting processes of (enantioselective) bioaccumulation is sufficiently high, can read-across of BCF-testing results be substantiated.

4.1 Characteristics of stereoisomeric chemicals

About 25% of all agrochemicals used in the world are chiral compounds (Williams, 1996), mostly in commerce as mixture of optical isomers, e.g. racemate: equimolar mixture of enantiomers. Only few pesticides are manufactured and used in the form of a pure enantiomeric compound. Further kinds of isomeric mixtures may be due to geometrical isomerism, e.g. cis/trans, whose composition depends on conditions of synthesis.

With regard to bioaccumulation potential, it is necessary to differentiate isomers and isomeric mixtures:

- **Enantiomers** have one chiral centre and do not differ in their physico-chemical properties under abiotic (achiral) conditions. If both enantiomers are stable, their BCF values are likely similar and amenable to read-across. If, however, stereoselective transformation in inherently chiral biota, e.g. biodegradation, metabolism, bioisomerization, takes place, their bioavailability will differ and consequently their BCF.

- **Diastereomers** have ≥ 2 chiral centres, i.e. ≥ 2 racemic mixtures, and differ in their physico-chemical properties. Therefore, it is likely that diastereomers have different BCF values. For read-across purposes they have to be regarded as different, not necessarily similar, substances.
- **cis-/trans-Isomers** are different substances with different physico-chemical properties and BCF values. Geometrical isomers are most likely dissimilar in terms of read-across.

The particularities of stereoisomers, i.e. differing 3-D geometries, are not covered by 2-D structural representations, e.g. SMILES. Because most QSAR-models and read-across operate with 2-D structures, they normally disregard differences between stereoisomers. Only if 3-D (dis)similarities are considered, can the modelling approaches be successful.

4.2 Functional similarity of (stereoisomeric) chemicals

Application of *in silico* tools, e.g. QSARs, read-across, is an alternative if experimental data are not available. However, estimated values can only be correct if appropriate models are used (Cronin et al. 2003a; Cronin et al. 2003b). Badly chosen models may deliver estimates that deviate from the true value by orders of magnitude. The key term in this context is similarity, particularly the concept of functional similarity. Most important criteria in model selection are the chemical domain (similarity of chemical structures and physico-chemical properties) (Jaworska et al. 2005; Netzeva et al. 2005) and the biological domain (functional similarity considering toxic mechanisms and modes of interaction with regard to rate-limiting processes) (Nendza and Müller, 2000; Nendza and Wenzel, 2006; Nendza and Müller, 2007b). Prior to application of *in silico* models, it is necessary to assess the similarity of the chemicals in question with regard to:

- similar chemical structures
- consistent physico-chemical properties
- diverse multiple, potentially specific, interactions with biotic (chiral) phases

Similarity (not only) of stereoisomers is context-dependent and with regard to bioaccumulation pathways it is particularly affected by processes in chiral biophases. For purposes of read-across of BCF-results, physico-chemical properties of stereoisomers in achiral (abiotic) phases are not necessarily identical, but can be regarded as sufficiently similar, e.g.:

- $\log K_{OW}$

- molecular size $D_{\max}$, D_{eff} , $D_{\min}$,
- dissociation

Significant differences in bioaccumulation between stereoisomers occur if selective reactions in chiral biophases take place, i.e. similarity is no longer substantiated. Among the possible processes,

- **stereoselective biotransformation, metabolism**

is by far the most relevant. Rates and extents of biotransformation (metabolism) can greatly differ between stereoisomers and if read-across is requested, it should be supported by experimental evidence for similar metabolic rates in fish, e.g. Cowan-Ellsberry et al. 2008. The relevance of functional dissimilarity with regard to biotransformation is illustrated with the example of indoxacarb isomers (Section 5.2), demonstrating that read-across of BCF-results between individual stereoisomers and different mixtures of stereoisomers is limited to those with similar metabolic rates.

Recommendation: If possible, multiple calculation results by different methods should be obtained, e.g. based on several independent $\log K_{\text{OW}}$ values for QSAR input, and read-across from a number of analogues (as to cover different aspects of structural and functional similarity in terms of lipophilicity, metabolic capacity, molecular size or dissociation) with similar BCF. After comparative analysis of the plausibility of the *in silico* results, the geometric mean of reliable estimates bears the best approximation to the true value.

4.3 Transferability of BCF-results between individual stereoisomers and different mixtures of stereoisomers

4.3.1 Stereoselective processes involved in bioaccumulation

The stereoselectivity of interactions between a substance and chiral biophases can cause differentiation of enantiomers with regard to rates of uptake and elimination, biodegradation, metabolism. If, due to such stereoselective processes, the bioavailability of enantiomers is modified to different extents, also their bioaccumulation must be different.

A review of current literature suggests two major stereoselective processes with potential effects on the extent of bioaccumulation of stereoisomers:

- **Biotransformation**

- **Bioisomerization**

Both processes result in elimination of the parent structure, thus reducing its bioavailability and apparent bioaccumulation, but formation of even more hazardous, here more accumulating, degradation products has to be considered and included in the respective assessment. Most studies start with racemic (equimolar) mixtures and evaluate enantiomer-specific transformation on the basis of enantiomeric ratios or enantiomer fractions (Hegeman and Laane, 2002):

An enantiomeric ratio (ER) is defined as the (+)-enantiomer concentration of a chiral compound divided by its (-)-enantiomer concentration (Vetter and Schurig, 1997):

$$\text{ER} = (+)\text{-enantiomer} / (-)\text{-enantiomer}$$

The enantiomeric ratio data is transformed into enantiomer fractions (EFs) as a standard descriptor (Harner et al. 2000). The EF can be calculated from ER by the formula:

$$\text{EF} = \text{ER} / (\text{ER} + 1)$$

Biotransformation: US EPA's OPP (2000) stated "*that biotic processes (e.g., soil and aquatic metabolism) may cause preferential degradation when compared to abiotic processes (e.g., hydrolysis and direct photolysis in water).*" Müller and Kohler (2004) confirmed that enantioselective degradation of chiral pollutants by (micro)organisms is rather the rule than the exception where transformation of the enantiomers may proceed along different avenues:

- Two enantioselective enzymes exist, each converting only one substrate enantiomer.
- Both enantiomers are simultaneously converted by one enzyme, but at different rates.
- Sequential conversion of the substrate enantiomers by one enzyme, i.e., the enzyme preferentially degrades one enantiomer. The other enantiomer is eventually also degraded, but only when the former one has been (completely) degraded.
- Enantioselective conversion of one enantiomer by one enzyme and isomerization of the other enantiomer by an isomerase.

Along this line, Hegeman and Laane (2002) reported minor deviations from the racemic composition (EF=0.5) for six chiral organochlorine pesticides (α -HCH, Mecoprop, *cis*-chlordane, *trans*-chlordane, oxychlordane, heptachlor *exo*-epoxide) in abiotic compartments

in the order: air < water < soil, while biotic compartments (whole organisms, tissues and organs) showed a higher deviation from EF=0.5, attributed to stereospecific metabolism and enzymatic transport processes. For the different compounds studied, lower trophic biota (i.e. mussels, cod, flounder) showed a smaller deviation from EF=0.5 than higher trophic organisms (i.e. seals, birds and terrestrial animals). High deviations from EF=0.5 were found for the different pesticides in specific organs in biota, for example in liver, kidney, brain tissue, and spinal marrow. For the biotic compartments a general trend in the deviation of EF=0.5 was found in the following sequence: lower trophic biota < higher trophic biota < liver/kidney < brain. This sequence was speculated to be the result of the combined effect of stereoselective degradation/metabolization, complexation, uptake and excretion within an organism or the organ of an organism.

Wiberg et al. (2000) made similar observations for α -HCH and chlordane related compounds in the polar bear food chain: While cod showed near racemic mixtures (ER=1), ERs in ringed seal and polar bear were frequently nonracemic (ER $\neq$ 1). These results are further supported by findings by Borga et al. (2007) that indicate higher accumulation of β -HCH as compared to α -HCH in arctic seabirds.

A study by Liu et al. (2004) on synthetic pyrethroids showed that the multiple stereoisomers of cypermethrin differ greatly in biological activity with only two (*1R-cis*-RS* and *1R-trans*-RS*) of the eight enantiomers being toxic to *Ceriodaphnia dubia*. The isomer selectivity in the measured aquatic toxicity was similar to that in insecticidal activity. The selectivity in biodegradation of the two biologically active enantiomers indicated that *1R-cis*-RS* was relatively persistent compared with the other stereoisomers, whereas *1R-trans*-RS* was likely the least persistent. The authors found that isomer selectivity may be compound and environment-specific, as selected bacteria preferentially degraded some diastereomers or enantiomers in both solution media and in sediment. Still, compared with the *cis* diastereomers, degradation of *trans* diastereomers was consistently more rapid, which resulted in relative enrichment of the *cis* diastereomers.

Significant differences in chronic toxicity and accumulation of the synthetic pyrethroid insecticide *cis*-bifenthrin have been observed in *Daphnia magna*, with the *1R-cis*-isomer being up to 80-fold more active than the *1S-cis*-isomer (Zhao et al. 2009). The differences were attributed to enantioselective uptake and biotransformation based on enantiomeric ratios measured during 4 days of exposure.

Against their expectations, Konwick et al. (2006a) observed no significant selectivity in biotransformation for most chiral triazole fungicides based on enantiomer fractions in rainbow trout compared to food values, likely due to very rapid transformation. In a second study, Konwick et al. (2006b) assessed the utility of using chiral analysis to provide insight into the dietary accumulation and enantioselective biotransformation of the chiral current use pesticide fipronil, along with a mixture of selected chiral [R-hexachlorocyclohexane (R-HCH), heptachlor epoxide (HEPX), polychlorinated biphenyls (PCBs) 84, 132, 174, o,p'-DDT, and

o,p'-DDD] and nonchiral (p,p'-DDT, p,p'-DDD) organochlorine compounds in juvenile rainbow trout (*Oncorhynchus mykiss*). Fish rapidly accumulated all compounds during the 32 d uptake phase, which was followed by varying elimination rates of the chemicals during the 96 d depuration period. The majority of the organochlorines examined showed no indication of enantiomerspecific biotransformation. Fipronil was rapidly biotransformed with rapid formation of fipronil sulfone, a known metabolite. Fipronil sulfone was found to persist longer ($t_{1/2} \sim 2$ d) than its parent compound fipronil ($t_{1/2} \sim 0.6$ d) and needs to be considered in fate studies of fipronil.

Tomy et al. (2008a) screened for a suite of metabolites of *syn*- and *anti*-isomers of Dechlorane Plus (DP) in the liver of juvenile rainbow trout exposed via diet, including dechlorinated, hydroxylated, methoxylated and methyl sulfone degradates. Even with the purposely high dose used in the uptake phase, none of these degradates could be detected in the extracts. This suggests that if metabolites of DP are detected in fish from aquatic food webs their presence is likely not from *in vivo* biotransformation of the parent compound. In a second study, Tomy et al. (2008b) examined the trophodynamics of seven brominated diphenyl ether (BDEs) and hexabromocyclododecane (HBCD) in a food web from the eastern Canadian Arctic. Both the BDE-47 and the R-isomer of HBCD showed a strong increase in concentration with increasing trophic level. Conversely, the BDE-209 showed a decrease in concentration with trophic level suggesting metabolic depletion of this congener or reduced assimilation up the food web. Interestingly, both enantiomers of R-HBCD increased with trophic level but the magnitude of the increase was slightly greater for the (-)R-enantiomer. Further, the EF values for the R-isomer showed an increase with trophic level implying that there is an overall preferential enrichment of the (-)R-enantiomer relative to the (+)R-enantiomer likely due to the greater bioaccumulation potential of the (-)R-enantiomer and/or to the greater susceptibility of the (+)R-enantiomer to metabolism.

From the literature sources evaluated, stereospecific transformation is evident in biotic phases with increasing trophic level, but relevant only to chemicals of intermediate transformation potential, while recalcitrant as well as rapidly degrading compounds appear to be concerned to a minor extent.

Bioisomerization: Isomer interconversion is frequently hypothesized, but experimental evidence in environmental biota is scarce. Only one study by Law et al. (2006) supports diastereomer interconversion during dietary accumulation of hexabromocyclododecane in juvenile rainbow trout. They found that fish exposed exclusively to the β -diastereomer showed statistically significant molar amounts of the α - and γ -diastereomer compared to the controls.

The combined expertise in the United Nations Environment Programme for the draft risk profile of Lindane (United Nations Environment Programme, 2006) concluded: "*The hypothesis that isomerization of γ -HCH to α -HCH in air emerged as a possible explanation for higher than expected α -HCH/ γ -HCH ratios in the Arctic. However no conclusive*

experimental evidence of isomerization taking place in air has been produced to date. Also, although there is evidence that bioisomerization of lindane can take place through biological degradation, it seems that this process may play an insignificant role in the overall degradation of γ -HCH.”

4.3.2 Illustrative example: Stereoselective bioaccumulation of indoxacarb isomer(mixture)s

The literature review has revealed ample evidence for stereoselective bioaccumulation and biotransformation (metabolism), but explicit data on individual isomers and their mixtures are scarce. The only example that could be retrieved concerns the R- and S-isomers of indoxacarb and their mixtures at two different ratios. According to the European Commission review report (European Commission, 2005), indoxacarb is used in four forms (Table 3).

Table 3: Measured BCF values for indoxacarb-isomer(mixture)s (European Commission, 2005).

Isomers	BCF values
IN-KN127 (insecticidally inactive R-isomer)	1848 l/kg
DPX-KN128 (pure insecticidally active S-isomer)	77.3 l/kg
DPX-JW062 (mixture of DPX-KN128 and IN-KN127 (1:1 ratio))	950.3 l/kg
DPX-MP062 (mixture of DPX-KN128 and IN-KN127 (3:1 ratio))	520 l/kg *

* estimated value (according to information from Umweltbundesamt)

To compare the BCF values of the indoxacarb-isomer(mixture)s relative to the TGD model eq. 74,

$$\log \text{BCF} = 0.85 \log K_{\text{OW}} - 0.70 \quad (\text{Veith et al. 1979})$$

$\log K_{\text{OW}}$ values were obtained from several sources (Table 4). It is noted that the available $\log K_{\text{OW}}$ values differ to some extent, but within 0.6 orders of magnitude. Keeping in mind that $\log K_{\text{OW}}$ is determined in achiral phases, no differentiation of isomers is made. Both $\log K_{\text{OW}}$ -programmes (SPARC, EPISuite) use as input 2-D structural representations, i.e. SMILES, and thus can produce only one $\log K_{\text{OW}}$ value for the two stereoisomers.

Table 4: Collected log K_{OW} values for indoxacarb (CAS 173584-44-6).

Source	log K_{OW} values
European Commission review report (European Commission, 2005)	4.60-4.65
SPARC	4.94
EPISuite	5.21

Figure 16 presents the relationship between the range of log K_{OW} values (Table 4) and log BCF for the indoxacarb-isomer(mixture)s (Table 3) relative to the TGD model eq. 74 (Veith et al. 1979). As the first observation, it is noted that the BCF value of the insecticidally inactive R-isomer IN-KN127 is well in agreement with the QSAR model, with a back-calculated log K_{OW} value of 4.67 for perfect fit. It can thus be assumed that the isomer IN-KN127 bioaccumulates by passive partitioning processes and it is not liable to metabolism in fish during the exposure period of bioaccumulation testing.

The second observation concerns the insecticidally active S-isomer DPX-KN128 which bioaccumulates to a much lesser extent than IN-KN127. It may be assumed that the isomer DPX-KN128 is metabolised and/or preferentially excreted during the exposure period of bioaccumulation testing, resulting in reduced bioaccumulation. Unfortunately, no isomer-specific metabolism data in fish are available.

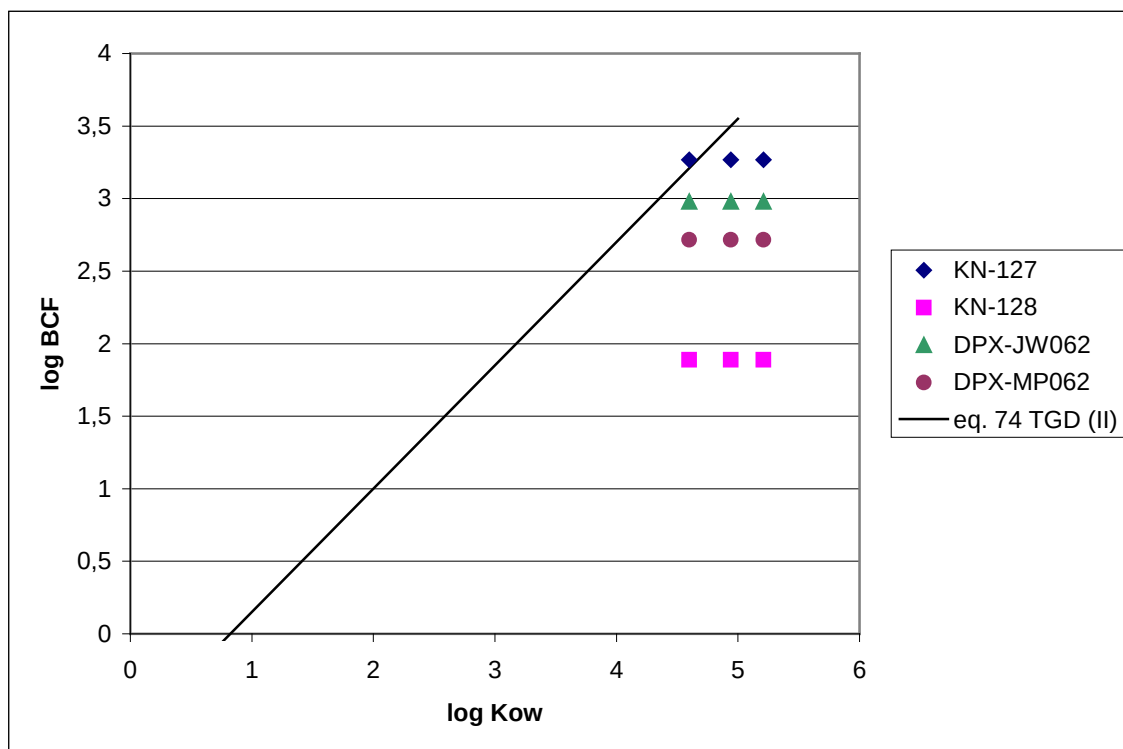


Figure 16: Relationship between the log K_{OW} values (Table 4) and log BCF for the indoxacarb-isomer(mixture)s (Table 3) relative to the TGD model eq. 74.

In support of the hypothesis that the insecticidally inactive R-isomer IN-KN127 is persistent at least for the time-course of bioaccumulation testing while the insecticidally active S-isomer DPX-KN128 is less bioavailable due to metabolism and/or preferential excretion, the mixture DPX-JW062 (mixture of DPX-KN128 and IN-KN127 (1:1 ratio)) shows a BCF value in-between those of the pure isomers (Figure 16). This evidence of additive bioaccumulation behaviour has been used to estimate the BCF value for DPX-MP062 (mixture of DPX-KN128 and IN-KN127 (3:1 ratio)) according to the fractional composition of the mixture. The lower fraction of the persistent isomer (IN-KN127) results in a lower BCF of the mixture (Table 5). The validity of this extrapolation implies no interaction between isomer mixture components in rate-limiting processes such that the BCFs of any mixtures of the two isomers can be calculated from the BCF values of the pure indoxacarb isomers and the respective molar fractions.

Table 5: Comparison of measured BCF values with estimated BCF values for indoxacarb-isomer mixtures from their fractional composition based on BCF values for DPX-KN128 (77.3 l/kg) and IN-KN127 (1848 l/kg).

	Estimated BCF values	Measured BCF values
DPX-JW062 (mixture of DPX-KN128 and IN-KN127 (1:1 ratio))	960 l/kg	950 l/kg
DPX-MP062 (mixture of DPX-KN128 and IN-KN127 (3:1 ratio))	520 l/kg	520 l/kg *

* estimated value (according to information from Umweltbundesamt)

The case of indoxacarb-isomer(mixture)s demonstrates that an analysis of functional similarity of substances with regard to bioaccumulation pathways is essential prior to transfer (read-across) of BCF-results. The significant differences in bioaccumulation between the two stereoisomers are likely due to **stereoselective biotransformation** (metabolism in fish) of the insecticidally active S-isomer DPX-KN128.

Conclusion: Transferability (read-across) of BCF-results between individual stereoisomers and different mixtures of stereoisomers is limited to those with similar metabolic rates (to be supported by (*in vitro*) experimental evidence).

Significant differences in bioaccumulation between stereoisomers occur if selective reactions in chiral biophases take place. Among the possible processes,

- **stereoselective biotransformation** (metabolism in fish)

is by far the most relevant. Rates and extents of biotransformation in fish can greatly differ between stereoisomers and it is recommended to include isomer-specific information about

biotransformation in bioaccumulation assessments. If read-across is requested, it should be supported by (experimental) evidence for similar metabolic rates in fish, e.g. Cowan-Ellsberry et al. (2008), Nichols et al. (2009).

5 Decision support on BCF testing requirements for (mixtures of) stereoisomers

Derogation of or request for experimental bioaccumulation studies for (mixtures of) stereoisomers may be supported by QSAR and read-across arguments, depending on three different conditions:

- Experimental data are available for mixtures of isomers;
- Experimental data are available for individual isomers;
- No experimental data are available.

Condition A: Experimental data are available for mixtures of isomers.

If data for mixtures of isomers are available and read-across is requested for an individual isomer, the following option may be considered:

A 1. Based on the assumption of additive bioaccumulation behaviour, a worst-case assessment of the BCF values of individual isomers is possible, provided that the fractional composition of the mixture is known:

Assume that all other isomers have $BCF = 0$ and divide the experimental values by the fraction of the isomer in question. The procedure is illustrated by two examples:

A 1.1: The 1:1 mixture of two isomers has a measured BCF value of 40. If an BCF value of zero is assumed for the other isomer, the BCF value of the isomer in question cannot exceed 80.

A 1.2: The equimolar mixture of four isomers has a measured BCF value of 500. If an BCF value of zero is assumed for the three other isomers, the BCF value of the isomer in question cannot exceed 2000.

Condition B: Experimental data are available for individual isomers.

If data for an individual isomer other than the one in question are available, two considerations with regard to read-across are recommended:

1. If the available experimental value corresponds to the worst-case QSAR estimate (eq. 4 of section 2.4) for the compound in question, read-across is acceptable. In the very unlikely

case of significant differences between the log K_{OW} values of the isomers, correction for different lipophilicity shall be applied.

2. If the available experimental value is less than the worst-case QSAR estimate (eq. 4 of section 2.4) for the compound in question, the mitigating factor shall be identified, e.g. metabolism in fish. In the next step, similarity between the isomers with regard to this property shall be substantiated, e.g. by experimental (*in vitro*) transformation data in fish (Cowan-Ellsberry et al. (2008), Nichols et al. (2009)). If, in this example, the relevant metabolic rates in fish are sufficiently similar, read-across may be acceptable.

Condition C: No experimental data are available.

Precautionary estimates may be obtained with the bilinear worst-case QSAR model (eq. 4 of section 2.2.1), where calculated values are frequently $\geq$ the actual BCF values.

The following limitations apply to any of the three conditions above:

- Any QSAR application requires explicit consideration of optimum prediction space (applicability domain);
- Any read-across requires (experimental) demonstration of sufficient similarity with regard to the relevant (rate-limiting) processes, e.g. lipophilicity, metabolic capacity, molecular size, dissociation;
- If evidence for low bioaccumulation is insufficient, an experimental study shall be requested.

6 Open questions and further research needs

The present study has reviewed the currently available methods and tools for estimating the BCF of pesticides and biocides with a special focus on the differential stereoselective accumulation of (mixtures of) stereoisomers.

For the specific application to (stereoisomeric) pesticides and biocides, comparative analyses have delivered several well established procedures, but also identified some open questions and further research needs:

1. Inherent uncertainties in $\log K_{OW}$ and $\log BCF$ estimates may produce propagated errors > 1 log unit. Acceptable ranges of errors relative to BCF thresholds need to be defined in the regulatory context for pesticides and biocides. As a starting point, multiple BCF values for representative compounds shall be evaluated with regard to comparable fish species, exposure regime, lipid normalisation, analytical method, etc. Across the regulatory relevant BCF scale (< 100 to > 5000), the acceptable variability of study results shall be differentiated for each of the thresholds.
2. Existing QSARs may be revisited to provide most accurate predictions in the range of BCF trigger values in pesticides and biocides regulation (e.g. $BCF = 100$ (if not readily degradable), 1000 (if readily degradable), 2000 (B compounds) or 5000 (vB compounds)).
3. Measures for different aspects of functional similarities with regard to bioaccumulation (e.g. lipophilicity, metabolic capacity, molecular size, dissociation) may require experimental (*in vitro*) testing in order to (dis)approve read-across.
4. The implicit consideration of metabolites via total radioactivity requires differential adaptation of data quality considerations and calls for particular QSAR and read-across applications.
5. The role of stereoselective uptake and elimination kinetics has been hypothesised, but systematic studies are absent. Here is a clear research need to fill this gap. A useful tool for this purpose may become PAMPA (Parallel Artificial Membrane Permeability Assays, Avdeef (2003)), a measure of uptake and elimination, i.e. bioavailability, and possibly paired with *in vitro* metabolism assays for extended ADME coverage (Nichols et al. (2009)).

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