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

Project-No. FKZ 3711 63 405 2
UBA-FB-00 [trägt die UBA-Bibliothek ein]

Weiterentwicklung der Kriterien zur Bioakkumulation unter REACH

Advancement of Bioaccumulation Criteria under REACH

by

Prof. Dr. Christian Schlechtriem, Dipl.-Biol. Rebecca Bebon
Fraunhofer IME, Schmallenberg

Prof. Dr. Gerrit Schüürmann, Dr. Ralph Kühne
UFZ, Leipzig

Dr. Thomas Preuß
RWTH, Aachen

Fraunhofer Institute for Molecular Biology and Applied Ecology (IME)
Auf dem Aberg 1
57392 Schmallenberg, Germany

Department Ecological Chemistry
Helmholtz Center for Environmental Research (UFZ)
Permoserstraße 15
04318 Leipzig, Germany

Institute for Environmental Research (Biology V)
RWTH Aachen University
Worringerweg 1
52074 Aachen

On behalf of the Federal Environment Agency

June 2015

Kurzbeschreibung

Ziel dieses Projekts war (1) die Entwicklung eines Modells, das den Vergleich von Fischbiomagnifikationsstudien, die gemäß der Testvorschrift OECD 305 durchgeführt wurden, mit REACH-Bioakkumulationskriterien erlaubt und (2) Möglichkeiten zur Integration organspezifischer Akkumulation von Substanzen in die PBT-Bewertung im Rahmen von REACH zu untersuchen.

Im ersten Arbeitspaket wurden zwei Datensätze, mit kinetischen BCF- und BMF-Werten, mithilfe einer Literaturrecherche zusammengestellt und überprüft. Zusätzlich stand ein bereits überprüfter Datensatz mit steady-state BCF-Werten zur Verfügung. Die kinetischen BCF-Werte wurden untersucht. Substanzklassen mit großen Abweichungen zwischen steady-state und kinetischen BCF wurden identifiziert. Dabei handelte es sich hauptsächlich um Thiophosphate und mehrfach halogenierte ungesättigte Kohlenwasserstoffe. Bereits existierende Modelle zur Ableitung von k_1 und k_2-Raten wurden angewendet und mit den experimentellen Daten verglichen. Obwohl in manchen Fällen Tendenzen zur Übereinstimmung der Daten erkennbar waren, konnte kein Modell als ausreichend bezeichnet werden. Die limitierte Anzahl an experimentellen Daten verhinderte die Ableitung eines neuen Modells. Die Untersuchung des BMF-Datensatzes zeigte zudem ein Ungleichgewicht zugunsten der halogenierten Kohlenwasserstoffe. Als Basis für die weitere Modellentwicklung sind weitere Studien unumgänglich. Der bestehende Datensatzes sollte um Substanzklassen mit Heteroatomen wie N, S und P erweitert werden.

Das zweite Arbeitspaket zeigte, dass PBTK-Modelle effektive Hilfsmittel für die erfolgreiche Vorhersage der Biokonzentration und kinetischer Geschwindigkeitskonstanten vieler organischer Substanzen in der Regenbogenforelle sind. Die besten prädiktiven Ergebnisse wurden für die Bioakkumulation moderat lipophiler Substanzen im Gesamtkörper sowie der Leber erzielt. Dagegen führten Simulationen im Blut im Allgemeinen zu einer Unter- oder Überschätzung der gemessenen Daten. Trotz der Annahme organspezifischer Akkumulation, konnte die Biokonzentration der in dieser Studie untersuchten PFAS verhältnismäßig gut vorhergesagt werden, was auf eine lipidgesteuerte Verteilung der meisten PFAS in der Leber und im Körper hindeutet. Zur weiteren Optimierung des eingesetzten PBTK-Modells sollte die Proteinsorption im Vordergrund stehen, wodurch die Vorhersagekraft des Modells bezüglich der Anreicherung im Blut erhöht würde.

Abstract

The aim of this project was to (1) derive a model that allows the comparison of results from fish biomagnification studies carried out according to the OECD 305 test guideline with the REACH bioaccumulation criteria and (2) to propose a way to integrate organ specific accumulation of substances in the PBT assessment under REACH.

In the first workpackage, two data sets have been established and validated from literature search, one for kinetic BCF together with the individual uptake and elimination rates, and another one for BMF together with individual elimination rates. Additionally, an already validated data set of steady-state BCF values was available. First, the collected kinetic BCF estimates were examined. Compound classes with large deviations between steady-state and kinetic BCFs were identified, which mainly represented thiophosphates and halogenated unsaturated hydrocarbons. Existing models for k_1 and k_2 were applied and compared to the experimental data. Even though in some cases at least trends were visible, none of them can be assumed to be sufficient yet. The limited number of experimental data did not allow for deriving a new model. Inspection of the BMF set revealed a serious imbalance toward halogenated hydrocarbons. Further studies, which are unavoidable with regard to the development of a sufficient data set for modeling, should focus on compounds classes containing heteroatoms as N, S, and P.

The second workpackage demonstrated that PBTK models are an effective tool for successful predictions of bioconcentration and kinetic rate constants of a wide range of organic compounds in rainbow trout. The highest predictive power was observed for simulations of moderately lipophilic compounds in whole body followed by liver whereas simulations in blood generally led to over- or underestimations of measured data. Despite assumptions of organ-specific accumulation, bioconcentration of investigated PFAS was reasonably well estimated, indicating a lipid triggered distribution of most PFAS in liver and whole body. In order to achieve further optimization of the PBTK model, particular emphasize should be placed on protein sorption, which would serve to increase the predictive power regarding blood simulations.

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AE	assimilation efficiency
BAF	bioaccumulation factor
BCF	bioconcentration factor
BCF_k	kinetic bioconcentration factor
BCF_{ss}	steady-state bioconcentration factor
BMF	biomagnification factor
CAS	chemical abstracts service
C_f	concentration of test substance in fish
CMR	carcinogenic, mutagenic or toxic for reproduction
C_w	concentration of test substance in water
DF	distribution factor
DSS	dioctyl sodium sulfosuccinate
EFSA	european food safety authority
EINECS	european inventory of existing commercial chemical substances
EU	european union
FABP	fatty acid binding protein
GI	gastrointestinal
GLP	good laboratory practice
HSA	human serum albumin
k_1	uptake rate constant
k_2	depuration rate constant
k_{2g}	growth-corrected depuration rate constant
K_M	metabolic transformation rate
K_{mw}	membrane/water partition coefficient
K_{ow}	octan-1-ol/water partition coefficient
K_{PW}	protein/water partition coefficient
LB	lipid content
LSER	linear solvation enthalpy relationship
OECD	organisation for economic co-operation and development
PBT	persistent, bioaccumulative and toxic
vPvB	very persistent and very bioaccumulative
PBTK-Model	physiology-based toxicokinetic model
PFAS	polyfluorinated alkyl substances
PFBS	perfluorobutanesulfonic acid

PFDA	perfluorodecanoic acid
PFDoA	perfluorododecanoic acid
PFHxS	perfluorohexane sulfonate
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctanesulfonic acid
PFTA	perfluorotetradecanoic acid
PFUnA	perfluoroundecanoic acid
REACH	registration, evaluation, authorisation and restriction of chemicals
SVHC	substance of very high concern
TG	test guideline
TGD	technical guidance document
TRR	transthyretin
UBA	federal ministry for the environment, nature conservation and nuclear safety
US EPA	united states environmental protection agency
UVCB	substances of unknown or variable composition, complex reaction products or biological materials
W	weight

Zusammenfassung

Das europäische Chemikalienrecht stellt durch die Verordnung zur Registrierung, Bewertung, Zulassung und Beschränkung chemischer Stoffe (REACH-Verordnung) das Vorsorgeprinzip für Mensch und Umwelt hinsichtlich besonders besorgniserregender Stoffe (substances of very high concern, SVHC) stärker in den Vordergrund. Stoffe mit (sehr) persistenten, (sehr) bioakkkumulierenden und toxischen Eigenschaften (PBT- und vPvB-Stoffe), karzinogen, mutagen und reproduktionstoxisch wirkende Substanzen (CMR-Stoffe) sowie Chemikalien, die in Einzelfallunterscheidungen als besonders besorgniserregend festgestellt werden (z.B. Stoffe mit endokriner Wirkung), können einem Zulassungsverfahren unterworfen werden.

In der REACH-Verordnung sind die Kriterien für die Identifizierung von PBT/vPvB Stoffen festgelegt. Stoffe, für die experimentell ein Biokonzentrationsfaktor (BCF) > 2000 ermittelt wird, sind Kandidaten für Stoffe mit PBT-Eigenschaften; Stoffe mit einem BCF > 5000 sind Kandidaten für vPvB-Stoffe (die P und ggf. T-Eigen-schaften müssen ebenfalls zutreffen). Biokonzentrationsfaktoren werden hauptsächlich in Durchflussstudien gemäß der OECD 305-Richtlinie durchgeführt (OECD 305, 2012). Durch den aquatischen Standardtest mit Fischen werden bislang die vielfältigen Aufnahme- und Eliminationsmechanismen in aquatischen Ökosystemen auf die respiratorische Absorption über die Kiemen und die Diffusion durch die Haut verkürzt.

Insbesondere für Chemikalien hoher Lipophilität (log k_{ow} >5) stellt die Durchführung von Biokonzentrationsstudien häufig ein Problem dar. Die schlechte Wasserlöslichkeit lipophiler Substanzen beeinträchtigt die Einstellung stabiler Testkonzentrationen und kann unter bestimmten Bedingungen zu unpräzisen Messungen der Testsubstanz im Medium führen. Zudem reichern sich Chemikalien in der Umwelt mit steigender Lipophilität verstärkt über die Nahrungskette an, so dass den Biomagnifikationsprozessen eine höhere Beachtung geschenkt werden müsste. Für Chemikalien mit schlechter Wasserlöslichkeit wird daher zukünftig ein alternatives Testdesign zur Durchführung von Bioakkumulationsstudien auf Basis von Fütterungsexperimenten zur Wahl stehen. Ziel dieser Studien ist die Bestimmung eines Biomagnifikationsfaktors (BMF). Gemäß der revidierten Richtlinie OECD 305 werden somit zukünftig zwei unterschiedliche Bioakkumulationsfaktoren in die Stoffbewertung eingehen. Die Berechnung des BMF erfolgt anhand der Geschwindigkeitskonstanten k_2 (BMF), welche analog zur BCF-Studie ermittelt wird, sowie der täglichen Fütterungsrate und der Assimilationseffizienz (α). Die so erhaltenen BMF-Werte sind allerdings nicht mit herkömmlichen BCF-Werten vergleichbar. Die Nutzung von BMF-Werten für die Identifizierung von PBT-Stoffen wird dadurch erschwert, dass entsprechende BMF-Schwellenwerte im Anhang XIII der REACH-Verordnung bislang fehlen. Die Ableitung von BCF-Werten aus Daten, die im Rahmen von Fischfütterungsstudien erhoben wurden, würde einen großen Vorteil darstellen und den regulatorischen Nutzen dieser Studien erheblich erhöhen. Für die Bestimmung eines kinetischen BCF-Werts wird jedoch neben der verfügbaren Eliminationsrate k_2 auch eine entsprechende Aufnahmekonstante k_1 benötigt, die jedoch in Fütterungsstudien nicht bestimmbar ist.

Zahlreiche Vorschläge und Modelle wurden entwickelt, um eine Abschätzung von k_1 zu erzielen. So wurde beispielsweise eine mathematische Beziehung zwischen der Fischgröße (Gewicht) und der Aufnahmekonstante für die Aufnahme hydrophober Substanzen über die Kiemen beschrieben (Sijm et al. 1993, 1994 and 1995). Andere Methoden beschreiben die Ableitung von BCF-Werten aus Depurationsdaten, die in Fütterungsstudien bestimmt wurden. Als Beispiel dafür sind Fugazitätsmodelle (Campfens and Mackay, 1997) oder Massenbilanzmodelle auf Basis von Octanol/Wasser-Verteilungskoeffizienten (Arnot and Gobas, 2003) zu nennen. Im Rahmen einer umfassenden Studie (Brooke et al. 2012) wurden die wesentlichen verfügbaren Modelle zur Bestimmung von Aufnahme-konstanten bewertet. Die Autoren kamen zu der Schlussfolgerung, dass die mit den getesteten Methoden erzielten Ergebnisse recht ungenau und somit nur bedingt geeignet für die Ableitung von BCF-Werten aus Fütterungsdaten sind. Die Ableitung eines Modells, welches den Vergleich von Ergebnissen aus Fischfütterungsstudien mit den REACH-Bioakkumulationskriterien erlaubt, wäre von hohem Wert.

Das Prinzip der durch Lipophilität verursachten Bioakkumulation ist für die Mehrheit der neutralen organischen Substanzen gültig. Es sind diverse Substanzgruppen bekannt, deren Bioakkumulationsverhalten jedoch nicht durch Lipophilität bestimmt wird. Poly- und perfluorierten Carbonsäuren und Siliconöle beispielsweise zeigen eine gleichermaßen repellente Wirkung gegenüber Wasser sowie nicht-polaren Lösungsmitteln. Amphiphile Substanzen wie oberflächenaktive Substanzen sind jedoch durch ihre Affinität für wässrige und organische Phasen gekennzeichnet und akkumulieren somit vorwiegend in Grenzschichten. Die Verwendung des log K_{ow} ist in diesem Fall für die Vorhersage des Bioakkumulationspotentials ungeeignet. Stattdessen können spezifische Interaktionen z.B. mit Proteinen einen wesentlichen Einfluss auf die Akkumulation dieser Substanzen haben. Ein Ansatz die Substanz/Protein-Interaktion auf Basis von Protein/Wasser Verteilungskoeffizienten (log K_{PW}) zu beschreiben wurde von Kelly et al. (2009) vorgeschlagen. Als Folge ihres ungewöhnlichen Verteilungsverhaltens bilden lipidhaltige Körperflüssigkeiten bzw. Gewebe nicht das bevorzugte Zielkompartiment dieser Substanzen. Wenn sich Substanzen in spezifischen Zielorganen verstärkt anreichern, kann dies jedoch zur Bildung erhöhter Konzentrationen führen, die zu toxischen Effekten in diesem Organ führen können. Die Bewertung von Chemikalien auf Basis der alleinigen Berücksichtigung der Lipophilie kann somit zu einem unvollständigen Bild bezüglich des Bioakkumulationsverhaltens von Stoffen in Mensch und Umwelt führen. Der Beitrag spezifischer, nicht-lipidbasierter Prozesse zur Bioakkumulation in aquatischen Lebewesen sollte daher in die Stoffbewertung einfließen.

In der Toxikologie und Pharmakologie haben sich computerbasierte Modellierungen zu einem wichtigen Hilfsmittel für die erfolgreiche Quantifizierung und Extrapolation der Toxikokinetik in Organismen entwickelt (Barton et al. 2007; Bois et al. 2011; Jager et al. 2011). Computermodelle bieten nicht nur eine zeit- und kosteneffiziente Simulation der chemischen Absorption, Distribution und Elimination im zeitlichen Verlauf, sondern bringen zusätzlich ethische Vorteile durch die Reduktion und Optimierung in der Verwendung von Tieren (Badyal et al. 2009; Stadnicka et al. 2012).

Allgemein können zwei toxikokinetische Modellgruppen unterschieden werden; das Einkompartimentenmodell, welches eine homogene Verteilung einer Chemikalie im Lebewesen annimmt und das Multikompartimentenmodell, in welchem die Akkumulation in spezifischen Organen und Geweben berücksichtigt wird (Stadnicka et al. 2012). Ein Beispiel für Letzteres ist das „Physiologically Based Toxicokinetic“ (PBTK) Modell, welches für Fische von Nichols und seinen Mitarbeitern entwickelt wurde (Nichols et al. 1990). Während Einkompartimentenmodelle auf simpler Kurvenanpassung basieren, ist das PBTK-Modell wesentlich detaillierter und komplexer. Es beschreibt einen Organismus unter Verwendung von mehreren anatomischen, physiologischen und biochemischen Komponenten in denen Organe und Gewebe als individuelle Kompartimente abgegrenzt sind (Abbas and Hayton 1997; Nichols et al. 1990; Nichols et al. 1998). Da spezies- und entwicklungsspezifische Parameter zusammen mit physikochemischen Daten der Substanz in die Modellstruktur eingefügt werden, ermöglicht das PBTK-Modell die Extrapolation zwischen verschiedenen Spezies und Expositionsregimes (Barron et al. 1990; Nichols et al. 1998; Schmitt 2008). Desweiteren werden im PBTK-Modell die toxikokinetischen Aspekte einer Chemikalie durch bestimmte Aufnahmepfade (z.B. branchial, dermal, oral oder injektiv) beschrieben, gefolgt von der Verteilung über den Kreislauf in die individuellen Kompartimente.

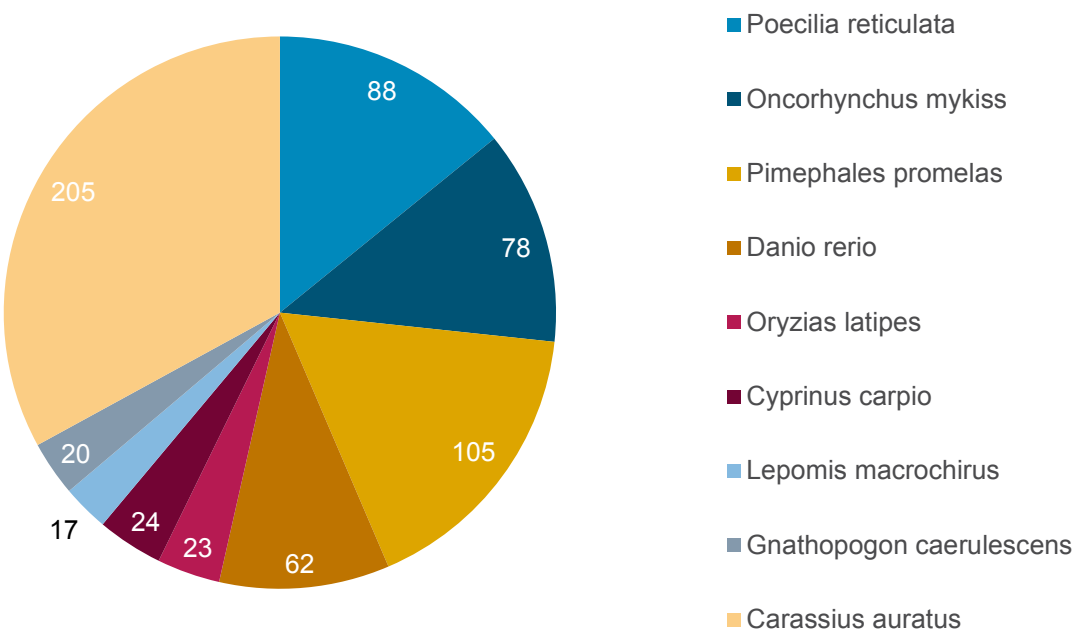
Das Prinzip der lipid-basierten Bioakkumulation trifft auf die meisten neutralen organischen Substanzen zu, wobei das Bioakkumulationsverhalten bestimmter Chemikaliengruppen basierend auf ihrem lipophilen Verhalten nicht entsprechend ermittelt werden kann. Poly- und perfluorierte Chemikalien sind für ihre amphiphilen Eigenschaften und für ihre Tendenz, sich bevorzugt an Luft/Flüssig-Grenzschichten als im Fettgewebe anzureichern, bekannt (EFSA 2008). Das Bioakkumulationsverhalten dieser Substanzen wird durch spezifische Interaktionen wie der Proteinbindung stark beeinflusst (EFSA 2008). Dies führt zu einer organspezifischen Akkumulation in Leber, Niere und Blut, was experimentell durch z.B. Goeritz et al. (2013) und Martin et al. (2003) gezeigt wurde. Folglich führt die Bewertung unter alleiniger Berücksichtigung der Lipophilität für PFAS zu einer Unterschätzung der Akkumulationsvorhersage in Umwelt und Mensch. Die dieser Studie zugrundeliegende Annahme ist, dass das PBTK-Modell einen guten Vertreter für die Biokonzentration und Bioakkumulation in Fischen darstellt und eine korrekte Vorhersage der Organverteilung erlaubt. Da die

chemische Verteilung im genutzten PBTK-Modell alleinig auf der Lipophilität basiert, sollte eine nicht-lipid-basierte Verteilung im Vergleich zum PBTK-Modell unterschiedliche Organverteilungsmuster zeigen. Diese Verteilungsmuster führen zu Biokonzentrationsfaktoren und Geschwindigkeitskonstanten, die von den nach OECD 305 gemessenen Größen abweichen.

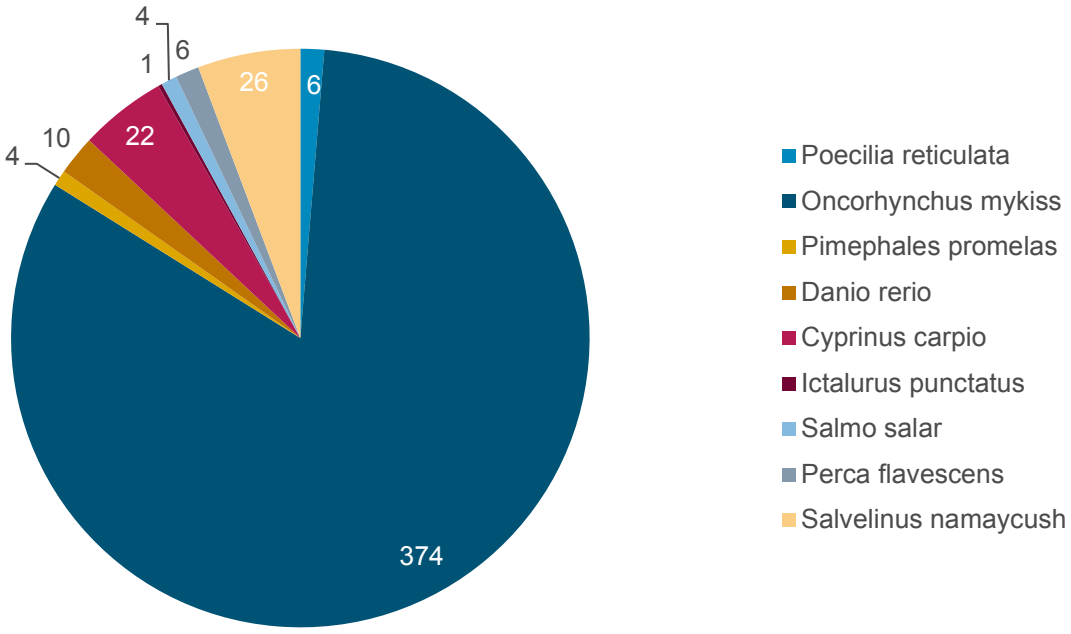
Daher ist es gefordert, die Durchführbarkeit eines PBTK-Modells zur Vorhersage lipid-gesteuerter Biokonzentration in Regenbogenforellen (*Oncorhynchus mykiss*) hervorzuheben und sein Potential spezifische Akkumulationsmuster, die nicht im Einklang mit der Lipophilität stehen, zu verdeutlichen. Für diesen Zweck muss die Leistungsfähigkeit des Modells mit Daten von kinetischen Raten und Biokonzentrationsfaktoren aus der Literatur und experimentell ermittelten Datern aus stoffregulatorischen OECD 305-Studien validiert werden.

Das Ziel des **ersten Arbeitspakets** war die Überprüfung mathematischer Korrelationen von kinetischen BCF und ihren jeweiligen k_2(BCF) für Substanzen, die aus der Literatur zusammengetragen wurden. Dasselbe Vorgehen fand bei BMF und ihrer jeweiligen k_2(BMF)-Werte Anwendung. Es wurde eine Literaturrecherche von Bioakkumulationsdaten (Biokonzentration und Biomagnifikation) im Fisch durchgeführt. Dabei wurden ausschließlich kinetisch ermittelte Biokonzentrations- und Biomagnifikationsfaktoren berücksichtigt. Die Suche beschränkte sich auf organische Testsubstanzen und Laborstudien.

Anzahl der Einträge pro Fischart im Biokonzentrationsdatensatz



Anzahl der Einträge pro Fischart im Biomagnifikationsdatensatz



Der Evaluierungsprozess war in zwei Teile getrennt. Im ersten Teil wurden alle Bioakkumulationsstudien analysiert und nach folgenden Kriterien evalutiert – Akkumulation im ganzen Fisch (nicht in spezifischen Geweben), nur gemessene Daten, Süßwasserfische (keine marinen Arten) und Alter der Testtiere. Für die Biomagnifikation wurden das Futter und die Fütterungmethode zusätzlich betrachtet. Zudem stellte das UBA kinetische Daten zu 42 weiteren, gemäß der OECD 305-Richtlinie durchgeführten BCF Studien, zur Verfügung. Diese Daten wurden zur Berechnung der Aufnahme- und Depurationsrate sowie der kinetischen und steady-state BCF für jede der 42 Substanzen genutzt.

Im zweiten Teil wurde die Datenqualität der Ergebnisse jeder Referenz mit der Zuordnung von Klimischfaktoren bewertet. Idealerweise sollten die Publikationen/Studien der OECD 305 Richtlinie folgen (OECD 305, 2012) und eine hohe Zuverlässigkeit gemäß Klimischfaktor 1 oder 2 aufweisen. Alle Publikationen wurden hinsichtlich der Validitätskriterien der OECD 305 evaluiert, welche für die Ermittlung der Klimischfaktoren genutzt wurden. Eine Zusammenfassung jeder Publikation und der jeweiligen Klimischfaktoren steht zur Verfügung. Daten, die mit Klimischfaktor 3 als unzuverlässig bewertet wurden, wurden aus dem Datensatz gelöscht, was zu einem bereinigten Datensatz mit ausschliesslich zuverlässigen Daten führte.

Experimentelle Daten aus der Literatur und vom UBA wurden eindeutigen organischen chemischen Strukturen zugeordnet. Die Daten waren bezüglich der unterschiedlichen chemischen Strukturen nicht gleichmäßig verteilt, weshalb eine Unterscheidung zwischen dem Rohdatensatz mit allen zusammengestellten Daten und dem kondensierten Datensatz mit einem Wert für die einzelnen Substanzen unterschieden werden musste.

Unstimmigkeiten zwischen berichteten BCF-Werten und dem Quotienten aus k_1 und k_2 innerhalb derselben Studie wurden untersucht. Falls Zweifel nicht beseitigt werden konnten, wurden die entsprechenden k_1 und k_2-Werte als invalide betrachtet, es sei denn die Werte konnten durch andere Studien mit derselben Substanz bestätigt werden. Desweiteren wurde beim Vorliegen von mehr als einem kompletten Eintrag (d.h. beiden k_1 und k_2 aus derselben Studie) die Spanne der BCFs geprüft. Unterschiede von mehr als zwei Größenordnungen wurden als unzuverlässig gewertet und die Einträge mit der höchsten Fehlerwahrscheinlichkeit in diesem Fall gelöscht. Falls erforderlich, wurde ein weiterer Validierungsschritt angewandt, indem die Ergebnisse des horizontalen (BCFs gemittelt) und vertikalen (k1 und k2 separat gemittelt und dann BCF berechnet) Mittelns

verglichen wurden. Hierbei wurden Unterschiede um eine Größenordnung geprüft und durch das Entfernen der zweifelhaftesten Einträge kuriert.

Das chemische Spektrum des Datensatzes wurde hinsichtlich der chemischen Elemente, Komplexität und Polarität untersucht. Diese Analysen wurden exklusiv, d.h. unter Zuordnung jeder Substanz zu exakt einer Gruppe durchgeführt. Im Falle von Widersprüchen wurde die Chemikalie, falls nicht anders erwähnt, der komplexeren Gruppe zugeordnet.

Falls verfügbar wurden steady-state BCFs mit den kinetisch ermittelten Werten verglichen. Entsprechende Daten wurden dem OSIRIS-Datensatz entnommen (OSIRIS 2007-2011), welcher innerhalb der ChemProp-Datenbank (UFZ 2014) öffentlich zugänglich ist. Für die Untersuchung des Einflusses der Lipidkorrektur wurden die berechneten BCF-Werte auf einen Lipidgehalt von 5% normalisiert.

Die Abhängigkeit des BCF von k_2(BCF) wurde untersucht. Wie erwartet nimmt k_2 mit steigendem BCF ab. Der Transport von Chemikalien zwischen Wasser und Fisch kann durch Hinzufügen von Resistenzen für wässrige Diffusion und Membranpermeation modelliert werden (Gobas et al. 1986). Einer dieser beiden Prozesse dominiert abhängig von der Chemikalie, wodurch die Gesamtrate entweder membrankontrolliert oder diffusionskontrolliert ist. Hydrophobie (modelliert durch K_{ow}) ist der entscheidende Faktor, der das Verhalten bestimmt. Membranpermeation wird bei niedriger Hydrophobie erwartet. Diffusionsschichtkontrolle wird bei hoher Hydrophobie erwartet. Bei der Elimination sollte k_2 mit dem K_{ow} bei niedriger Hydrophobie steigen. Es konnte jedoch nur ein schwacher Zusammenhang beobachtet werden. Bei hoher Hydrophobie wird erwartet, dass k_2 invers vom K_{ow} abhängt. Dieser Zusammenhang wird durch die Ergebnisse dieser Studie im Ansatz bestätigt.

Um die kinetischen BCFs mit den modellierten steady-state Werten zu vergleichen, wurden mehrere verschiedene Modellansätze mit unterschiedlicher Komplexität angewandt. Zusätzlich wurden mehrere K_{ow}-Modelle verglichen, die jedoch zu keinen signifikant unterschiedlichen Resultaten führten. Zuletzt wurde das ChemProp-Konsensus-Modell (UFZ 2014) angewandt, sofern experimentelle Daten vorhanden waren. Für die Metabolismusrate (k_M) wurde die ChemProp-Anwendung von Arnot et al. (2009) genutzt. Die Schätzung des BCF wurde mit und ohne k_M ermittelt.

Die Beziehungen der Aufnahmeraten (k_1) zum BCF und zur Hydrophobie wurden untersucht. Um zwischen Membranpermeationskontrolle und Diffusionskontrolle unterscheiden zu können, wurden die jeweiligen Hydrophobiebereiche separat inspiziert. Der Oktanol/Wasser-Koeffizient wurde als Hydrophobiekriterion angewendet. Bei der Aufnahme sollte k1vom Kow abhängig sein im Falle von Membrankontrolle bei niedriger Hydrophobie. Im Gegensatz dazu resultieren hohe Hydrophobie und somit Diffusionskontrolle theoretisch in einer Ratenunabhängigkeit vom Kow. Jedoch kann k1 sogar mit steigendem Kow sinken, da Diffusionskoeffizienten für einen Abfall bei steigender Hydrophobie bekannt sind.

Die Kalkulation von k_1 wurde kürzlich ausgiebig geprüft (Brooke et al. 2012; Brooke & Crookes 2007). Aus einer großen Anzahl an berichteten Möglichkeiten, hat der Autor ein paar wenige Schlüsseltechniken, basierend auf Gewicht (W), K_{ow} oder beidem, extrahiert. Diese Modelle fanden im aktuellen Datensatz Anwendung.

Zusätzlich wurden alternative Ansätze getestet. Der K_{ow} wurde durch den Membran/Wasser-Koeffizienten k_{mw} ersetzt, welcher durch ein Schätzungsmodell, das in der hausinternen Version von ChemProp (bisher nicht öffentlich zugänglich) anwendbar ist. Alternativ wurde die Verteilung zwischen Wasser und Protein verwendet, die als Bindung zum humanen Serum Albumin-nach Valko et al. 2003 aus einer LSER-Gleichung berechnet wurde.

Genauso wie für k_1, wurden die Eliminationskonstanten k_2 mit dem BCF und der Hydrophobie anhand entsprechender Graphen verglichen. Desweiteren wurden Aufnahme- und Eliminationskonstanten gegeneinander aufgetragen.

Für die Abschätzung von k_2 stehen verschiedene Modelle, die auch die Biotransformation (d.h. metabolische Elimination) beinhalten, zur Verfügung. Um deren Einfluss zu untersuchen, wurde die Gleichung mit und ohne Berücksichtigung der Biotransformation angewendet. Für die Abschätzung der Biotransformationsraten wurde das Modell von Arnot et al. 2009 verwendet.

Die chemische Untersuchung der Substanzen, zu denen BMF-Daten vorliegen, wurde ausgeführt. Die chemischen Eigenschaften der untersuchten Stoffe wurden mit den BMF-Daten verglichen. Zu Beginn wurde dafür das chemische Spektrum des Datensatzes hinsichtlich der chemischen Elemente, Komplexität und Polarität untersucht. Durch grafische Darstellung gefolgt von visueller Betrachtung wurde, falls möglich, die Beziehung der experimentell ermittelten Eliminationsraten k_2(BMF) zum BMF und den aus kinetischen BCF-Ermittlungen stammenden k_2 untersucht.

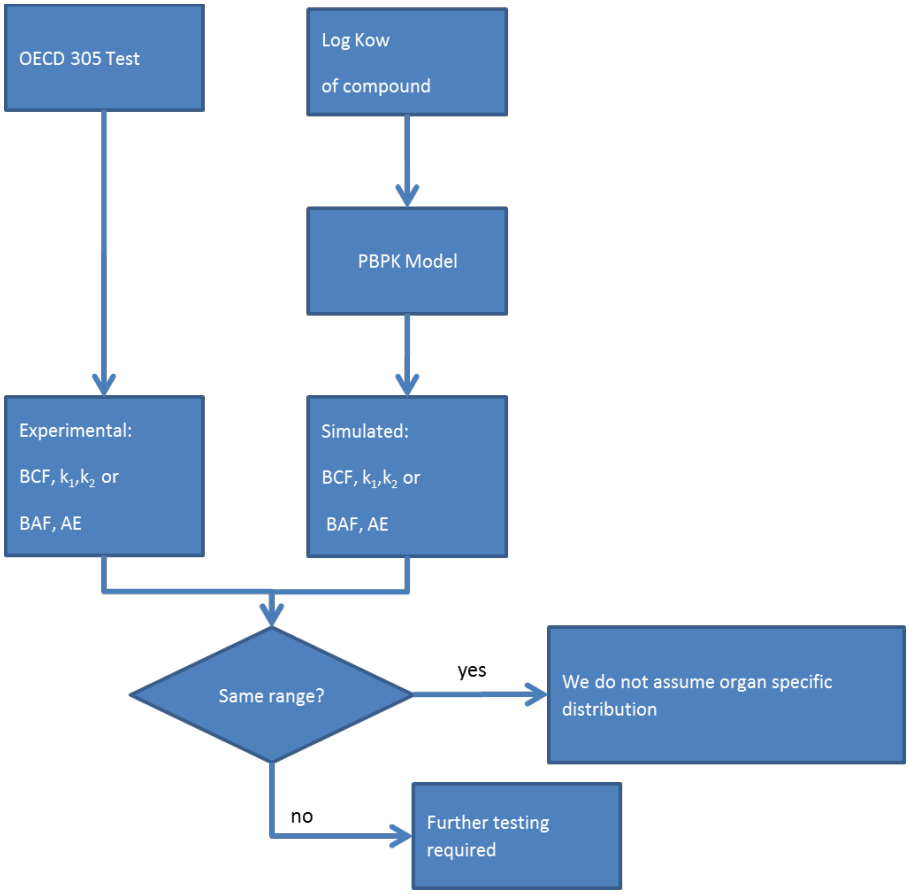
Das Ziel dieses Arbeitspakets war es, ein neues Modell zu entwickeln, das den Vergleich der Ergebnisse aus Fischfütterung und Biokonzentrationsstudien ermöglicht. Allerdings hat die Analyse der verfügbaren Daten ergeben dass eine Ableitung eines vertrauenswürdigen Modells nicht möglich war. Der Hauptgrund dafür liegt in der begrenzten Anzahl verfügbarer Daten. Zudem waren die verfügbaren Daten z.T. recht ungenau, worauf nicht zuletzt der große Aufwand zur Datenaufbereitung hinweist. Ein nicht unerheblicher Anteil der Daten konnte keiner genauen chemischen Struktur zugeordnet werden. Ein großer Anteil der noch verbliebenen Daten konnte zudem aufgrund der begrenzten Vertrauenswürdigkeit nicht verwendet werden. Die im endgültigen Datensatz enthaltenen 604 Daten beziehen sich auf nur 237 unterschiedliche organische Stoffe. Die Analyse der Daten zeigte dann, dass nur schwache Trends beobachtet werden konnten.

Um diese Einschränkungen zu überwinden und einen brauchbaren Datensatz für die Modellentwicklung zu akquirieren, sind zusätzliche Messungen erforderlich. Um den dafür erforderlichen Aufwand insbesondere aus ethischen und ökonomischen Gründen zu minimieren, sollten die erforderlichen Studien sich auf spezielle Kriterien bei der Substanzauswahl beziehen:

1. Der Fokus sollte auf hydroprobe Chemikalien gelegt werden.
2. Die Experimente sollten mit einzelnen Chemikalien mit bekannten und spezifischen Strukturen ausgeführt werden.
3. Wenn möglich sollten Stoffe selektiert werden, für die zuverlässige BCF-Werte vorhanden sind.
4. Studien für Stoffe mit bereits bekannten k_1 und/oder k_2 aus kinetischen Messungen sind zu bevorzugen.
5. Studien mit halogenierten Kohlenwasserstoffen sollten vermieden werden, weil diese Substanzklasse bereits den vorhandenen Datensatz dominiert. Insbesondere Stoffe, die Heteroatome wie N, S, und P enthalten, sind bislang im Datensatz nicht ausreichend enthalten.

Das Ziel des **zweiten Arbeitspakets** war es zu untersuchen, ob PBTK Modelle für die Ableitung nicht lipid induzierter Organverteilung von Stoffen im Rahmen von OE CD 305 Studien, bei denen nur Gesamtkörperkonzentrationen gemessen werden, verwendet werden können.

Konzept zur Identifikation von Stoffen mit spezifischer Organverteilung aus OECD 305 Daten



Zur Anwendung des PBTK Modells sind Parameter wie der log K_{ow} der Testsubstanz sowie die Simulationszeit und die Expositionszeit in Stunden erforderlich. Andere Parameter, die für die Durchführung des Modells erforderlich sind, sind das Körpergewicht der Versuchstiere in Kilogramm, die chemische Konzentration in Mikrogramm pro Liter, Wassertemperatur in Grad Celsius, die gelöste Sauerstoffkonzentration in Milligramm pro Liter und der Lipidgehalt der Fische. Wenn die erzielten Endpunkte, nach Berechnung aus dem Experiment oder simuliert durch das Modell, im gleichen Rahmen liegen wie bei der Studie nach OECD 305, kann von einer lipidabhängigen Kinetik ausgegangen werden. Sollte dies nicht der Fall sein, wären weitere Untersuchungen erforderlich. Das beschriebene Konzept wurde in diesem Projekt validiert.

Eine Literaturrecherche wurde durchgeführt um Studien zu identifizieren, in denen die Organverteilung von Stoffen in Regenbogenforellen untersucht wurde. Für alle Stoffe wurden Distributionsfaktoren berechnet und die folgenden Schritte durchgeführt:

1. Die Organverteilungsmuster lipophiler Chemikalien wurden untersucht.
2. Die Organverteilungsmuster nicht lipophiler Chemikalien wurden untersucht.
3. Ein PBTK-Modell wurde selektiert, implementiert und getestet.
4. Simulierte und gemessene Organverteilungsmuster wurden verglichen.

Ein PBTK Modell wurde implementiert, um die Aufnahme und Distribution chemischer Substanzen basierend auf ihrer Lipophilität vorherzusagen. Der dafür erforderliche Rahmen wurde der Veröffentlichung von

Nichols et al. (1990) entnommen, welche die Disposition von Chemikalien in Regenbogenforellen (*Oncorhynchus mykiss*) nach Aufnahme über die Wasserphase beschreibt. Das Modell enthält eine auf den Durchfluss begrenzte Beschreibung des Chemikalienflusses an Fischkiemen (Erickson & McKim 1990) und bezieht sich auf die Verteilung zwischen den folgenden Kompartimenten (Organen): Adipöses Gewebe, gering durchströmtes Gewebe (Muskel), Nieren, reich durchströmte Kompartimente und Leber. Zusätzliche Anpassungen bezüglich der Beziehung von Lipidfraktion im Gesamtkörper und dem Fettkörpervolumen, die von Stanicka et al. (2012) zusammengefasst wurden, wurden in das Modell integriert. Die relevanten Modellparameter und Gleichungen wurden von Stanicka et al. (2012) beschrieben. Das Modell wurde schließlich unter Verwendung von Embarcadero Rad Studio XE2 (Embarcadero Technologies, San Francisco, USA) implementiert.

Desweiteren wurde das Modell unter Berücksichtigung von zwei zusätzlichen Aufnahmepfaden erweitert: 1. Aufnahme über die Haut und 2. Aufnahme über die Nahrung. Die Aufnahme über die Haut wurde gemäß Nichols et al. (1996) implementiert. Die Haut wurde als separates Kompartiment modelliert, wo Aufnahme und Elimination von Chemikalien als Funktion der chemischen Permeabilität beschrieben werden konnte. Die Aufnahme über die Nahrung wurde von Nichols et al. (2003) präsentiert inklusive der Futteraufnahme im Gastrointestinaltrakt. Das Modell geht ausschließlich von einer lipidbasierten Organverteilung aus. Aufnahme, Elimination und Verteilung von Chemikalien im Fisch sind dabei abhängig vom Lipidgehalt der Organe und dem K_{ow} der Testsubstanz. Um Verteilungsmuster von Chemikalien in Abhängigkeit ihrer log K_{ow} zu erlangen, wurden Modellsimulationen durchgeführt. Die Ergebnisse wurden als Distributionsfaktoren (DFs) ausgedrückt, die als Quotient aus Konzentration der Chemikalie im Gewebe und der Konzentration im Gesamtfisch im Gleichgewichtszustand berechnet wurden.

Die durch das PBTK Modell erzielten Vorhersagen wurden mit gemessenen Biokonzentrationsfaktoren und Aufnahme- und Eliminationsraten organischer Schadstoffe in Regenbogenforellen aus der Literatur verglichen. Titel und Abstract der ausgewählten Artikel wurden auf ihre Relevanz hinsichtlich der eingesetzten Versuchstierart, der Aufnahmeroute und der eingesetzten Testsubstanz untersucht. Studien wurden ausgeschlossen wenn: 1. andere Arten als Regenbogenforellen eingesetzt wurden, 2. keine organischen Substanzen untersucht wurden, 3. die Aufnahme der Testsubstanz nicht über den Wasserpfad erfolgte oder 4. keine der folgenden Kompartimente/Organe berücksichtigt wurden: Blut, Fett, Muskel, Leber, Niere, Haut, reichlich durchströmtes Gewebe oder Gesamtkörper. Neben Biokonzentrationsfaktoren (BCFs), kinetischen Aufnahmeraten (k_1) und kinetischen Eliminationsraten (k_2), wurden verfügbare Daten zum Körpergewicht, Fisch Lipidgehalt, der Sauerstoffkonzentration, Temperatur und Metabolismus recherchiert.

Die Literaturrecherche ergab insgesamt 320 Studien (Pubmed:66; Web of Science:254) von denensich 18 Veröffentlichungen (mit 77 unterschiedlichen Substanzen) als geeignet für die weitere Verwendung herausstellten. Für diese 77 Chemikalien wurden K_{ow} Werte als Input für die PBTK Modelle aus der Literatur verwendet. Zusätzlich zu den Daten aus begutachteten Veröffentlichungen, wurden BCF Daten aus 29 anonymisierten Studien nach OECD Richtlinie 305, vom Umweltbundesamt zur Verfügung gestellt, die in die Modelle integriert und mit den Modellvorhersagen verglichen wurden.

Ein generelles Verteilungsmuster für die Profilstoffe konnte im Rahmen der Untersuchungen zur verfügbaren Literatur nicht identifiziert werden. In fast allen Studien ergaben sich für das Muskelgewebe niedrige oder gar die niedrigsten DF-Werte. Die höchsten DF-Werte wurden hingegen für Fett, Leber, Galle und den Gastrointestinaltrakt, in Abhängigkeit von den jeweils in den Veröffentlichungen beschriebenen Organen/Geweben, berechnet. Es war auffällig, dass die höchsten DF-Werte in fast allen Studien für Leber bestimmt wurden. Es könnte in diesem Zusammenhang diskutiert werden, ob Metabolismus eine wesentliche Rolle bei der Gewebsverteilung spielt, da hierdurch Veränderungen in der Fettlöslichkeit denkbar wären (Phase I, Phase II Metabolismus).

Das PBTK Modell ermöglichte die Vorhersage gemessener BCF Werte. Eine Einschränkung der Vorhersagestärke wurde jedoch für extrem fettlösliche Substanzen (log K_{ow} >6) beobachtet, für die gemessene BCFs überbestimmt wurden. Die erzielten Ergebnisse sind auf einer Linie mit zuvor durchgeführten Studien, wel-

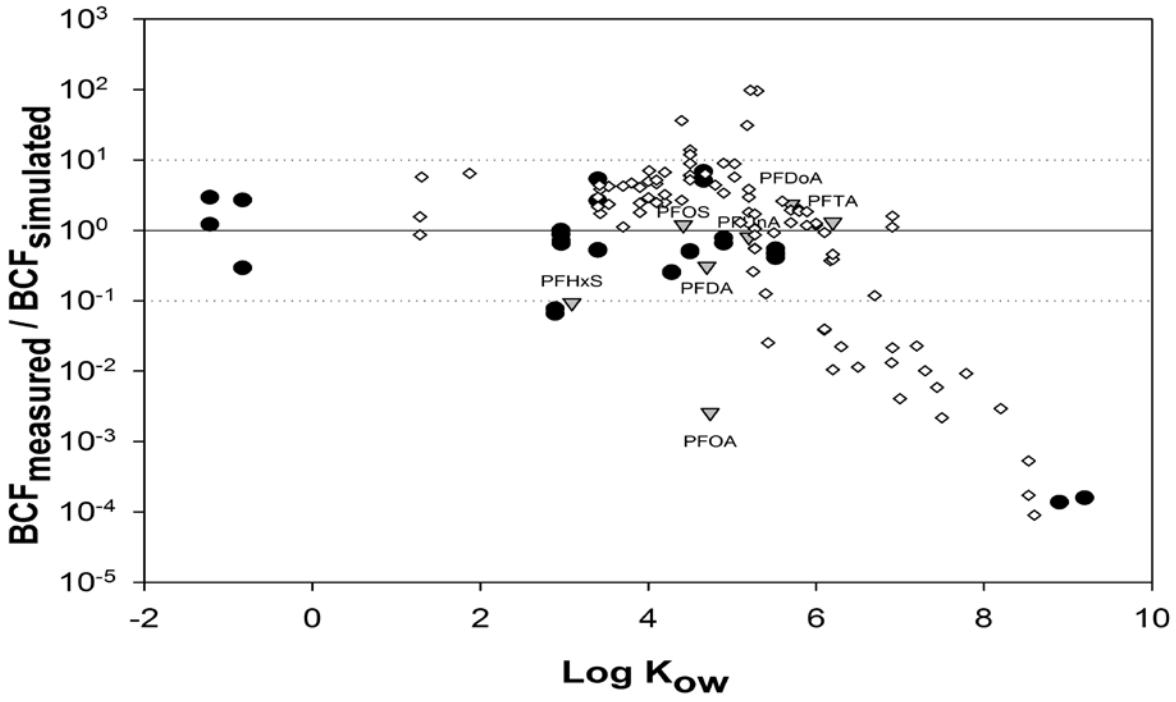
che die mangelnde Eignung einfacher Verteilungsmodelle zur Vorhersage der Biokonzentration für Stoffe mit erhöhter Lipophilität (log K_{ow} >5,5 und 6,5) (Connell and Hawker, 1988; Dimitrow et al. 2002) zeigten. Die Diskrepanz zwischen Biokonzentration und Oktanol/Wasser Verteilungskoeffizient, die für extrem lipophile Substanzen beobachtet wurde, kann mit den unterschiedlichen Einflussfaktoren wie Molekulargröße, Fettlöslichkeit und Zeitperiode zur Erlangung von Gleichgewichtsbedingungen erklärt werden (Hawker and Connell, 1985; Connell and Hawker, 1988; Dimitrov et al. 2002). Die Hauptroute der Substanzexposition von Stoffen mit einem log K_{ow} >5 ist die Nahrung (Bruggeman et al. 1984) und Modelle, welche die direkte Aufnahme über das Wasser simulieren, mögen daher für extrem lipophile Substanzen nicht geeignet sein.

Chemikalien, für die das Modell keine adäquaten Vorhersagen für gemessene BCFs treffen konnte, waren Musk xylene, Pentachlorobenzene, 2,5-Dichlorbiphenyl und 2,4,6-Tritertbutylphenol (Adolfsson-Erici et al. 2012). Wir haben herausgefunden, dass für diese Chemikalien gemessene BCFs mit einem Faktor von bis zu 26 unterbestimmt wurden. Diese Ergebnisse stimmen mit der kürzlich veröffentlichten Arbeiten von Stadnicka et al. (2012) überein, wo das gleiche PBTK Modell zu einer Unterbestimmung interner Konzentrationen für zwei polare organische Substanzen im Fisch führte; Phenol und 2,4,5-Trichlorphenol. Wie von den Autoren beschrieben, könnte vermutlich ein Grund dafür im chemischen Verhalten polarer Substanzen liegen, welches nicht mit dem jeweiligen Oktanol/Wasser Verteilungskoeffizienten korreliert (Ramos et al. 1997). Diese Erklärung passt jedoch nicht zu den weiteren sieben polaren Substanzen, die von Adolfsson-Erici et al. (2012) untersucht wurden und für welche die gemessenen BCFs durch unser Modell korrekt vorhergesagt wurden.

Bezüglich der amphiphilen Eigenschaften von PFAS, werden K_{ow} basierte Modelle generell als nicht geeignet für die Vorhersage des Akkumulationsverhaltens angesehen (Houde et al. 2006). Die klare Übereinstimmung zwischen simulierten und gemessenen BCFs für PFOS, PFHxS, PFDA, PFTA, PFDoA und PFUnA (Martin et al. 2003), welche in dieser Studie beobachtet wurde, erfolgte daher recht unerwartet. Im Bezug auf den Datensatz der hier untersuchten PFAS wurde nur PFOA als „Ausreißer“ identifiziert, was in einem deutlich überschätzten BCF-Wert zum Ausdruck kommt. Vermutlich wirkt sich mit abnehmender aliphatischer Kettenlänge die Anlagerung an Proteine stärker aus und beginnt die Anreicherung durch Fettlöslichkeit zu überlagern. Im Gegensatz zu PFOS, wurde PFOA mit einem niedrigen Biokonzentrationspotenzial im Fisch in Verbindung gebracht (Review by Fujii et al. 2007; Inoue et al. 2012). Eine mögliche Erklärung für diese Beobachtungen könnte die hohe Wasserlöslichkeit von PFOA (3,4 bis 9,5 g/L; US EPA, 2005), welche im Vergleich zu PFOS (519 bis 680 mg/L; OECD, 2002) eine effektive Exkretion über die Kiemen ermöglicht, sein (Vierke et al. 2012).

Im Gegensatz zur erfolgreichen Vorhersage gemessener BCFs, war eine deutliche Unterbestimmung der Biokonzentration im Blut für alle PFAS, außer für PFOA and PFHxS, zu beobachten. Im Gegensatz zu anderen persistenten organischen Schadstoffen, akkumulieren PFAS auch in Körperkompartimenten mit hohem Proteingehalt (MacManus-Spencer et al. 2010). In einer Studie von Van den Heuvel et al. (1992), konnte gezeigt werden, dass PFAS kovalent an Plasmaproteine binden, welche als wichtige Senke für PFAS erkannt wurde (Ng und Hungerbühler 2013).

Beziehung zwischen gemessenen und simulierten BCFs vs. logarithmierte Oktanol/Wasser Verteilungskoeffizienten für 29 anonymisierte Substanzen (Kreise), sieben PFAS (Dreiecke; Daten aus Martin et al. 2003) und ausgewählte Daten aus der Literatur (Diamanten) für Regenbogenforelle (*Oncorhynchus mykiss*). Durchgezogene und gestrichelte Linien repräsentieren jeweils 1:1 Übereinstimmung und ± 1 log Einheit.



In Anbetracht der Tatsache, dass albuminartige Proteine in Regenbogenforellen identifiziert wurden (Manera und Britti, 2006), kann die deutliche Unterschätzung gemessener Konzentrationen im Blut, wie in dieser Studie im Modell beobachtet, mit großer Wahrscheinlichkeit durch die effektive Sorption von PFAS an Blutproteine erklärt werden (Luebker et al. 2002; Jones et al. 2003). Im Gegensatz zu PFAS, wurde die Blutkonzentration einer anderen oberflächenaktiven Substanz, Natriumdioctylsulfosuccinat (DSS), durch unser Modell leicht überschätzt. Als Grund für diese Überschätzung könnte der DSS-Metabolismus genannt werden, wie zuvor von Goodrich et al. 1991 beschrieben. Die Überschätzung von Blutkonzentrationen wurde auch bei vier Pharmazeutika beobachtet: Naproxen, Ibuprofen, Carbamazepine und Bisoprolol. Die schnelle Konjugierung und Elimination über die Leber/Gallenroute, wie zuvor von Ferreira-Leach und Hill (2001) and Lahti et al. (2011) beschrieben, spielt dabei vermutlich eine wesentliche Rolle.

Im Hinblick auf den hohen Proteingehalt der Leber war die hohe Übereinstimmung zwischen simulierten und gemessenen Leberkonzentrationen für die Mehrzahl der untersuchten PFAS nicht überraschend. In Übereinstimmung mit einer Studie von Mortensen et al. (2011), war die gemessene Akkumulation von PFOS in der Leber geringfügig höher als vorhergesagt. Wie zuvor bereits für Blut diskutiert, kann die Unterschätzung gemessener PFAS BCF höchstwahrscheinlich durch Sorption an Proteine erklärt werden. Im Gegensatz zu PFOS und den anderen Substanzen, wurde das Biokonzentrationspotenzial von PFOA hingegen deutlich überbewertet. Eine schnelle Ausscheidungsrate und die hohe Wasserlöslichkeit, wie bereits angemerkt, könnten helfen die niedrige Vorhersagekraft des Modells für PFOA zu erklären.

Auch wenn sich das Modell generell für die Vorhersage von BCFs als geeignet erwiesen hat, führte die Vorhersage von Aufnahme – und Eliminationskonstanten jedoch zu deutlichen Abweichungen. Der größte Unterschied war die reversive Beziehung zwischen dem Modellwert und dem Oktanol/Wasser Verteilungskoeffizient, wie er für die kinetische Depurationsrate beobachtet wurde. Während BCF und kinetische Aufnah-

meraten für hoch lipophile Substanzen (log K_{ow}>6) nur unzureichend vorhergesagt wurden, zeigten simulierte und gemessene Eliminationsraten eine gute Übereinstimmung für alle Substanzen, abgesehen von denen mit hoher Wasserlöslichkeit (log K_{ow}<0). Wie zuvor dargestellt, ist die Kinetik von Chemikalien im Fisch stark durch den Metabolismus, die Sekretion und aktive Transportsysteme (Nichols et al.1990), ebenso wie die Physiologie der Organismen und das Passieren der Organe und Membranen (z.B. Kiemen) beeinflusst. Die Physiologie der Organismen ist bislang weit weniger verstanden bzw. nicht messbar wie der Lipidgehalt der Organe, der den Hauptfaktor im BCF Modell darstellt.

Für die Mehrzahl der PFAS stellte sich das Modell als geeignet für die Vorhersage von Aufnahme und Elimination in Gesamtkörper und Leber von Regenbogenforellen dar. PFHxS and PFOA wurden als Ausreißer identifiziert, da die gemessenen Aufnahmeraten (k_1) für beide Kompartimente überschätzt wurden.

Schlussfolgernd kann festgestellt werden, dass das im zweiten Arbeitspaket angewendete PBTK Modell ein effektives Werkzeug für die erfolgreiche Abschätzung der Biokonzentration sowie kinetischer Aufnahme- und Eliminationsraten in Regenbogenforellen für ein breites Spektrum organischer Substanzen darstellt. Die größte Vorhersagestärke wurde für die Simulation moderat lipophiler Substanzen im Gesamtkörper gefolgt vonLeber beobachtet, wohingegen die Simulation der Anreicherung im Blut generell zu einer Über- oder Unterschätzung der gemessenen Daten führte. Trotz der vermuteten organspezifischen Akkumulation kann die Biokonzentration der untersuchten PFAS im Gesamtkörper ausreichend genau über das Modell bestimmt werden, was auf eine lipidgeleitete Verteilung der meisten PFAS in Leber und Gesamtkörper deutet. Um eine weitere Optimierung des PBTK Modells zu erzielen, sollte ein besonderes Augenmerk auf die Proteinsorption gelegt werden, wodurch die Vorhersagestärke im Bezug auf die Anreicherung im Blut erhöht werden kann. Eine weitere zukünftige Herausforderung stellt die Abschätzung der Bioakkumulation von polaren Substanzen dar.

Summary

The European chemicals legislation places by the REACH regulation greater emphasis on the precautionary principle for humans and the environment with regard to substances of very high concern (SVHC). Substances with (very) persistent, (very) bioaccumulative and toxic properties (PBT and vPvB substances), carcinogenic, mutagenic and toxic for reproduction (CMR substances) as well as chemicals categorized in case-by-case decisions as substances of very high concern (e.g. endocrine disruptors) might be subject to an authorization procedure.

Criteria for identification of PBT/vPvB substances are defined in the REACH regulation. Substances with experimentally determined bioconcentration factors (BCF) > 2000 are classified as substances with bioaccumulative (B) properties; a BCF > 5000 identifies candidates for substances with very bioaccumulative (vB) properties. Bioconcentration factors are mainly determined in flow-through fish tests following the OECD 305 guideline (OECD 305, 2012). Aquatic standard tests in fish reduce the diverse uptake and elimination processes in aquatic ecosystems to respiratory absorption through gills and dermal diffusion.

Especially for highly lipophilic chemicals (log K_{ow} > 5) bioconcentration studies are often difficult to perform. Lipophilic substances are poorly water soluble, which makes the adjustment of stable test concentrations difficult and, under certain conditions, may cause inexact measurements of the test substance in the medium. Moreover, chemicals in the environment tend to show an increasing accumulation via the food chain with increasing lipophilicity. As a consequence, biomagnification processes should receive more attention. For poorly soluble chemicals an alternative test design for the performance of bioaccumulation studies based on feeding experiments is therefore offered. The aim of these studies is the determination of the biomagnification factor (BMF). In the scope of the revised OECD guideline 305, two different bioaccumulation factors will be evaluated in future. Analogous to the conventional BCF study, an elimination rate constant ($k_{2(BMF)}$) is determined which will be used together with daily feed ration and assimilation efficiency for calculation of BMF. The BMF value obtained is not directly comparable to the conventional BCF value. Usage of BMF values for identification of PBT substances is difficult due to missing definition of thresholds in Annex XIII of the REACH regulation. Derivation of BCF values from data obtained in feeding studies would be advantageous especially regarding their value in a regulatory framework. In addition to the available elimination rate constant k_2, an uptake rate constant k_1 is required for derivation of a kinetic BCF, which cannot be determined in feeding studies.

Several suggestions and models were developed to estimate k_1 such as the mathematical relationship between fish weight and uptake rate constant for uptake of hydrophobic compounds through gills (Sijm et al. 1993c, 1994 and 1995). Other mehods describe derivation of BCF values from depuration data determined in feeding studies. Examples are fugacity models (Campfens & Mackay 1997) or mass balance models based on the octanol/water partition coefficient (Arnot & Gobas 2003). Available models for derivation of the uptake rate constant were assessed by Brooke et al. (2012). However, tested methods yielded imprecise data and are therefore restrictedly suitable for derivation of BCF values from feeding data. The derivation of a model that allows the comparison of results from fish feeding studies to REACH bioaccumulation criteria would be of high value.

The principle of lipophilicity-induced bioaccumulation applies to most neutral organic substances. However, for several groups of substances, bioaccumulation is not determined by lipophilicity. For example poly- and perfluorinated carbonic acids and silicone oils show equally repellent effects on water as non-polar solvents. However, amphiphilic substances like surface-active substances are characterized by their affinity for water and organic phases and therefore accumulate primarily in boundary layers. Therefore, application of log K_{ow} for prediction of the bioaccumulation potential is not appropriate. Instead, specific interactions with e.g. proteins may have a significant influence on accumulation of these substances. Kelly et al. 2009 described a concept for inclusion of substance/protein interactions based on the protein/water partition coefficient (log K_{PW}). As a consequence of the substances' unusual partitioning behavior, lipid-containing body fluids and tissues are not their preferred compartments. Possible accumulation of substances in specific target organs

may lead to higher tissue concentrations resulting in toxic effects in affected organs. The assessment of chemicals based on consideration of lipophilicity only may result in an incomplete picture concerning the bioaccumulation behavior of substances in humans and the environment. Hence, specific non-lipidbased bioaccumulation processes in aquatic organisms should be incorporated into substance evaluation.

In toxicology and pharmacology, computer based modeling has developed into an essential tool for successful quantification and extrapolation of toxicokinetics in organisms (Barton et al. 2007; Bois et al. 2011; Jager et al. 2011). Computer models do not only provide a time- and cost efficient simulation of chemical absorption, distribution and elimination over time, but also bring ethical advantages in the reduction and refinement of animal use (Badyal et al. 2009; Stadnicka et al. 2012).

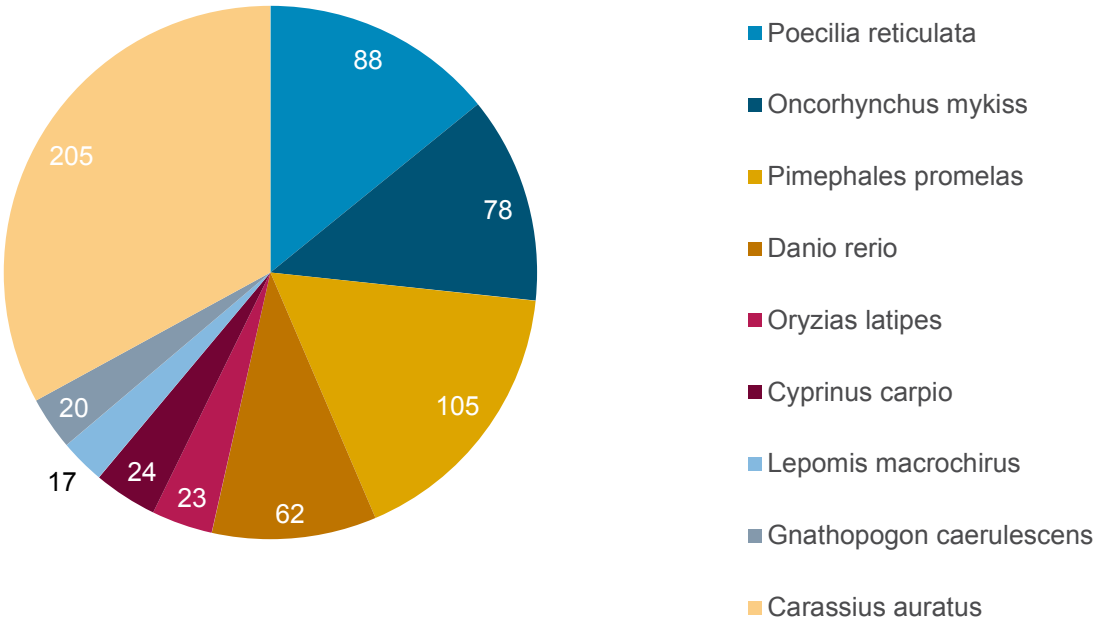
In general, two toxicokinetic model groups can be distinguished; the one-compartment model assuming a homogenous distribution of a chemical in biota, and the multi-compartment model in which accumulation in specific organs and tissues is taken into consideration (Stadnicka et al. 2012). An example of the latter is the Physiologically Based Toxicokinetic (PBTK) model, which was developed for fish by Nichols and coworkers (Nichols et al. 1990). Whereas one-compartment models are based on simple curve fitting (Barron et al. 1990), the PBTK model is considerably more detailed and complex, describing an organism with use of multiple anatomical, physiological and biochemical components in which organs and tissues are defined as individual compartments (Abbas and Hayton 1997; Nichols et al. 1990; Nichols et al. 1998). As species- and life-stage specific parameters together with physiochemical data of a compound are integrated in the model structure, the PBTK model offers the possibility to extrapolate between various species and exposure regimes (Barron et al. 1990; Nichols et al. 1998; Schmitt 2008). In the PBTK model, toxicokinetic aspects of a chemical are furthermore described by certain uptake paths (e.g. branchial, dermal, dietary or injection) followed by its circulatory distribution to the individual compartments.

The principle of lipid based bioaccumulation is applicable for most neutral organic compounds, there are certain groups of chemicals whose bioaccumulation potential cannot be appropriately determined based on their lipophilic behavior. Poly- and perfluorinated chemicals are known for their amphiphilic properties and for their tendency to partition in air/liquid interface rather than in fatty tissue (EFSA, 2008). Bioaccumulation potential of these compounds has been shown to be greatly influenced by specific interactions such as protein binding (EFSA, 2008), leading to organ specific accumulation in liver, kidney and blood, as experimentally shown by e.g., Goeritz et al. (2013) and Martin et al. (2003). Hence, assessments relying solely on lipophilicity may for PFAS lead to underestimated predictions regarding environmental and human accumulation.The assumption underlying this study is that the PBTK model is a good representative for bioconcentration and bioaccumulation in fish allowing a correct prediction of organ distribution. As chemical partitioning in the used PBTK model is soley based on lipophilicity, non-lipid distribution should show different organ distribution patterns which in turn will result in differences in bioconcentration factors and rate constants measured according to OECD 305.

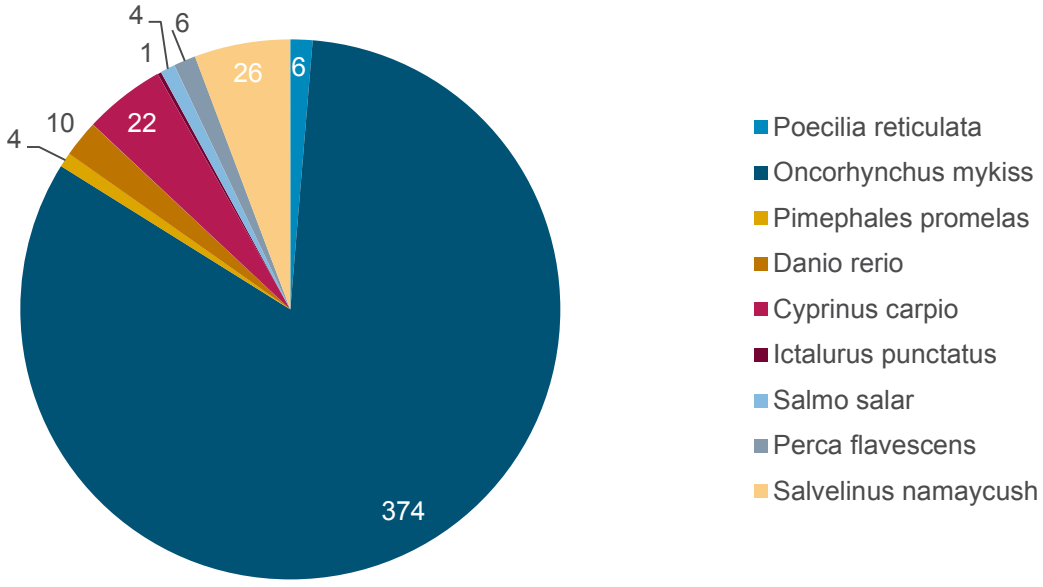
It is thus required to highlight the feasibility of a PBTK model to predict lipid triggered bioconcentration in rainbow trout (*Oncorhynchus mykiss*) and to elucidate its potential to identify specific accumulation pattern not in line with lipophilicity. For this purpose model performance needs to be validated with data on kinetic rates and bioconcentration factors collected from peer-reviewed literature and from measured data available from OECD 305 studies carried out for substance regulation.

The aim of the **first workpackage** was to examine mathematical correlations of kinetic BCF and their corresponding $k_{2(BCF)}$ values for substances collected from the literature. The same procedure was applied to BMF and their corresponding $k_{2(BMF)}$ values. A literature search for bioaccumulation data (bioconcentration and biomagnification) in fish was conducted. Only kinetically derived bioconcentration and biomagnification factors were considered. The search was focused on organic test compounds and laboratory studies.

Number of bioconcentration data set entries per fish species



Number of biomagnification data set entries per fish species



The evaluation process was divided into two parts. In the first part, all collected publications on bioaccumulation studies were analyzed and evaluated with respect to the criteria-accumulation in whole fish (not in specific tissues), no predicted data, freshwater fish (no marine species) and age of test animals. For biomagnification, food and feeding method were additionally regarded. In addition to that 42 fish BCF studies carried out according to OECD 305 were provided by the UBA. The data were used to calculate uptake and depuration rate constants as well as a kinetic and steady-state BCF value for each of the 42 test compounds.

In part two, data quality of the results presented in each reference was assessed. Ideally, publications/studies should follow the OECD 305 test guideline (OECD 305, 2012) and show a high reliability according to Klimisch score 1or 2. All publications were evaluated with respect to the validity criteria defined in OECD 305 in which were used to establish the Klimisch system. A summary of every publication and the respective Klimisch scores are provided. Data ranked with Klimisch score 3, not reliable, were excluded from the main data set resulting in a filtered data set containing reliable data only.

Experimental data collected from the literature and provided by the UBA were assigned to unique organic chemical structures. Data were not distributed equally to the different chemical structures, and it was thus necessary to distinguish between the raw data set containing all exploitable data and a condensed data set with one value for each chemical.

Disagreements between reported BCF values and the ratio of k_1 and k_2 within the same study were examined. If the doubts could not be clarified, the corresponding k_1 and k_2 were both considered as invalid unless they could be justified by an agreement to other studies for the same compound. Furthermore, when more than one complete item (i.e. both k_1 and k_2 from the same study) were available, the range of obtained BCFs was inspected. Differences larger than 2 orders of magnitudes have been treated as unreliable, and the data entries with the highest probability to be erroneous were removed in these cases. If required, a further validation step was applied by comparing the results of horizontal (taking average of BCFs)and vertical (taking average of k1 and k2 prior to BCF calculation) averaging. Here, any differences larger than one order of magnitude were inspected and curated by removing the most doubtful items.

The chemical domain of the data set with regard to the chemical elements, complexity and polarity was investigated. These analyses were performed exclusively, i.e. each compound belongs to exactly one group. In case of ambiguities, unless otherwise stated explicitly, a chemical was assigned to the most complex class.

When available, steady state BCFs were compared with the kinetically derived values. Respective data were taken from the OSIRIS data set (OSIRIS 2007-2011) that is publically available within the ChemProp database (UFZ 2014). To examine the influence of lipid correction, the calculated BCF values were normalized to a lipid content of 5%.

The BCF dependency on k_2 (BCF) was investigated. As expected k2 is decreasing with increasing BCF. The transport of chemicals between water and fish can be modelled by the addition of resistances for aqueous diffusion and membrane permeation (Gobas et al. 1986). Depending on the chemical, one of these processes dominates, and thus the total rates can either be membrane controlled or diffusion controlled. Hydrophobicity (modeled through K_{ow}) is the key factor determining this behavior. Membrane permeation is expected for low hydrophobicity. Diffusion layer control is expected for higher hydrophobicity. For the elimination, k_2 should increase with K_{ow} for low hydrophobicity. With high hydrophobicity, k_2 is expected to inversely depend on K_{ow}.

In order to compare the kinetic BCFs to modeled steady state values, several different respective model approaches with different levels of sophistication have been applied. In addition to that several K_{ow} models were explored, but did not yield significant differences. Finally, the ChemProp consensus model (UFZ 2014) was applied, unless experimental data were available. For k_M, the ChemProp implementation of the Arnot et al. (2009) was used. Since the original model does include k_M but uses a default value of 0, also the BCF estimation without k_M was explored.

The relationships of the uptake rates (k_1) to the BCF and to the hydrophobicity have been examined. In order to distinguish between membrane permeation control and diffusion control, respective hydrophobicity ranges were inspected separately. The octanol/water partition coefficient has been applied as the hydrophobicity criterion. Regarding uptake, k_1 should depend on K_{ow} in the case of membrane control for lower hydrophobicity. In contrast, high hydrophobicity and thus diffusion control would result in a rate independent from K_{ow}. However, since diffusion coefficients are known to decrease with increasing hydrophobicity, k_1 may even decrease with increasing K_{ow}.

The estimation of k_1 has been recently reviewed extensively (Brooke et al. 2012, Brooke & Crookes 2007). From a large number of reported opportunities, the authors extracted a few key techniques, based on weight (W), K_{ow}, or both of them. These models have been applied to the current data set.

In addition, alternative approaches were tested. K_{ow} was replaced by the membrane/water partition coefficient K_{mw} obtained by an estimation model implemented in the in-house version of ChemProp (not publically available yet) and by the partitioning between water and proteins in terms of the Human serum albumin partitioning. The latter was calculated by the LSER equation of Valko et al 2003.

In the same manner as for k_1, the elimination rates k_2 were compared against BCF and hydrophobicity by respective plots. Furthermore, the uptake and elimination rates were plotted against each other.

There are different models available to estimate k_2, which also include biotransformation (i.e. metabolic elimination). To examine its influence, the equations were applied with and without considering biotransformation. To estimate the biotransformation rates, the model of Arnot et al 2009 was used.

Chemical investigations of BMF data were carried out. As a first step, the chemical domain of the data set with regard to the chemical elements, complexity and polarity was investigated. By plotting and following visual inspection, the relation of the experimental elimination rates $k_{2(BMF)}$ to BMF and to the k_2 obtained by kinetic BCF measurements was investigated, if possible.

The aim of this workpackage was to derive a new model that allows the comparison of results from fish feeding and bioconcentration studies. However, the analysis of the available data did not allow deriving a reliable model. The main reason is the limited number of data. Moreover, the available data were rather uncertain, as indicated by the large effort of data curation required. A rather high part of the (originally more than 700) data did not correspond to unique chemical structures at all, and from the remaining data a lot of them could not be used due to insufficient reliability. Even more, the 604 data in the final set corresponding to 237 different organic compounds (i.e. roughly one duplicate for each chemical in average) still contained a large variability and uncertainty, e.g. with regard to species, protocols, etc. In result, only some weak trends could be observed from the data analyses.

To overcome these shortcomings and to step forward for achieving a data set useful for model developments, additional measurements seem to be unavoidable. In order to minimize such efforts, mainly for ethical reasons but also for economic reasons, respective studies should focus on particular criteria to select the test compounds:

1. Since the concern is bioaccumulation, test focus should be on hydrophobic chemicals.

2. Experiments should be carried out with single chemicals with known and unique structure.

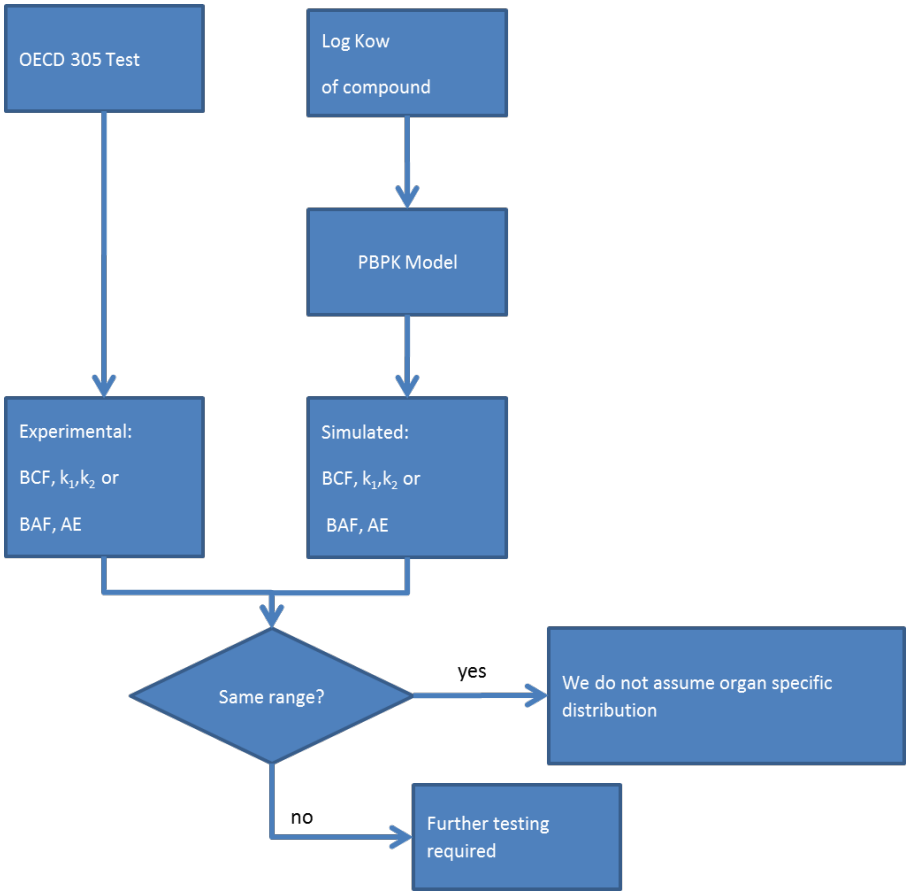
3. Preferably compound should be selected with reliably known steady state BCF.

4. Studies for compounds with already known k_1 and/or k_2 from kinetic measurements are appreciated.

5. Studies should avoid halogenated hydrocarbons, because this compound class already dominates the overall data set. Particularly, compounds containing heteroatoms as N, S, and P are missing in the data set.

The aim of the **second workpackage** was to elucidate the possibility to use a PBTK model for identifying possible non lipid triggered organ distribution of compounds from an OECD 305 experiment in which only full body concentrations are measured.

Approach for identification of compounds with specific organ distribution from OECD 305 data



To run the PBPK model, parameters as the log K_{ow} of the compound as well as the simulation time and exposure time in h are required. Other parameters needed to run the model are body wet weight in kg, chemical concentration in µg/l, water temperature in °C, dissolved oxygen concentration in mg/l and lipid content of the whole fish as a fraction of body weight. If endpoints calculated from the experiment and simulated by the model are within the same range as obtained by OECD 305 studies, it can be assumed that kinetics are lipid dependent. If not, further investigations are required. The approach was validated in this project.

A literature search was conducted to find studies in which organ distribution of rainbow trout was investigated. For all compounds distribution factors were calculated and the following steps carried out:

1. Organ distribution patterns for lipophilic chemicals were investigated
2. Organ distribution patterns for non-lipophilic compounds were investigated
3. A PBTK model was selected, implemented and tested
4. Simulated and measured organ distribution patterns were compared

A PBTK model was implemented in order to predict uptake and distribution of chemical substances based on their lipophilicity. The framework was taken from a publication by Nichols et al. (1990), describing disposition of chemicals after waterborne uptake in rainbow trout (*Oncorhynchus mykiss*). The model includes a flow-limited description of chemical flux at fish gills (Erickson & McKim 1990) and distribution between the following compartments (organs): adiposis tissue, poorly perfused tissues (muscles), kidney, richly perfused compartment and liver. Additional adjustments, summarized by Stadnicka et al. (2012), were made regarding the relationship between lipid fractions in the whole body and the volume of fat compartment. For model parameters and equations see the supporting information of Stadnicka et al. (2012). The model was finally implemented using Embarcadero Rad Studio XE2 (Embarcadero Technologies, San Francisco, USA).

Moreover, the model was further extended by considering two additional uptake pathways: 1. Dermal absorption and 2. Dietary uptake. Dermal uptake was implemented according to Nichols et al. (1996). The skin was modeled as a discrete compartment where uptake and elimination of chemicals could be described as a function of chemical permeability and the concentration gradient between exposed water and skin tissue. Dietary uptake was presented by Nichols et al. (2003) incorporating intake of food into the gastrointestinal (GI) tract. The model assumes lipid based organ distribution only. Uptake, elimination and distribution of chemicals in the fish are triggered by the lipid content of the organs and the K_{ow} of the compound. To achieve distribution patterns of chemicals depending on their log K_{ow}, model simulations were performed. Results are expressed as Distribution Factors (DFs), which were calculated by dividing the concentration of a chemical inside the tissue by the concentration inside the whole fish at equilibrium.

Predictions of the PBTK model were compared with measured bioconcentration factors and rate constants for organic pollutants in rainbow trout (*Oncorhynchus mykiss*) available from peer-reviewed literature. Title and abstract of the collected articles were screened for relevance by assessing the study specimen, exposure route and compounds tested. Studies were excluded if they: (1) did not include rainbow trout, (2) did not investigate organic compounds, (3) did not assess water as route of exposure or (4) did not take any of the following compartments into account: blood, fat, muscle, liver, kidney, skin, richly perfused tissues or whole body. Along with bioconcentration factors (BCFs), kinetic uptake rate constants (k_1) and kinetic depuration rate constants (k_2), available data on body weight, fish lipid content, oxygen concentration, temperature and metabolism were extracted. In case no information on body weight, lipid content, oxygen concentration and temperature was given, the model was run with the following standard settings.

The literature search yielded a total of 320 studies (Pubmed: 66; Web of Science: 254) of which 18 publications (with 77 different chemicals) were eligible for inclusion. For this 77 chemicals measured K_{ow} as input for the PBPK model were taken from literature. In addition to peer-reviewed data, 29 anonymized BCF values measured according to the OECD guideline and provided by the UBA were integrated in the model calibrationcompared to model predictions.

A general distribution pattern for lipophilic compounds could not be found after investigating the available literature. One aspect that almost all studies had in common was that the muscle tissues usually had a very low, if not the lowest DF. As for the highest DF, fat, liver, bile and the GI tract were the compartments for which it could be calculated, depending on the organs/tissues investigated by the authors. It was noticeable, however, that liver was found to have the highest or among the highest DF in amost studies. It could be discussed whether metabolism was an important factor here, since changes in lipophilicity could be possible (Phase I, Phase II metabolism).

In general, the PBTK model was able to predict measured whole body BCFs. A decrease in predictive power was seen for extremely lipophilic compounds (log $K_{ow} > 6$) for which measured BCFs were overestimated with up to four orders of magnitude. The obtained results are in line with previous studies demonstrating the inadequacy of simple partitioning models to predict bioconcentration of compounds possessing log $K_{ow} > 5.5$ and 6.5, respectively (Connell and Hawker, 1988; Dimitrov et al., 2002). The discrepancy between bioconcentration and octanol-water partition coefficient seen for extremely lipophilic compounds has been related to influencing factors such as molecular size, lipid solubility and time period required to attain equilibrium (Hawker and Connell, 1985; Connell and Hawker, 1988; Dimitrov et al., 2002). Moreover, it has generally been stated that the main route of exposure for compounds possessing log K_{ow} above 5 is dietary uptake (Bruggeman et al., 1984), hence for extremely lipophilic compounds, models simulating direct uptake via water may not be appropriate.

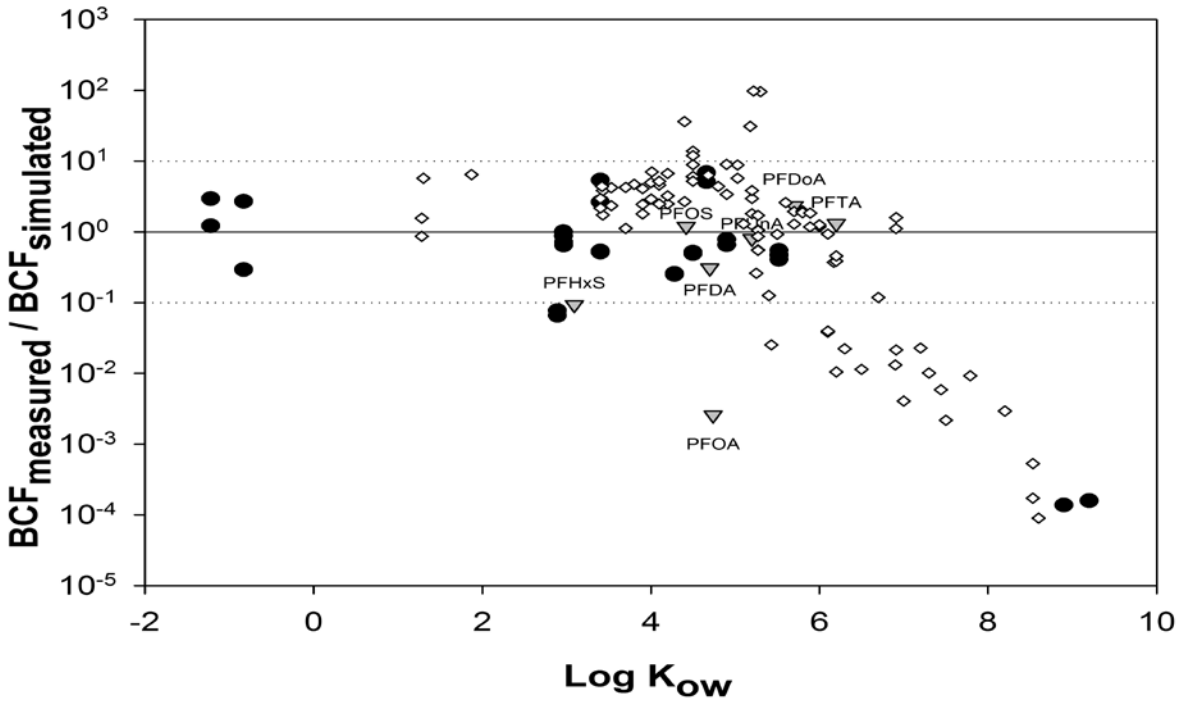
Chemicals for which the model failed to adequately predict measured whole body BCF were musk xylene, pentachlorobenzene, 2,5-dichlorobiphenyl and 2,4,6-tri-tert-butylphenol (Adolfsson-Erici et al. 2012). We found that measured BCFs were underestimated by a factor of up to 26, results which are consistent with a recent study by Stadnicka et al. (2012), where the same PBTK model was shown to generate underestimated internal concentrations of two polar organic compounds in fish; phenol and 2,4,5-trichlorophenol. As dis-

cussed by Stadnicka and coworkers, a putative reason could be that chemical behavior of polar compounds does not correlate well with the corresponding octanol/water partitioning coefficient (Ramos et al. 1997). This explanation does however not hold for the additional seven polar compounds investigated by Adolfsson-Erici et al. (2012) for which the measured BCFs were accurately predicted by our model. Understanding the mechanism of bioconcentration for these groups of chemicals clearly needs further experiments and will enlarge our overall understanding of bioconcentration and bioaccumulation.

Given the amphiphilic properties of PFAS, K_{ow} based models are generally not considered suitable for predictions of environmental fate (Houde et al. 2006). The fine agreement between simulated and measured whole body BCFs for PFOS, PFHxS, PFDA, PFTA, PFDoA and PFUnA (Martin et al. 2003) obtained in the present study was therefore rather unexpected. Of the PFAS, only PFOA was identified as an "outlier" (see Figure below) as reflected by a clear overestimation of the measured BCF. It can be assumed that with shorter aliphatic chain length binding to protein will increase and potentially dominate the accumulation process. In contrary to PFOS, PFOA has repeatedly been associated with a low bioconcentration potential in fish (reviewed by Fujii et al. 2007; Inoue et al. 2012). A possible explanation to these observations is related to the high water solubility of PFOA (3.4 to 9.5 g/L; U.S. EPA, 2005) which compared to PFOS (519 to 680 mg/L; OECD, 2002), allows for an effective excretion via gill permeation (Vierke et al. 2012).

In contrast to the successful predictions of measured whole body BCFs, bioconcentration in blood was for all PFAS, but PFOA and PFHxS, underestimated by more than one order of magnitude. Unlike other persistent organic pollutants, PFAS are also distributed in body compartments with high protein content (MacManus-Spencer et al. 2010). In a study by Van den Heuvel et al. (1992), PFAS were shown to covalently bind proteins in plasma of rats following in vivo administration. Particularly plasma albumin has been recognized as an important sink for PFAS (references in Ng and Hungerbühler 2013).

Relationship of measured BCF over simulated vs. logarithmized octanol/water partition coefficients for twenty-nine anonymized compounds (circles), seven perfluorinated acids (triangles; measured data from Martin et al. 2003) and selected literature data points (diamonds) for whole body of rainbow trout (*Oncorhynchus mykiss*). Solid and dashed/dotted lines represent 1:1 agreement and ± 1 log unit, respectively.



Given the fact that albumin-like proteins have been identified in rainbow trout (Manera and Britti, 2006), the strong underestimation of measured blood BCFs by modeling observed in the present study is most likely related to the effective sorption of PFAS to blood proteins (Luebker et al. 2002; Jones et al. 2003). In contrast to the PFAS, blood BCF of another surfactant, dioctyl sodium sulfosuccinate (DSS), was slightly overestimated by our model. The reason behind this overprediction might be related to DSS metabolism, as confirmed by the authors (Goodrich et al., 1991). Overpredicted blood BCFs were furthermore seen for four pharmaceuticals; naproxen, ibuprofen, carbamazepine and bisoprolol. Rapid conjugation and elimination via the liver/bile route as reported in Ferreira-Leach and Hill (2001) and Lahiti et al. (2011) is assumed to play a major role.

With respect to the high protein content of the liver, the high agreement between simulated and measured liver BCFs for the majority of investigated PFASs was not surprising. Consistent with a study by Mortensen et al. (2011), measured accumulation of PFOS in liver was found to be slightly higher than predicted. As previously discussed for blood, underestimation of measured PFOS BCF can most likely be related to protein sorption. Contrary to PFOS and the other compounds, the bioconcentration potential of PFOA was clearly overpredicted by nearly two orders of magnitude. Fast clearance rate as to a high water solubility may, as already hypothesized, help to explain the weak predictive power for PFOA.

Even if the model was able to predict whole body BCFs satisfactorily, the prediction of rates was much more scattered. The most prominent difference was the reversed relationship between model performance and octanol-water partition coefficient observed for the kinetic depuration rate. Whereas whole body BCF and kinetic uptake rate were poorly predicted for extremely lipophilic compounds ($\log K_{ow} > 6$), simulated and measured whole body elimination showed good agreement for all compounds but for those with high water solubility ($\log K_{ow} < 0$). As previously stated, chemical kinetics in fish are strongly affected by metabolizing, secretory and active transport systems (Nichols et al., 1990) so by the physiology of the organism and passing of organs and membrans (e.g. gills). The physiology of the organisms are much less understood or not as adequate and generic measurable as the lipid content of organs which is the main factor affecting the BCF in this model. Consistent with the observed underestimation of whole body BCFs for pentachlorobenzene, 2,4,6-tri-tert-butylphenol and 2,5-dichlorobiphenyl, predicted elimination rates for these polar compounds were overestimated by more than one order of magnitude. In the case of pentachlorophenol, the underestimated elimination can most likely be explained by metabolic transformation as previously confirmed for pentachlorophenol both in vitro and in vivo (Stehly and Hayton, 1989; Cravedi et al. 1999).

For the majority of PFAS, the model proved to be a suitable tool for predictions of uptake and elimination in both whole body and liver of rainbow trout. PFHxS and PFOA were identified as “outliers” as measured k_1 were overpredicted by more than one order of magnitude in both compartments.

In conclusion, the second workpackage demonstrated that the PBTK model is an effective tool for successful predictions of bioconcentration and kinetic rate constants of a wide range of organic compounds in rainbow trout. The highest predictive power was seen for simulations of moderately lipophilic compounds in whole body followed by liver whereas simulations in blood generally led to over- or underestimations of measured data. Despite assumptions of organ-specific accumulation, whole body bioconcentration of investigated PFAS was reasonably well estimated, indicating a lipid triggered distribution of most PFAS in liver and whole body. In order to achieve further optimization of the PBTK model, particular emphasize should be placed on protein sorption, which would serve to increase the predictive power regarding blood simulations. Future challenges also include successful predictions of very polar compounds.

1 Introduction

The European chemicals legislation places by the REACH regulation greater emphasis on the precautionary principle for humans and the environment with regard to substances of very high concern (SVHC). Substances with (very) persistent, (very) bioaccumulative and toxic properties (PBT and vPvB substances), carcinogenic, mutagenic and toxic for reproduction (CMR substances) as well as chemicals categorized in case-by-case decisions as substances of very high concern (e.g. endocrine disruptors) might be subject to an authorization procedure.

Criteria for identification of PBT/vPvB substances are defined in the REACH regulation. Substances with experimentally determined bioconcentration factors (BCF) > 2000 are classified as substances with bioaccumulative (B) properties; a BCF > 5000 identifies candidates for substances with very bioaccumulative (vB) properties. Bioconcentration factors are mainly determined in flow-through fish tests following the OECD 305 guideline (OECD 305, 2012). Aquatic standard tests in fish reduce the diverse uptake and elimination processes in aquatic ecosystems to respiratory absorption through gills and dermal diffusion.

Especially for highly lipophilic chemicals (log K_{ow} > 5) bioconcentration studies are often difficult to perform. Lipophilic substances are poorly water soluble, which makes the adjustment of stable test concentrations difficult and, under certain conditions, may cause inexact measurements of the test substance in the medium. Moreover, chemicals in the environment tend to show an increasing accumulation via the food chain with increasing lipophilicity. As a consequence, biomagnification processes should receive more attention. For poorly soluble chemicals an alternative test design for the performance of bioaccumulation studies based on feeding experiments will therefore be offered. The aim of these studies is the determination of the biomagnification factor (BMF). In the scope of the revised OECD guideline 305, two different bioaccumulation factors will be evaluated in future. Analogous to the conventional BCF study, an elimination rate constant ($k_{2(BMF)}$) is determined which will be used together with daily feed ration and assimilation efficiency for calculation of BMF. The BMF value obtained is not directly comparable to the conventional BCF value. Usage of BMF values for identification of PBT substances is difficult due to missing definition of thresholds in Annex XIII of the REACH regulation. Derivation of BCF values from data obtained in feeding studies would be advantageous especially regarding their value in a regulatory framework. In addition to the available elimination rate constant k_2, an uptake rate constant k_1 is required for derivation of a kinetic BCF, which cannot be determined in feeding studies.

Several suggestions and models were developed to estimate k_1 such as the mathematical relationship between fish weight and uptake rate constant for uptake of hydrophobic compounds through gills (Sijm et al. 1993c, 1994 and 1995). Other mehods describe derivation of BCF values from depuration data determined in feeding studies. Examples are fugacity models (Campfens & Mackay 1997) or mass balance models based on the octanol/water partition coefficient (Arnot & Gobas 2003). Available models for derivation of the uptake rate constant were assessed by Brooke et al. (2012). However, tested methods yielded imprecise data and are therefore only suitable for derivation of BCF values from feeding data.

The principle of lipophilicity-induced bioaccumultation applies to most neutral organic substances. However, for several groups of substances, bioaccumulation is not determined by lipophilicity. For example poly- and perfluorinated carbonic acids and silicone oils show equally repellent effects on water as non-polar solvents. However, amphiphilic substances like surface-active substances are characterized by their affinity for water and organic phases and therefore accumulate primarily in boundary layers. Thus, application of log K_{ow} for prediction of the bioaccumulation potential may not generally be appropriate. Instead, specific interactions with e.g. proteins may have a significant influence on accumulation of these substances. Kelly et al. 2009 described a concept for inclusion of substance/protein interactions based on the protein/water partition coefficient (log K_{PW}). As a consequence of the substances' unusual partitioning behavior, lipid-containing body fluids and tissues are not their preferred compartments. Possible accumulation of substances in specific target organs may lead to higher tissue concentrations resulting in toxic effects in affected organs. The assessment of chemicals based on consideration of lipophilicity only may result in an incomplete picture concerning the

bioaccumulation behavior of substances in humans and the environment. Hence, specific non-lipidbased bioaccumulation processes in aquatic organisms should be incorporated into substance evaluation.

In toxicology and pharmacology, computer based modeling has developed into an essential tool for successful quantification and extrapolation of toxicokinetics in organisms (Barton et al. 2007; Bois et al. 2011; Jager et al. 2011). Computer models do not only provide a time- and cost efficient simulation of chemical absorption, distribution and elimination over time, but also bring ethical advantages in the reduction and refinement of animal use (Badyal et al. 2009; Stadnicka et al. 2012).

In general, two toxicokinetic model groups can be distinguished; the one-compartment model assuming a homogenous distribution of a chemical in biota, and the multi-compartment model in which accumulation in specific organs and tissues is taken into consideration (Stadnicka et al. 2012). An example of the latter is the Physiologically Based Toxicokinetic (PBTK) model, which was developed for fish by Nichols and coworkers (Nichols et al. 1990). Whereas one-compartment models are based on simple curve fitting (Barron et al. 1990), the PBTK model is considerably more detailed and complex, describing an organism with use of multiple anatomical, physiological and biochemical components in which organs and tissues are defined as individual compartments (Abbas and Hayton 1997; Nichols et al. 1990; Nichols et al. 1998). As species- and life-stage specific parameters together with physiochemical data of a compound are integrated in the model structure, the PBTK model offers the possibility to extrapolate between various species and exposure regimes (Barron et al. 1990; Nichols et al. 1998; Schmitt 2008). In the PBTK model, toxicokinetic aspects of a chemical are furthermore described by certain uptake paths (e.g. branchial, dermal, dietary or injection) followed by its circulatory distribution to the individual compartments.

The assumption underlying this study is that the PBTK model is a good representative for bioconcentration and bioaccumulation in fish allowing a correct prediction of organ distribution. As chemical partitioning in the used PBTK model is soley based on lipophilicity, non-lipid distribution should show different organ distribution patterns which in turn will result in differences in bioconcentration factors and rate constants measured according to OECD 305. Thus, the objective of this study was to highlight the feasibility of a PBTK model to predict lipid triggered bioconcentration in rainbow trout (*Oncorhynchus mykiss*) and to elucidate its potential to identify specific accumulation pattern not in line with lipophilicity. Model performance was validated with data on kinetic rates and bioconcentration factors collected from peer-reviewed literature and from measured data available from Fraunhofer IME, Schmallenberg . The comparison was done for lipophilic compounds as well as for PFAS.

2 Part I: Derivation of a model to compare the results of fish feeding to bioconcentration studies

2.1 Objectives

The aim of this workpackage was to examine mathematical correlations of kinetic BCF and their corresponding $k_{2(BCF)}$ values for substances collected from the literature. The same procedure was applied to BMF and their corresponding $k_{2(BMF)}$ values. Here, the influence of factors like α (assimilation efficiency) and physiological differences between test species had to be elucidated. Ultimately, it was intended to derive a model which defines a critical $k_{2(BCF)}$ value that corresponds to a BCF of 2000 and 5000. In case k_2 values are comparable, a critical BMF value based on $k_{2\,(BMF)}$ had to be derived. Reliability and limitations of such a model are discussed.

2.2 Material and Methods

2.2.1 Literature search and data collection and evaluation

A **literature search** for bioaccumulation data (bioconcentration and biomagnification) in fish was conducted. Different databases, e.g. google scholar, Pubmed and Web of Science, were used. The following search items were defined in varying combinations:

For bioconcentration: Bioconcentration, fish, rainbow trout, kinetic, elimination, OECD 305

For biomagnification: Biomagnification, dietary accumulation, fish, rainbow trout, kinetic, elimination

Additional literature was obtained by checking the references of the collected literature. Only kinetically derived bioconcentration and biomagnification factors were considered. The search was focused on organic test compounds and laboratory studies. The **evaluation process** was divided into two parts. In the **first part**, all collected publications on bioaccumulation studies were analyzed and evaluated with respect to the following criteria:

Accumulation in whole fish (not in specific tissues), no predicted data, freshwater fish (no marine species) and age of test animals. For biomagnification, food and feeding method were additionally regarded.

From the collected literature, separate data sets for bioconcentration and biomagnification were created in Excel containing the following parameters (Data Set 1 (unfiltered)):

- Substance group
- Substance name
- CAS
- Log k_{ow}: Experimental or calculated value (EpiSuite)
- Species
- Weight of animals
- Test concentration
- Uptake rate constant (BCF only)
- Assimilation efficiency (BMF only)
- Elimination half-life
- Depuration rate constant
- Bioconcentration/Biomagnification factor
- Fish lipid content (+ analysed fishpart in BMF)

- Reference

A separate entry for bioconcentration/biomagnification estimates of a single test substance were created when the data were derived under unequal conditions including differences in water/food concentration, species or weight of fish. Because many publications cover studies on more than one chemicals, the total number of entries is therefore much higher than the number of publications. All references were included into an Endnote file (Bioconcentration_Biomagnification.enl) which is part of this report.

In **part two**, data quality of the results presented in each reference was assessed. Ideally, publications should follow the OECD 305 test guideline (OECD 305, 2012) and show a high reliability according to Klimisch score 1or 2. All publications were evaluated with respect to the validity criteria defined in OECD 305 in which were used to establish the Klimisch system. A summary of every publication and the respective Klimisch scores are given in Annex 1(Chapter 6). Data ranked with Klimisch score 3, not reliable, were excluded from Data Set 1 resulting in the filtered Data Set 2 containing reliable data only.

Validity criteria for aqueous exposure studies according to OECD 305:

- Mortality or other adverse effects/disease was less than 10% at the end of the test
- Water temperature variation was less than ± 2°C
- Concentration of dissolved oxygen did not fall below 60% saturation
- Concentration of the test substance was maintained within ± 20% of the mean of the measured values during the uptake phase
- Concentration of the test substance was below its limit of solubility in water
- During the uptake phase a clear test item concentration plateau was reached with confidence margins (standard error) less than ± 20%

Validity criteria for dietary exposure studies according to OECD 305:

- Mortality or other adverse effects/disease was less than 10% at the end of the test
- Water temperature variation was less than ± 2°C
- Concentration of dissolved oxygen did not fall below 60% saturation
- Concentration of the test substance in fish food collectedbefore and at the end of the uptake phase was within a range of ± 20%
- Homogeneity of substance in food at test start did not vary more than ± 15% from the mean (at least three samples)
- Concentrations of test substance were detected in unspiked food or control fish tissues

For chemical investigation and modeling, data set 1 (unfiltered) was used due to limited entries regarding modeling purposes.

2.2.2 Bioconcentration data provided by the UBA

Data sets of 42 fish BCF studies carried out according to OECD 305 were provided by the UBA including data from studies on zebra fish, rainbow trout and fathead minnow. The data were used to calculate uptake and depuration rate constants as well as a kinetic and steady-state BCF value for each of the 42 test compounds.

Ratios of test item concentrations in fish (C_f) and in water (C_w) were determined for the exposure concentration in order to estimate uptake and depuration rates, and to determine the BCF at the equilibrium between uptake and elimination. The BCF was determined as steady state BCF (BCF_{ss}) and as kinetic BCF (BCF_k).

The steady-state bioconcentration factor (BCF_{ss}) was estimated by calculating the ratio of the concentration of the test substance in the fishs tissue (C_f) to the concentration of the test substance in the water (C_w) under steady state conditions. The steady state bioconcentration factor is found when the BCF does not change significantly over a prolonged period of time, under constant exposure conditions.

$$BCFss = \frac{Cf\ at\ steady\ state\ (mean)}{Cw} \qquad \text{(Equation 1)}$$

According to OECD Test Guideline 305, the elimination of accumulated test substance in fish over time is calculated by fitting the following exponential function to the concentrations measured in fish, resulting in the depuration rate constant (k_2).

$$Cf(t) = Cf(t0) * e^{-k2*t} \qquad \text{(Equation 2)}$$

where $C_f(t)$ is the concentration in fish at sampling time in % of $C_f(t0)$ and $C_f(t0)$ is the concentration in fish at start of depuration phase (=100%).

The k_2 value was used to calculate the uptake rate constant (k_1) by fitting a non-linear regression ($Cf = \frac{k1}{k2} * Cw * (1 - e^{-k2*t})$). The kinetic bioconcentration factor (BCF_k) was calculated by using the formula:

$$BCFk = \frac{k1}{k2} \qquad \text{(Equation 3)}$$

The kinetic bioconcentration parameters were calculated using Microsoft Excel 2010® and SigmaStat®.

2.2.3 Chemical investigation of BCF data

Experimental data collected from the literature and provided by the UBA were assigned to unique organic chemical structures. Data are not distributed equally to the different chemical structures, and it is thus necessary to distinguish between the **raw data set** containing all exploitable data and the **condensed data set** with one value for each chemical. In the analyses, the term data set is used for the condensed data set. Any reference to the original values is denoted as raw data set. In order to distinguish the BCF from the BMF data, the term BCF data set is applied for the condensed set.

Disagreements between reported BCF values and the ratio of k_1 and k_2 within the same study were examined. If the doubts could not be clarified, the corresponding k_1 and k_2 were both considered as invalid unless they could be justified by an agreement to other studies for the same compound. Furthermore, when more than one complete item (i.e. both k_1 and k_2 from the same study) were available, the range of obtained BCFs was inspected. Differences larger than 2 orders of magnitudes have been treated as unreliable, and the data entries with the highest probability to be erroneous were removed in these cases. If required, a further validation step was applied by comparing the results of horizontal and vertical averaging as explained below. Here, any differences larger than one order of magnitude were inspected and curated by removing the most doubtful items.

The chemical domain of the data set with regard to the chemical elements, complexity and polarity was investigated. These analyses were performed exclusively, i.e. each compound belongs to exactly one group. In case of ambiguities, unless otherwise stated explicitly, a chemical was assigned to the most complex class, which is the bottommost group in the respective legend.

To proceed with the data exploration, average values for k_1, k_2, and BCF for each chemical are required. Supposing there are no serious differences between species, this is straightforward for k_1 and k_2. However, there are basically two opportunities to obtain the BCF as the ratio of k_1 and k_2. (1) For each raw data item, the BCF can be calculated if both k_1 and k_2 are reported, and then the BCF can be averaged (horizontal averaging). (2) For each chemical, average values for k_1 and k_2 can be calculated individually (vertical averaging), and the BCF then is obtained from the ratio of these averages. Moreover, either the arithmetic mean or the geometric mean, i.e. the arithmetic mean of the logarithmic values, could be applied.

When available, steady state BCFs were compared with the kinetically derived values. Respective data were taken from the OSIRIS data set (OSIRIS 2007-2011) that is publically available within the ChemProp database (UFZ 2014). To examine the influence of lipid correction, the calculated BCF values were normalized to a lipid content of 5%, i.e. the data were multiplied by the ratio of 0.05 (lipid fraction).

2.2.4 BCF dependency on k_2 (BCF)

2.2.4.1 Theoretical background of uptake and elimination kinetics

The transport of chemicals between water and fish can be modelled by the addition of resistances for aqueous diffusion and membrane permeation (Gobas et al. 1986). Depending on the chemical, one of these processes dominates, and thus the total rates can either be membrane controlled or diffusion controlled. Hydrophobicity (modeled through K_{ow}) is the key factor determining this behavior.

Membrane permeation is expected for low hydrophobicity. Diffusion layer control is expected for higher hydrophobicity.

Regarding uptake, k_1 should depend on K_{ow} in the case of membrane control for lower hydrophobicity. In contrast, high hydrophobicity and thus diffusion control would result in a rate independent from K_{ow}. However, since diffusion coefficients are known to decrease with increasing hydrophobicity, k_1 may even decrease with increasing K_{ow}.

For the elimination, again k_2 should increase with K_{ow} for low hydrophobicity. With high hydrophobicity, k_2 is expected to inversely depend on K_{ow}.

2.2.4.2 BCF modeling

In order to compare the kinetic BCFs to modeled steady state values, several different respective model approaches with different levels of sophistication have been applied.

The mechanistic BCF model derived from the BAF approach of Arnot & Gobas (2003)

$$BCF = (1 - LB) + \frac{k1\varphi}{k2} \qquad \text{(Equation 4)}$$

with the lipid content LB taken from the experimental data and the bioavailable fraction φ set to 1 was applied to estimate the BCF. In this approach, k_1 (L/kg/d) is estimated from the octanol/water partition coefficient K_{ow} and the fish weight W in kg by

$$k_1 = \frac{1}{\left(0.01 + \frac{1}{K_{ow}}\right) \cdot W^{0.4}} \qquad \text{(Equation 5)}$$

and $k_{2(diffusion)}$ (d^{-1}) is obtained then from k_1 by

$$k_{2(\text{diffusion})} = \frac{k_1}{L_B} \cdot K_{ow} \qquad \text{(Equation 6)}$$

As a consequence of this model, both k_1 and k_2 increase with increasing K_{ow}. Note, some confusion may arise when looking into the original paper. The authors denote $k_{2(diffusion)}$ as k_2. The denominator of Equation 4 (k_2 in our nomenclature) is then obtained by adding up $k_{2(diffusion)}$ with the growth dilution rate k_G

$$k_G = 0.0005 \cdot W^{-0.2} \qquad \text{(Equation 7)}$$

and the metabolic transformation rate k_M in d^{-1}.

Several K_{ow} models were explored, but did not yield significant differences. Finally, the ChemProp consensus model (UFZ 2014) was applied, unless experimental data were available. For k_M, the ChemProp implementation of the Arnot et al. (2009) was used. Since the original model does include k_M but uses a default value of 0, also the BCF estimation without k_M was explored.

2.2.4.3 Uptake rates

The relationships of the uptake rates (k_1) to the BCF and to the hydrophobicity have been examined. In order to distinguish between membrane permeation control and diffusion control, respective hydrophobicity ranges were inspected separately. The octanol/water partition coefficient has been applied as the hydrophobicity criterion.

2.2.4.4 Models for k_1

The estimation of k_1 has been recently reviewed extensively (Brooke et al. 2012, Brooke & Crookes 2007). From a large number of reported opportunities, the authors extracted a few key techniques, based on weight (W), K_{ow}, or both of them. These models have been applied to the current data set.

The models of Thomann & Connolly (1984)

$$\ln k_1 = -0.165 \cdot \ln W + 4.88 \qquad \text{(Equation 8)}$$

and Barber (2003)

$$\ln k_1 = -0.197 \cdot \ln W + 6.098 \qquad \text{(Equation 9)}$$

are based on W (in g).

Key models employing log K_{ow} are for instance the simple models of Hawker & Connell (1985)

$$\log k_1 = 0.337 \cdot \log K_{\mathrm{ow}} - 0.373 \qquad \text{(Equation 10)}$$

Spacie & Hamelink (1982)

$$\log k_1 = 0.147 \cdot \log K_{\mathrm{ow}} + 1.98 \qquad \text{(Equation 11)}$$

and Tolls & Sijm (1995)

$$\log k_1 = 0.122 \cdot \log K_{\mathrm{ow}} + 2.192 \qquad \text{(Equation 12)}$$

together with the more complex model of Hawker & Connell (1988)

$$\log k_1 = \frac{0.04 \cdot K_{\mathrm{ow}}}{0.00142 \cdot K_{\mathrm{ow}} + 12.01} \qquad \text{(Equation 13)}$$

Finally, there is the k_1 approach taken from the Arnot & Gobas (2003) model (Equation 5) as well as a model of Barber (2003) (with W in g)

$$k_1 = 0.401 \cdot \left(\frac{1.4 \cdot W^{-0.4} \cdot K_{\mathrm{ow}}}{100 + K_{\mathrm{ow}}}\right)^{1.025} \qquad \text{(Equation 14)}$$

In addition, alternative approaches were tested. K_{ow} was replaced by the membrane/water partition coefficient K_{mw} obtained by an estimation model implemented in the in-house version of ChemProp (not publically available yet) and by the portioning between water and proteins in terms of the Human serum albumin partitioning. The latter was calculated by the LSER equation of Valko et al 2003:

$$\log \mathrm{KHSA} = -1.28 + 0.82\,\mathrm{E} + -0.36\,\mathrm{S} + 0.18\,\mathrm{A} + -1.97\,\mathrm{B^H} + 1.62\,\mathrm{V} \qquad \text{(Equation 15)}$$

with the Abraham compound descriptors, where E is the excess molar refraction, S is the dipolarity/polarisability, A is the H-bond acidity, B^H is H-bond basicity, and V is the McGowan volume.

2.2.4.5 Elimination rates

In the same manner as for k_1, the elimination rates k_2 were compared against BCF and hydrophobicity by respective plots. Furthermore, the uptake and elimination rates were plotted against each other.

2.2.4.6 Models for k_2

There are two models available to estimate k_2, the one from Equation 4 and 6, and the equation used in the OECD TG305

$$\log k_2 = -0.414 \cdot \log K_{\mathrm{ow}} + 1.47 \qquad \text{(Equation 16)}$$

In this study, the respective equation taken from the Arnot & Gobas 2003 model (eq. 6 with input from eq. 5) was tested for its applicability as an alternative approach. Since this model includes biotransformation (i.e. metabolic elimination), the latter needs to be estimated in turn. To examine its influence, the equations were applied with and without considering biotransformation. To estimate the biotransformation rates, the model of Arnot et al 2009 was used.

2.2.5 Chemical investigation of BMF data

The chemical domain of the data set with regard to the chemical elements, complexity and polarity was investigated. These analyses were performed exclusively, i.e. each compound belongs to exactly one group. In case of ambiguities, unless otherwise stated explicitly, a chemical is assigned to the most complex class, which is the bottom-most group in the respective legend.

2.2.6 Relationship of BMF to the kinetic BCF and to the respective rates

By plotting and following visual inspection, the relation of the experimental elimination rates $k_{2(BMF)}$ to BMF and to the k_2 obtained by kinetic BCF measurements was investigated, if possible. With regard to modeling of k_2, the respective TGD model (eq. 16) was inspected. Due to the limited number of experimental data, a new model could not be derived.

2.3 Results and Discussion

2.3.1 Literature search and data collection and evaluation

2.3.1.1 Bioconcentration

Step 1

After the first evaluation step, 91 publications were identified as relevant. All of them were available except for the three following: Coenen (1988), Topcuoglu & Birol (1982) (Both cited in Tolls et al. 1994) and Cook et al. (1990) (Cited in Schmieder et al. 1995). Those could not be further analyzed. Based on the available literature, a total of 705 entries were included in Data Set 1.

Several publications were excluded during Step 1 due to the following reasons: Bioconcentration was not investigated in whole fish but in specific tissues like muscle or liver (e.g. Petersen et al. 1985, Goodrich et al. 1991, Tsuda et al. 1990a, Tsuda et al. 1989a, Wu et al. 2001), bioaccumulation in fish was predicted using an *in vitro* extrapolation model (e.g. Dyer et al. 2008), bioconcentration was modeled without acquiring experimental data (e.g. Gobas et al. 1986, Brooke et al. 2012), marine instead of freshwater species were used as test organisms (e.g. Álvarez-Muñoz et al. 2007, Tollefsen et al. 1998, Shaw & Connell 1987) and early life stages of fish rather than juvenile or adult animals were chosen for testing (e.g. Galassi & Calamari 1983).

Kinetic bioconcentration data should, as announced in the proposal, preferably be taken from studies on frequently used species such as rainbow trout, *Oncorhynchus mykiss*. Unfortunately, only a limited number of studies was available, so other species were considered as well. Some species are recommended by the OECD 305, others are not. An overview of test species used in the collected publications is given in the following:

- *Oncorhynchus mykiss*
- *Pimephales promelas*
- *Lepomis macrochirus*
- *Poecilia reticulata*
- *Danio rerio*
- *Gasterosteus aculeatus*
- *Cyprinus carpio*

- *Oryzias latipes*

as well as

- *Carassius auratus*
- *Ictalurus punctatus*
- *Gambusia holbrooki*
- *Gambusia affinis*
- *Leuciscus idus melanotus*
- *Hyphessobrycon bifasciatus*
- *Aristichthys nobilis*
- *Salmo salar*
- *Jordanella floridae*
- *Gnathopogon caerulescens*
- *Proterorhinus marmoratus*

The first part of the listing above mentions species that are recommended by the OECD 305 guideline, all freshwater species suggested by the guideline can be found in the listing. Additionally, 11 other species were used for testing due to different reasons. Studies on adult individuals of Atlantic salmon, *Salmo salar*, live in marine waters and were not considered as mentioned above however; studies using juveniles were included due to living in freshwater habitat (e.g. Zitko 1980).

Step 2

Publications identified as relevant were compared to validity criteria according to OECD 305 test guideline in the next step. Only 8 publications followed the 305 guideline as noted by the authors. All other studies did not refer to any guideline which underlines the necessity for evaluation of data quality.

A prime example of a study illustrating high reliability is Memmert et al. (2013). Reasons are choice of an OECD 305 recommended species (*Oncorhynchus mykiss*), fulfillment of all validity criteria according to the guideline and results are presented in detail including a graph showing uptake and depuration of the test substance in fish and calculated curves for the complete study period.

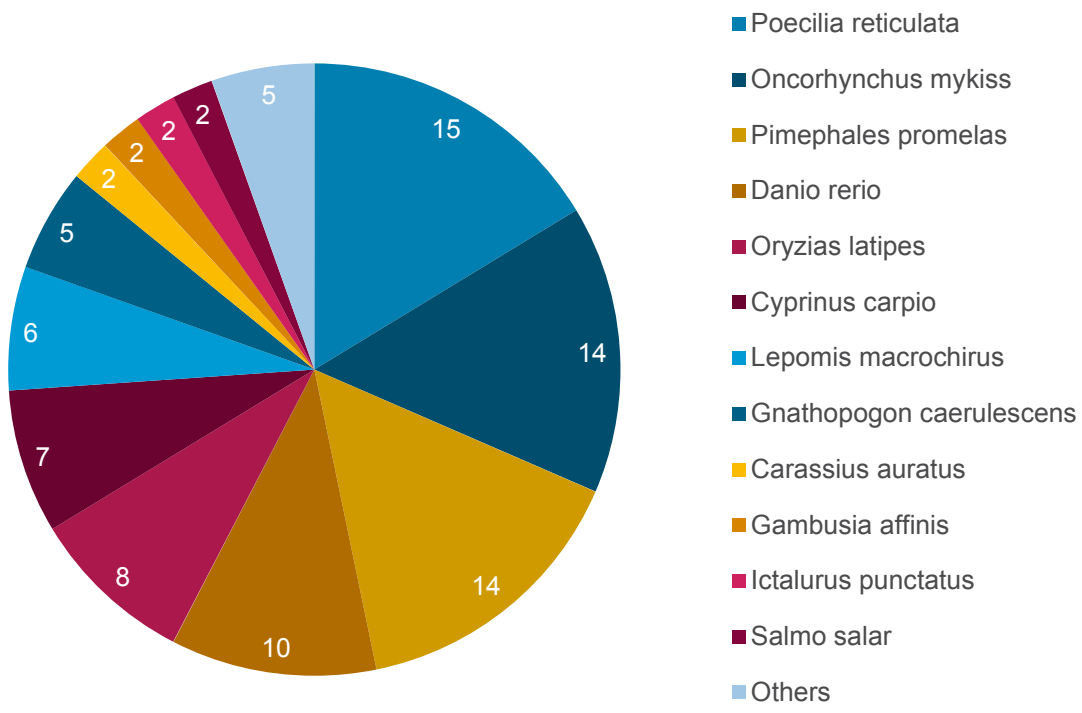
In total, 81 publications were classified as relevant after step 2. They account for 655 entries in Data Set 2. An overview of the data set is given in the following, highlighting different aspects of the collected literature and giving more detailed information on the exclusion criteria applied.

2.3.1.1.1.1 Test species

A listing of species used in the unfiltered data set (Data Set 1) is given below. The total number of fish species used in the reliable data set is 18, including all of the 8 species recommended by OECD 305 test guideline. Only data obtained by one species, the yellow tetra *Hyphessobrycon bifasciatus,* cannot be found in Data Set 2. Specific species were chosen to investigate differences in bioaccumulation of different fish species (e.g. Ensenbach & Nagel 1991) or due to regional interest (e.g. Hoang et al. 2011, Tsuda et al. 1988, Tsuda et al. 1989b, Tsuda et al. 1990b, Tsuda et al. 1992a, Tsuda et al. 1992b). Bradford et al. (2006) stated specific reasons for their choice of eastern mosquitofish, *Gambusia holbrooki*. These include the wide distribution of this species in the United States, its ecological importance, the large amount of available literature and the fact that field studies demonstrated uptake of the test substance from environmental media. The distribution of species used in the collected literature on bioconcentration is represented in Figure 1 and Figure 2. With respect to the number of publications, the guppy *Poecilia reticulata*, the rainbow trout *Oncorhynchus*

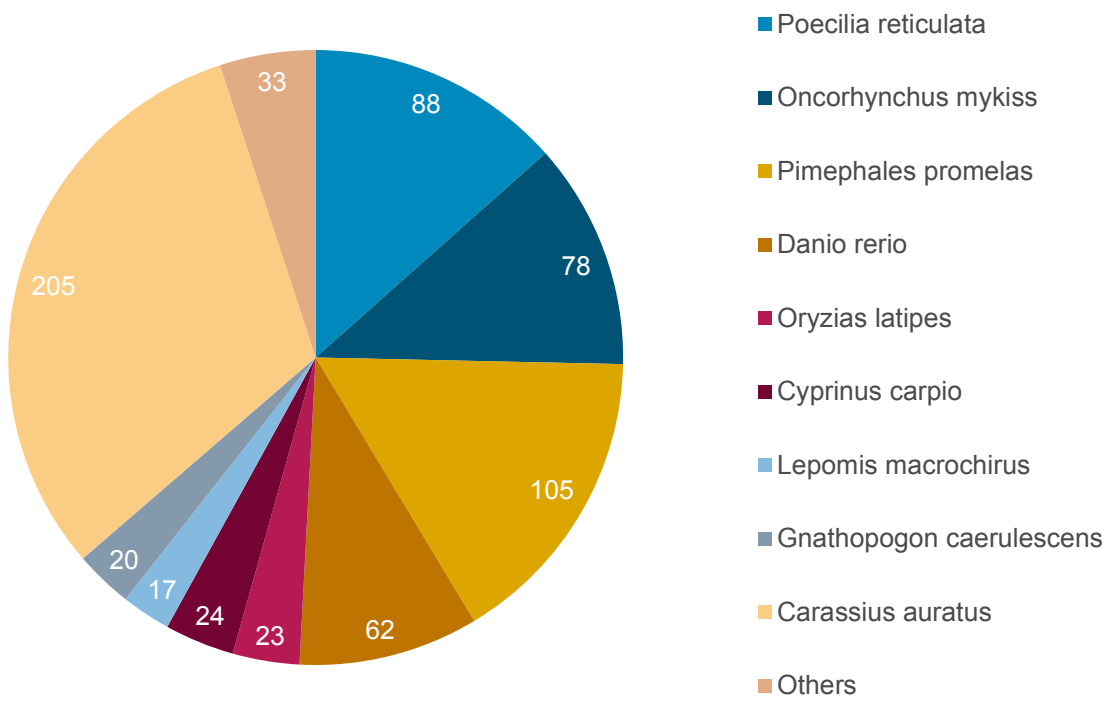
mykiss, the fathead minnow *Pimephales promelas* and the zebrafish *Danio rerio* were used most frequently (Figure 1). Some studies included more than one species (e.g. Muir et al. 1983, Muir et al. 1985, Muir et al. 1986, Keizer et al. 1991, Sijm et al. 1993a, Ensenbach et al. 1996), leading to a higher number of publications per species in Figure 1.

Figure 1: Number of bioconcentration publications per fish species



Species used only once (*Aristichthys nobilis, Gambusia holbrooki, Gasterosteus aculeatus, Jordanella floridae, Leuciscus idus melanotus*) were not included in the graph.

Figure 2: Number of bioconcentration data set entries per fish species



Species with less than 10 data set entries (*Gambusia affinis, Ictalurus punctatus, Salmo salar, Aristichthys nobilis, Gambusia holbrooki, Gasterosteus aculeatus, Jordanella floridae, Leuciscus idus melanotus*) were not included in the graph.

Comparing both graphs, the distribution patterns reflect the high number of entries for the guppy, rainbow trout, fathead minnow and zebrafish. However, the high number of data set entries for the goldfish, *Carassius auratus*, originates only from two publications (Al-Ansari et al. 2013, Sijm et al. 1993b), whereof 204 of the 205 entries were extracted from Sijm et al. (1993b).

According to the guideline, the selection of fish species should consider the ready availability, the obtainment in convenient sizes and the maintainability in the laboratory. Additionally, other criteria like regional and ecological importance etc. are stated. Other species can be used for bioconcentration testing as long as the test conditions are adapted to the selected fish species to provide suitable test conditions and the rationale and method for the chosen species is reported. Therefore, selection of a fish species not recommended by the guideline does not reduce the quality of a publication, which should be kept in mind with respect to the classification of publications according to the Klimisch system.

2.3.1.1.1.2 Calculations

Most studies followed the kinetic approach describing uptake and depuration (first order kinetics, one compartment) as recommended by the OECD 305 test guideline (see Equation 17).

$$\frac{dCf}{dt} = k1Cw - k2Cf \quad \text{(Equation 17)}$$

C_f represents the concentration in fish, C_w the concentration of test substance in water and k_1 and k_2 represent the uptake and depuration rate constants, respectively. The kinetic bioconcentration factor is subsequently calculated using Equation 18.

$$BCFk = \frac{k1}{k2} \quad \text{(Equation 18)}$$

Many publications, especially the ones older than 1993, refer to the BIOFAC program, based on the publication of Blau et al. (1975), Ecokinetics: a Study of Kinetics of Ecological Systems. This program is based on the same equations included in OECD 305 test guideline.

In some cases, e.g. Ensenbach et al. (1996), a one-compartment model was not sufficient to describe elimination kinetics. In this case, data were fitted to a two-compartment model (Equation 3). The possible need for more complex models than the one described above is also outlined in the 305 test guideline.

$$Cf(t) = A * e^{-k(a)*t} + B * e^{-k(b)*t} \qquad \text{(Equation 19)}$$

where the constants A, B reflect the sizes of the two compartments expressed as percent of C_f at t=0 and k(a) and k(b) the corresponding elimination rate constants. In case of a biphasic elimination the uptake rate constant k01 of xenobiotics was calculated by

$$k01 = \big(A * k(a) + B * k(b)\big) * BCF/(A + B) \quad \text{(Equation 20)}$$

Alternatively, Andreu-Sánchez et al. (2012) applied a more realistic model that includes the dissipation rate k3, which describes the disappearance of the test substance in water through physical, chemical and biological processes. The water concentrations of the test item can be described by $Cw(t) = Cw0 * e^{-k3t}$ with Cw0 representing the initial water concentration of the test substance in water. Concentrations of the test substance in fish were calculated according to Equation 21.

$$Cf(t) = \frac{Cw0k1(e^{-k3t} - e^{-k2t})}{k2 - k3} \qquad \text{(Equation 21)}$$

Bradford et al. (2006) calculated the uptake rate constant k1 from $k1 = k2 * \frac{Css}{Cw}$ with Css being the steady-state concentration in fish tissues. The uptake rate constant could also be estimated by a tangent to the initial uptake curve (Spacie et al. 1983, Muir et al. 1983, Dewolf et al. 1993, Ownby et al. 2005) or by concentration factor increment per hour (Kikuchi et al. 1980).

In Gobas et al. (1989), elimination of test substance into the feces was considered. Equation 17 was modified according to $\frac{dCf}{dt} = k1Cw - k2Cf - keCf$ where ke is the rate constant for chemical elimination into the feces. The total elimination rate constant kt (k_2+k_e) was derived from the slope of the log C_f versus time plot. No separate value of ke was calculated.

A modification of the simple one compartment, first order equilibrium model was described by Keizer et al. (1991). It takes the effects of metabolism into account assuming an elimination rate of the parent compound made up of two components, a physical excretion rate (passive diffusional processes) and a metabolic elimination rate (enzymatic processes within the organism). It was also assumed that the production of the major metabolite represented the total metabolism of the parent compound, and was linearly dependent on the concentration of parent compound in the fish. Bioconcentration and metabolism were described by $\frac{dCf(p)}{dt} = k1Cw - k2(p)Cf(p) - \frac{Vmax}{km} * Cf(p)$ and $\frac{dCf(p)}{dt} = \frac{Vmax}{km} * Cf(p) - k2(m) * Cf(m)$ with the assumption that the uptake of the metabolite from water is negligible. Meanings of abbreviations are: $C_{f(p)}$= concentration of parent compound in fish, k_2(p)= rate constant for excretion of parent compound, V_{max}= maximal velocity of the metabolism producing metabolite 1, km= Michaelis constant for parent compound, $k_{2(m)}$= rate constant for excretion of metabolite 1, $C_{f(m)}$= concentration of metabolite 1 in fish. Metabolism of the parent compound was also considered in Opperhuizen & Voors (1987a), Southworth et al. (1980) and Southworth et al. (1981).

In static exposure systems, concentrations in water are often not constant. In this case, Linder et al. (1985) circumvented this problem by expressing the water concentration as $\frac{dCf}{dt} = -\frac{Mw}{Mf}\frac{dCw}{dt}$ where Mf is the mass of fish and Mw is the mass of exposure water. When substituting in Equation 17 the uptake and depuration constants can be calculated from the resulting equation:

$\frac{dCw}{dt} = \frac{Mf}{Mw}(k2Cf - k1Cw)$.

Bioconcentration parameters calculated by using different methods are not comparable to those estimated according to the guideline. In consequence, those publications were classified as additional information. The single exception is Tolls et al. (1997), where k1 was estimated from the linear part of the uptake curve, but all the other validity criteria were satisfied.

2.3.1.1.1.3 Flow-through, semi-static, static

The exposure method recommended in the OECD guideline for bioconcentration experiments is a flow-through system, although semi-static systems are also permissible, provided that the validity criteria are satisfied (OECD 305, 2012). In this context the concentration in water has to be maintained within 20 % of the mean of the measured value during the uptake phase. An overview of the different test procedures applied in the collected literature is given in Table 1.

Table 1: Test procedures used in bioconcentration studies

Exposure method	Number of studies	Thereof ± 20% during uptake
Flow-through	51	26
Semi-static	8	3
Static	13	3
Static using Chromosorb	6	1
Not specified	3	Not specified

In most studies flow-through systems were used. More than 50% of the studies concentration of the test substance could be maintained within the mean of the measured value during the uptake phase and therefore met the validity criterion.

Generally, all exposure systems were acceptable as long as stable water concentrations could be maintained. However, usage of semi-static or static exposure methods reduced the percentage of studies fulfilling this criterion.

2.3.1.1.1.4 Validity criteria

Further validity criteria for bioconcentration studies included low mortality, stable test item concentrations in water and reaching a clear plateau of tissue concentrations during uptake phase. Publications that reported mortalities >10% were excluded during step 2 (e.g. Adams et al. 1986, Branson et al. 1985, Bruggeman et al. 1984, Gobas et al. 1989 Experiment I, Opperhuizen et al. 1985). Additionally, publications were excluded where test item's concentration in water were not analyzed (e.g. Toledo & Jonsson 1992, Jonsson & Toledo 1993) or fish material (Banerjee et al. 1984).

2.3.1.1.1.5 Klimisch Code

To evaluate data, publications were ranked within the Klimisch system: Score 1 is reliable without restriction, Score 2 reliable with restriction, Score 3 not reliable and Score 4 not assignable (Annex 1; Table 4). Classification and rationale for individual papers can be found in Annex 1 (Chapter 6.1 and 6.2).

For classification, validity criteria were regarded (stability of test substance, mortality of fish, reaching ofplateau). If a validity criterion, e.g. reaching a clear plateau, was not fulfilled in a study, the criterion was assessed as not fulfilled. Acceptable water quality parameters included temperature variation during the test within ± 2°C and oxygen saturation above 60% throughout the study. For every species, the recommended temperature range was considered. Slight deviations did not reduce the quality of the paper [e.g. Deneer 1994, Kimerle et al. (1981), Könemann & van Leeuwen (1980)].

Figure 3: Number of publications on bioconcentration per Klimisch Code

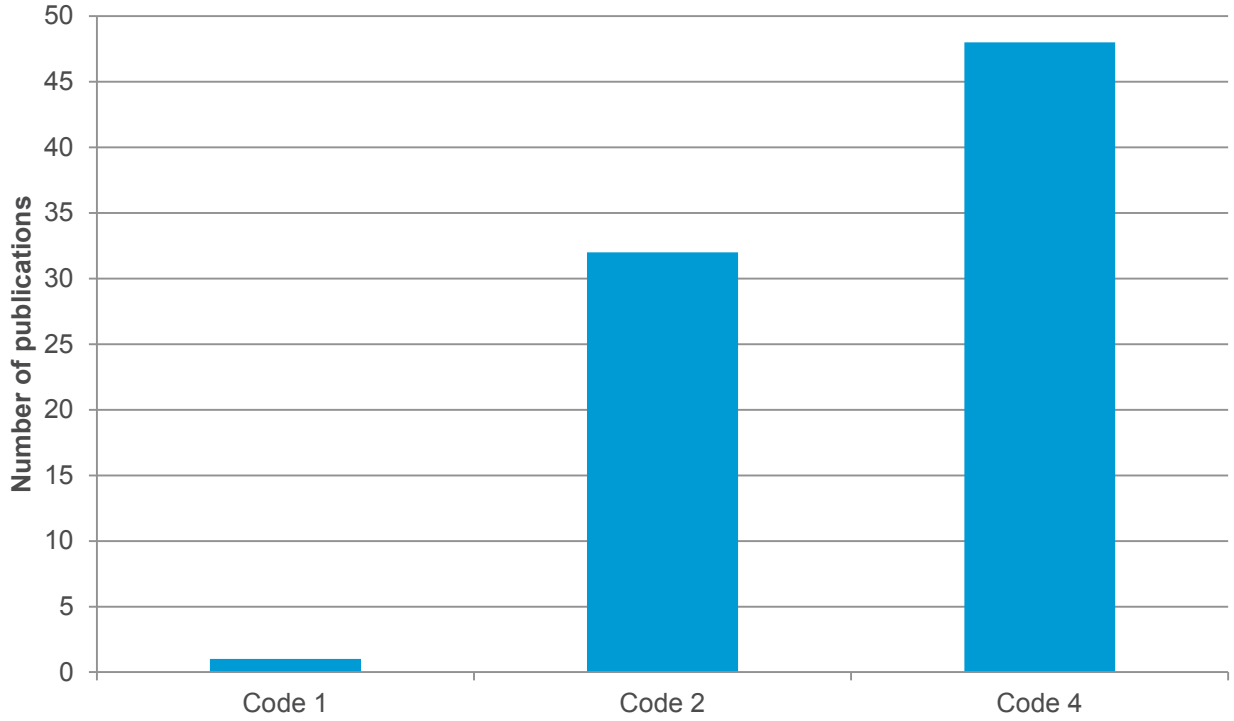
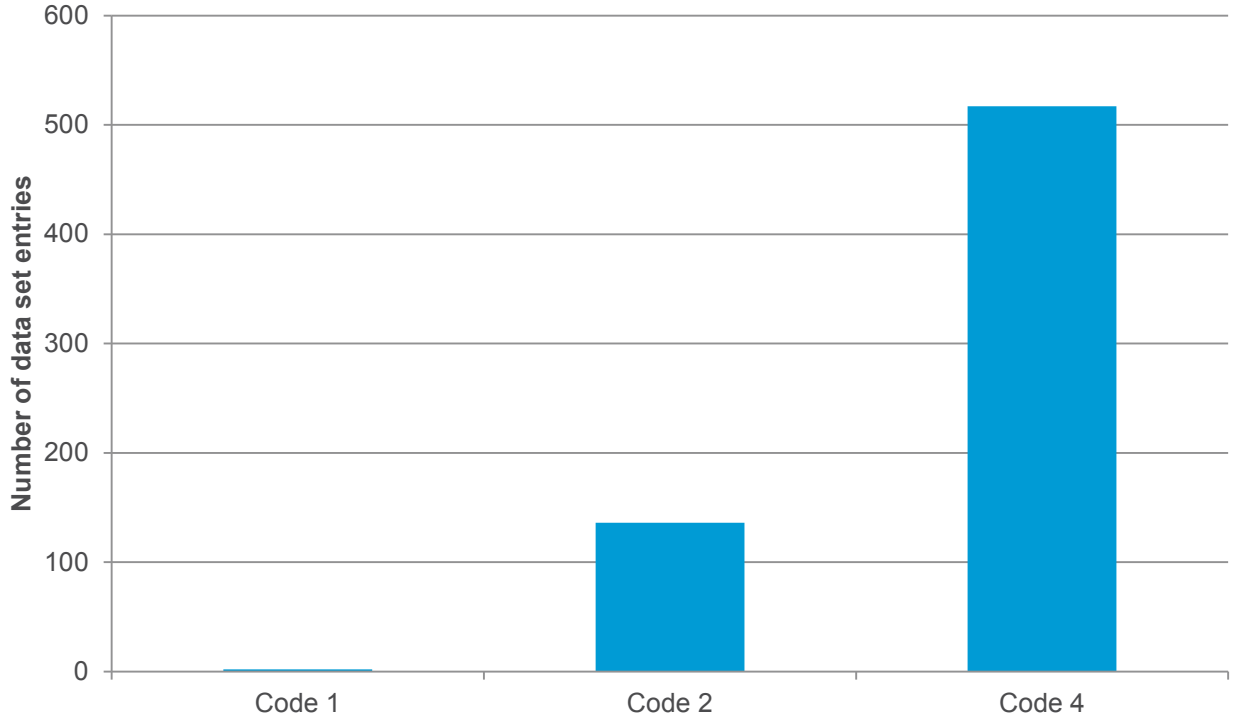


Figure 4: Number of bioconcentration data set entries per Klimisch Code



About 60% of the 81 publications identified as relevant fall into Klimisch Code 4, not assignable (Figure 3). About 40% are categorized as Code 2, reliable with restrictions, and only 1 publication is reliable without any restrictions or Klimisch Code 1. Regarding the number of database entries per Klimisch Code, almost 80% of entries are classified as Klimisch Code 4 and 20% as reliable with restrictions or Klimisch Code 2 (Figure 4). Only 2 out of 655 data set entries are reliable without restrictions (Klimisch Code 1).

Klimisch Code 4 was applied to publications, when water concentrations of the test substance during the uptake phase decreased significantly. This was found in several papers that selected static exposure systems (e.g. Andreu-Sánchez et al. 2012, De Voogt et al. 1991, McLeese et al. 1981 etc.), but this could be also observed using semi-static (e.g. Linder et al. 1985, Southworth et al. 1981) or flow-through (e.g. Muir et al. 1986) tests. If variation of the test substance during the uptake phase was greater than 20%, no information on stability is available and other validity criteria are not met or information of them is missing, papers were classified as not assignable (Burke et al. 1991, Butte et al. 1991, Martin et al. 2003a etc.). In case stable concentrations of the test substance in water during the uptake phase were reported and no information on mortality was available, papers that did not meet the other validity criterion (reaching a plateau) were classified as Klimisch 4 (e.g. Bradbury et al. 1993). Some did reach a plateau during uptake, but applied an alternative method to calculate kinetic parameters, those were not reliable as well (e.g. Bradford et al. 2006).

Klimisch Code 2 was assigned to publications missing different aspects stated by the guideline. For instance, in some studies, the stability of the test substance during the uptake phase exceeded the variation of 20% stated by the guideline. Those publications were classified as relevant with restriction as long as all the other validity criteria were fulfilled (Paterson & Metcalfe 2008, Tsuda et al. 1988 etc.). Many papers reported stable water concentrations during uptake, but did not offer information on the mortality of fish during the experiment, therefore no classification as Klimisch Code 1 was possible. If the last validity criterion (reaching a plateau) was fulfilled, the paper was ranked as Klimisch Code 2 (e.g. Görge & Nagel 1990, Seo et al. 2002). This applied also to studies where test species not recommended by the test guideline were used, no information on mortality of fish is given but the validity criteria were principally fulfilled (e.g. Ensenbach et al. 1996, Hoang et al. 2011). Al-Ansari et al. (2013), Kalsch et al. (1991) and Smith et al. (1980) offered information on all validity criteria except for mortality and water quality criteria (oxygen saturation), they were classified as Klimisch 2 as well. In case, both, stability of the test substance and mortality of fish were satisfied, publications were ranked as Klimisch Code 2: Tolls et al. (1997) used alternative calculation methods (details above) and others (e.g. Belden et al. 2005) did not reach a plateau during uptake.

Publications categorized as reliable without restrictions (Klimisch Code 1) met all validity criteria and other instructions recommended by the guideline, e.g. feeding of fish during the experiment, running a control in parallel to treatment in order to elucidate possible effects not caused by the test substance, selecting the number of fish per sampling date adequately high to ensure proper curve fitting and usage of fish in recommended weight range. Only Memmert et al. (2013) satisfied all criteria. Examples for publications meeting all validity criteria but still not classified as Klimisch 1 are mentioned in the following: Debruijn & Hermens (1991) was not assigned to Klimisch Code 1, because no control was run in parallel to treatments and 3 fish were taken per sampling date, the guideline however recommends at least four. A control was also missing in Tolls & Sijm (1999), therefore it was not characterized as reliable without restrictions. Call et al. (1980) did use too small individuals of the fathead minnow for bioconcentration testing.

2.3.1.2 Biomagnification

Step 1

In step 1, 27 publications were identified as relevant including the OECD ring test for the dietary exposure bioaccumulation fish test (OECD 2012). Based on those papers, 464 entries were included in Data Set 1.

During the evaluation process, the following criteria prevented data from being included in the data set: Biomagnification in specific tissues, not in whole fish was investigated (Zeng et al. 2014, De Silva et al. 2009, Amlund et al. 2006, Gemmill et al. 2011, Sanborn et al. 1977, Burreau et al. 2000, Lazartigues et al. 2011, Lazartigues et al. 2013, Jones et al. 2001, Law et al. 2006, Tomy et al. 2007, Van Veld et al. 1984), modeling of biomagnification without the acquisition of experimental data (Berntssen et al. 2013, Berntssen et al. 2011, Clark et al. 1990, Thomann 1989, Mackay & Hughes 1984) and using marine organisms (Stehlik & Merriner 1983, Zhang et al. 2011, Serrano et al. 2003, Berntssen et al. 2008). Additionally, the experimental diet and feeding of test organisms were evaluated. Studies using the following approaches were excluded: Spiking of other organisms as a food source for test animals (Dabrowska et al. 1996, Andersson et al. 2001, Burreau et al. 1997), injecting the test compound intraperitoneally (Seubert & Kennedy 2000, Norheim & Roald 1985, Coristine et al. 1996, Nagel & Urich 1980), administering a single oral dose via a capsule directly into the stomach (Niimi & Oliver 1983, Niimi & Oliver 1986, Niimi & Oliver 1988, Niimi 1986) and feeding only a single oral dose at the beginning of the experiment (Smith et al. 1980).

As mentioned in the bioconcentration paragraph, the preferred fish species for bioconcentration and biomagnification testing is the rainbow trout, *Oncorhynchus mykiss*. However other species of fish were incorporated in the data set as well. A list of species besides *Oncorhynchus mykiss* is given in the following:

- *Pimephales promelas*
- *Poecilia reticulata*
- *Danio rerio*
- *Cyprinus carpio*

as well as

- *Coregonus clupeaformis*
- *Ictalurus punctatus*
- *Perca flavescens*
- *Salmo salar*
- *Salvenlinus namaycush*

The same list arrangement as in the bioconcentration paragraph was maintained, displaying OECD 305 recommended species in the first part of the list and others in the second part. In total, 10 species were used in the collected dietary accumulation studies. In contrast to bioconcentration testing, biomagnification studies were conducted using only a limited number of species.

Step 2

An evaluation of Data Set 1 with regard to the OECD 305- validity criteria was undertaken. Three studies were conducted more or less according to the 305 guideline as noted by the author (Goeritz et al. 2013, OECD 2012, Woodburn et al. 2013). The limited number of guideline-compliant studies was expected due to recent revision of the guideline in 2012. Therefore, validity criteria for dietary accumulation tests could not be accessed in numerous publications because of missing information.

A prime example of a reliable study is Goeritz et al. (2013). Reasons are: Choice of an OECD 305 recommended species (*Oncorhynchus mykiss*), fulfillment of all validity criteria and detailed presentation of results including graphs visualizing uptake and depuration of test items in fish.

The number of relevant publications was reduced to 26 after step 2. Data Set 2 contains 453 entries. The only excluded publication was Sijm et al. (1992) due to high mortality of fish during the test. Additionally, values from Fisk et al. (1997) were excluded because of the same reason.

An overview of Data Set 2 is given in the following, highlighting different aspects of the collected literature.

2.3.1.2.1.1 Test species

A listing of species used in the unfiltered data set is given above. The only species that cannot be found in the filtered data set is the lake whitefish, *Coregonus clupeaformis*. The total number of species used in dietary accumulation testing is 9, including 5 of the recommended species. The distribution of species based on publications and data set entries is shown in Figure 5 and Figure 6. Unlike the bioconcentration studies, in more than half of the publications the rainbow trout was used as the selected test species (Figure 5). Based on database entries, more than 75% of the studies were carried out with rainbow trout *Oncorhynchus mykiss* followed by the lake trout *Salvelinus namaycush* (6%) and the common carp *Cyprinus carpio* (5%).

As mentioned in the bioconcentration paragraph, using a species not recommended by the guideline for biomagnification testing does not necessarily reduce the quality of a publication.

Figure 5: Number of publications on biomagnification per fish species

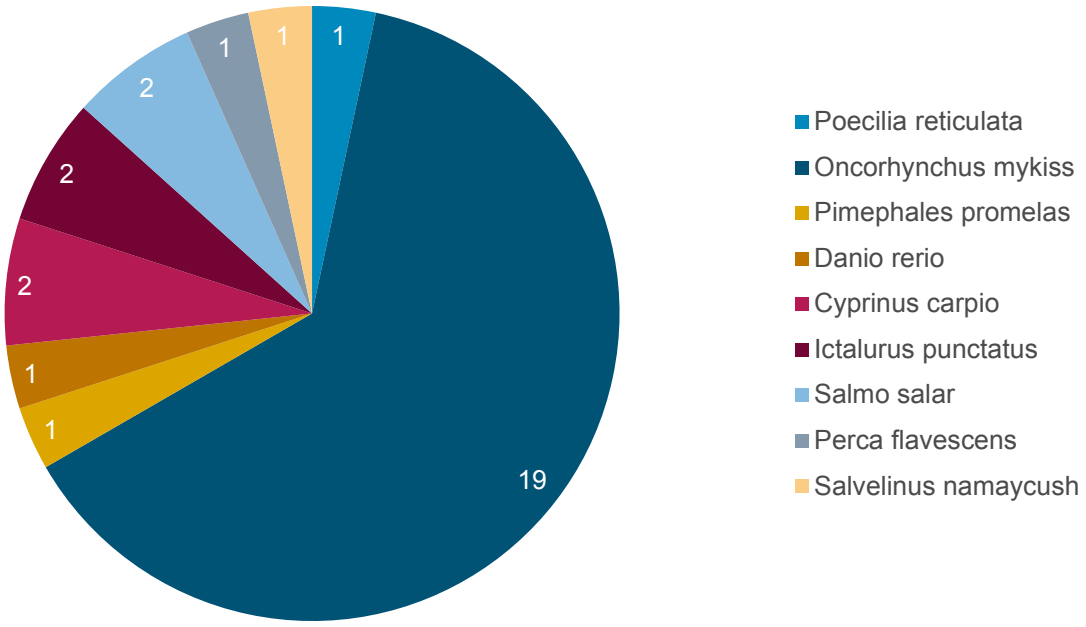
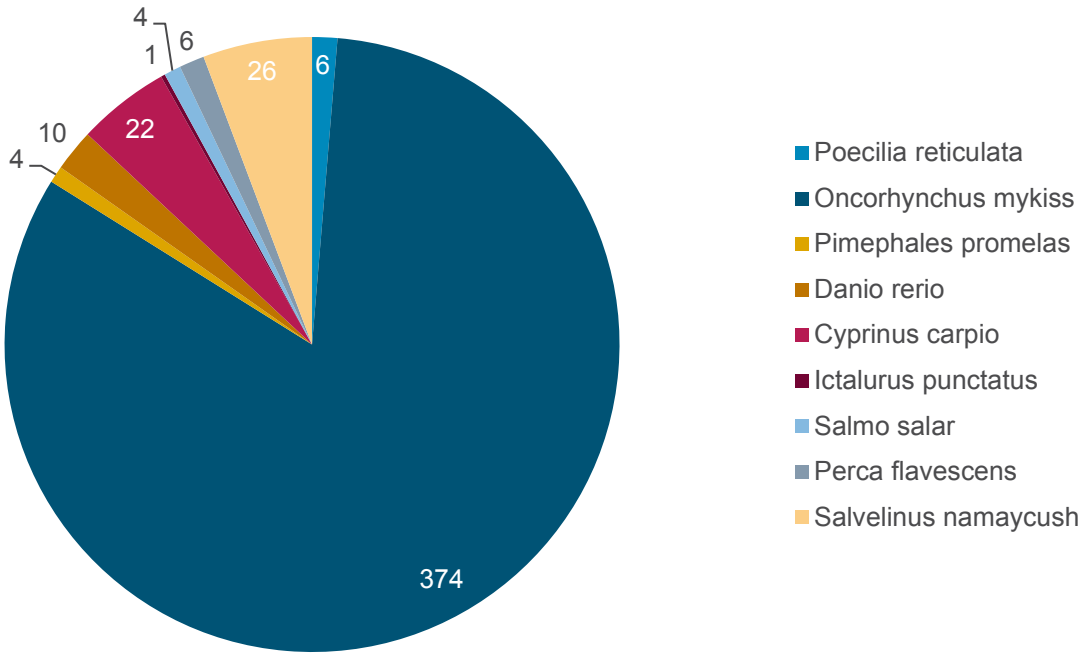


Figure 6: Number of biomagnification data set entries per fish species



2.3.1.2.1.2 Calculations

According to OECD 305 test guideline, the chemical assimilation efficiency α is the absorption of the test substance across the gut and is calculated by

$$\propto = \frac{C0,d * k2}{I * Cfood} \frac{1}{1 - e^{-k2t}} \qquad \text{(Equation 22)}$$

where C0,d is the derived concentration in fish at time zero of the depuration phase, k_2 is the overall depuration rate constant, I is the food ingestion rate constant, Cfood is the concentration in food and t is the duration of the feeding period. k_2 is calculated by plotting ln (concentration) versus time and performing a linear regression. The slope is an estimate of the depuration rate constant. The biomagnification factor, BMF, can subsequently be calculated according to

$$BMF = \frac{I * \propto}{k2} \qquad \text{(Equation 23)}$$

In most publications, equations mentioned above were used, however deviations concerning the calculation of α could be observed. Bruggeman et al. (1984) calculated α using k_2 and measured fish concentrations. Dabrowska et al. (1999) determined the assimilation efficiency from the slope of the linear relationship between the test item's concentration in fish and dietary exposure time, and the amount of test item in the food. Alternatively, Stapleton et al. (2004) calculated α using equation 24:

$$\propto = \frac{body\ burden\ in\ fish\ at\ time\ t}{cumulative\ mass\ exposed\ to\ fish\ upt\ to\ time\ t} \qquad \text{(Equation 24).}$$

In this study calculation of biomagnification was also deviant from the guideline with $BMF = \frac{(\frac{U}{F})}{k}$ where U is the net rate of assimilation calculated from the slope of concentration versus time, F is the concentration in food and k is the depuration rate.

Tomy et al. (2004) as well as Tomy et al. (2008) used the equation 25 for calculation of the assimilation effidiency. In both publications, the BMF was calculated according to OECD test guideline 305.

$$\propto = \frac{(control-corrected\ concentration\ in\ fish)*(mass\ of\ fish)}{(control\ concentrated\ in\ food)*(mass\ of\ eaten\ food)} \qquad \text{(Equation 25)}$$

Woodburn et al. (2008) included the change in fish size in Equation 17 (bioconcentration) for calculation of the uptake rate constant, k_1, leading to $Cfish = Cfood * k1 * \frac{1-e^{-((k2+kg)*t)}}{(k2+kg)}$ where kg is the fish growth rate constant. Subsequently, the assimilation efficiency α can be calculated from k1 divided by the feeding rate. For derivation of the biomagnification factor, the uptake rate constant was divided by the elimination rate constant, both solved from the equation mentioned above. Growth dilution was considered following the OECD test guideline by correcting k_2 for growth. Therefore, the growth rate constant kg was calculated from fish weight data and subtracted from the depuration rate constant. The growth-corrected depuration rate constant (k_{2g}) is used in Equation 23. In fast growing fish, additionally the feeding rate I can be corrected for growth.

2.3.1.2.1.3 Flow-through, semi-static, static

Flow-through conditions are recommended by the OECD test guideline to limit potential exposure of test item via water due to leaching of the substance from spiked food or faeces. Most studies (19 out of 26 publications) applied a flow-through system. Alternatively, recirculation and carbon-filtration to remove any contaminant residues in water was chosen as an exposure system (Konwick et al. 2006a, Konwick et al. 2006b, Bruggeman et al. 1984). 3 publications offered no information on their employed conditions.

2.3.1.2.1.4 Validity criteria

Several validity criteria according to the guideline could not be evaluated due to missing information. This includes homogeneity of substance in food. 10 out of 26 studies demonstrated homogenous spiking of food with the test substance at the test start. Stability of test substance in food throughout the study period was checked in 4 publications only and was found to be within 20%. Control food and fish have to be analyzed for test substance concentrations, which was done in several studies for fish or food or both.

2.3.1.2.1.5 Klimisch Code

To evaluate data, publications were ranked within the Klimisch system (Annex 1; Table 4) as described above for bioconcentration data: Score 1 - reliable without restriction, Score 2 - reliable with restriction, Score 3 - not reliable and Score 4 - not assignable (Annex 1; Table 4). Classification and rationale for individual papers can be found in Annex 1 (Chapter6.1 and 6.2).

The distribution of 26 publications identified as relevant is shown in Figure 7. As can be seen, most publications were classified as not assignable (Klimisch Code 4). Klimisch Code 4 was applied, when not enough information on the validity criteria for biomagnification tests was available which means in detail: no information on stability of the test substance during the study, homogeneity of spiked food and substance in control food and fish is available. 14 publications were classidied as Klimisch Code 4 on this basis. Other reasons were limited information on methodology (Brown et al. 2002) or measured temperature outside the range recommended by the test guideline 305 (Buckman et al. 2004). Some publications show a combination of both, limited information and missing of validity criteria (Fisk et al. 1998b, Tomy et al. 2004, Wong et al. 2002).

6 publications were assessed as reliable with restrictions (Klimisch Code 2). They do not comply totally with the test guideline and differ in weight of test animals (Goeritz et al. 2013) or calculation method of biomagnification parameters (Stapleton et al. 2004, Tomy et al. 2008, Woodburn et al. 2013). Some did not offer information on all validity criteria (Konwick et al. 2006a, Konwick et al. 2006b) and could therefore not be classified as Klimisch Code 1.

The only reliable publication (Klimisch Code 1) was the ring test for the dietary exposure fish accumulation test (OECD 2012). It followed the 305 guideline and met all validity criteria however it was not conducted to the principles of GLP (OECD 1998).

All in all, 7 publications were classified as reliable (Klimisch Code 1 or 2) corresponding to 94 out of 453 data set entries (Figure 8). The rest of the data set entries was classified as not assignable (Klimisch Code 4), corresponding to 79% of the data set entries. As mentioned before, the 305 testguideline was revised recently, which explains the lack of information on validity criteria.

Figure 7: Number of publications on biomagnification per Klimisch Code

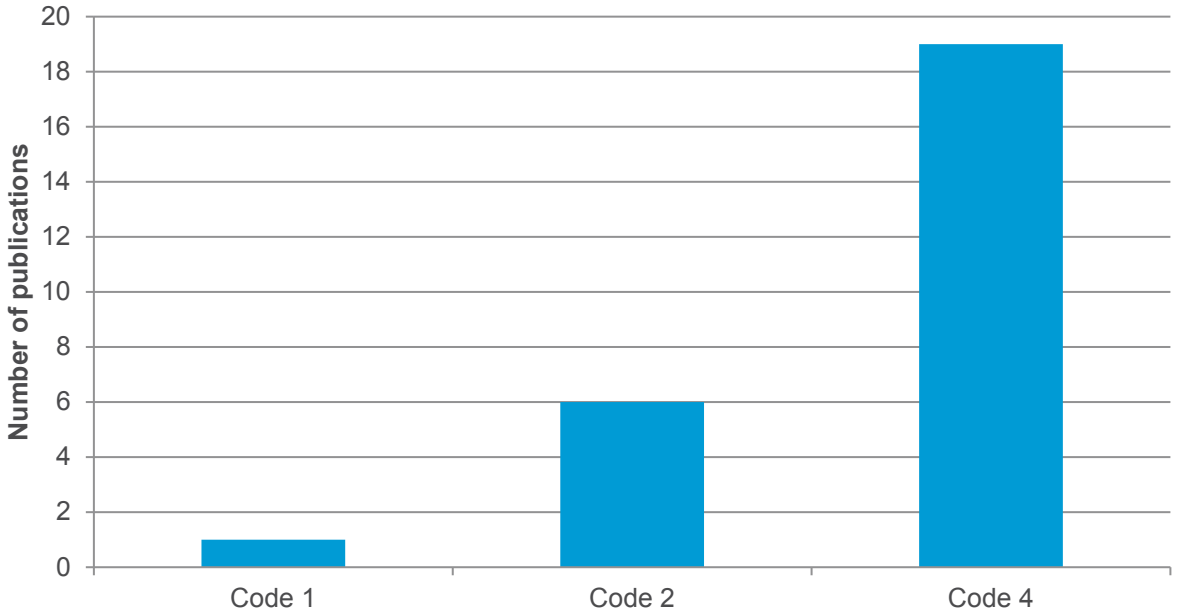
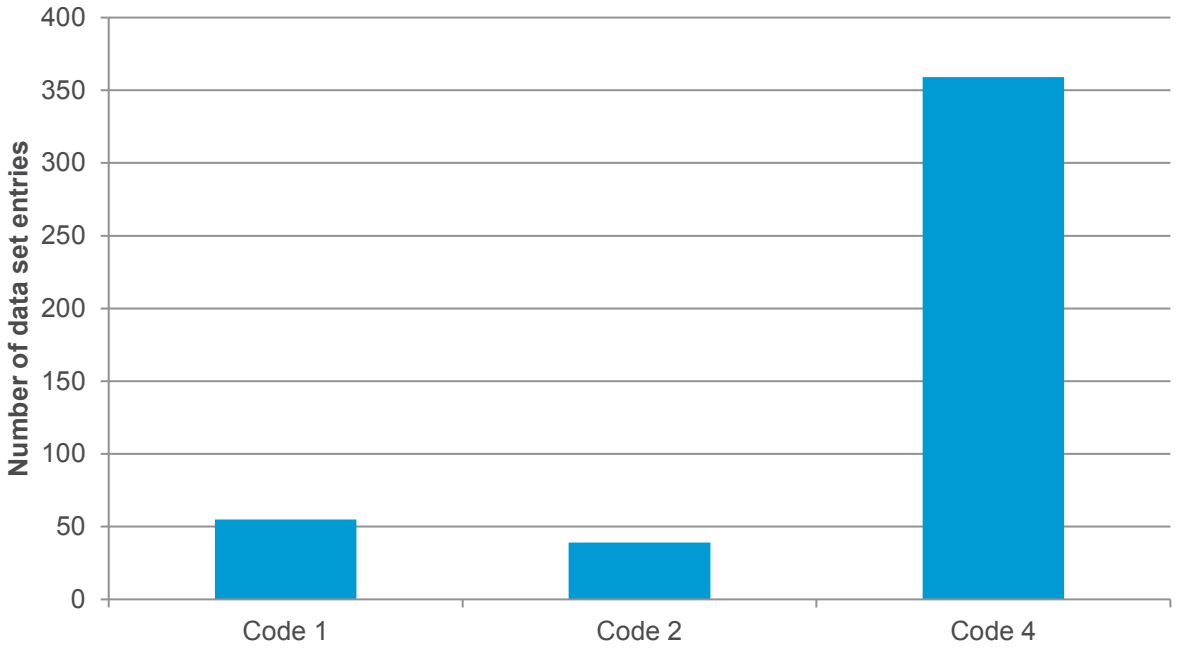


Figure 8: Number of biomagnification data set entries per Klimisch Code



2.3.2 Chemical investigation of BCF data

2.3.2.1 Compounds

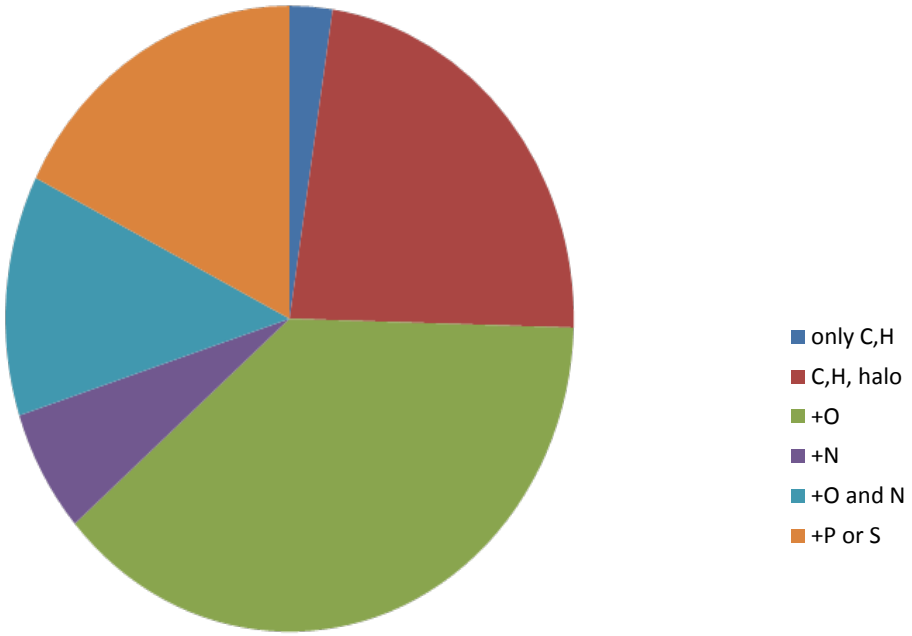
From the experimental data, 709 could be assigned to unique organic chemical structures. 1 single item for an inorganic chemical, 47 values for tensides of unknown exact composition, 66 data corresponding to UVCB (Substances of Unknown or Variable composition, Complex reaction products or Biological materi-

als) compounds, and the remaining data without any published chemical identity at all, had to be excluded from the further analyses. The 701 data correspond to 330 different individual chemical structures. Since the 709 values are not distributed equally to the 330 chemicals, it was necessary to distinguish between the raw data set with respect to the number of 709 exploitable raw data, and the condensed data set with one value of each type corresponding to the 330 chemicals.

2.3.2.2 Chemical domain

The chemical domain of the data set with regard to the chemical elements, complexity and polarity (as e.g. used in Schüürmann et al 2006) is presented in Figure 9 to Figure 11.

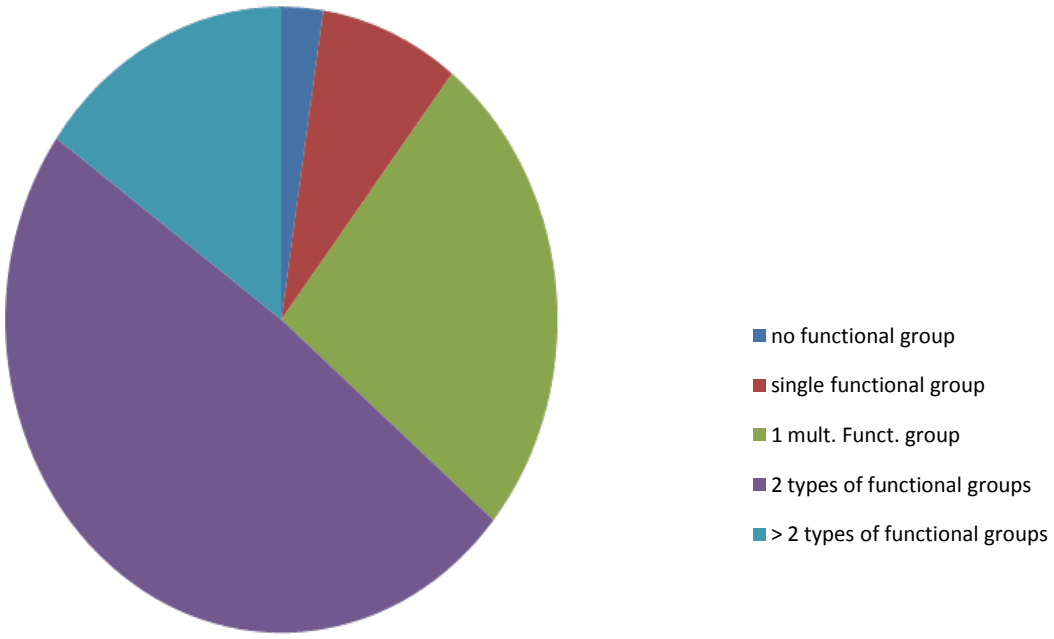
Figure 9: Chemical composition of the BCF data set.



First, Figure 9 shows the chemical composition. A large part (38%) of the compounds contains C, H, and O; followed by 23% of halogenated hydrocarbons; 18% substances with either P or S or both of them (and may include halogens, O, S); and 12.5% with C, H, O and N. Additionally, there were 6.5% substances with C, H, N and 2% hydrocarbons. Remarkably, 90% of the chemicals are aromatic compounds. This is rather different in comparison to other data sets, as e.g. available for physico-chemical properties or the large compound set of EINECS, that may be assumed to more or less represent the composition of the chemical universe in general. Such sets are typically almost balanced with a slight majority of aromatics. Particularly, there are no nonaromatic hydrocarbons in the data set.

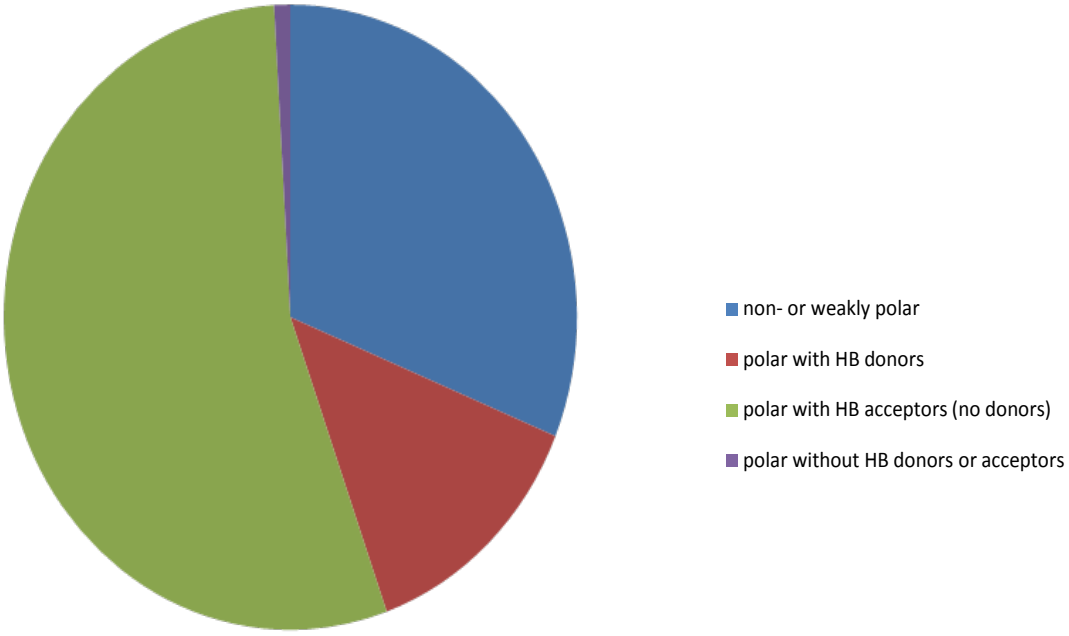
With regard to complexity (Figure 10), almost half of the chemicals (49%) contain 2 different functional groups; 26% have one functional group with multiple occurrences of it, and even 15% contain more than 2 different functional groups. The remaining 10% are less complex substances; either hydrocarbons (2%) or compounds with one single functional group (8%).

Figure 10: Complexity analysis of the BCF data set.



Looking at the polarity, the data set is dominated by hydrogen bond acceptors (55%). There are 13% hydrogen bond donors (that may also act as acceptors) and 1% polar compounds that do not participate in hydrogen bonding. The remaining 31% are non-polar or only weakly polar.

Figure 11: Polarity analysis of the BCF data set.



2.3.2.3 Averaging

After the data curation, the number of individual values for each compound ranged from 1 to 21, and the mean value was 2.1. The curated raw data correspond to experiments with 1 to 6 different species per chemical. The average was 1.3 in this case. Not in all cases k_1 and k_2 were reported, 448 individual raw k_1 data could be found for 234 chemicals, and 457 raw k_2 data for 239 substances.

The horizontal averaging restricted the number of available BCFs to the 207 compounds with respective pairs available. With the vertical approach, 209 BCF could be calculated.

Figure 12 compares the approaches in terms of log BCF obtained from arithmetic means, leaving out chemicals with only one value available. The application of the vertical approach (2) is plotted against the results of the horizontal one (1). Fortunately, there is not much difference in general. However, occasionally the horizontal approach (1) yields higher BCF, i.e. is more conservative. There are only a few cases when higher BCF results are obtained from vertical averaging (2). The total bias towards (1) is 0.44 in logarithmic units. Thus, the following procedure is recommended to combine maximum precaution with maximum applicability:

1. If individual BCF are available, their average should be used (horizontal averaging).

2. Even in case of only one individual BCF, it should be used.

3. If no individual BCF is given, but there are k_1 and k_2 for this compound, the BCF should be calculated from their average values (vertical averaging).

Figure 12: Plot of log BCF means from averaged k_1 and k_2 (y) against the logarithm of averaged BCF values (x).

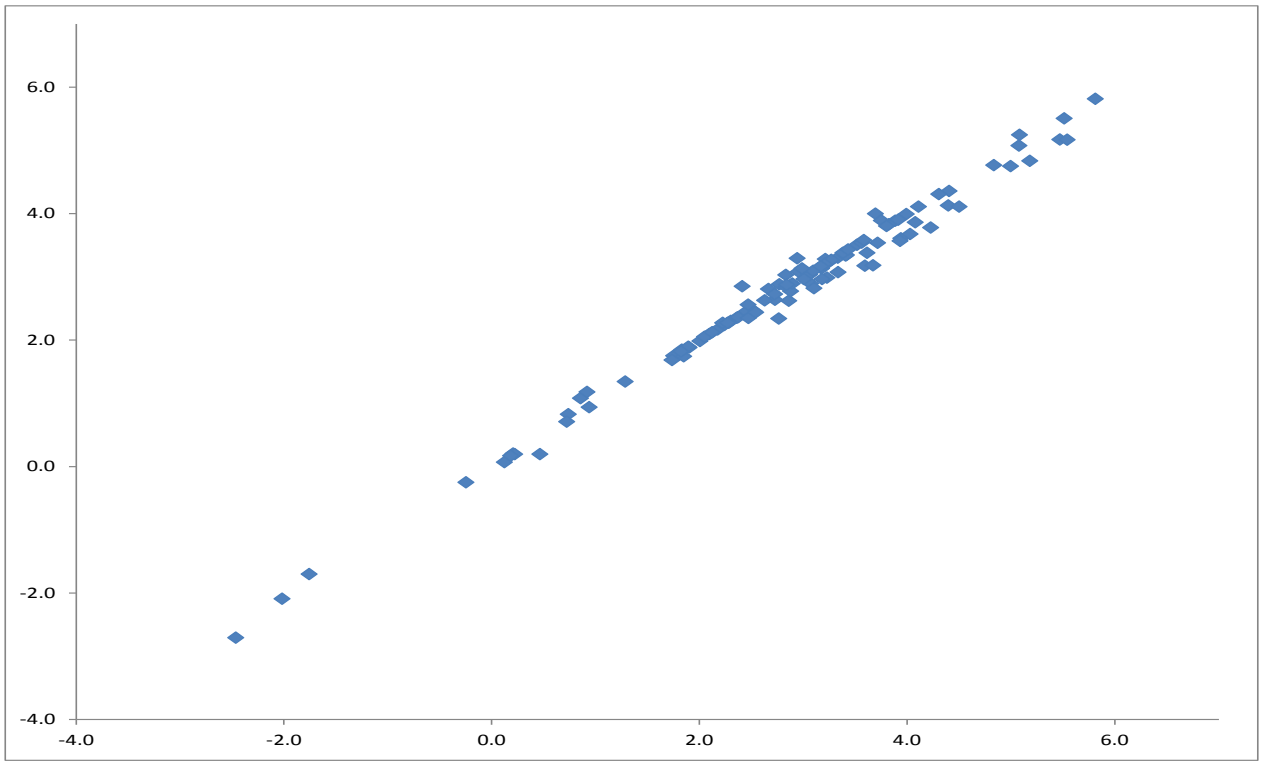


Figure 13: Difference of horizontal and vertical averaging (y) against the number of individual k_1 (blue diamonds), k_2 (red squares), and single study BCF (green crosses) values (x).

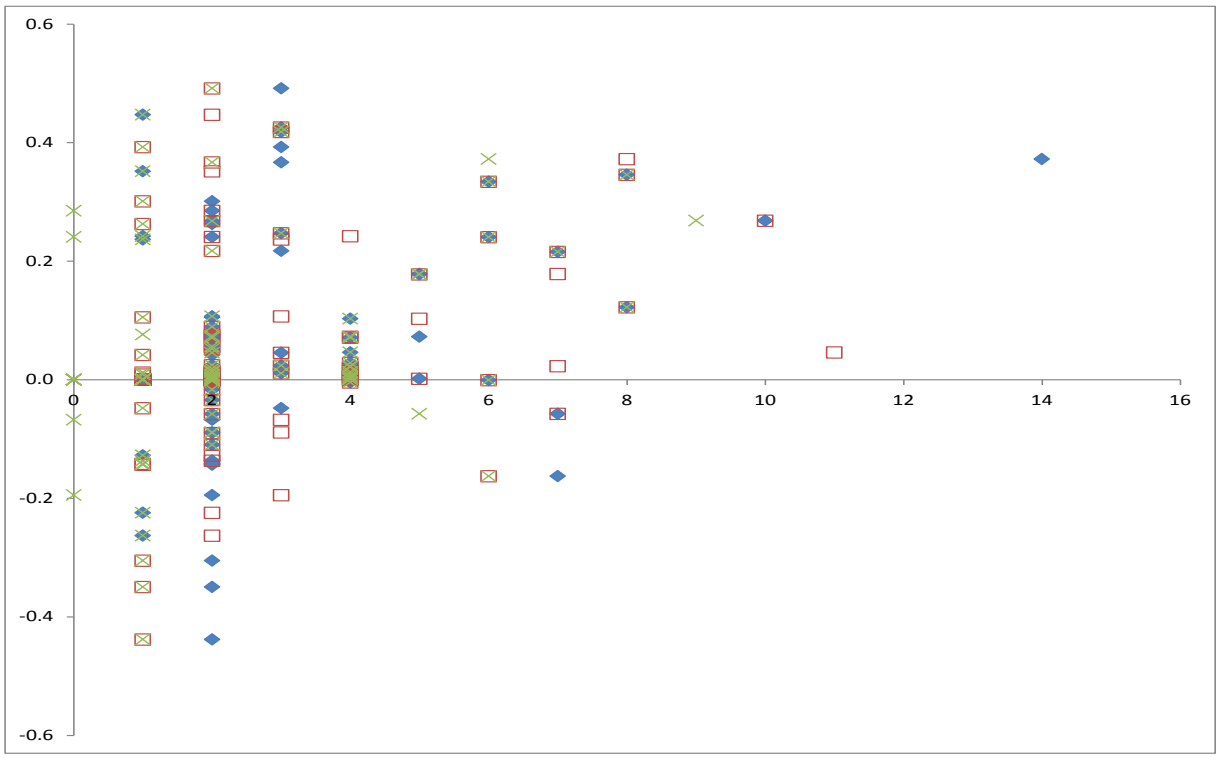


Figure 13 and Figure 14 show that the difference between both approaches does not depend on the number of individual data nor the number of different species involved. Even when looking at the minimum and maximum individual BCF (Figure 15), there is no real relationship to the number of values. The same applies to the number of fish species (Figure 16). Only a subtle increase in the range of individual results from one to more than one species occurs.

Figure 14: Difference of horizontal and vertical averaging (y) against the number of different species (x).

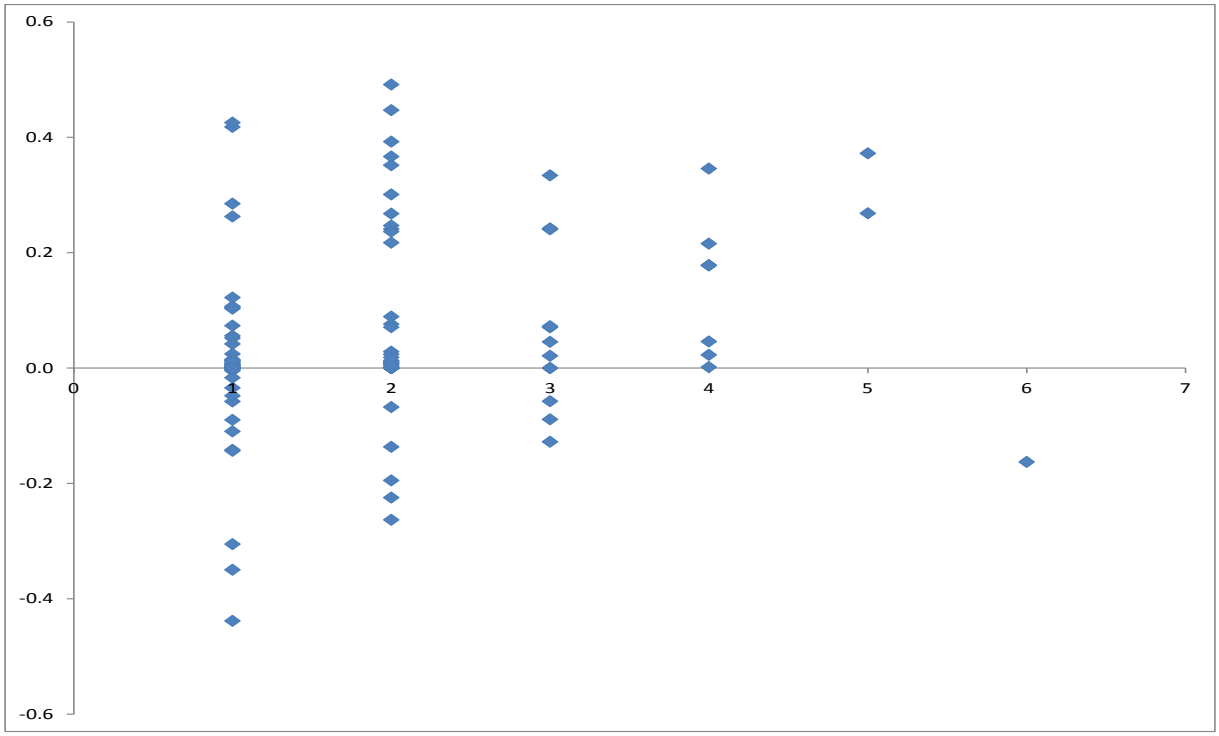


Figure 15: Difference between the maximum and minimum individual BCF for each compound (y) against the number of individual k_1 (blue diamonds), k_2 (red squares), and single study BCF (green crosses) values (x).

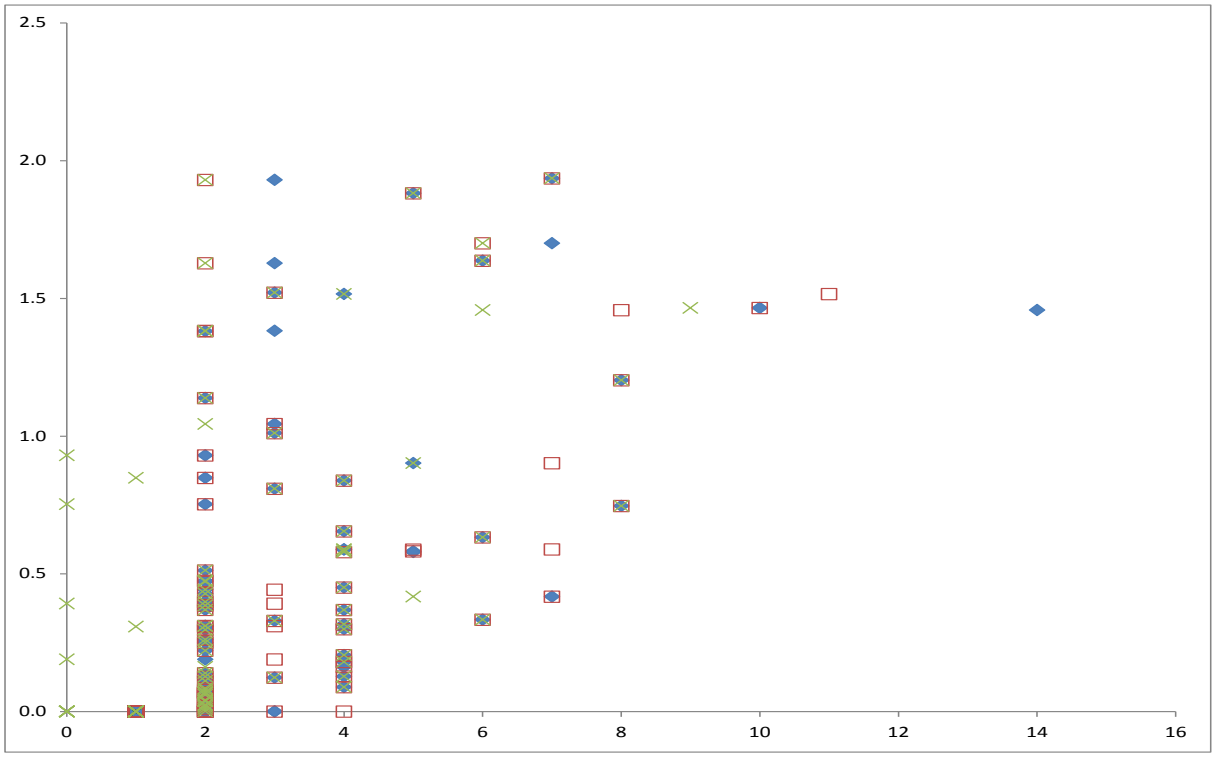
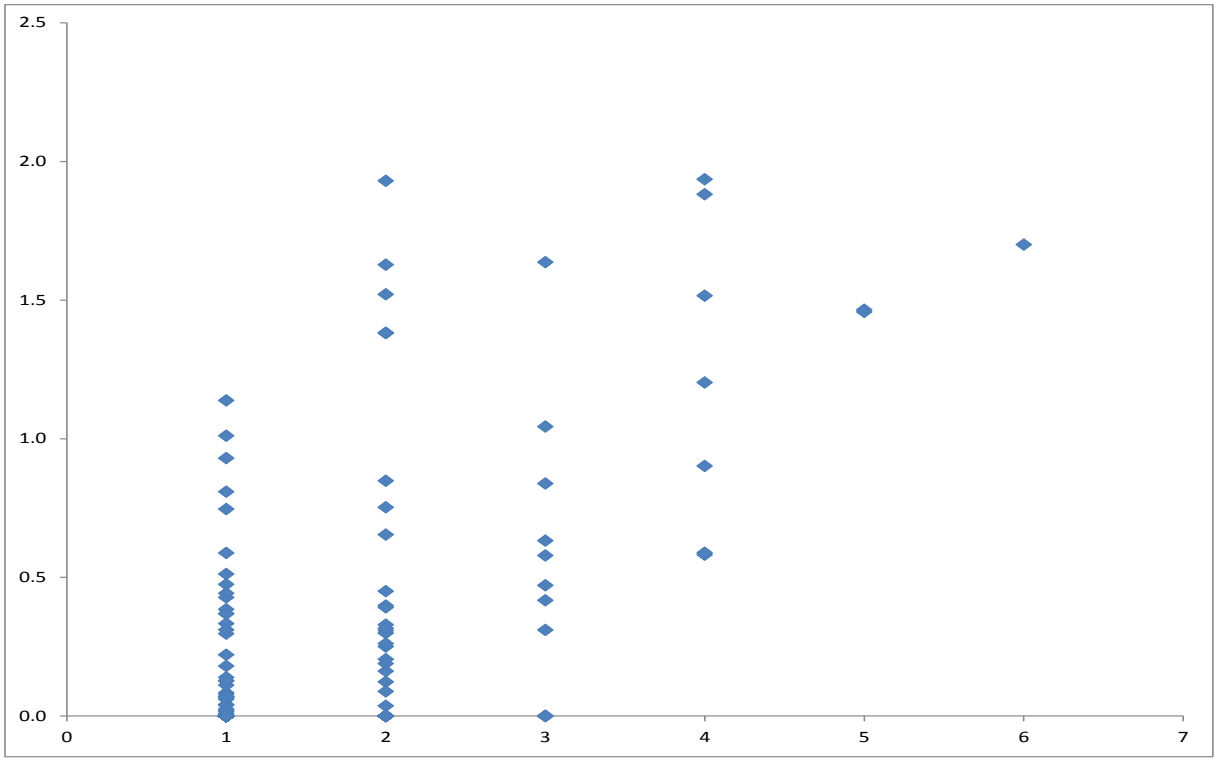


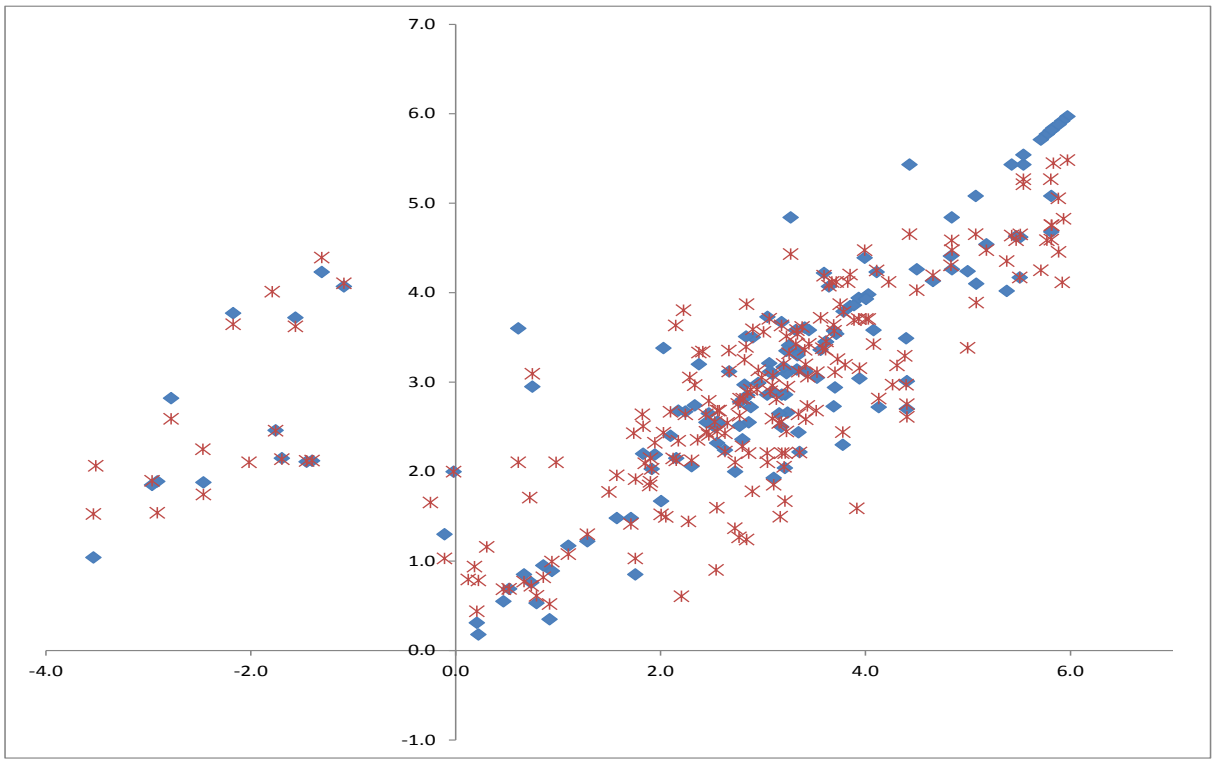
Figure 16: Difference between the maximum and minimum individual BCF for each compound (y) against the number species involved (x).



2.3.2.4 Comparison to steady state BCF

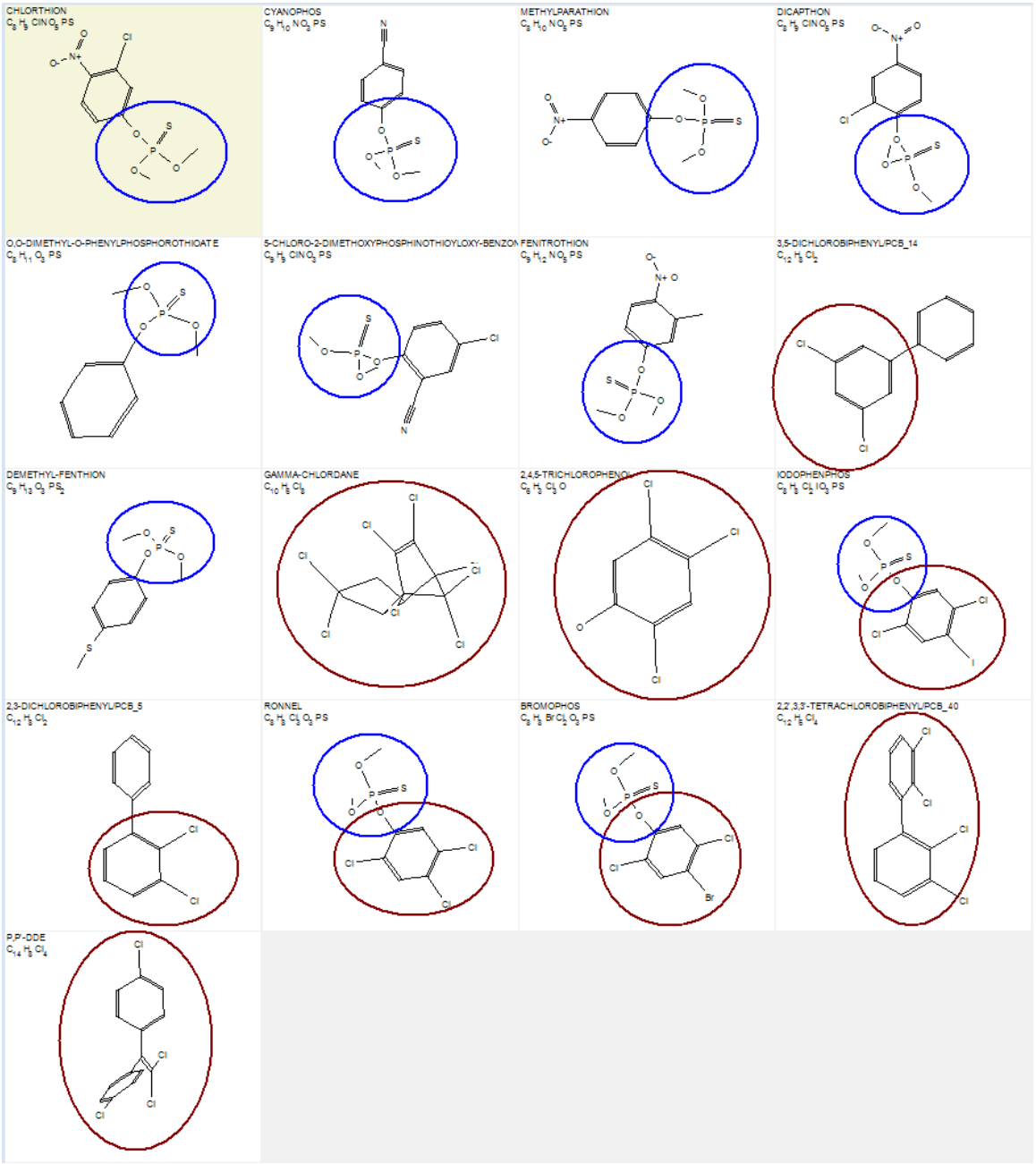
A validated set of steady state BCFs was available from OSIRIS (OSIRIS 2007-2011) and implemented in the database of ChemProp (UFZ 2014). While for 204 compounds of the data set experimental BCFs were there, only for 144 chemicals pairs of steady state and kinetic BCFs were available. To extend the comparison, a read-across model from ChemProp (UFZ 2014) was applied in addition for the steady state BCF. The model was applicable for all compounds. This yielded 209 pairs of kinetic and steady state BCF.

Figure 17: Plot of experimental (blue) and read-across estimated (red) log BCF (y) vs. kinetic log BCF (x).



Generally, there is agreement of steady state and kinetic BCFs. However, for some compounds very small kinetic BCFs < 1 (log BCF < 0) could be observed (Figure 17), and this is not reflected in the steady state BCFs. A detailed look at them revealed, the difference is due to extremely small k_1 in the kinetic approach, while their k_2 are within the usual range. Interestingly (Figure 18), chemicals with kinetic BCFs below 0.1 (log BCF < -1) belong to two compound classes only: Multiply halogenated (particularly chlorinated) hydrocarbons are knownto deviate from normal bioaccumulation behavior. In this study, the respective halogens all were attached to unsaturated rings (aromatic rings with one exception). Organophosphates (in particular, thiophosphates here) are hydrolyzing rather rapidly. At least in the latter case, this may yield significantly decreased concentrations in water and thus confine uptake rates, while on the other hand equilibrium (steady state BCF) is still possible but needs a longer time to be reached. Further targeted investigations might bring more insight for a mechanistic explanation here.

Figure 18: Compounds with large differences between steady state and kinetic BCF values. Highlighted are thiophosphate (blue) and multiply halogenated unsaturated hydrocarbon ring (brown) substructures.



2.3.2.5 Lipid correction

To exclude the influence of the lipid content in the animal, lipid correction of the BCF is suggested. With lipid contents from the experimental data available, this correction was possible for 210 chemicals. Lipid correction is only possible together with the averaging approach (1).

For the studies data set, even though lipid correction does have some effect (Figure 19), it does not notably influence the differences to the steady state BCF (Figure 20).

Figure 19: Plot of kinetic uncorrected log BCF (y) vs. lipid corrected kinetic log BCF (x)

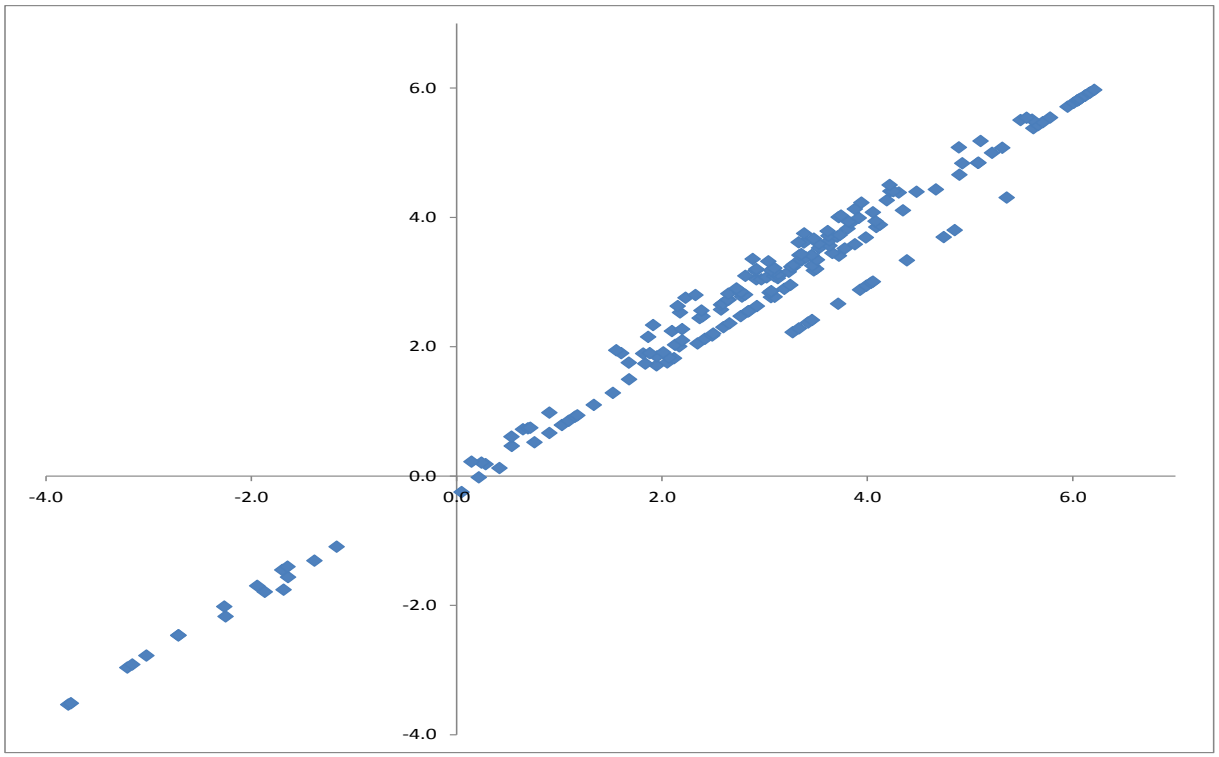
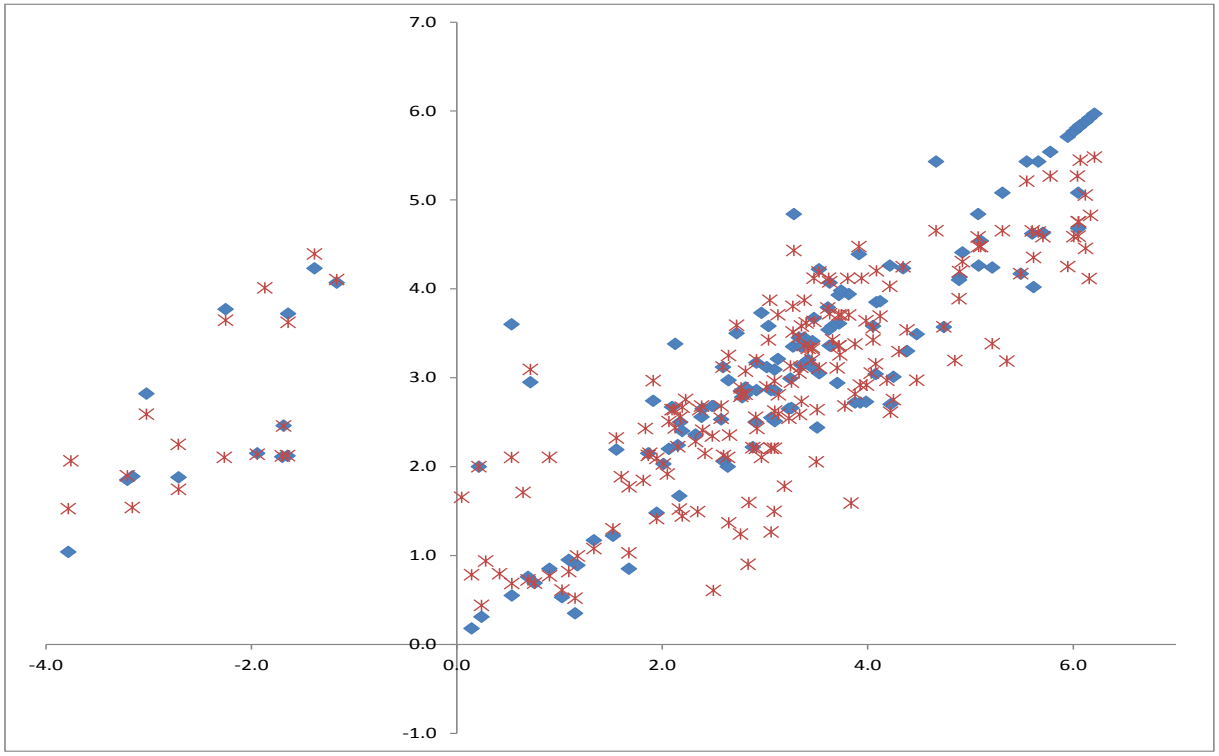


Figure 20: Plot of experimental (blue) and read-across estimated (red) log BCF (y) vs. lipid corrected kinetic log BCF (x).



2.3.3 BCF dependency on k_2 (BCF)

2.3.3.1 BCF modeling

The results of the model predictions in comparison to the kinetic BCF values are shown in Figure 21. In spite of the outliers discussed already, the inclusion of estimated biotransformation rates yields a consistent under-

estimation of the kinetic BCF which is unfavorable in terms of the precautionary principle. On the other hand, disregarding biotransformation yields overestimation in more cases, what is principally more tolerable. However, there is still some underestimation which seems to be of more systematic nature in particular for experimental log BCF above 4.

Figure 21: Log BCF estimated from Arnot & Gobas (2003) without (blue) and with (red) consideration of biotransformation (y) vs. kinetic log BCF (x).

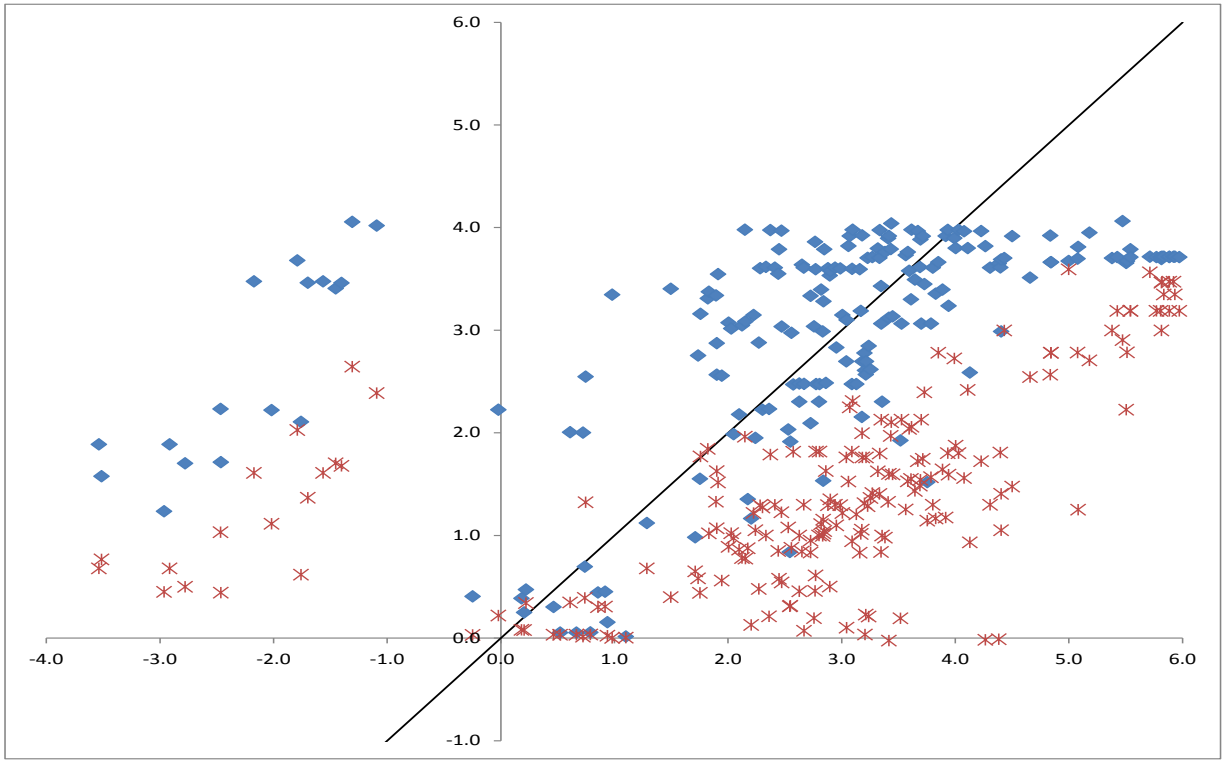
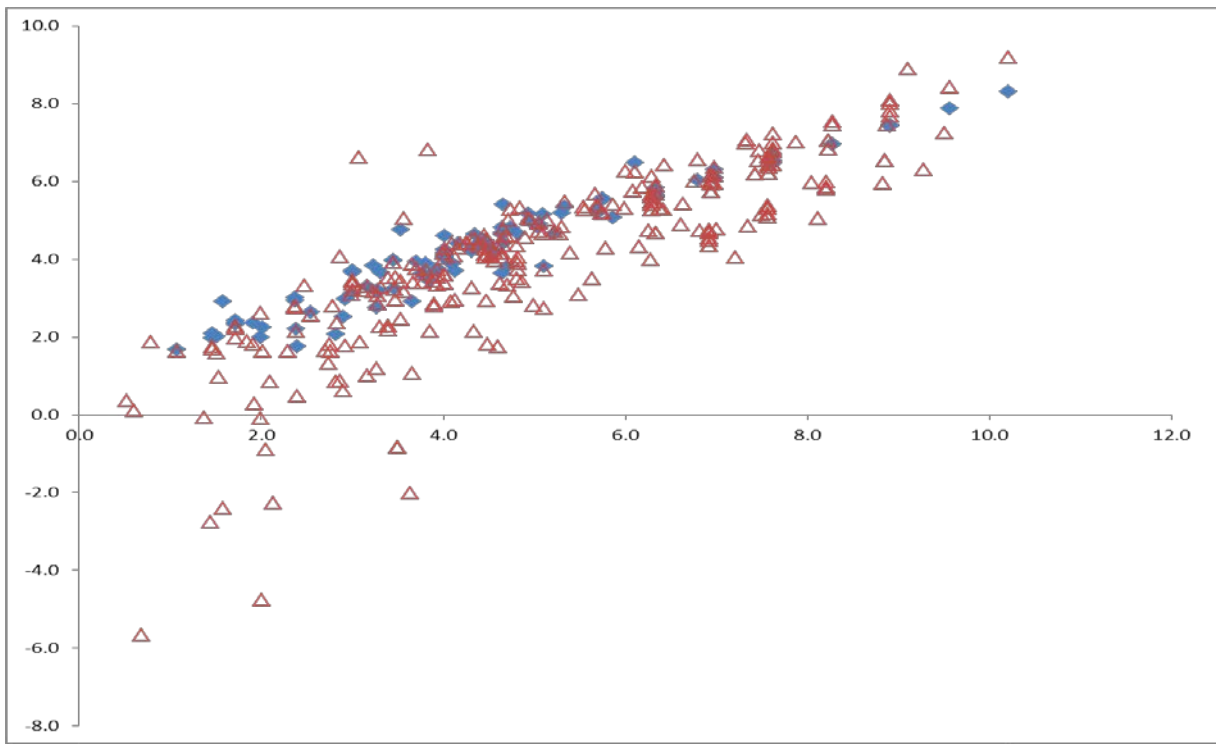


Figure 22: Log K_{mw} estimated experimental (blue) and calculated (red) Abraham parameters (y) vs. log K_{ow} (x).



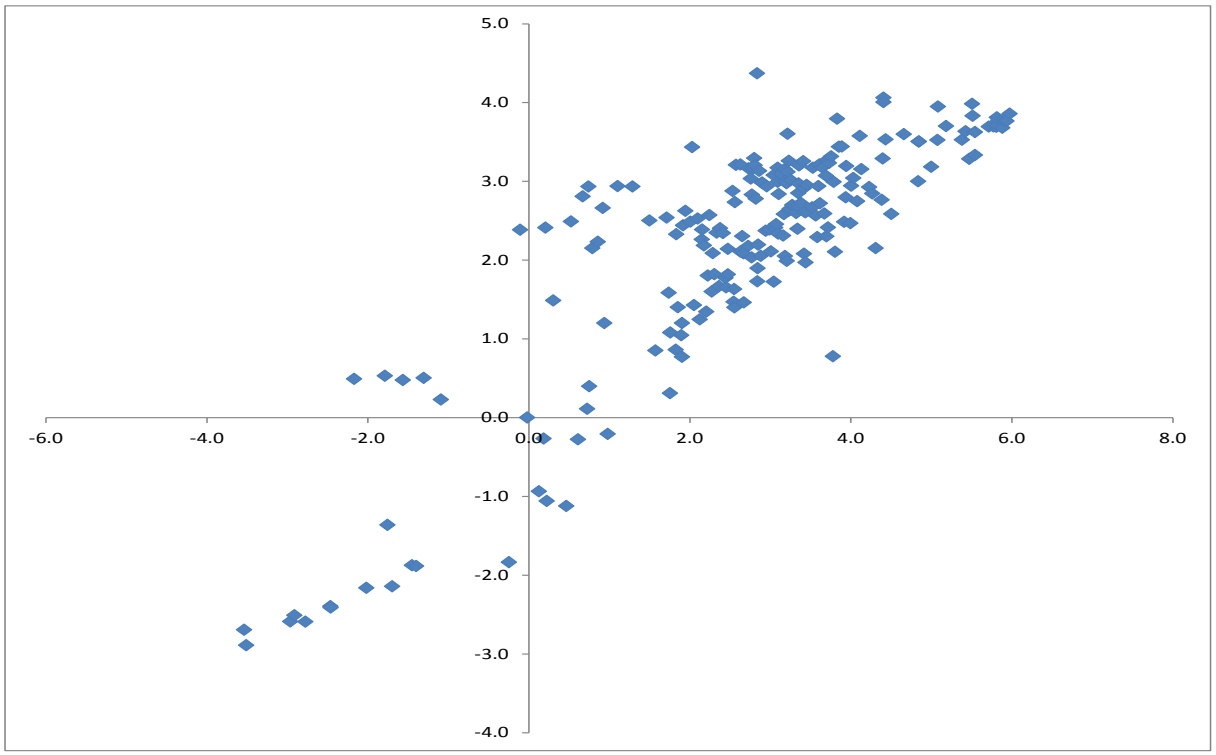
2.3.3.2 Membrane partition coefficient K_{mw}

The octanol/water partition coefficient is usually applied to model membrane partitioning. Actually, this may introduce an additional error, and membrane/water partition coefficients K_{mw} should be more suitable. On the other hand, only few experimental K_{mw} data are available, and estimation models are rather limited. ChemProp offers an estimation model for K_{mw} from Abraham parameters. A comparison of these estimations for log K_{mw} applying both experimental (if available) and calculated descriptors to the log K_{ow} is shown in Figure 22. There is a strong correlation between both partition coefficients.

2.3.3.3 Uptake rates

The relationship of the experimental log k_1 to log BCF is plotted in Figure 23. As expected, k_1 increases with the BCF.

Figure 23: Experimental log k_1 (y) vs. kinetic log BCF (x).



Looking at the relationship to hydrophobicity, Figure 24 shows the general dependence. More detailed, Figure 25 focusses on lower hydrophobicity (membrane control expected, and Figure 26 on higher hydrophobicity (diffusion control expected). Both the octanol/water partition coefficient and the membrane/water partition coefficient are applied as the hydrophobicity measure.

To separately investigate membrane permeation control and diffusion control, Figure 25 is restricted to compounds with log $K_{ow} < 3$, and Figure 26 is restricted to log $K_{ow} > 4$. Furthermore, thiophosphates are left out because of their rapid hydrolysis.

No obvious trends can be observed, and in particular no indication of different behaviors for membrane permeation and partition control can be seen for both partition coefficients.

Figure 24: Dependence of the uptake rate (y: log k_1) to hydrophobicity (x: blue log K_{ow} and red log K_{mw}).

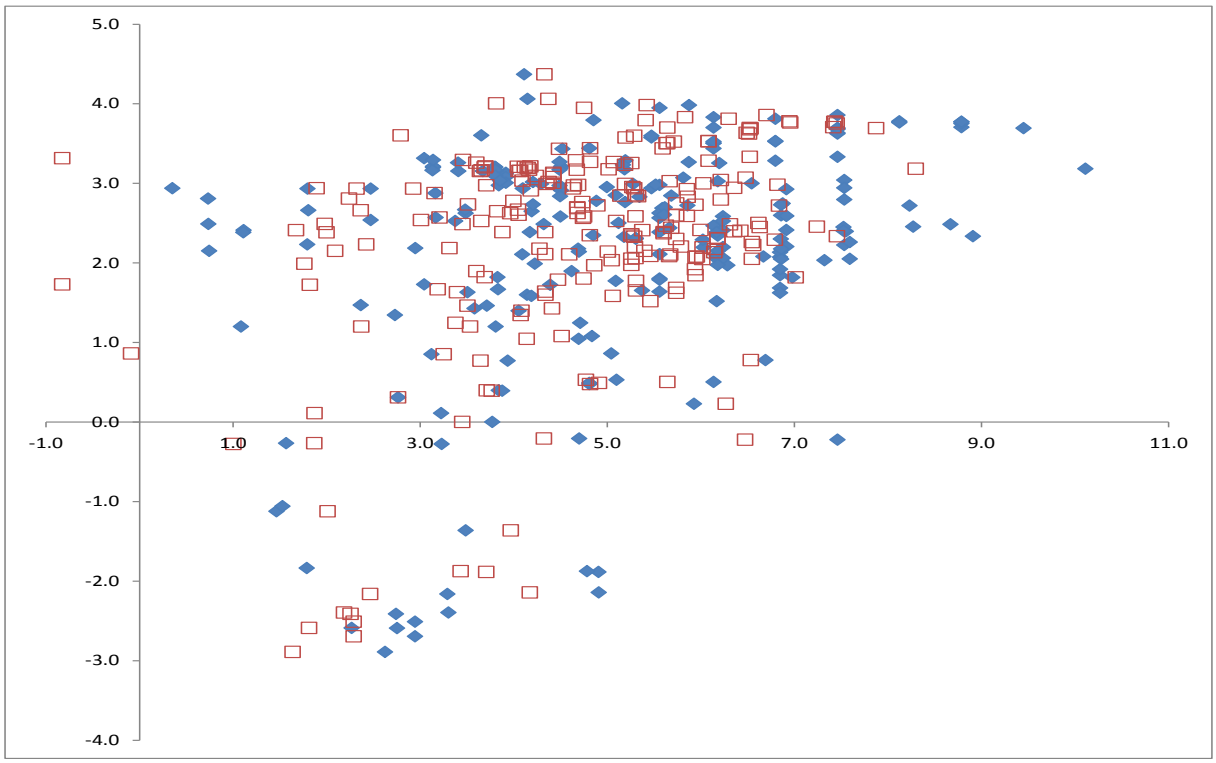


Figure 25: Log K_{ow} < 3 (membrane permeation control): Dependence of the uptake rate to hydrophobicity(x: blue log K_{ow} and red log K_{mw}).

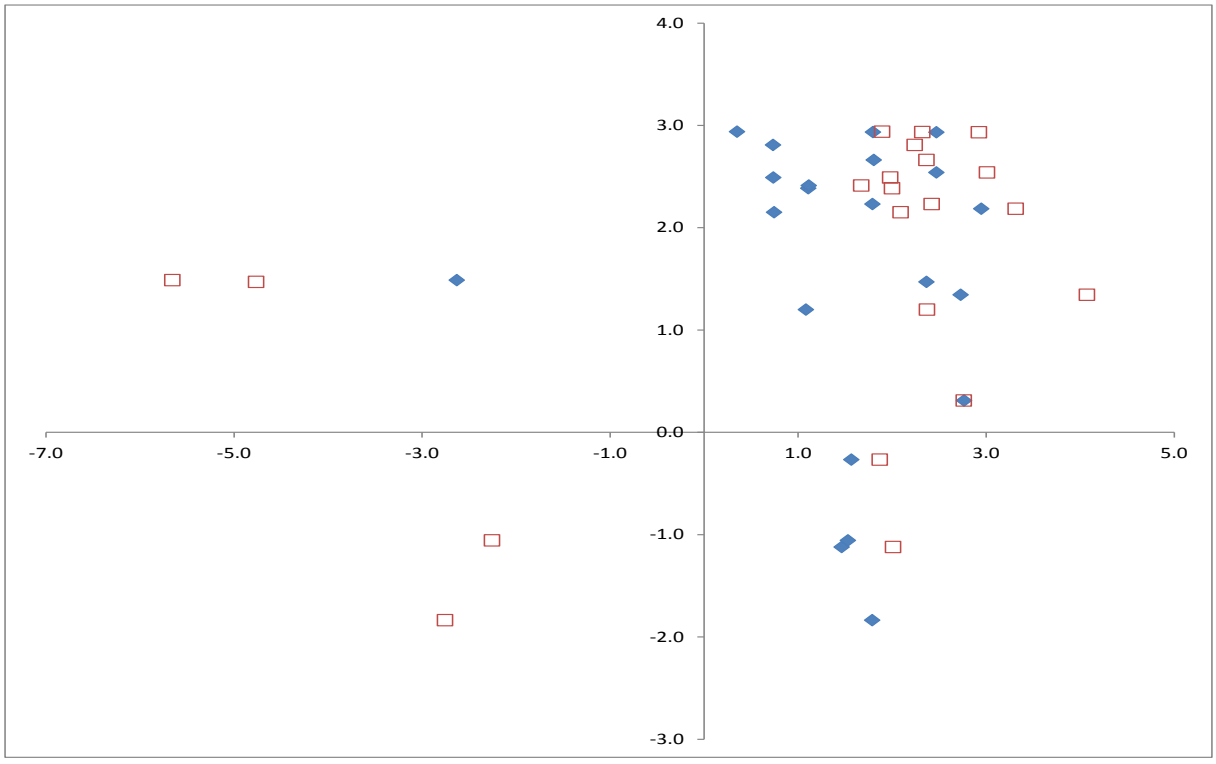
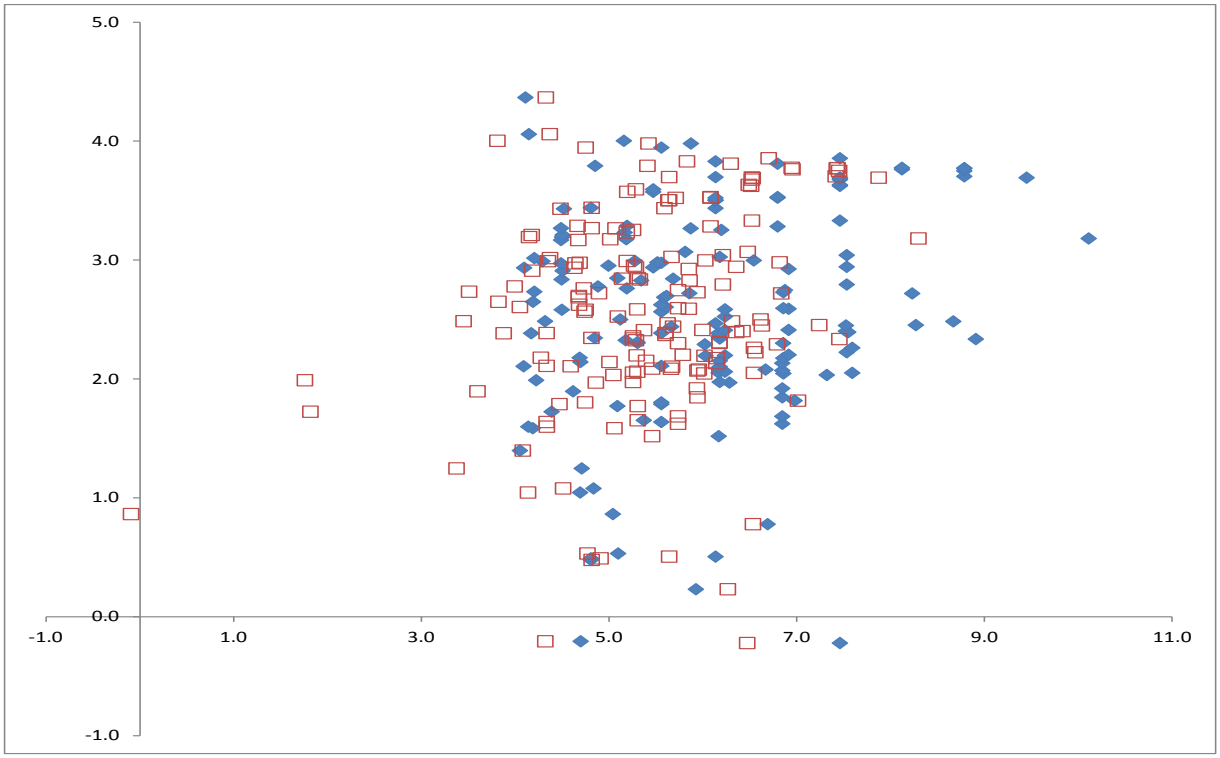


Figure 26: Log K_{ow} > 4 (diffusion layer control): Dependence of the uptake rate to hydrophobicity (x: blue log K_{ow} and red log K_{mw}).



2.3.3.4 Models for k_1

In agreement with the study, none of the models yielded sufficient results.

Actually, the model predictions (Figure 27) are in the center range of the experimental data, but since no dependency of the structure or properties of the chemicals is included, the predictions are not really useful.

Looking at the results presented before, all of these models include only a rather slight slope with increasing hydrophobicity. This is confirmed when plotting the results (Figure 28). Again, none of the models can be considered as a reliable estimation model for k_1.

The same holds true for models combining W and K_{ow} (Figure 29). The one extreme outlier (hexahydro-1,3,5-trinitro-1,3,5-triazine) obviously is an artefact of the consensus model for K_{ow}. Applying other K_{ow} approaches would remove it but introduce other artefacts. This further emphasizes the insufficient performance of the k_1 models from K_{ow}.

Figure 27: Log k_1 estimated via Equation 13 (blue) and Equation 14 (red) (y) vs. experimental data (x).

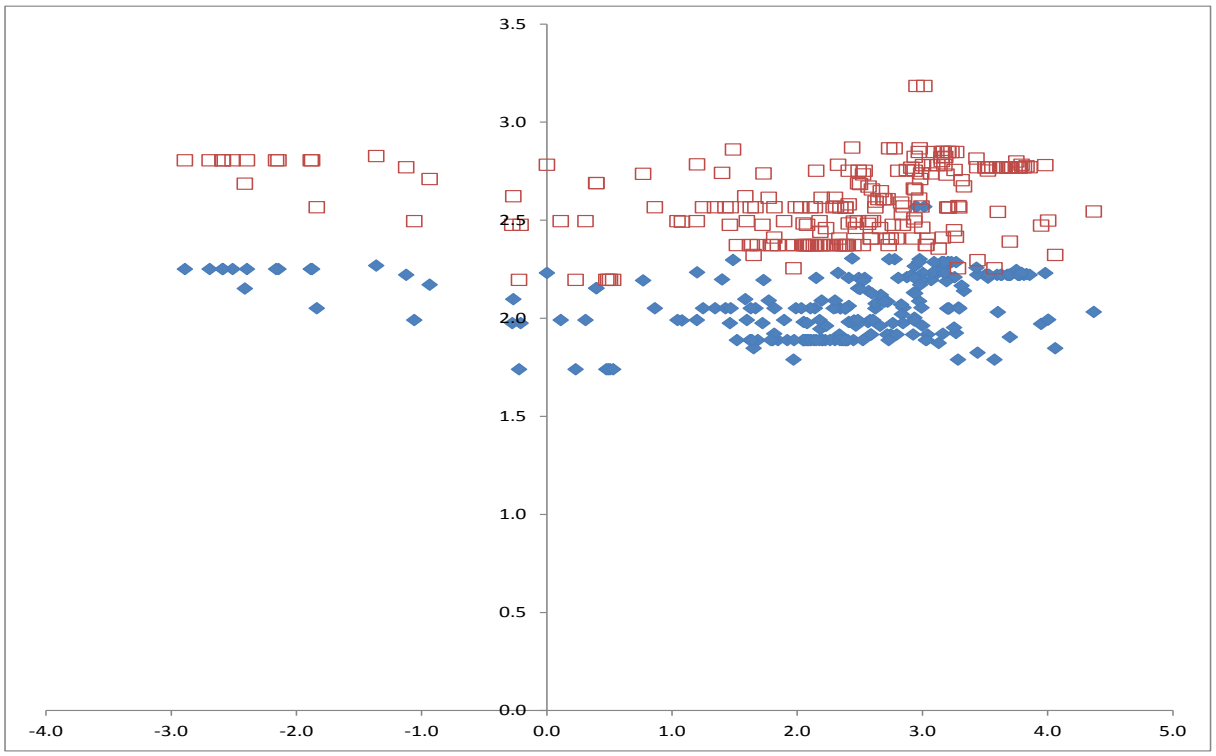


Figure 28: Log k_1 estimated via Equation 15 (blue), 16 (red), 17 (green), and 18 (crosses) (y) vs. experimental data (x).

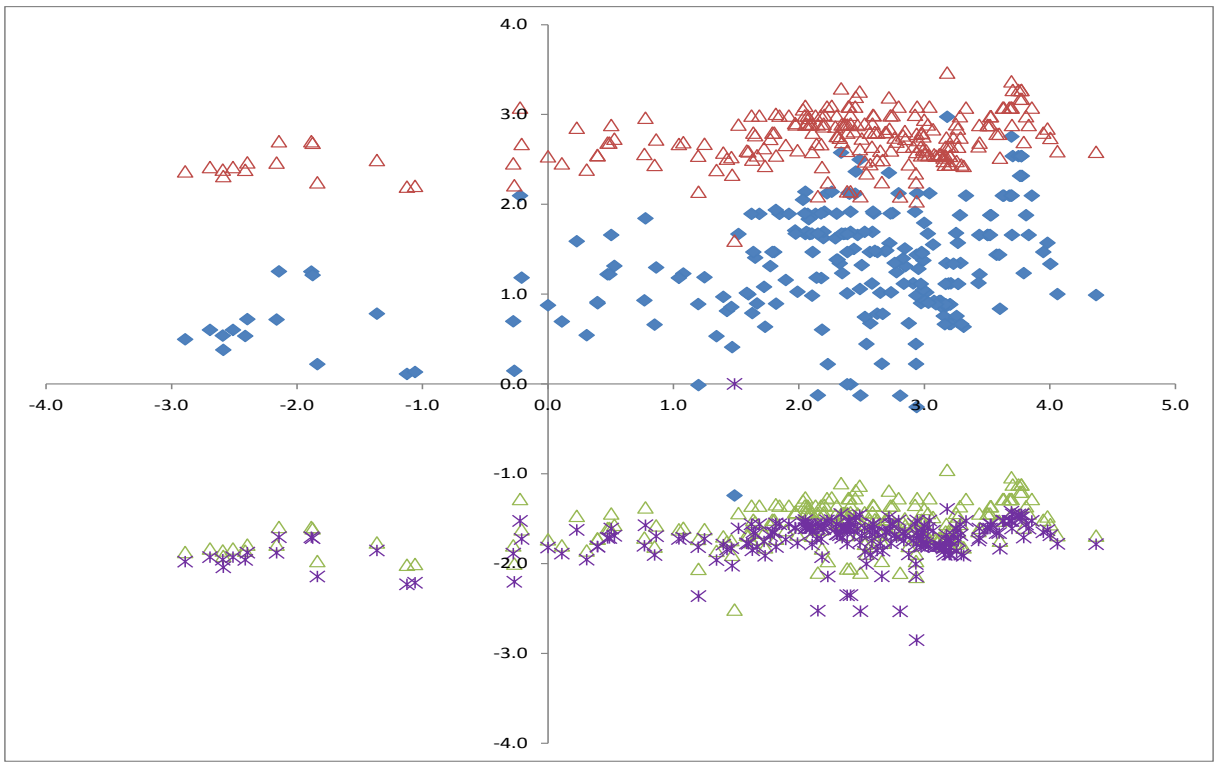


Figure 29: Log k_1 estimated via Equation 19 (blue) and 10 (red) (y) vs. experimental data (x).

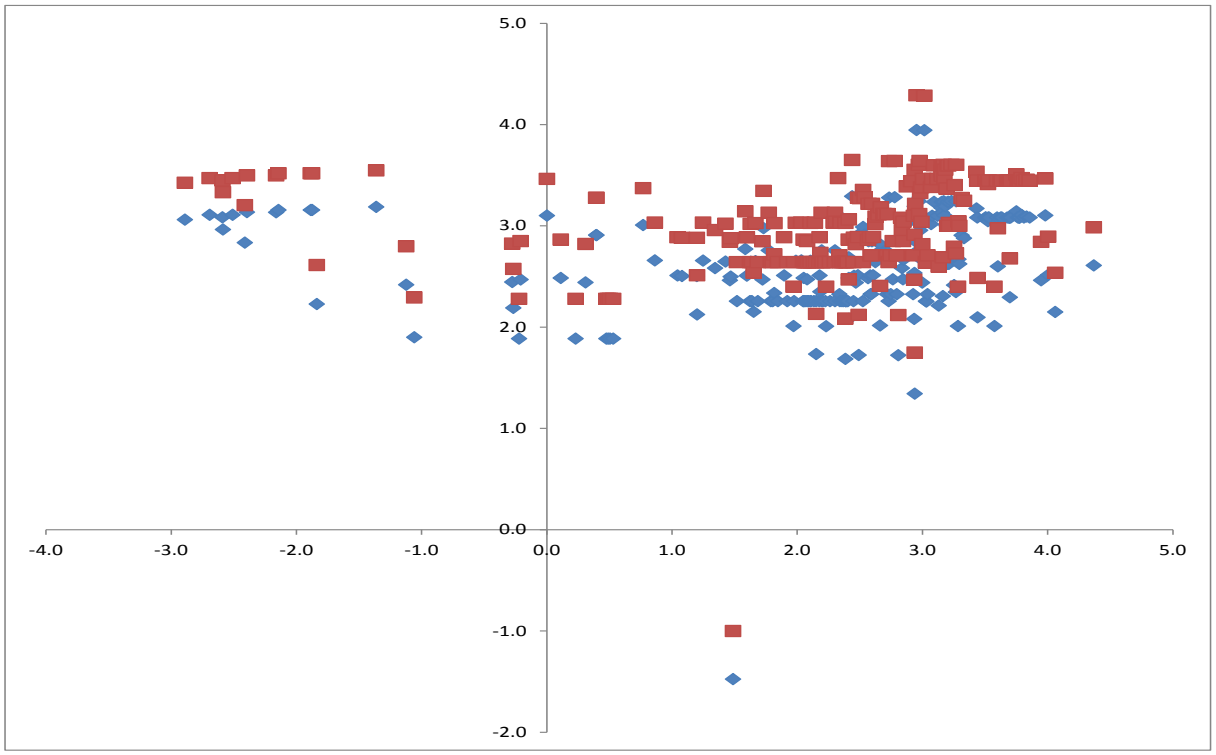
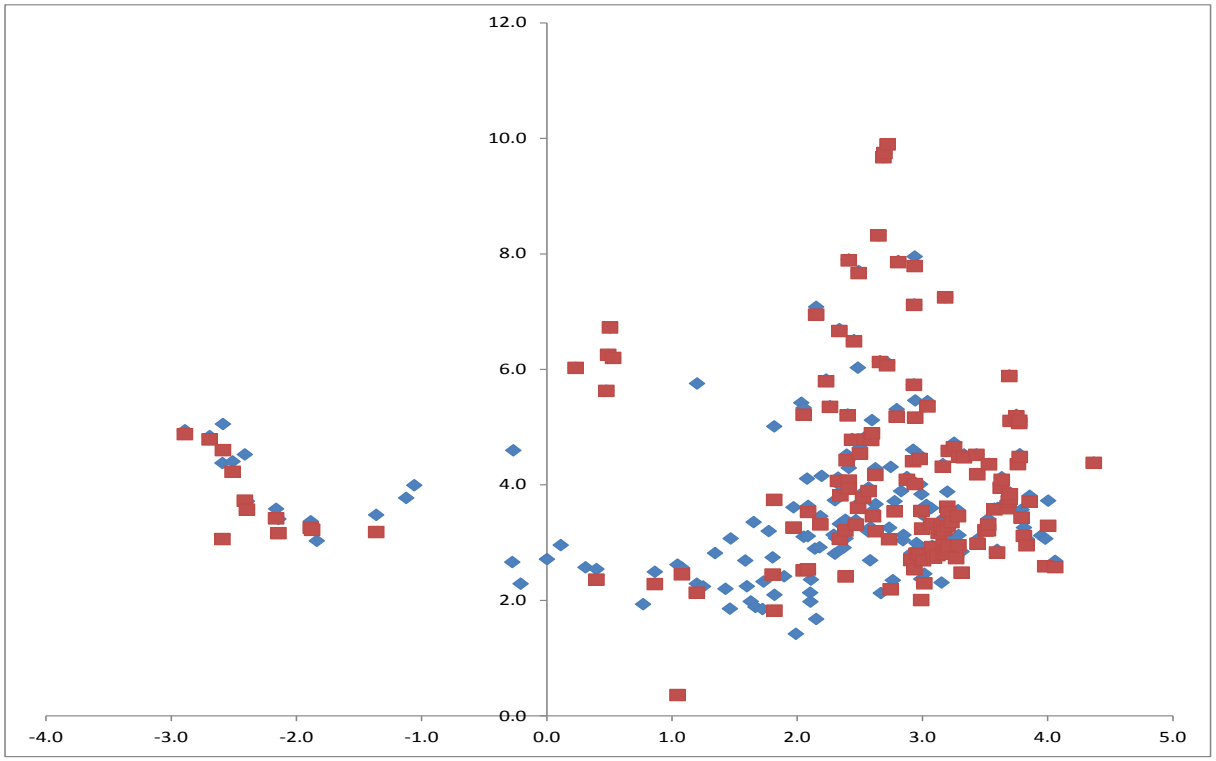


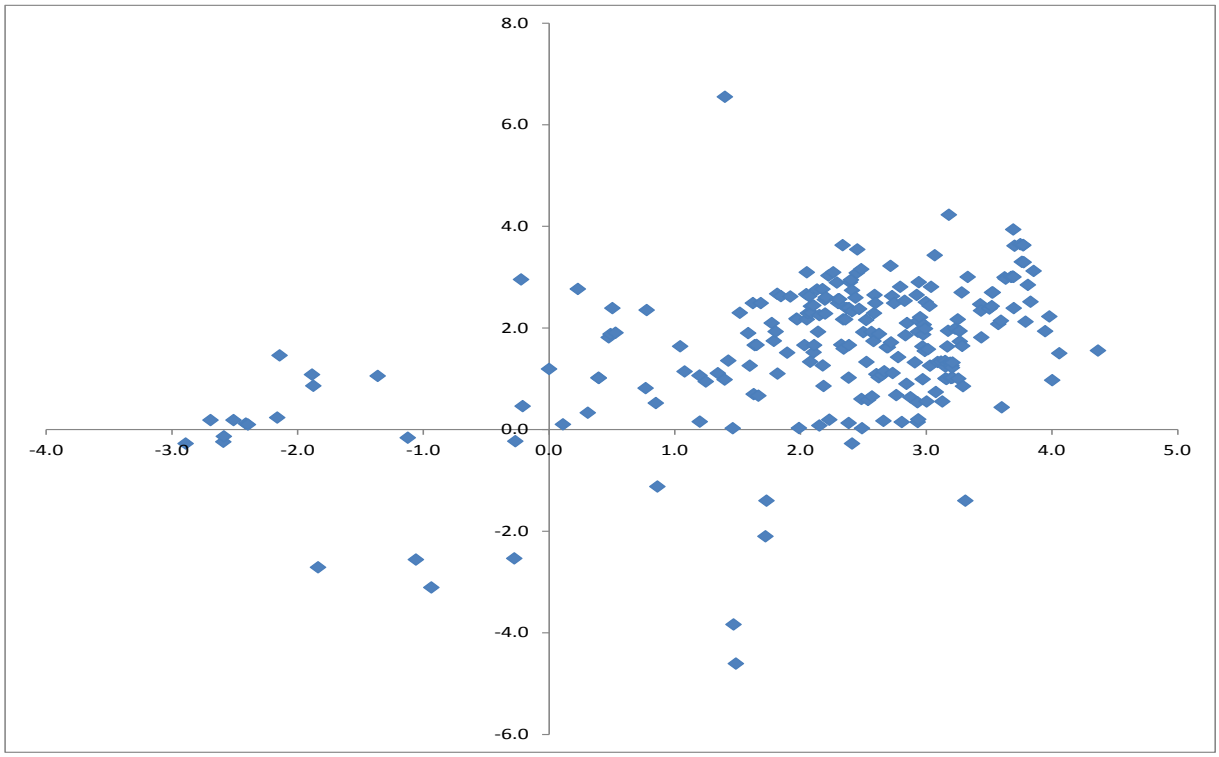
Figure 30: Log k_1 estimated from experimental k_2 through reversing Equation 6 without (blue) and with (red) consideration of biotransformation (y) vs. experimental data (x).



2.3.3.5 Alternative approaches

Some alternatives for predicting k_1 have been examined. Replacing K_{ow} by K_{mw} (with a new fit, indeed) does not really improve any model due to the strong correlation of them. When using the experimental k_2 together with an estimation of k_1 from k_2, LB and K_{ow} by reversing (Equation 6), at least a trend correspondence can be obtained, as shown in Figure 30. Regarding biotransformation does not yield any improvement here.

Figure 31: Log HSA by Valko et al (2003) (y) vs. experimental data of log k_1 (x).



Furthermore, the correlation to human serum albumin bonding (HSA) estimated by a Abraham equation (Valko et al 2003) was examined (Figure 31).The figure indicates there is a trend indeed, however it is too weak to be exploited for modeling. Direct modeling from Abraham parameters did not yield any useful result.

2.3.3.6 Elimination rate k_2

In Figure 32, the experimental log k_2 are plotted against the log of BCF. As expected, k_2 generally decreases with increasing BCF. The expected trend of increasing k_2 with increasing k_1 (Figure 33) is less obvious.

Figure 32: Experimental log k_2 (y) vs. experimental log BCF (x).

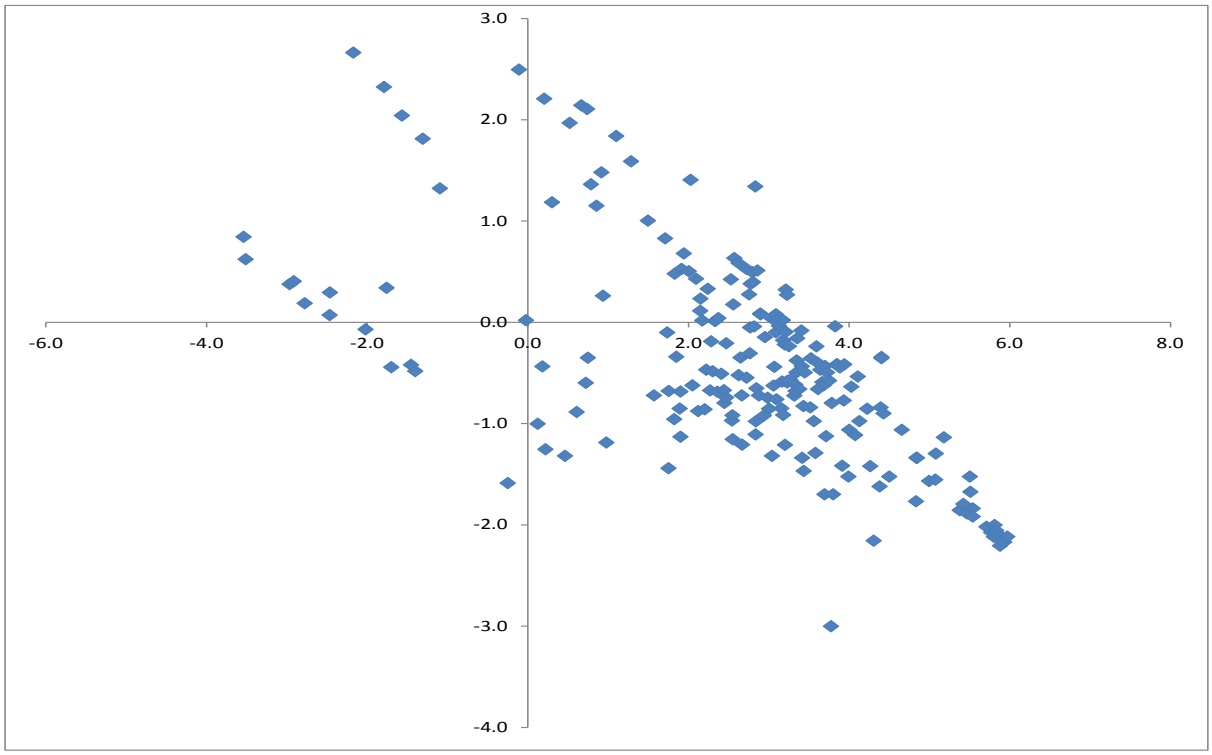
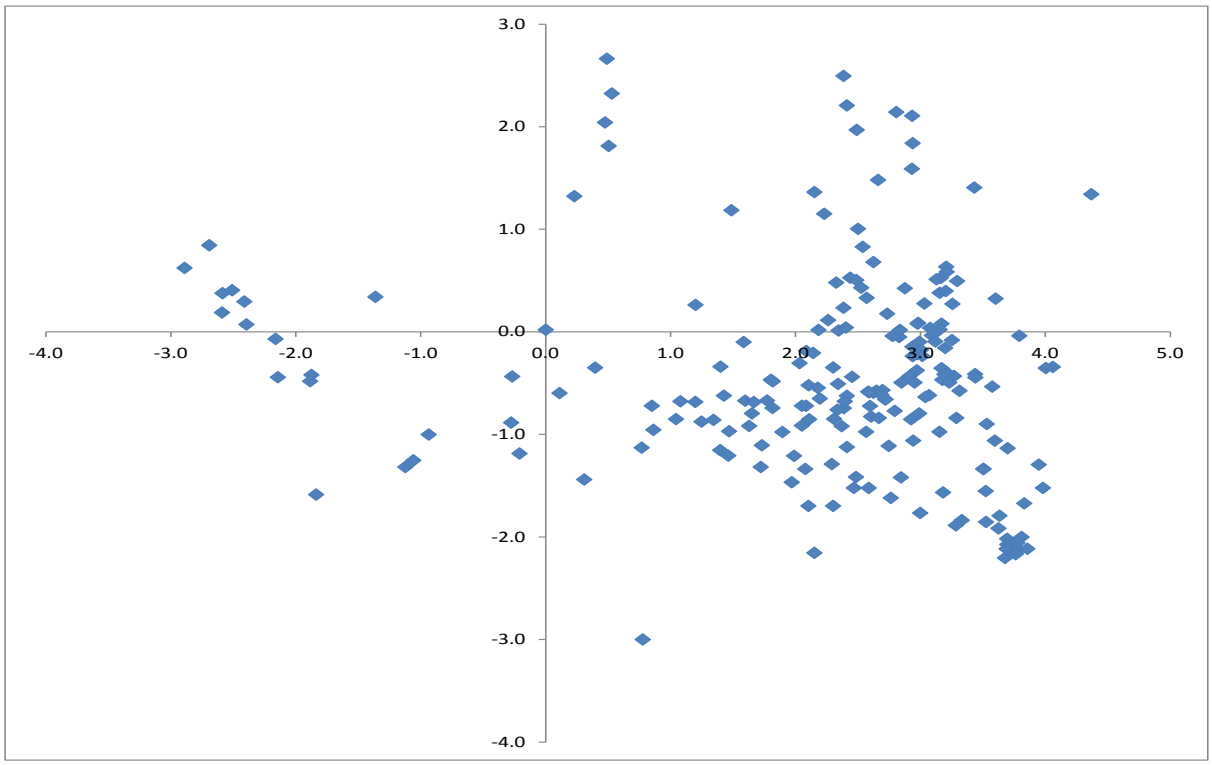


Figure 33: Experimental log k_2 (y) vs. experimental log k_1 (x).



When looking to K_{ow} and K_{mw} (Figure 34), again separation into membrane control (Figure 35) and diffusion control (Figure 36) was done to inspect these ranges separately. No correlation between k2 and hydrophobicity is observed when K_{ow} or K_{mw} are used for the membrane permeation controlled range. In the diffusion controlled range, decreasing k_2 with hydrophobicity is observed. However, a stronger correlation to K_{ow} or K_{mw} cannot be seen due to a high scatter.

Figure 34: Experimental log k_2 (y) vs. log K_{ow} (blue) and log K_{mw} (red) (x).

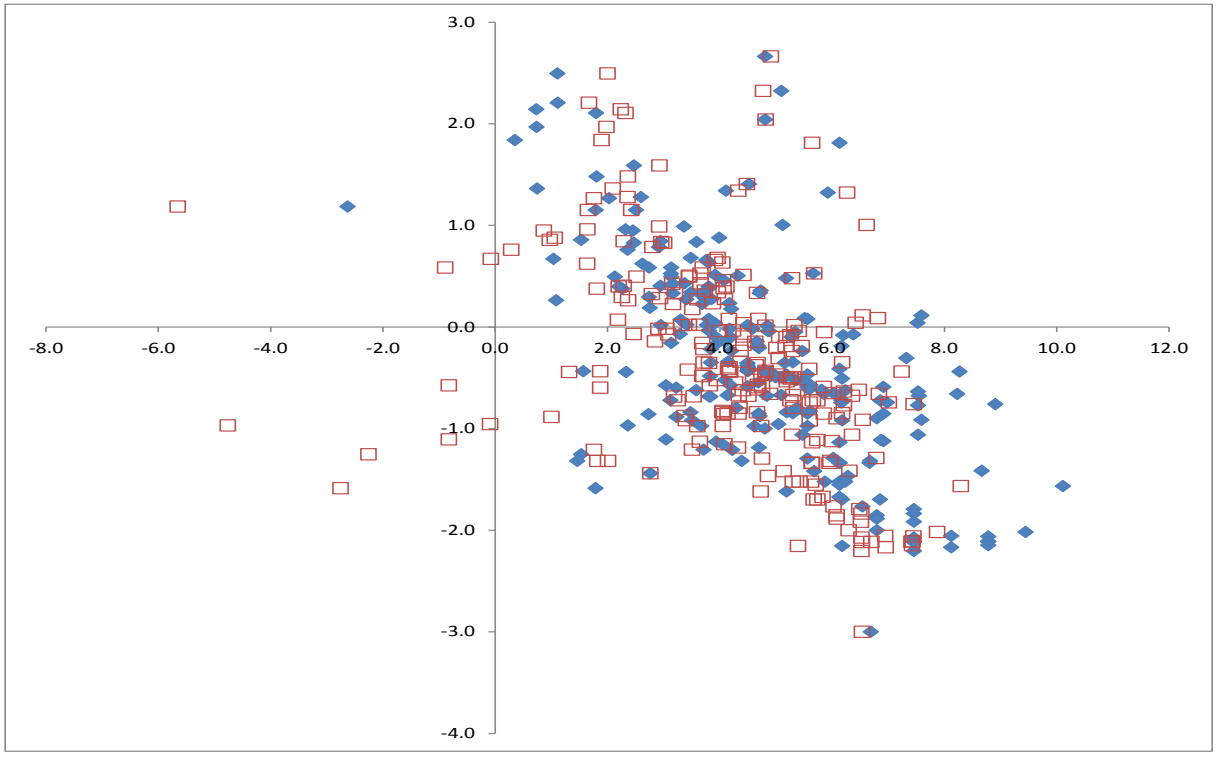


Figure 35: For log K_{ow} < 3, experimental log k_2 (y) vs. log K_{ow} (blue) and log K_{mw} (red) (x).

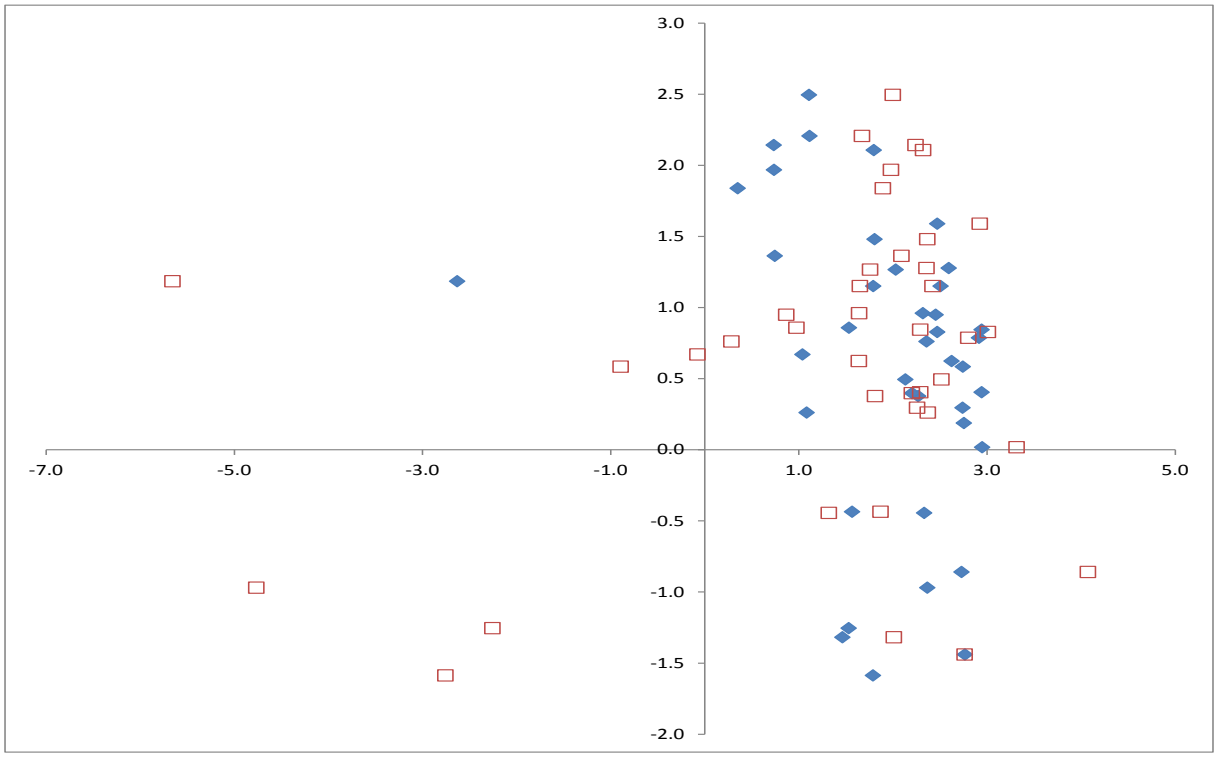
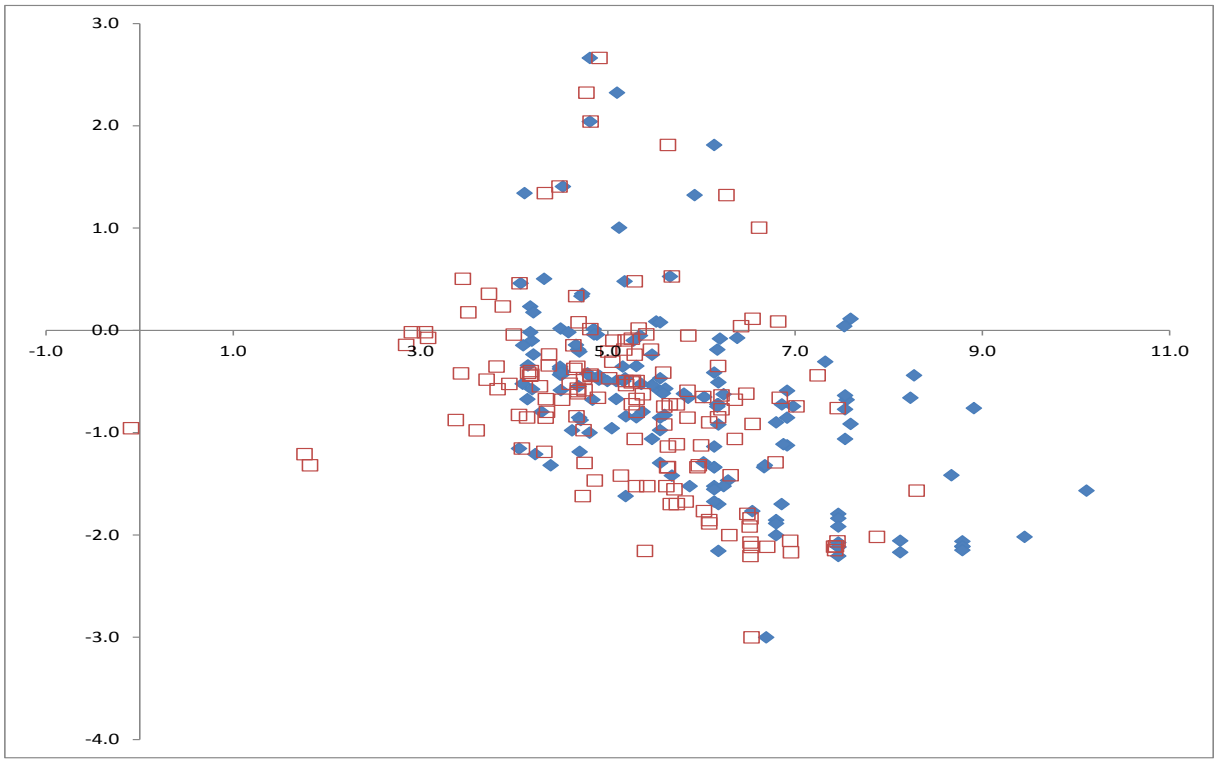


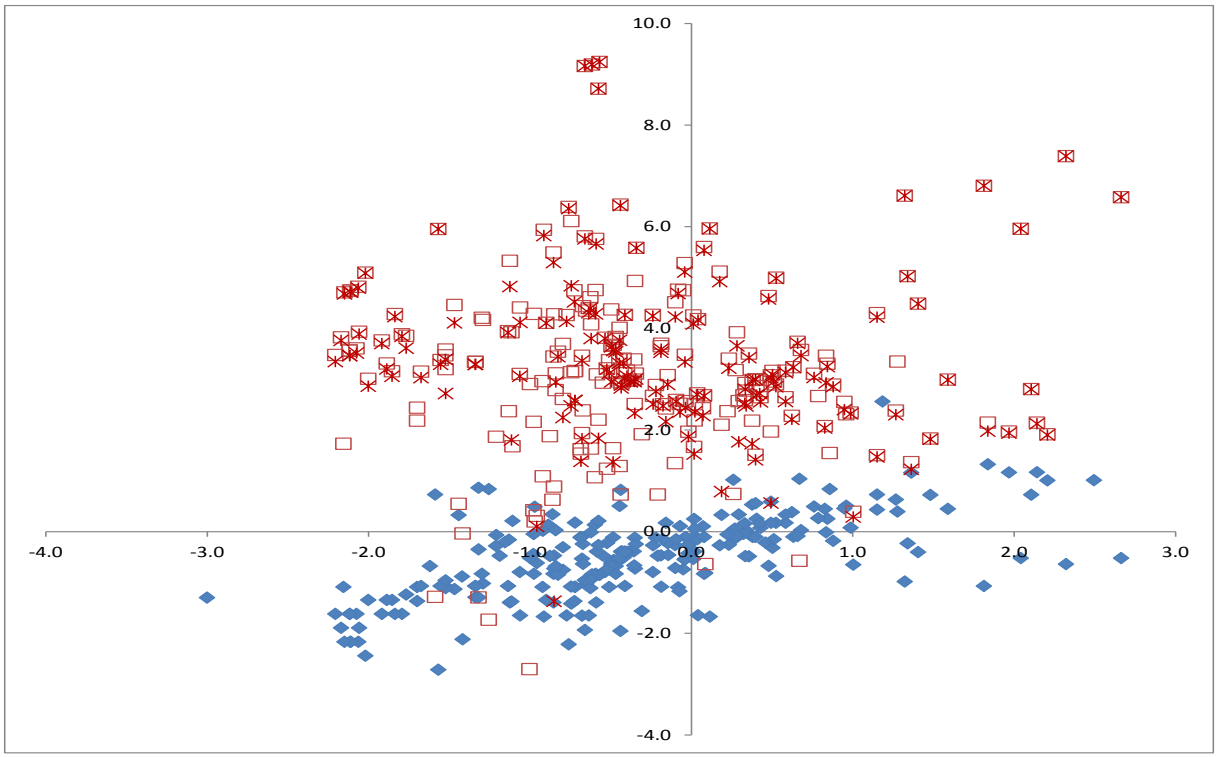
Figure 36: For log K_{ow} > 4, experimental log k_2 (y) vs. log K_{ow} (blue) and log K_{mw} (red) (x).



2.3.3.7 Modeling k_2

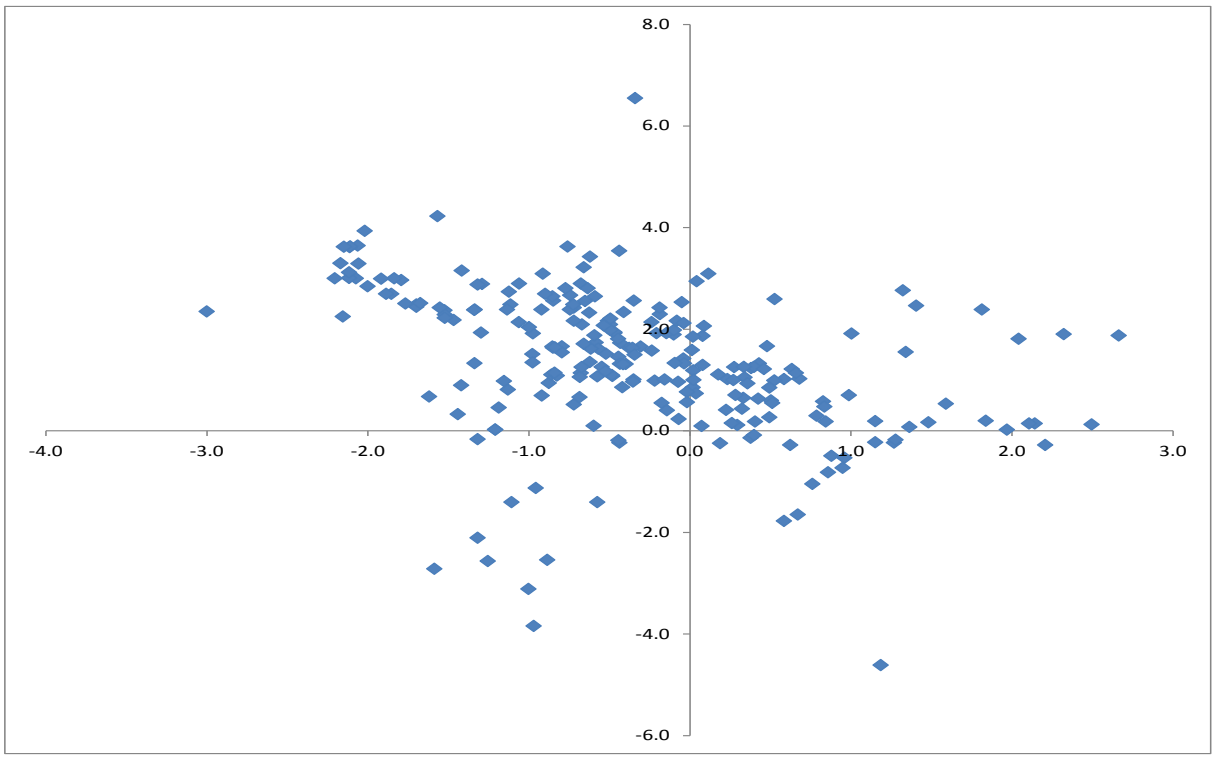
The application of these models is shown in Figure 37. At least a trend can be obtained from the Arnot & Gobas approach, interestingly with superiority of the version including biotransformation here.

Figure 37: Estimation of log k_2 by Equation 16 (blue), 4 and 6 without (red squares) and with (red crosses) consideration of biotransformation (y) vs. experimental values (x).



Furthermore, the relationship to the HSA model is shown in Figure 38. The elimination rate obviously decreases with increasing HSA bonding, however the correlation is not strong enough to justify a quantitative model. Direct modeling from Abraham parameters again did not yield any useful result.

Figure 38: Log HSA by Valko et al (2003) (y) vs. experimental data of log k_2 (x).

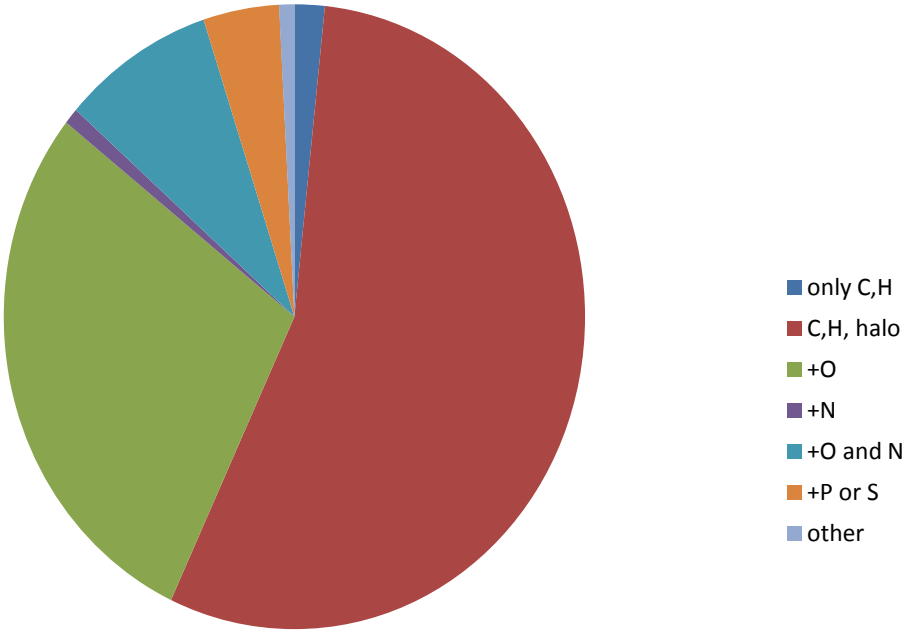


2.3.4 Chemical investigation of BMF data

2.3.4.1 Compounds

The BMF data set was condensed to unique chemical structures in the same manner as described for the BCF data set. From the experimental values, 604 of them could be assigned to 237 unique organic chemical structures. 32 values corresponded to polymers, and the remaining 113 to other UVCBs. Averaging was straightforward here. Eventually, 90 BMF data could be calculated from 236 individual measurements, and 131 k_2 data from 331 listed values. For 33 compounds, BMF as well as kinetic BCF from the BCF data set were available, and for 48 chemicals k_2 from both sets.

Figure 39: Chemical composition of the BMF data set



2.3.4.2 Chemical domain

The respective analyses in the same manner as with the kinetic BCF set are shown in Figure 39 to Figure 41. With regard to the goal of feeding studies, the selection of chemicals is shifted towards hydrophobic substances. More than half of the chemicals (56%) were halogenated hydrocarbons, followed by 29% of compounds containing oxygen. The remaining groups are rather marginal, 9% with N and O, almost 4% with P and/or S, <1% with N, 2% hydrocarbons, and <1% other. Again, the fraction of aromatic compounds (86%) is very high.

There is also less complexity than in the BCF set. More than half of the chemicals (almost 57%) contain only one type of functional groups, even though with multiple occurrences. Mainly, these substances belong to the group of halogenated hydrocarbons. There are 33% of chemicals with two different functional groups, and 8% with more than 2. <2% of the chemicals had only one single functional group and the remaining <2% are the hydrocarbons.

Figure 40: Complexity analysis of the BMF data set

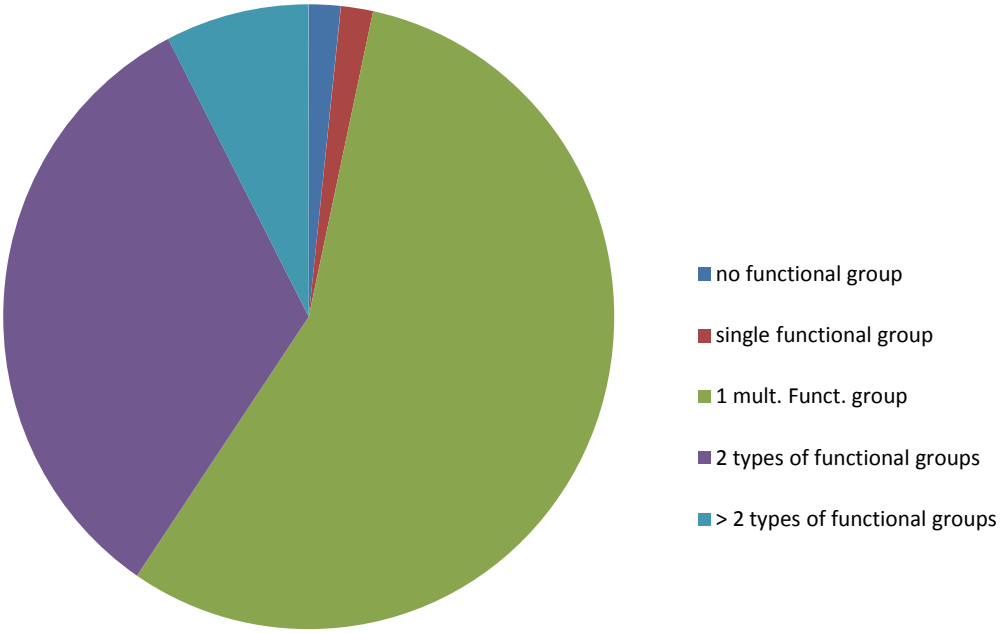
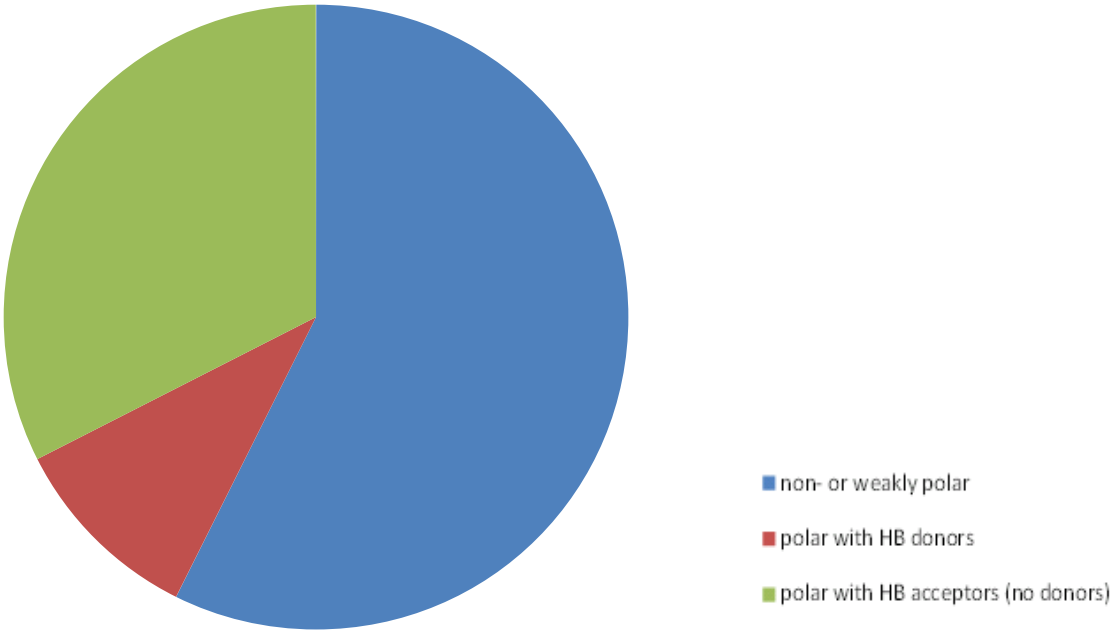


Figure 41: Polarity analysis of the BMF data set



Correspondingly, 58% of the chemicals are non-polar or weakly polar only. There are 33% with hydrogen bond acceptors without donors, and almost 10% with donors. There are no polar chemicals in the set without hydrogen bonding.

2.3.5 BMF dependency on k_2 (BMF)

From Figure 42 can be seen that k_2 does not correlate to hydrophobicity in terms of K_{ow} nor to the membrane/water partion coefficient K_{mw}.

Figure 42: Comparison of log k_2 (BMF) (y) to log K_{ow} (blue) and log K_{mw} (red) (x).

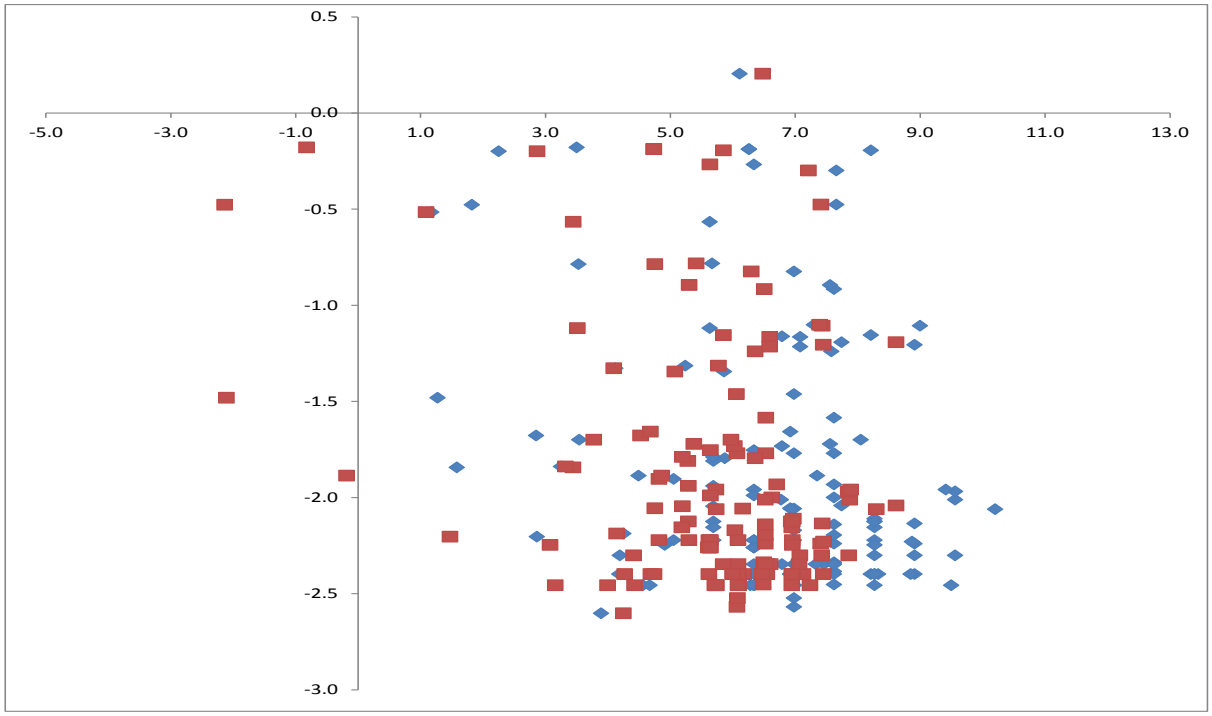
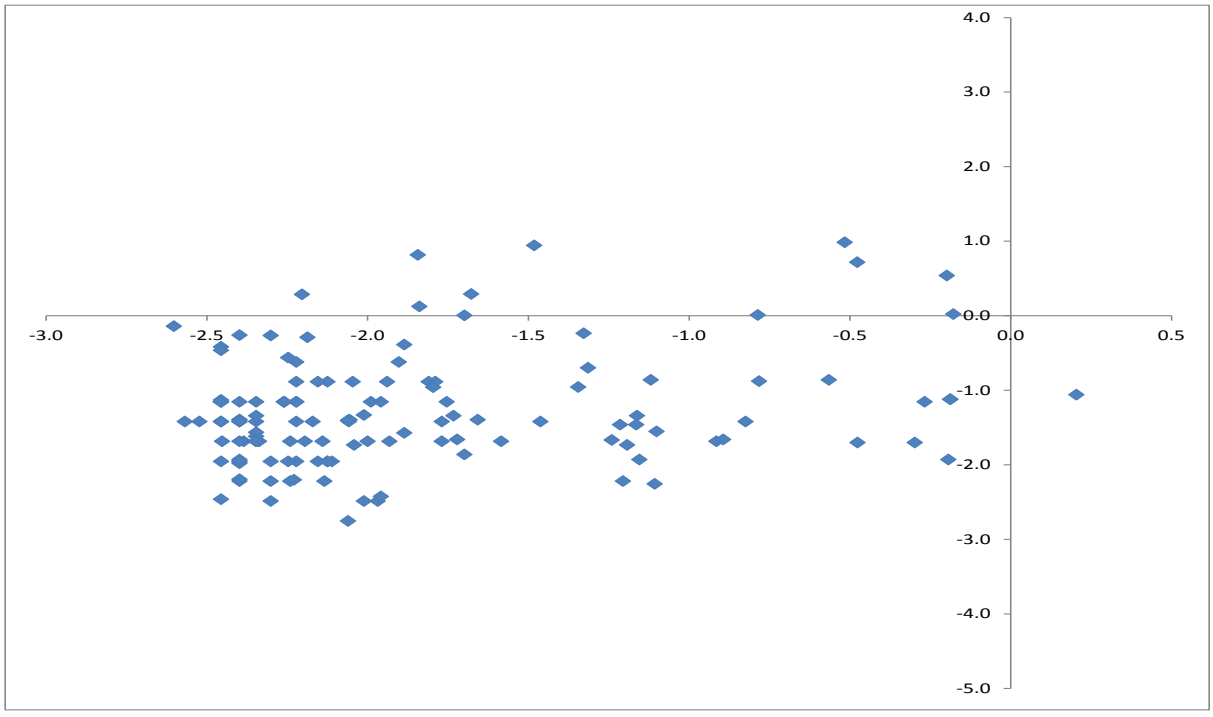


Figure 43: Comparison of estimated (Equation 20) log k_2 (BCF) (y) to experimental log k_2 (BMF) (x).



2.3.5.1 Modeling k_2

The OECD TG305 approach from K_{ow} (Figure 43) is not appropriate here. A direct model from Abraham parameters did not yield any useful results. Estimated log k_2 (BCF) compared to experimental log k_2 (BMF) showed no correlation.

2.3.6 Comparison of kinetic BCF and BMF

As can be seen from Figure 44, for the few chemicals with both kinetic BCF and BMF available, there is some correspondence regarding the trend. However, no general trend of the elimination rates can be observed due to (Figure 45), some scatter. One must keep in mind, that absolute numerical agreement cannot be expected here. Even though both k_2 values are measured as whole body data, the different uptake routes alter the occurrence of the chemical in the body compartments, and thus may yield a shift in the ratio of the individual elimination processes.

Figure 44: Comparison of log BMF (y) to the kinetic log BCF (x). One outlier (PCB 26) is not shown because of the probably unreliable kinetic BCF.

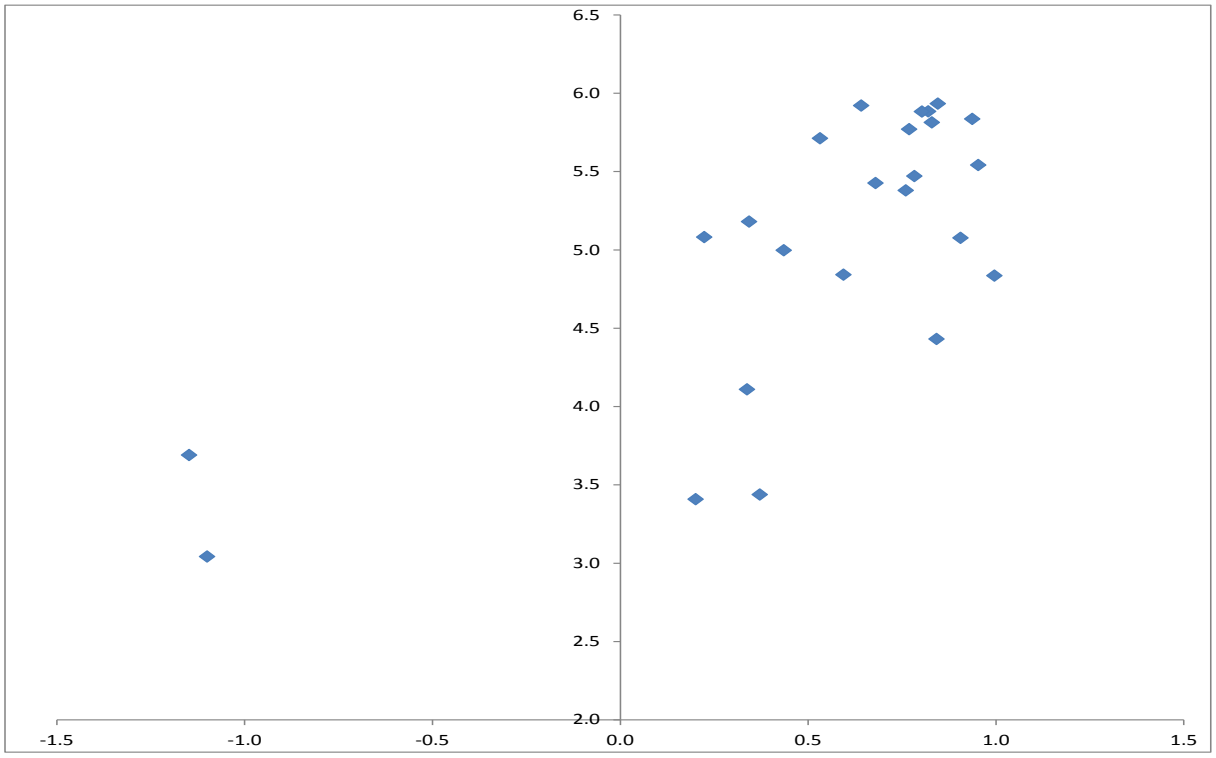
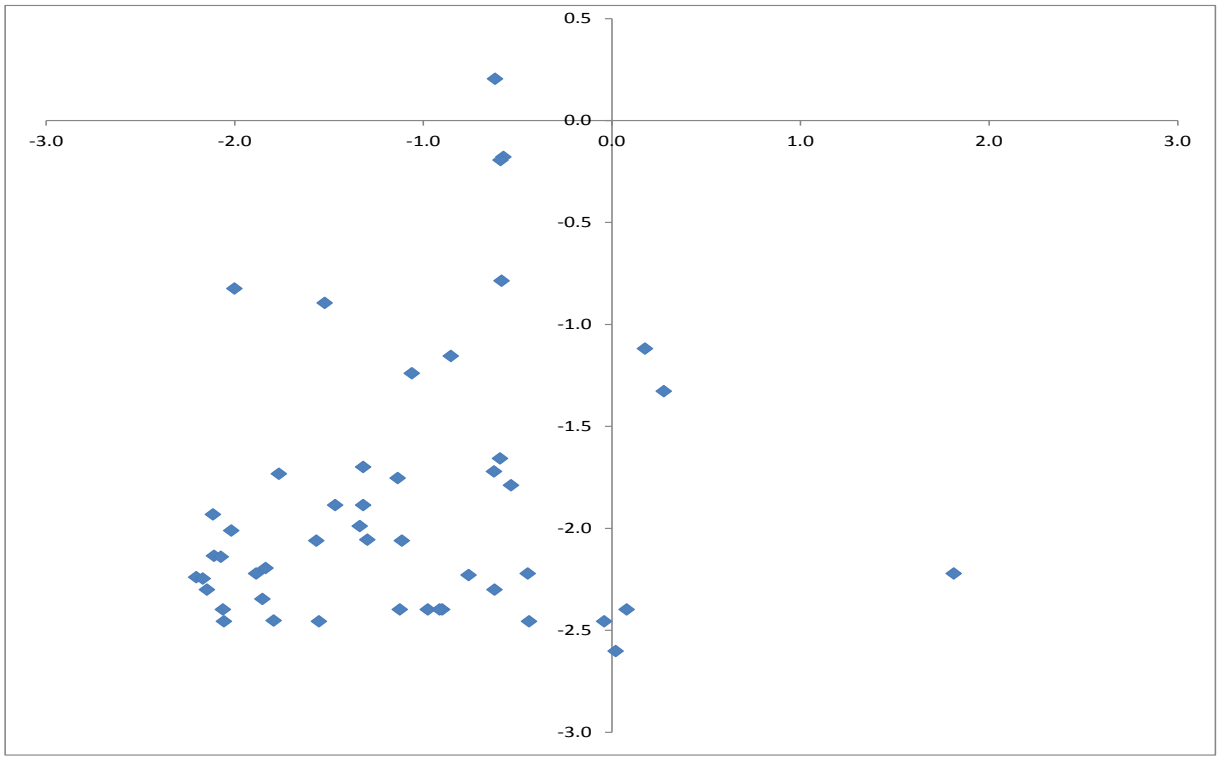


Figure 45: Comparison of log k_2 (BMF) (y) to k_2 (BCF) (x).



2.3.7 Summary

The analysis of the available data did not allow deriving a reliable model. The main reason is the limited number of data. Moreover, the available data were rather uncertain, as indicated by the large effort of data curation required. A rather high part of the (originally more than 700) data did not correspond to unique chemical structures at all, and from the remaining data a lot of them could not be used due to insufficient reliability. Even more, the 604 data in the final set corresponding to 237 different organic compounds (i.e. roughly one duplicate for each chemical in average) still contained a large variability and uncertainty, e.g. with regard to species, protocols, etc. In result, only some weak trends could be observed from the data analyses.

To overcome these shortcomings and to step forward for achieving a data set useful for model developments, additional measurements seem to be unavoidable. In order to minimize such efforts, mainly for ethical reasons but also for economic reasons, respective studies should focus on particular criteria to select the test compounds:

1. Since the concern is bioaccumulation, test focus should be on hydrophobic chemicals.

2. Experiments should be carried out with single chemicals with known and unique structure.

3. Preferably compound should be selected with known steady state BCF.

4. Studies for compounds with already known k_1 and/or k_2 from kinetic measurements are appreciated.

5. Studies should avoid halogenated hydrocarbons, because this compound class already dominates the overall data set. Particularly, compounds containing heteroatoms as N, S, and P are missing in the data set.

2.4 General Summary

Two data sets have been established and validated from literature search, one for kinetic BCF together with the individual uptake and elimination rates, and another one for BMF together with individual elimination rates. Additionally, an already validated data set of steady-state BCF values was available.

First, the kinetic BCF were examined. Compound classes with large deviations between steady-state and kinetic BCF were identified, these were mainly thiophosphates and multiply halogenated unsaturated hydrocarbons. Existing models for k_1 and k_2 were applied and compared to the experimental data. Even though in some cases at least trends were visible, none of them can be assumed to be sufficient yet. The limited number of experimental data did not allow for deriving a new model.

Inspection of the BMF set revealed a serious imbalance toward halogenated hydrocarbons. Further studies, that turned out to be unavoidable with regard to a sufficient set for modeling, should focus on compounds classes containing heteroatoms as N, S, and P.

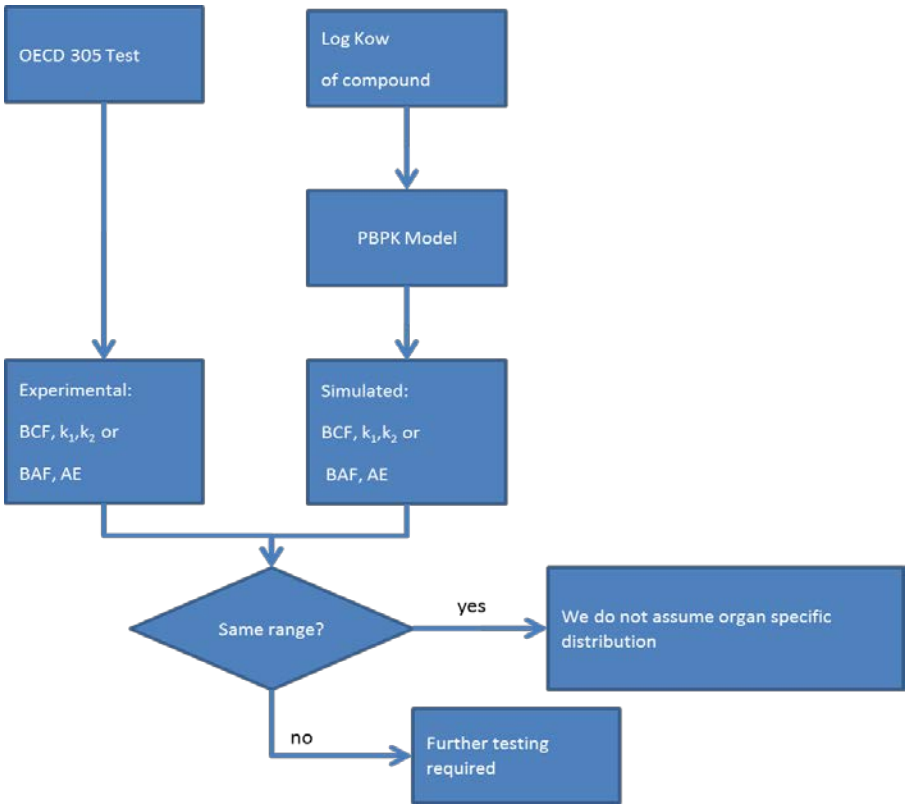
3 Part II: Proposal to take account of organ specific accumulation

3.1 Objective

A Physiologically Based Toxicokinetic (PBTK) model describes a whole organism using physiological components, in which several organs are each considered as a compartment and are connected through the blood system (Abbas & Hayton1997, Nichols et al. 1998). In contrast to the often used compartment models, a PBTK approach is considerably more detailed and complicated (OECD TG 305). Toxicokinetic aspects are described by certain uptake paths (e.g. branchial, dermal, dietary or injection) and distribution of a chemical to the several compartments by the blood system. Therefore, a PBTK model consists of species- and life-stage specific parameters such as weight and lipid content, but also more substance-specific data like tissue/organ distribution (Nichols et al. 1998, Schmitt 2008). In turn this means that it is possible to estimate the distribution pattern of a chemical by its physico-chemical properties instead of generating data directly with laboratory experiments.

The aim of this workpackage was to elucidate the possibility to use a PBTK model for identifying possible non lipid triggered organ distribution of compounds from an OECD 305 experiment in which only full body concentrations are measured. The assumption behind is that a PBTK model is a good representative of bioconcentration and bioaccumulation for fish and predicts the organ distribution correctly. Since organ distribution in a PBTK model is lipid based, non-lipid distribution should show different organ distribution patterns and this difference in organ distribution also result in differences of bioconcentration factors and kinetic constants measured in OECD 305 experiments. The application of this approach, if successful, is shown in Figure 46, to test the applicability of the concept several steps have to be fulfilled. The crucial step is to show the validity of the PBPK model for lipophilic compounds and differences in the organ distribution for non-lipophilic compounds resulting in differences in toxicokinetic endpoints.

Figure 46: Approach for identification of compounds with specific organ distribution from OECD 305 data



To run the PBPK model, parameters as the log K_{ow} of the compound as well as the simulation time and exposure time in h are required. Other parameters needed to run the model are body wet weight in kg, chemical concentration in µg/l, water temperature in °C, dissolved oxygen concentration in mg/l and lipid content of the whole fish as a fraction of body weight. If endpoints calculated from the experiment and simulated by the model are within the same range as obtained by OECD 305 studies, it can be assumed that kinetics are lipid dependent. If not, further investigations are required. The approach is validated in this project.

3.2 Material and methods

The following approach was selected to test this concept:

A literature search was conducted to find studies in which organ distribution of rainbow trout was investigated. Literature was investigated in Web of Science as well as in the ECOTOX database of US EPA. For all compounds distribution factors were calculated and the following steps carried out:

1. Organ distribution patterns for lipophilic chemicals were investigated
2. Organ distribution patterns for non-lipophilic compounds were investigated
3. A PBTK model was selected, implemented and tested
4. Simulated and measured organ distribution patterns were compared
5. The PBTK model was used to simulate OECD 305 experiments for non-lipophilic and lipophilic compounds and toxicokinetic endpoints were compared.

Within the following chapters the results for the different steps are presented.

Comparison of organ distribution was based on distribution factor (DF) to compensate for different concentrations, exposure times and bioconcentration factors.

Distribution factors were calculated for all approaches using Eq. 26:

$$DF = \frac{Corg}{Cint} \qquad \text{(Equation 26)}$$

C_{org}: Concentration of the chemical in the organ of interest [mg/kg wet weight]

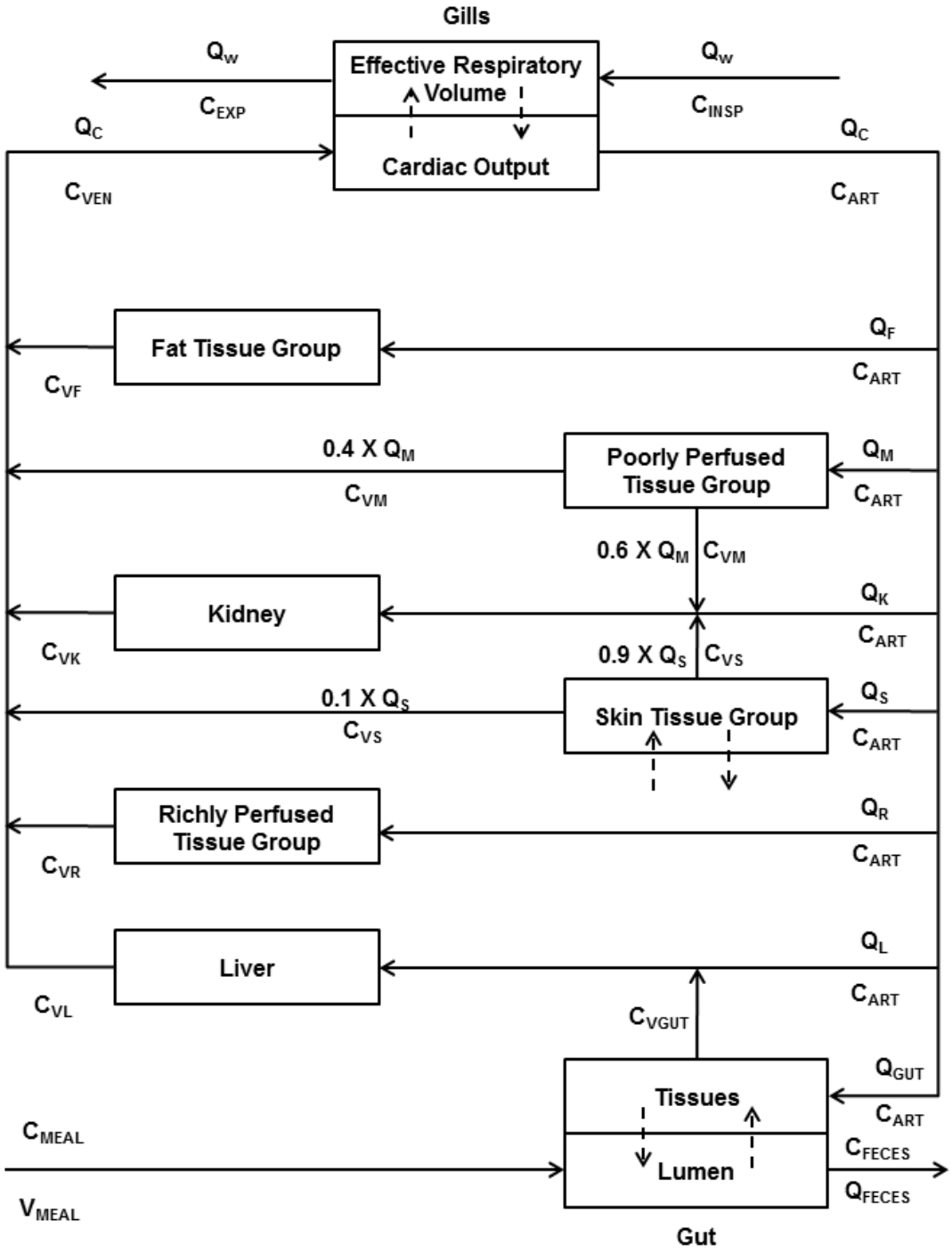
C_{int}: Concentration of the chemical in the whole fish [mg/kg wet weight]

3.2.1 Physiologically Based Toxicokinetic (PBTK) Model for fish

3.2.1.1 Modeling: Organ distribution of lipophilic and non-lipophilic chemicals

A PBTK model was implemented in order to predict uptake and distribution of chemical substances based on their lipophilicity (Figure 47). The framework was taken from a publication by Nichols et al. (1990), describing disposition of chemicals after waterborne uptake. The PBTK model was the identical version used in Brinkman et al. (2014). Currently, only rainbow trout (*Oncorhynchus mykiss*) has been included. The model included a flow-limited description of chemical flux at fish gills (Erickson & McKim 1990) and distribution between the following compartments (organs): adiposis tissue, poorly perfused tissues (muscles), kidney, richly perfused compartment and liver. Additional adjustments, summarized by Stadnicka et al. (2012), were made regarding the relationship between lipid fractions in the whole body and the volume of fat compartment. For model parameters and equations see the supporting information of Stadnicka et al. (2012). The model was implemented using Embarcadero Rad Studio XE2 (Embarcadero Technologies, San Francisco, USA).

Figure 47: Schematic representation of a PBTK model for fish incorporating branchial, dermal and dietary routes of exposure. Symbols and abbreviations are given in Nichols et al. (1990), Nichols et al. (1996) and Nichols et al. (2003a).

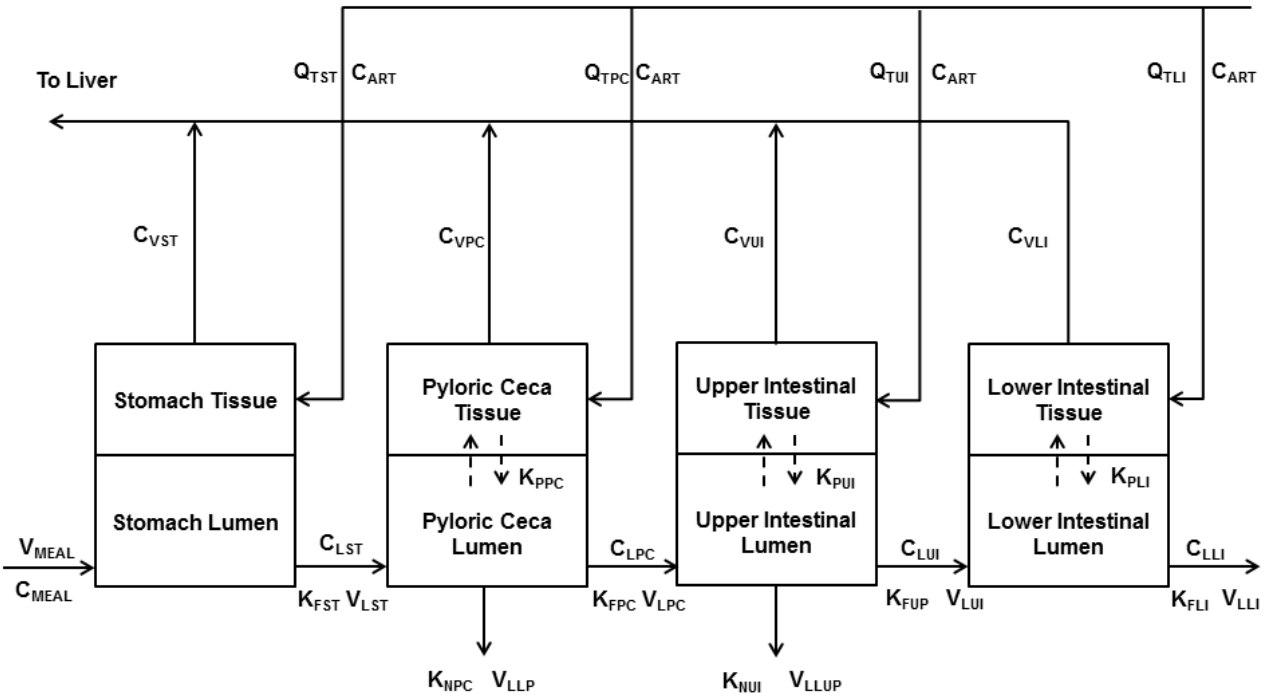


Moreover, the model was further extended by considering two additional uptake pathways: 1. Dermal absorption and 2. Dietary uptake. Dermal uptake was implemented according to Nichols et al. (1996). The skin was modeled as a discrete compartment where uptake and elimination of chemicals could be described as a function of chemical permeability and the concentration gradient between exposed water and skin tissue.

Dietary uptake was presented by Nichols et al. (2003) and incorporated intake of food into the gastrointestinal (GI) tract (Figure 48). The GI tract consisted of four compartments: stomach, pyloric ceca, upper intestinal and lower intestinal. These compartments were each divided into lumenal contents and tissue. The volume of lumenal contents was allowed to change in time as a function of bulk flow down the GI tract and nutrient uptake (for pyloric ceca and upper intestine). Chemical flow was described by the change of lumenal volume with time as well as diffusion between lumenal contents and GI tissues using permeability coefficients.

The model assumes lipid based organ distribution only. Uptake, elimination and distribution of chemicals in the fish are triggered by the lipid content of the organs and the K_{ow} of the compound. To achieve distribution patterns of chemicals depending on their log K_{ow}, model simulations were performed. Results are expressed as Distribution Factors (DFs), which were calculated by dividing the concentration of a chemical inside the tissue by the concentration inside the whole fish at equilibrium.

Figure 48: Schematic representation of the gut description by Nichols et al. (2003a). Symbols and abbreviation are given in the corresponding publication.



Predictions of the PBTK model were compared with measured bioconcentration factors and rate constants for organic pollutants in rainbow trout (*Oncorhynchus mykiss*) available from peer-reviewed literature. Relevant studies were identified by searching PubMed and Web of Science from September to December 2013with a combination of the key words bioconcentration and rainbow trout. In addition, reference lists of eligible studies were searched manually. Title and abstract of the collected articles were screened for relevance by assessing the study specimen, exposure route and compounds tested. Studies were excluded if they: (1) did not include rainbow trout, (2) did not investigate organic compounds, (3) did not assess water as route of exposure or (4) did not take any of the following compartments into account: blood, fat, muscle, liver,

kidney, skin, richly perfused tissues or whole body. Along with bioconcentration factors (BCFs), kinetic uptake rate constants (k_1) and kinetic depuration rate constants (k_2), available data on body weight, fish lipid content, oxygen concentration, temperature and metabolism were extracted. In case no information on body weight, lipid content, oxygen concentration and temperature was given, the model was run with the following standard settings: 0.1 kg, 8.5 %, 8.87 mg/L and 11° C, respectively. The literature search yielded a total of 320 studies (Pubmed: 66; Web of Science: 254) of which 18 publications (with 77 different chemicals) were eligible for inclusion. For this 77 chemicals measured K_{ow} as input for the PBPK model were taken from literature. In addition to peer-reviewed data, 29 anonymized BCF values measured according to the OECD guideline and provided by the UBA were integrated in the model calibrationcompared to model predictions.

3.3 Results

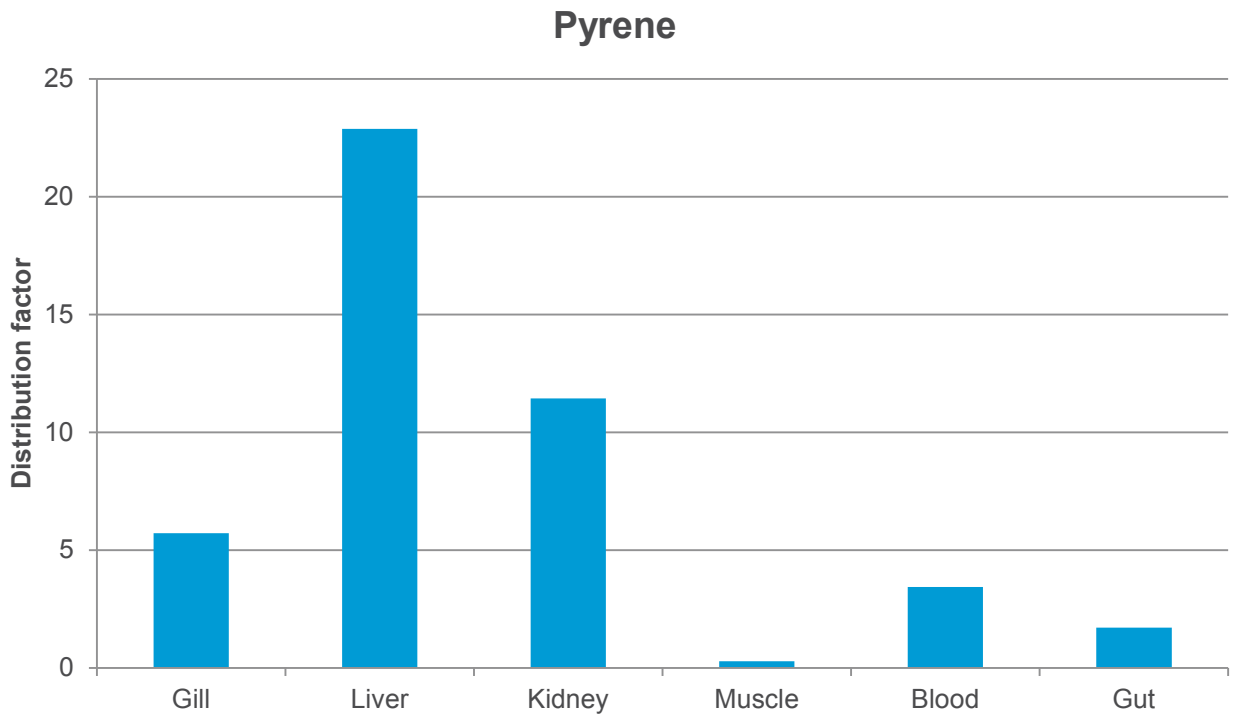
3.3.1 Organ distribution pattern for lipophilic chemicals

In the literature, eight studies were found and investigated in more details. The investigated compounds and their properties are given in Table 2.

Table 2: Investigated lipophilic chemicals

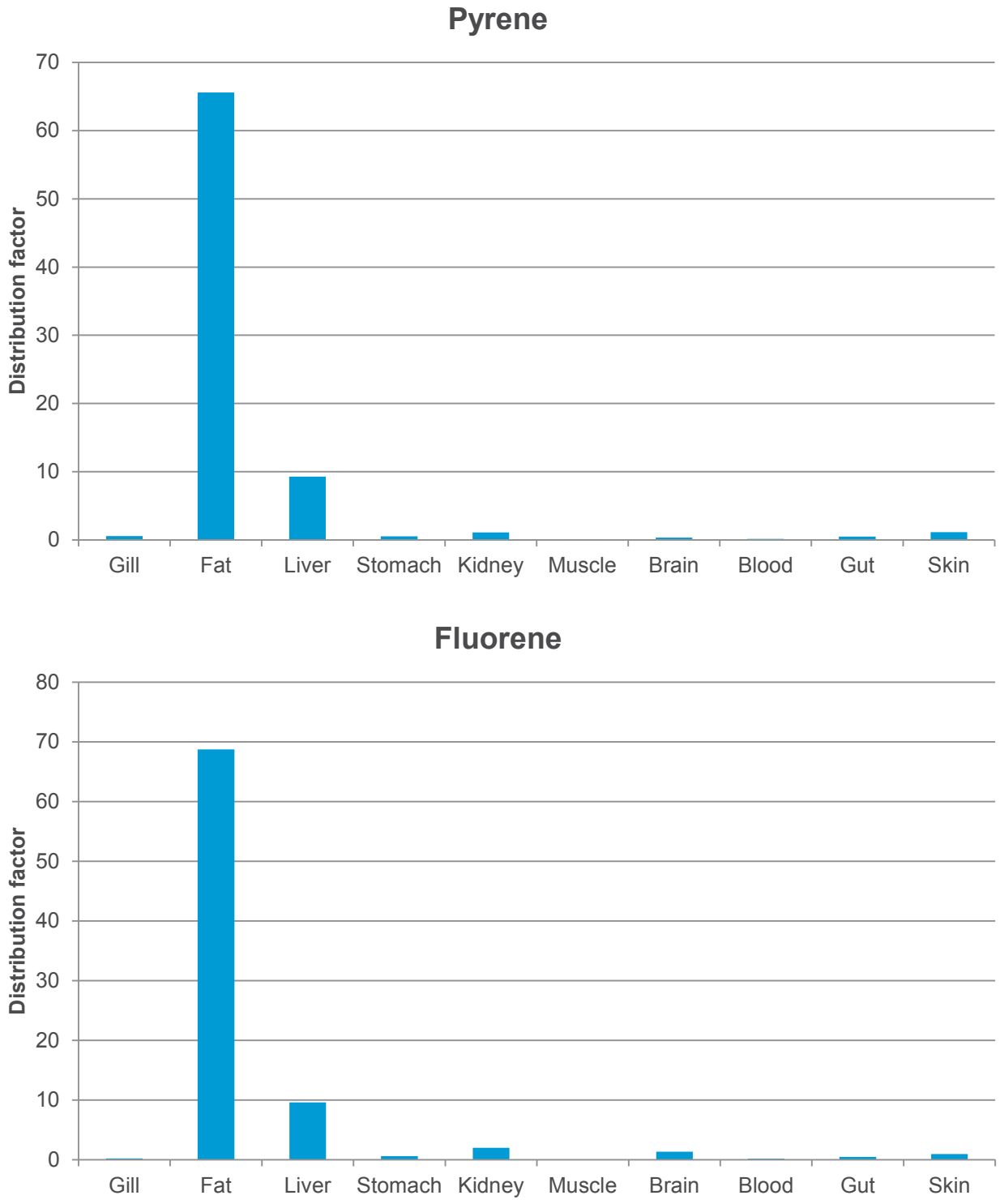
Compound	Log K_{ow}	Exposure	Exposure time	Reference
Pyrene	4.88	Intraarterial injection	140 days	Law et al. 1991
Pyrene	4.88	Intraarterial injection	6 days	Kennedy & Law 1990
Fluorene	4.18	Intraarterial injection	6 days	Kennedy & Law 1990
2-Methylnaphthalene	3.86	Intraarterial injection	6 days	Kennedy & Law 1990
Benzo[a]pyrene	6.06	Intraarterial injection	2 days	Seubert & Kennedy 2000
Nonylphenol	4.2	Static exposure in water	2 days	Lewis & Lech 1996
Bisphenol A	3.2	Exposure in water	7 days	Lindholst et al. 2000
4-tert-octylphenol	4.12	Exposure in water	10 days	Ferreira-Leach & Hill 2001
Rotenone	4.1	Injection	3 days	Gingerich 1986
Oxytetracycline	-1.12	Intravenous injection	4 days	Black et al. 1991

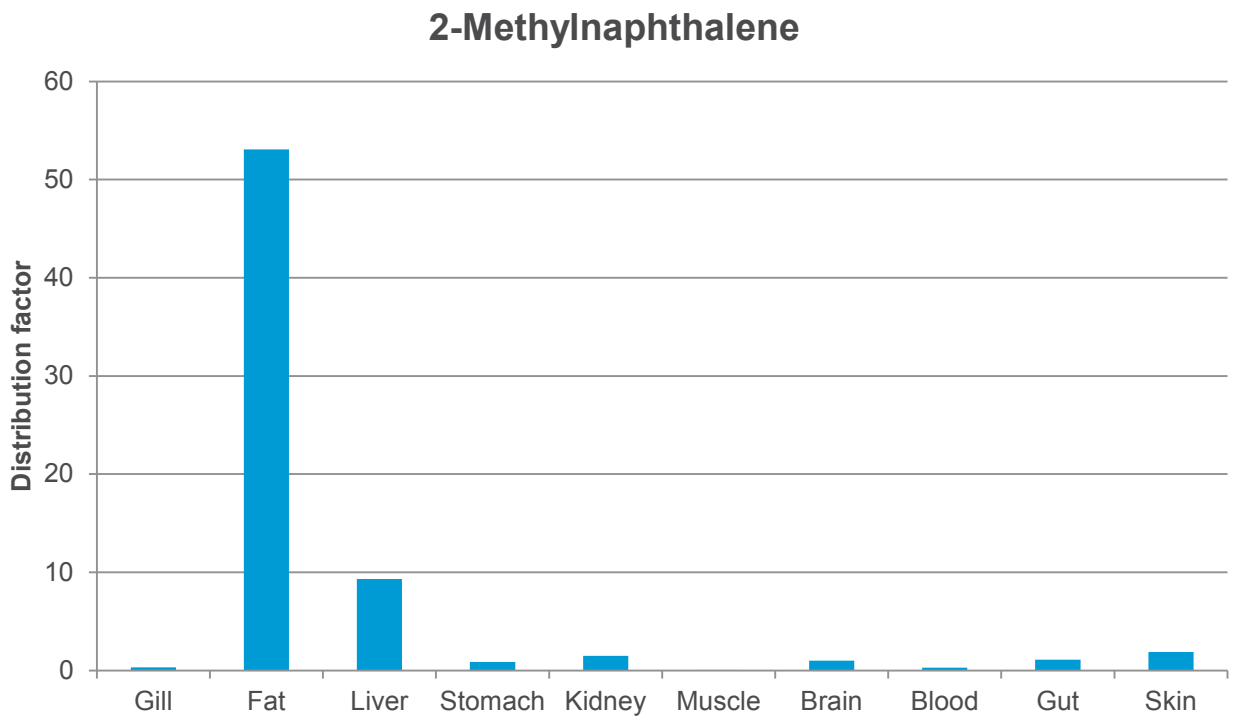
Figure 49: Distribution factors for different tissue compartments for rainbow trout taken from experimental data by Law et al. (1991)



Pyrene was investigated in two studies. Figure 49 represents the results of a study performed by Law et al. (1991) which estimated DFs for Pyrene. The values range from less than 1 (muscles) to about 23 (liver). The kidney showed the second highest DF of about 10, followed by the gills with 5 as well a blood with 3 and gut with 2. These values were different from the model results and generally higher than simulated. The DF ranking for the compartments were also different as the liver showed a higher DF than the kidney. Comparability was limited, however, as the study did not include a fat compartment and additionally considered concentrations in gill, gut and blood. Another publication by Kennedy & Law (1990) indicated different patterns and distribution of pyrene and other chemicals (Figure 50). This study covered fat tissue and showed very high DFs for this compartment. For the investigated chemicals, fat showed the highest DF of approximately 65, 69 and 52 for Pyrene, Fluorene and 2-Methylnaphthalene respectively. The second highest DF was calculated for liver and was about 10 for all three substances. Moreover, the third highest DF of about 1 for the kidney was observed for Pyrene and Fluorene. Other compartments had lower DFs of a bit less or strongly lower than 1 for 2-Methylnaphthalene. The tissues of kidney and skin had about the same DFs, which were a bit higher than for Pyrene and Fluorene. Stomach, brain and gut tissue also presented slightly higher DFs than for the other two chemicals. Figure 51 compares DFs for different tissues in rainbow trout resulting from experiments by Seubert & Kennedy (2000). The fish were exposed by intraarterial injection with Benzo[a]pyrene (Figure 51). Data from this publication showed a different pattern of distribution. The trend of DFs was: liver > blood > kidney > fat > gill > stomach > gut > gill > muscle.

Figure 50: Distribution factors of Pyrene, Fluorene and 2-Methylnaphthalene for different tissue compartments for rainbow trout taken from experimental data by Kennedy & Law (1990).





Because the exposure time was only 48 hours, it could be assumed that equilibrium conditions were not reached yet. Therefore, a model simulation was performed using the same experimental conditions as Seubert & Kennedy (2000). According to the model output, equilibrium conditions could be expected after 48 hours (Figure 52), which indicates no change of DFs after that point. Furthermore, the model showed a higher DF for fat throughout the simulations time except for the first few hours, which was different to the experimental results even when non-equilibrium conditions were assumed for the experimental data (Figure 51).

Data for Nonylphenol (Figure 53) suggest similar DFs of about 10 for liver, kidney and fat. The muscle, heart and gills showed lower DFs of 1-2. As above, these results might be influenced by a rather short exposure time of 48 hours. Nonylphenol however, is a difficult chemical to generate distribution and accumulation data with due to its fast metabolization rate (Preuss et al. 2008).

Figure 51: Distribution factors for different tissue compartments for rainbow trout taken from experimental data by Seubert & Kennedy (2000).

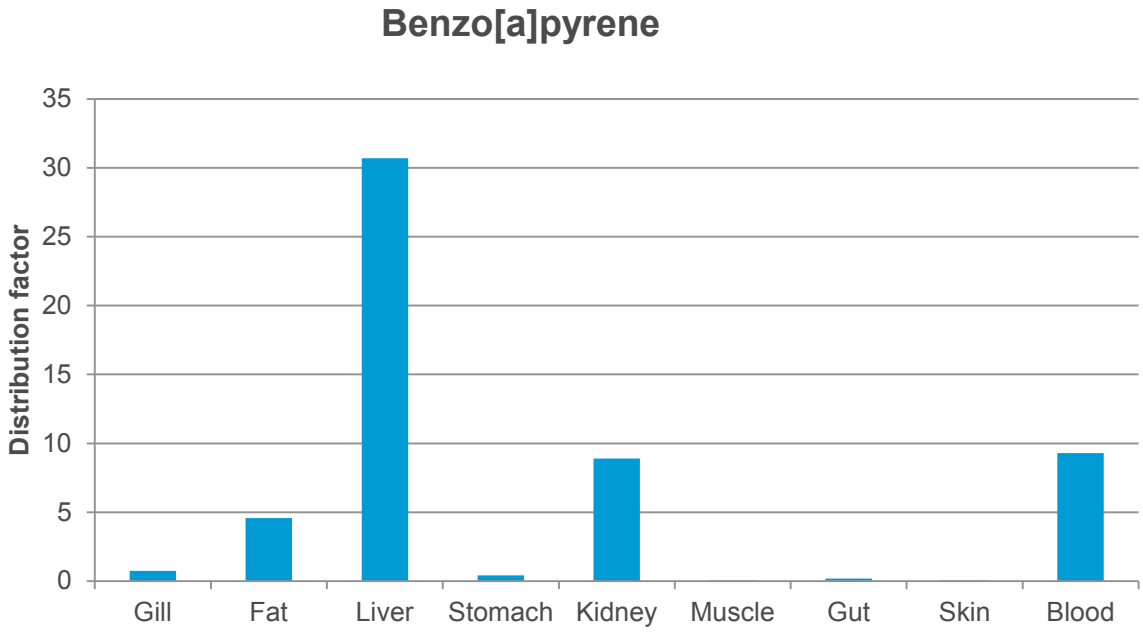


Figure 52: Model output showing distribution factors against time for different rainbow trout tissue compartments according to experimental conditions by Seubert & Kennedy (2000).

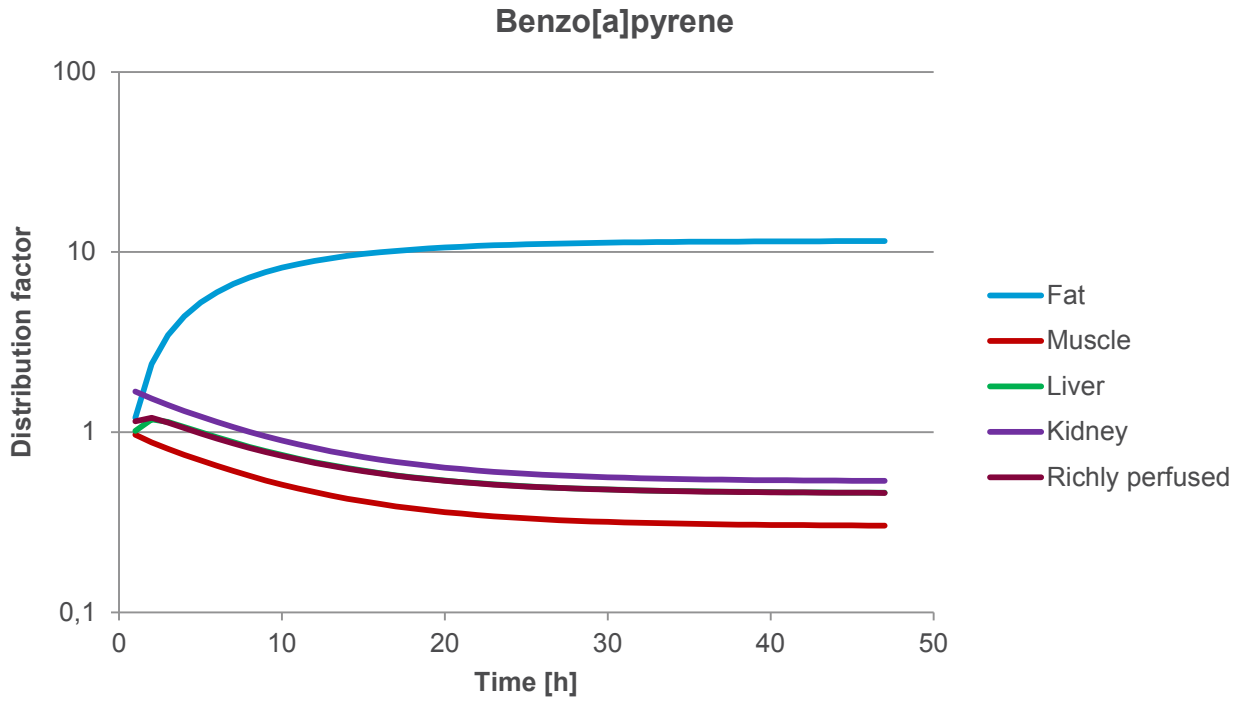
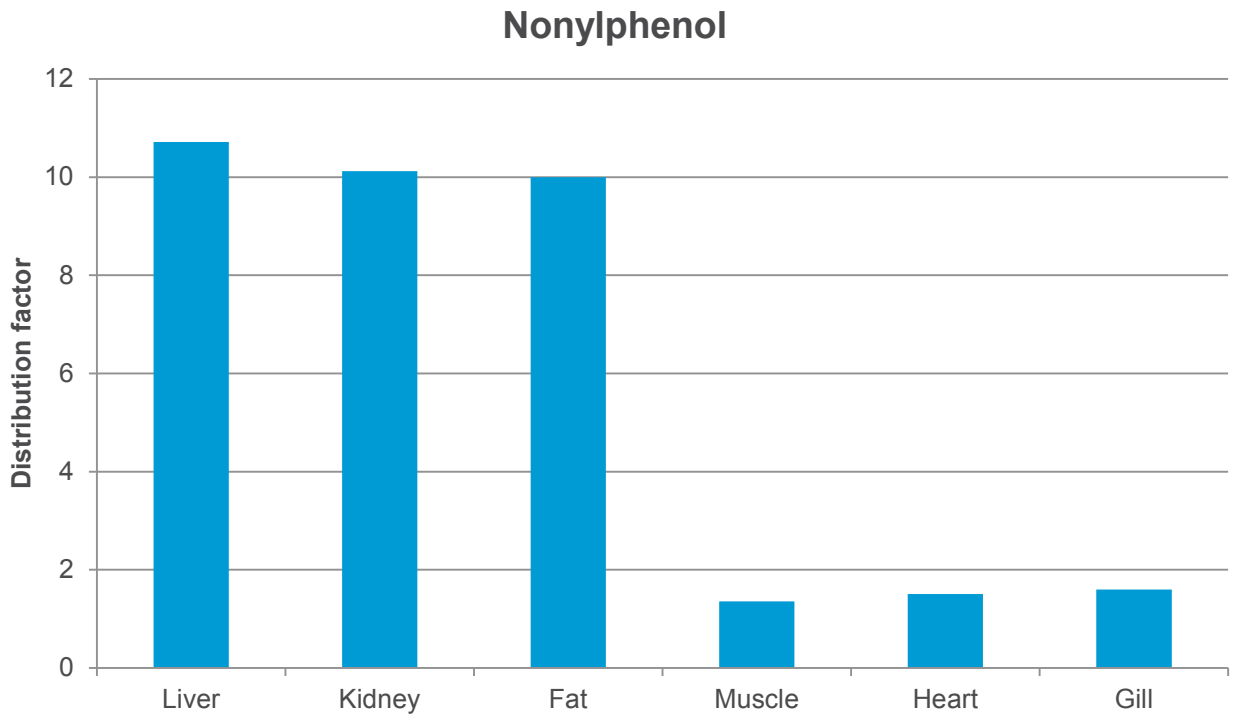
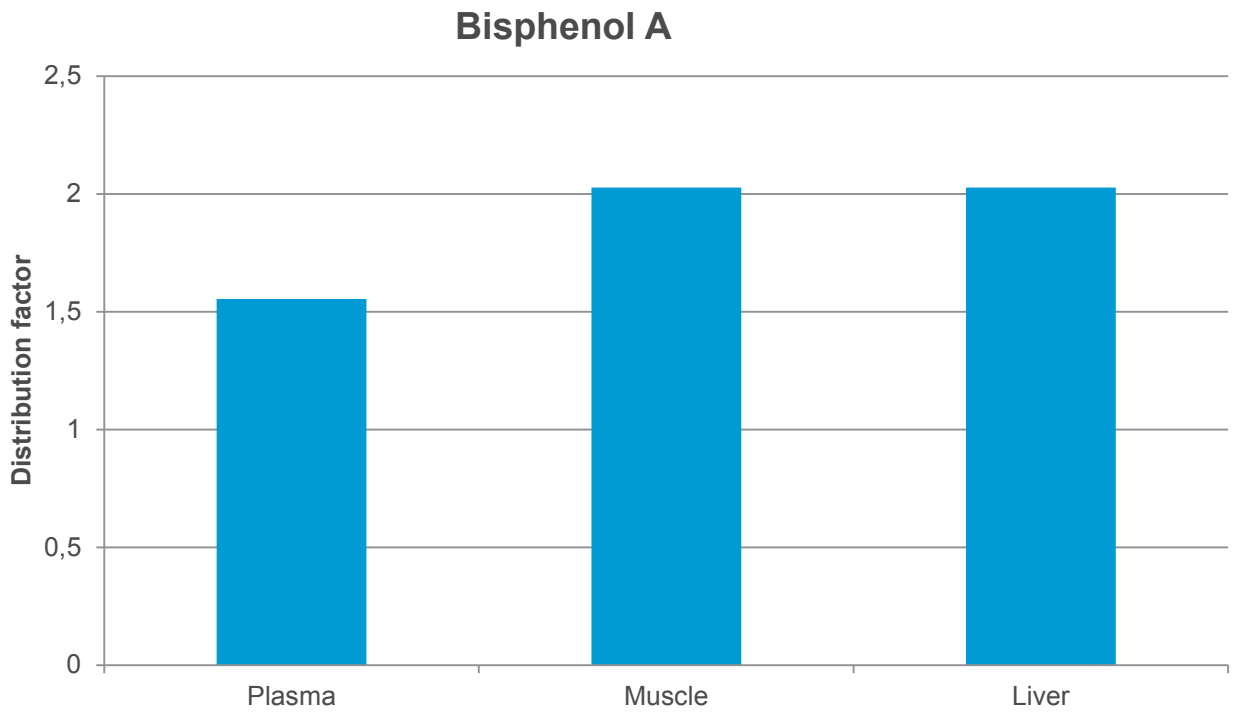


Figure 53: Distribution factors for different tissue compartments for rainbow trout taken from experimental data by Lewis & Lech (1996).



DFs for Bisphenol A from a study by Lindholst et al. (2000) were 1.5, 2 and 2 for plasma, muscle and liver respectively (Figure 54). Unfortunately, the authors only considered those 3 compartments, so a comparison is certainly difficult.

Figure 54: Distribution factors for different tissue compartments for rainbow trout taken from experimental data by Lindholst et al. (2000).



Ferreira-Leach & Hill (2001) compared tissue distribution for 4-tert-octylphenol (Figure 55 and Figure 56), showing very high DFs for bile and faeces (140 and 30 respectively) and lower DFs for fat, liver, intestines and pyloric caeca ranging from 1.5 to 2.5. Other tissues had DFs of < 0.5.

Figure 55: Distribution factors for different tissue compartments for rainbow trout taken from experimental data by Ferreira-Leach & Hill (2001).

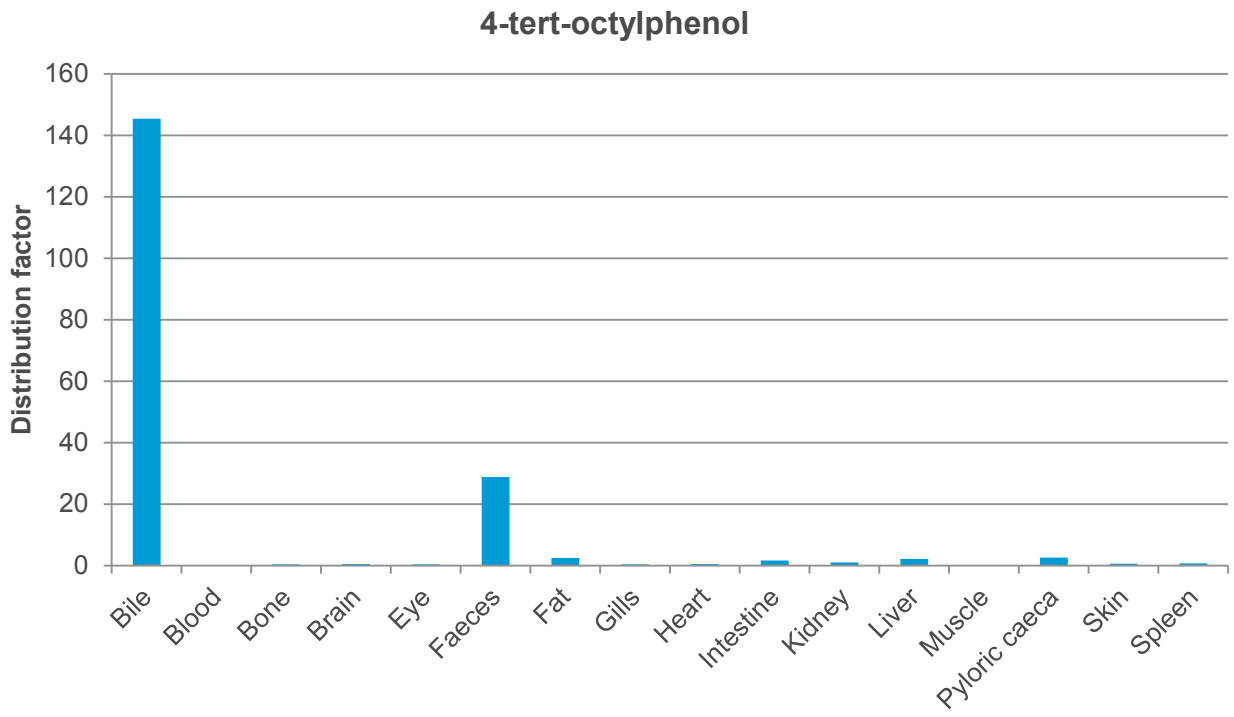
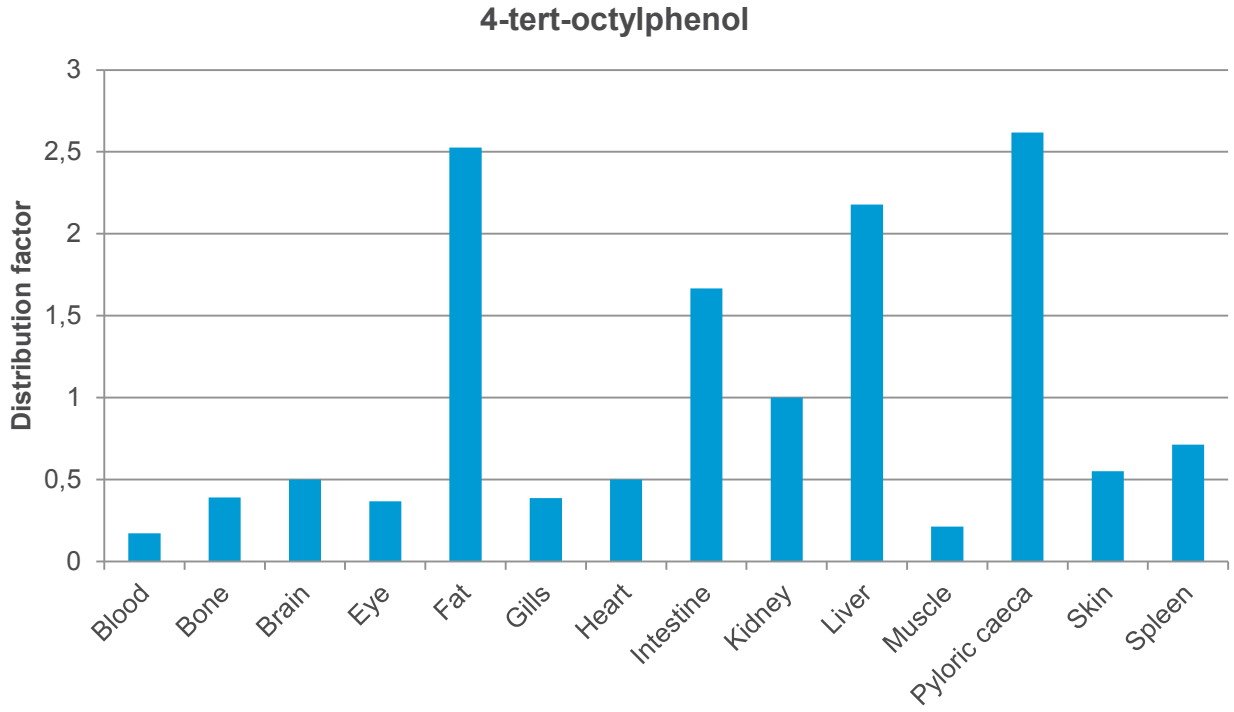


Figure 56: Distribution factors for different tissue compartments for rainbow trout taken from experimental data by Ferreira-Leach & Hill (2001).



DFs for the pesticide Rotenone (Gingerich 1986) were high for the heart, intestines, pyloric caeca and red muscles (4.5 – 8.5) and low for other tissues (Figure 57), including fat, liver, kidney and white muscles (0.5 - 2).

Figure 57: Distribution factors for different tissue compartments for rainbow trout taken from experimental data by Gingerich (1986).

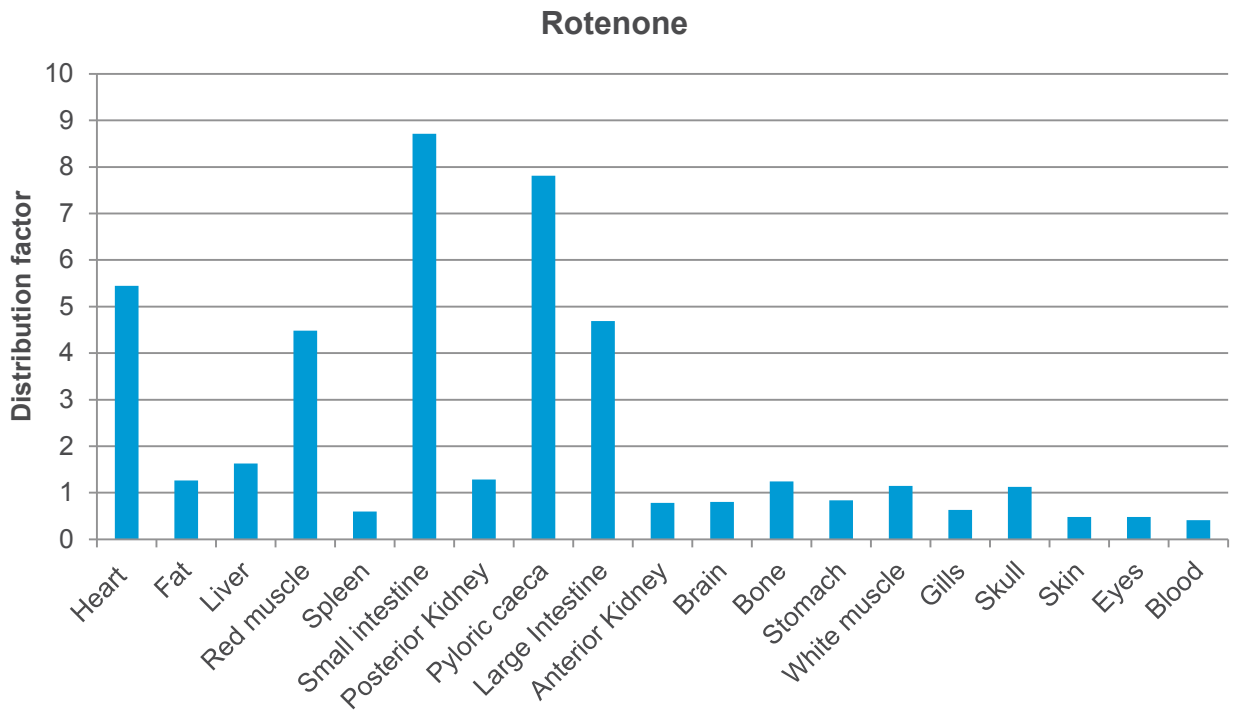
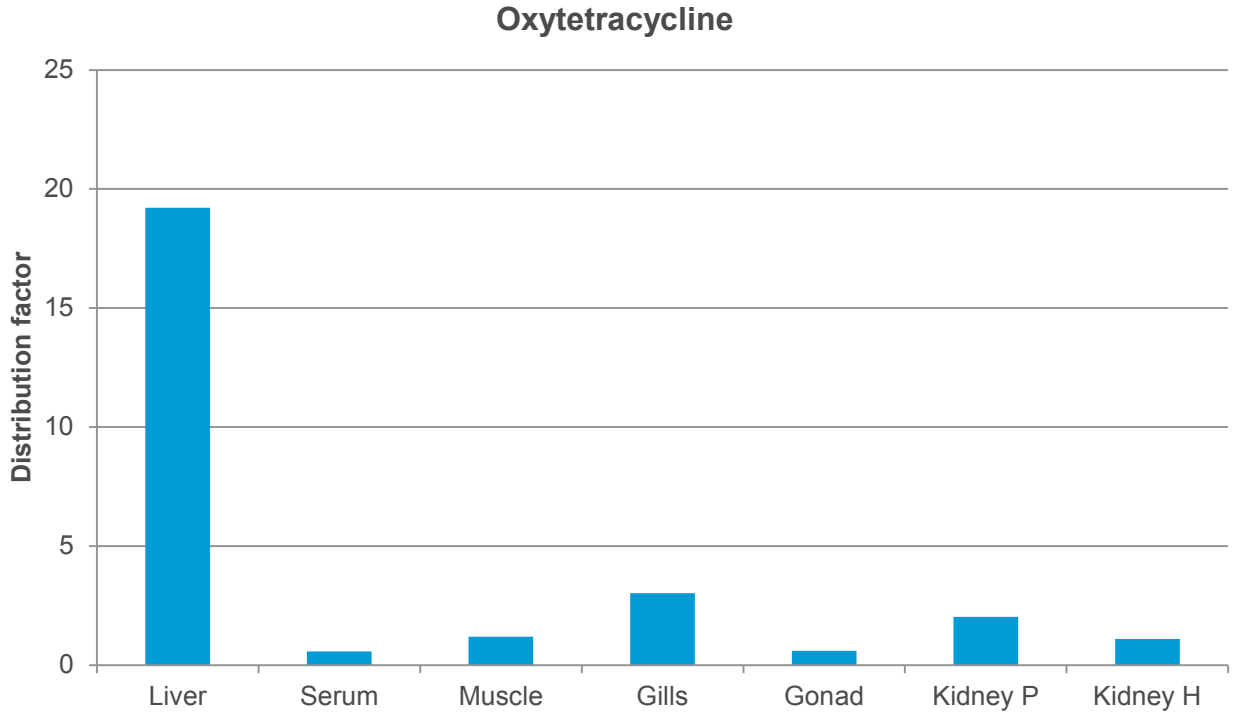


Figure 58: Distribution factors for different tissue compartments for rainbow trout taken from experimental data by Black et al (1991).



Oxytetracycline, analyzed by Black et al. (1991), showed a DF of around 20 for liver, followed by gills, posterior kidney, anterior kidney, muscles, gonads and serum (Figure 58). Apart from the liver tissue, all other DFs range from 0.5 to 3.

The presented studies show very different results with only data by Kennedy & Law (1990) agreeing closely with model predictions. The discrepancies were no doubt partly due to inclusion or exclusion of different tissues and organs. Since the model is based on Nichols et al. (1990), it cannot predict concentrations or even simulate the influence of compartments like bile or brain. Another source of inaccuracy is the calculation of DFs for the experimental data. Many, if not all presented publications did not show DFs directly and presented concentrations in tissues instead. One difficulty was to determine the concentration of a chemical in the whole fish for those studies since it was not given but had to be calculated from the total amount of chemicals, which in turn has to be determined from the sum of chemical-amounts in all tissues. Because this had to be calculated from the concentration in tissues (mostly given) and volume of tissues (mostly notavailable), an estimation of tissue volumes was required (Table 3).

Table 3: Estimation of tissue volumes in fraction of whole body weight.

Tissue	Fraction of whole body weight	Reference
Gill	0.039	Law et al. 1991
Fat	0.01	Nichols et al. 1990
Liver	0.0116	Law et al. 1991
Stomach	0.015	Nichols et al. 2003a
Kidney	0.008	Law et al. 1991
Muscle	0.465	Law et al. 1991
Brain	0.00154	Abbas & Hayton 1997
Blood	0.0411	Law et al. 1991
Skin	0.1	Nichols et al. 1996
Gut/Intestines	0.0852	Law et al. 1991
Heart	0.00172	Abbas & Hayton 1997
Carcass	0.35	Law et al. 1991
Anterior Kidney	0.003	Gingerich 1986
Posterior Kidney	0.0076	Gingerich 1986
Gonads	0.0152	Weatherley & Gill 1983

Also, the influence of different uptake paths has to be considered as well as the exposure length / sample time, which ideally should be performed until / at equilibrium conditions. As shown by the model, it is very important to consider whole body lipid contents because that parameter can heavily influence the DF of a tissue compartment. The studies above did not give lipid content data so, apart from pattern comparisons, absolute values were also affected by an uncertainty factor.

3.3.2 Organ distribution factors for non-lipid based compounds

The literature search showed that no tissue specific distribution factors for fish are available for non-lipid based compounds. Therefore, data obtained from a study on the biomagnification of perfluoralkyl substances (PFAS) in market size rainbow trout (*Oncorhynchus mykiss*) carried out by Fraunhofer IME during the course of this project were used to calculate organ specific distribution factors.

Distribution factors express concentrations of a chemical in different tissues in relation to the average concentration estimated for the whole fish. The highest DFs for PFAS were calculated for the liver (19, 16, 9, 8, and 6 for PFHxS, PFBS, PFOS, PFOA and PFNA) followed by the blood (13-5), kidney (5-0.4), skin (4-2),

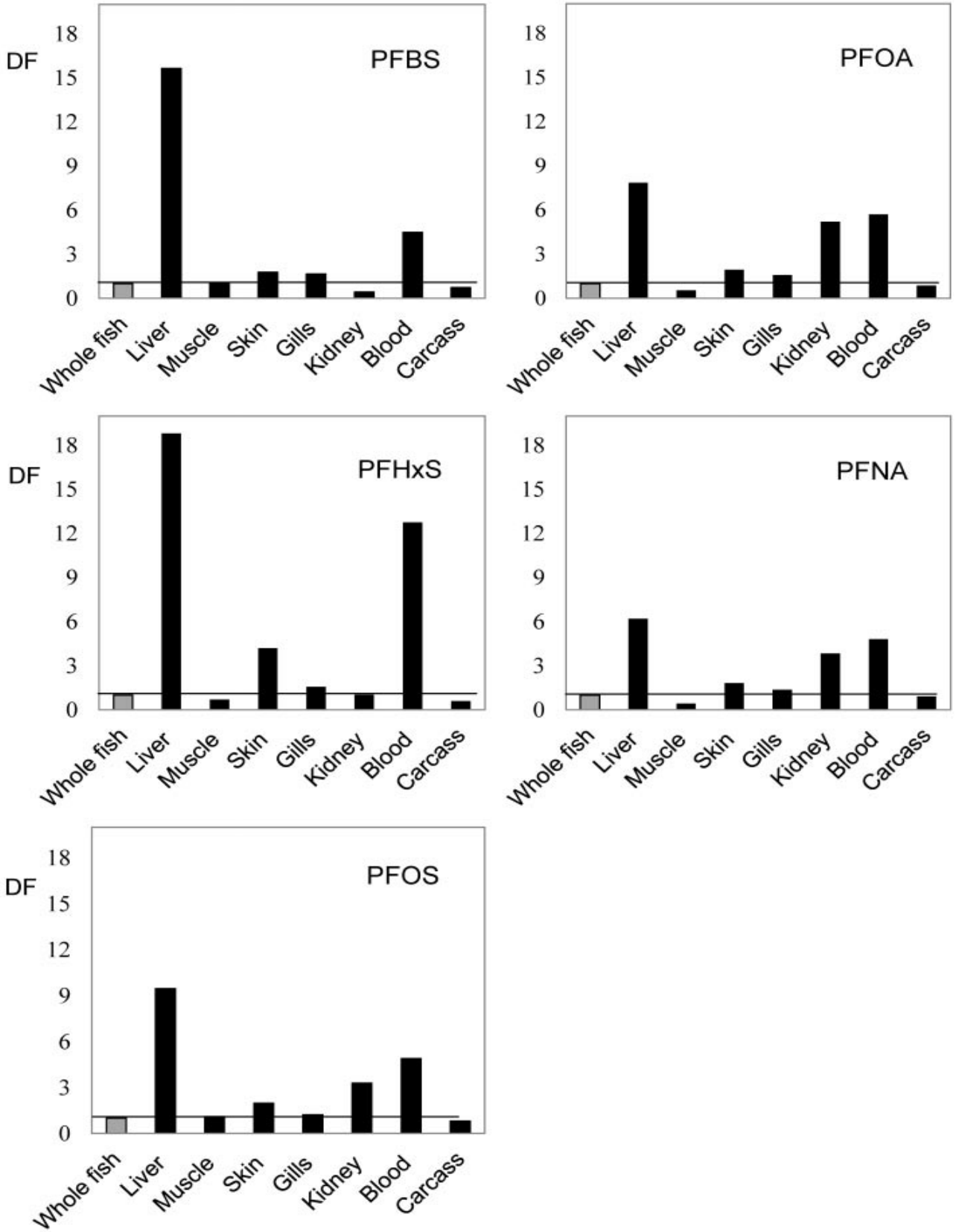
gills (2-1) and muscle (1-0.4) (Figure 59). The results were presented by Goeritz et al. (2013). One of the reasons for the preferential accumulation of PFASs in liver is the effective binding to plasma albumin (Jones et al., 2003). The liver is the main site of plasma albumin synthesis and therefore providing potential binding sites for PFASs. PFASs can also bind to various proteins from the liver cytosol, nuclei, and mitochondria fractions, as revealed in a study on the subcellular distribution and protein binding of PFOA in rat by ligand blotting analysis (Han et al. 2005). In rat, PFOA and PFOS were also shown to bind to liver fatty acid binding proteins (FABPs) abundant in the cytosol of hepatocytes (Luebker et al. 2002). A further binding site for PFASs might be proteins and lipids in the membrane fraction of liver cells, as shown for PFOA in a further study on rats (Kudo et al. 2007). All mechanisms may also contribute to the accumulation of PFASs in fish liver; however, precise information on the binding mechanisms of PFASs in fish liver tissue is limited.

In our study on market-size rainbow trout the perfluoroalkyl sulfonates generally showed a higher affinity for liver compared to the tested perfluoroalkyl carboxylates, which might be explained by a different binding affinity to serum proteins and FABPs abundant in liver (Luebker et al. 2002, Jones et al. 2003). The affinity of PFASs to accumulate in the liver seems to be higher for shorter chain perfluoroalkyl sulfonates such as PFHxS and PFBS, as reflected in the higher DF values, compared to their longer chain homologue PFOS. However, this has not been observed in previous studies investigating the binding characteristics of PFASs. Furthermore, enterohepatic recirculation is assumed to occur in fish rather for PFOS and longer chain perfluoroalkyl carboxylates that show relatively slow elimination rates (Martin et al. 2003b). We therefore assume that there might be further, less examined binding sites in the liver of rainbow trout favouring the binding of smaller molecules like PFBS and PFHxS leading to an increased disposition of those compounds.

After liver, PFAS were mostly distributed to blood, which can be explained by the effective binding to various blood proteins such as plasma albumin and FABPs (Luebker et al. 2002, Jones et al. 2003). Plasma albumin accounts for > 50% of total plasma protein content and is considered the primary binding protein for PFASs in plasma as shown for perfluoroalkyl carboxylates (Han et al. 2003). The high disposition of PFHxS in liver might be explained by an exceptionally binding affinity for plasma proteins as reflected in the high DF value for blood. In human blood, for instance, PFHxS showed an increased binding potency for the plasma protein transthyretin (TRR) compared to other PFASs such as PFOS, PFOA, and PFNA (Weiss et al. 2009). However, the binding affinity of PFHxS to human plasma protein can significantly differ from that in fish and the results should therefore not be generalized. Further studies are required to clarify the mechanisms of PFAS-retention in fish blood dependent on their functional group and chain length.

The binding mechanisms involved in the retention of PFASs in kidney tissue also need to be further elucidated. In our study on market-size rainbow trout the shorter chain homologues, PFBS and PFHxS, showing the highest affinity for liver, exhibited the lowest affinity for kidney compared to the other PFASs. Martin et al. (2003a) suggested that the preferential disposition of PFASs in kidney simply reflects the high perfusion of kidney tissue with blood. However, this is in contradiction to our findings where exceptionally high PFHxS concentrations in blood did not lead to a significant disposition in kidney.

Figure 59: Distribution factors (DF) for PFASs in single organs and tissues of test animals sampled at day 28. DF values for single organs and tissues are presented in relation to the whole fish (DF=1). Perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA). (Goeritz et al. 2013)



3.3.3 Physiologically Based Toxicokinetic (PBTK) Model for fish

3.3.3.1 Organ distribution in the model

The organ distribution for a compound with a log K_{ow} of 4 after waterborne exposure is shown in Figure 60. In steady state conditions, the model predicts a very high DF of 11 in fat and a lower DF of below 1 in other tissues. Figure 61 describes Distribution Factors (DFs) of several compartments in rainbow trout over a log K_{ow} of -2 to 5 considering different whole body weights of the fish. For these simulations, only branchial exposure was allowed and dietary uptake as well as dermal absorption was not considered. It was observed that, regardless of compartment and weight, the distribution factor changed over the selected range of log K_{ow} before reaching a stable value for a log K_{ow} of 3-5. In general, DF values were the highest for fat, followed by the kidney. The liver and richly perfused compartment had practically identical values and was ranked behind kidney, showing higher DFs than the muscles, which had the lowest values. For fat, the distribution factor started from below 1 and increased with a sigmoidal pattern to a maximum of approximately 11 at log K_{ow} = 3. Other compartments (muscle, liver, kidney and richly perfused tissue) showed a sigmoidal decrease with increasing log K_{ow} instead. These tissues started with a DF slightly above 1 and reached a minimum of below 1. The minimum values were 0.3, 0.5 and about 0.6 for muscle, liver / richly perfused tissue and kidney respectively. The graphs also indicate no changes in DFs depending on whole body wet weights from 0.1 to 1 kg.

Figure 60: Distribution factors of a chemical with log K_{ow} = 4 for rainbow trout fat, muscle, liver, richly perfused tissue and kidney for a whole body wet weight of 0.25 kg. The simulation time was 2400 hours and the exposure concentration was set to 10 µg/l. Water temperature was 11 °C and the dissolved oxygen concentration 8.87 mg O_2/l. The whole body lipid content was set to 8.5 %.

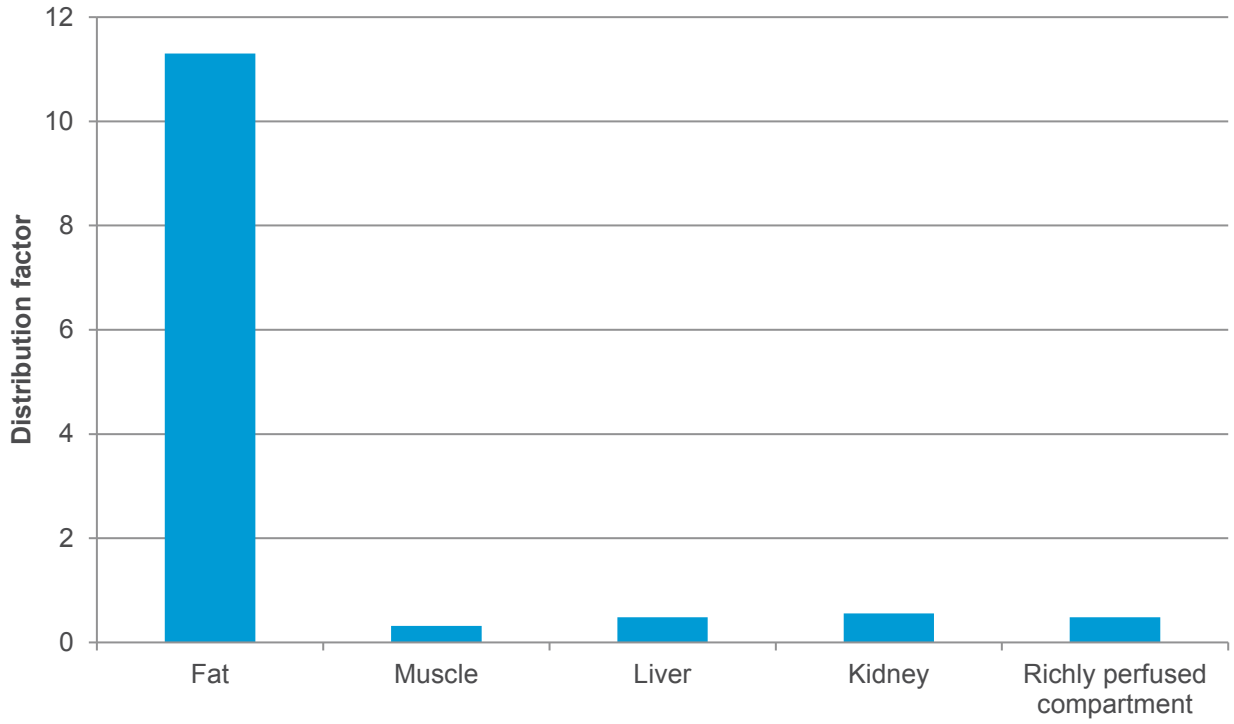
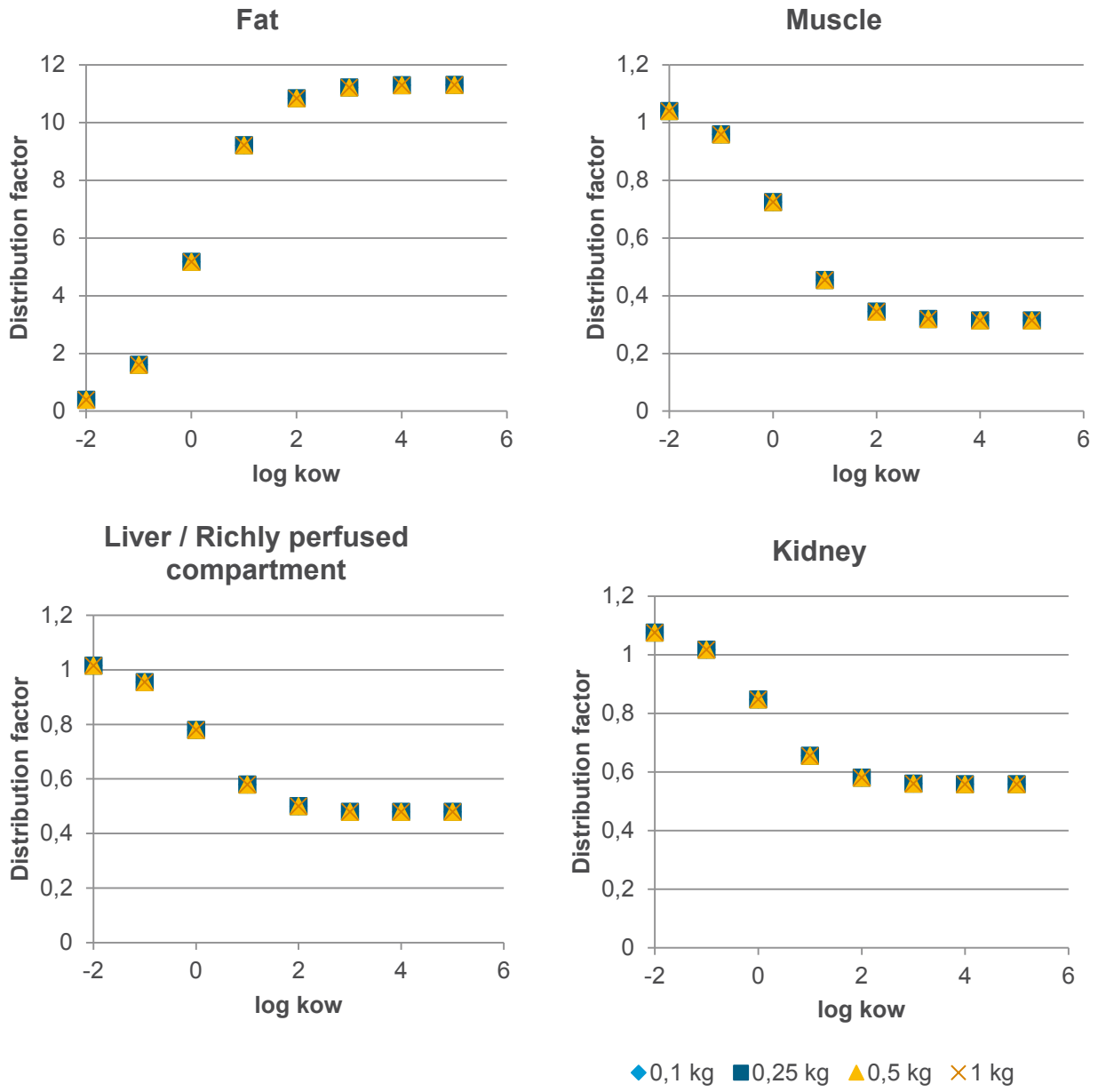


Figure 61: Distribution factors of rainbow trout fat, muscle, liver, richly perfused tissue and kidney plotted against log K_{ow} of chemicals for a whole body wet weight of 0.1, 0.25, 0.5 and 1 kg. The simulation time was 2400 hours and the exposure concentration was set to 10 µg/l. Water temperature was 11 °C and the dissolved oxygen concentration 8.87 mg O_2/l. The whole body lipid content was set to 8.5 %.



Since the simulations so far did not consider other uptake paths besides branchial exposure, additional simulations were performed comparing DFs for branchial exposure, branchial and dermal exposure, injection into the muscle tissue and dietary uptake (Figure 62). In case of branchial / dermal uptake, a concentration of 10 µg/l was used for a 48 h exposure. This was done for an easier comparison with dieteray uptake, which also occurs for 48 hours. The results show that, independent of uptake path, the highest DF was found in fat, followed by kidney, liver, richly perfused tissues. The lowest concentration was predicted for the muscle tissue or skin in case of dermal uptake. For branchial and branchial + dermal uptake, DF patterns were very similar and the concentration in fat was about a factor 10 higher than the other tissues in equilibrium. The inclusion of the skin tissue resulted in slightly faster uptake as the equilibrium condition was reached faster compared to just branchial exposure although the DF for skin itself was low and about a factor 1000 lower than for muscle, liver, kidney and richly perfused tissue. Uptake via injection showed almost the exact same pattern

to branchial exposure with just a faster uptake, which was expected since uptake was instantaneous. For dietary exposure, the simulation time was reduced to 96 hours because the uptake occurs over a very short period (dietary uptake) followed by elimination at the gills. Changes in DF occurred slower compared to other uptake paths, which also could be expected due to the complex mechanism of gastrointestinal (GI) uptake. The absolute number as well as difference between DF for fat and other tissues were very similar, if not almost the same as other exposure paths. However, the ranking was slightly different as the model showed fat > liver/richly perfused compartment > kidney > muscle instead of fat > kidney > liver/richly perfused compartment > muscle like seen on other uptake paths. This could be explained by Figure 48, which shows that uptake of chemicals occur in the GI tract followed by transport to the liver compartment. Therefore, a higher concentration and thus DF could be assumed for liver.

Since according to the model, weight had no influence of the distribution pattern of a chemical, the effect of different whole body lipid contents was investigated next (Figure 63). In contrast to body weight, whole body lipid content had a strong influence on DFs of different tissues. The ranking from highest to lowest DF was found to be the same as above (adiposis tissue > kidney > liver/richly perfused tissues > muscles). The adiposis compartment showed the same trend as observed above with increasing log K_{ow}, but with a lower lipid content the maximum DF increased up to 6-fold. The same pattern was observed for muscles, where the lowest lipid content resulted in a minimum DF of almost the value as at the start, showing almost no substantial decrease of DFs with increasing log K_{ow}. For liver, richly perfused compartment and kidney, a decrease of DF with increasing log K_{ow} even changed into an increase of DF with higher log K_{ow} values. This shows that according to model results, whole body lipid content is an important parameter and should always be measured and included in experiments.

Figure 62: Organ distribution for different uptake paths of PCB 52. Fish weight was set to 0.25 kg, the exposure concentration was to 10 µg/l for all exposure pathways. Water temperature was 11 °C and the dissolved oxygen concentration 8.87 mg O_2/l. The whole body lipid content was set to 8.5 %. Log K_{ow} = 6.1.

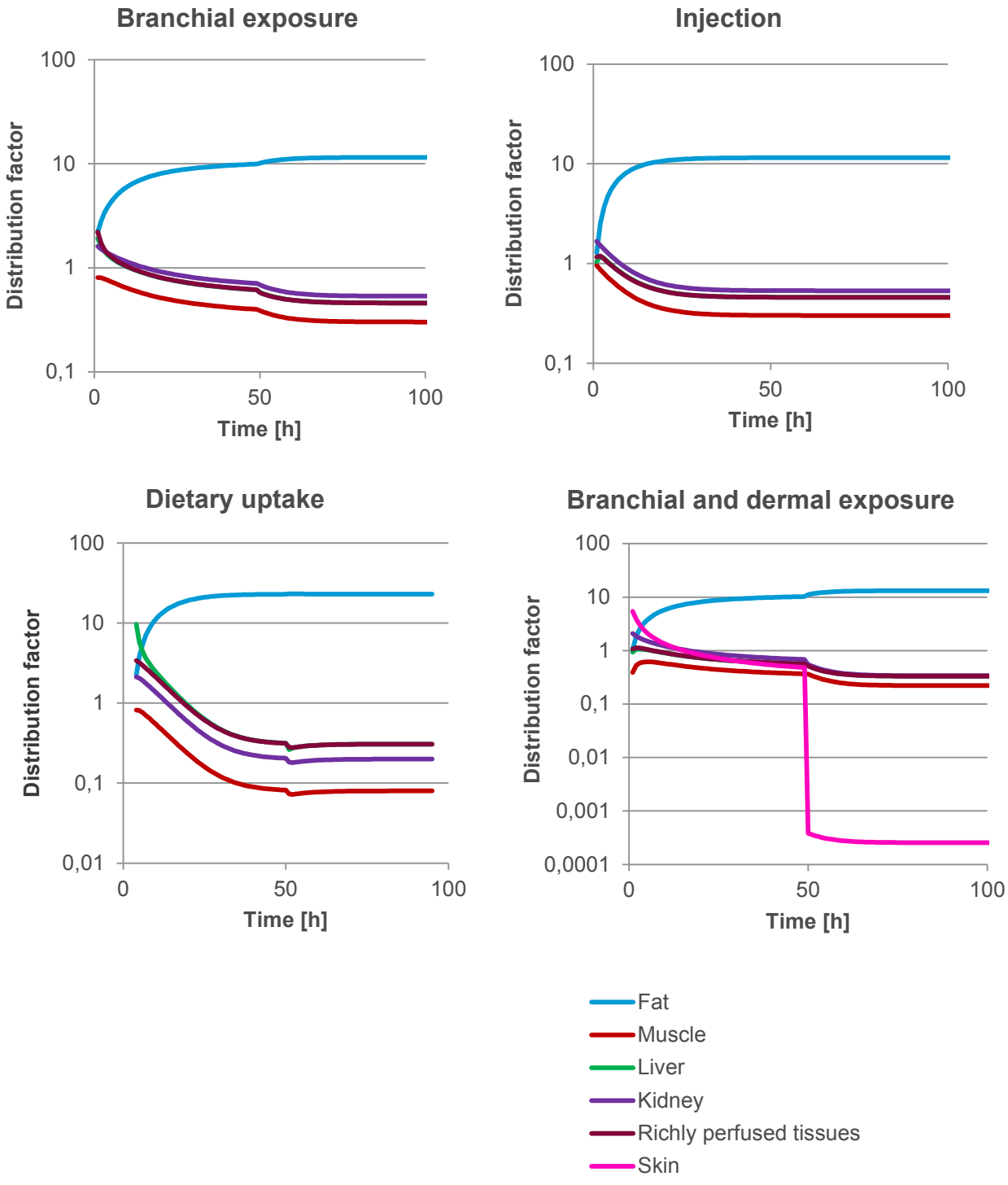
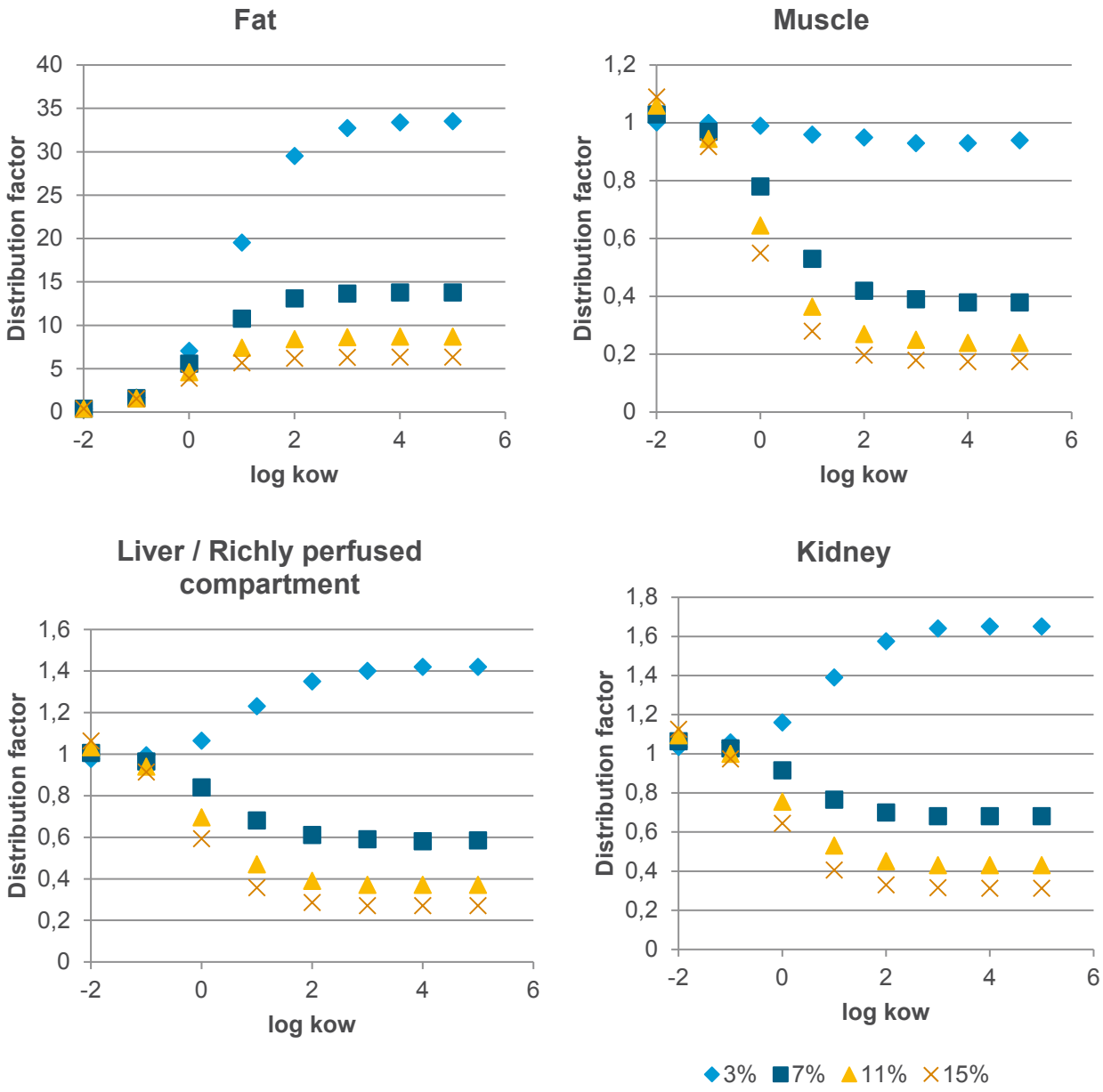


Figure 63: Distribution factors of rainbow trout fat, muscle, liver, richly perfused tissue and kidney plotted against log K_{ow} of chemicals for a whole body lipid content of 3, 7, 11 and 15 %. The simulation time was 2400 hours and the exposure concentration was set to 10 µg/l. Water temperature was 11 °C and the dissolved oxygen concentration 8.87 mg O_2/l. The whole body wet weight was set to 0.25 kg.

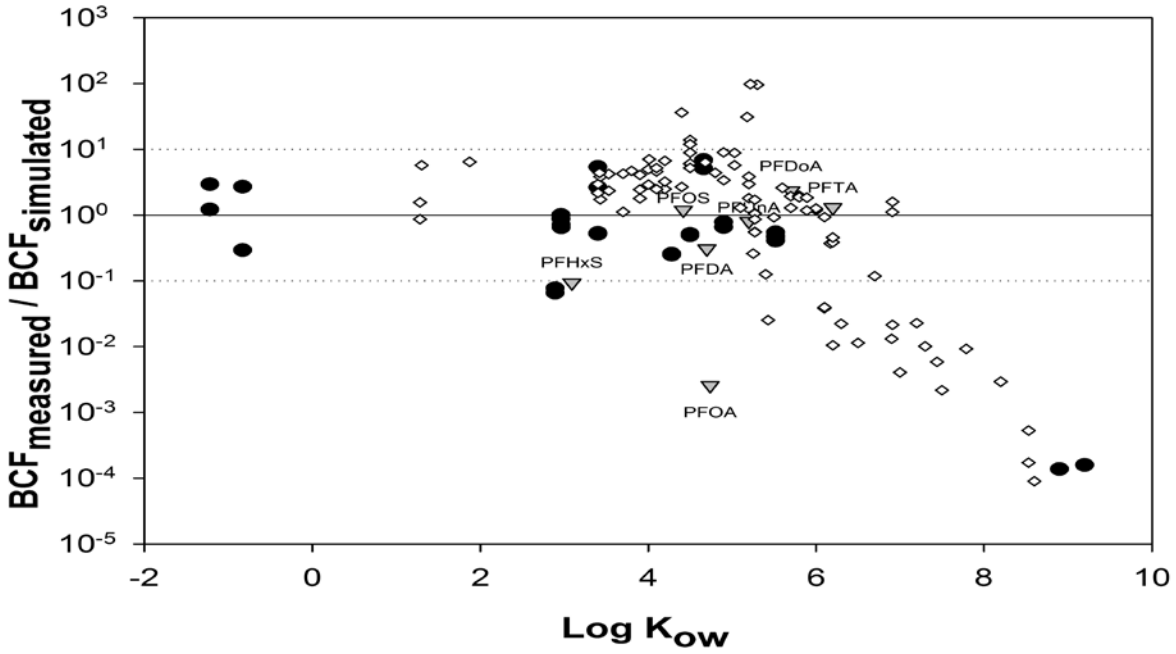


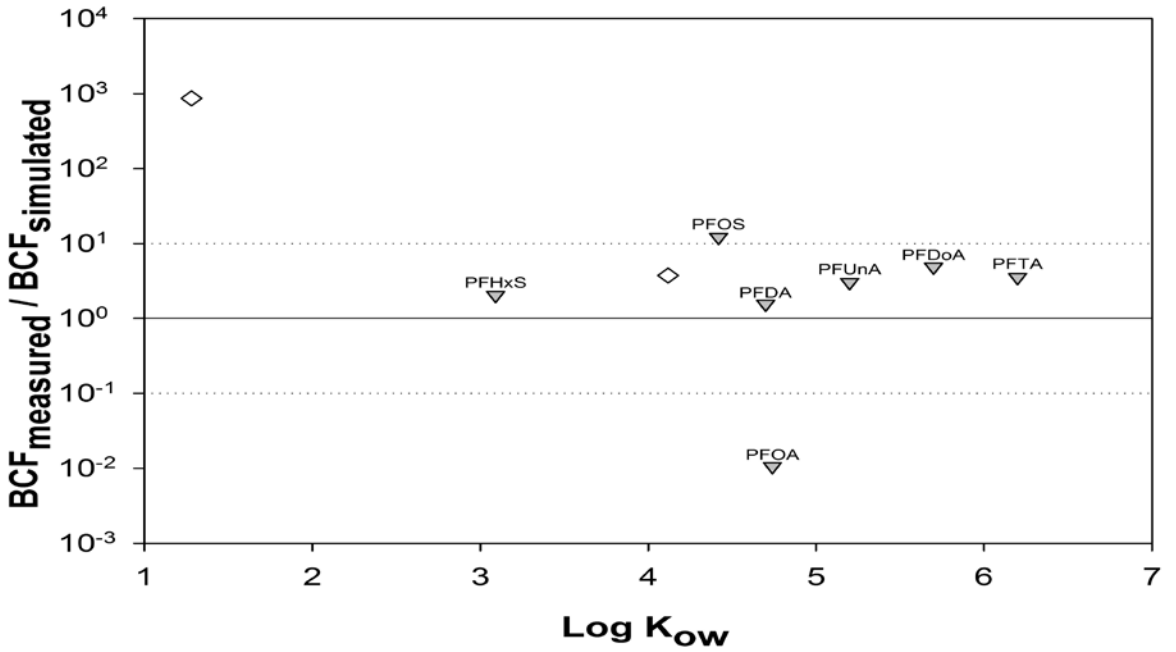
3.3.4 Comparison of measured and simulated organ distribution patterns

3.3.4.1 Measured and simulated bioconcentration in whole body, liver and blood

There was generally good agreement between simulated and measured whole body BCF with a prediction within one order of magnitude (Figure 64). A decrease in predictive power was seen for extremely lipophilic compounds (log K_{ow} > 6) for which the model overestimated whole body BCFs with up to four orders of magnitude. For the PFAS, bioconcentration in whole body was predicted well with the exception of PFOA for which an overestimation by more than two orders of magnitude was seen (predicted: 1558, measured: 4.0).

Figure 64: **A.** Relationship of measured BCF over simulated vs. logarithmized octanol/water partition coefficients for twenty-nine anonymized compounds (circles), seven perfluorinated acids (triangles; measured data from Martin et al. 2003) and selected literature data points (diamonds) for whole body of rainbow trout (*Oncorhynchus mykiss*). Solid and dashed/dotted lines represent 1:1 agreement and ± 1 log unit, respectively. **B.** Relationship of measured BCF over simulated BCF vs. logarithmized octanol/water partition coefficients for seven perfluorinated acids (measured data from Martin et al. 2003) and selected literature data points for liver in rainbow trout (*Oncorhynchus mykiss*). Solid and dashed/dotted lines represent 1:1 agreement and ± 1 log unit, respectively.

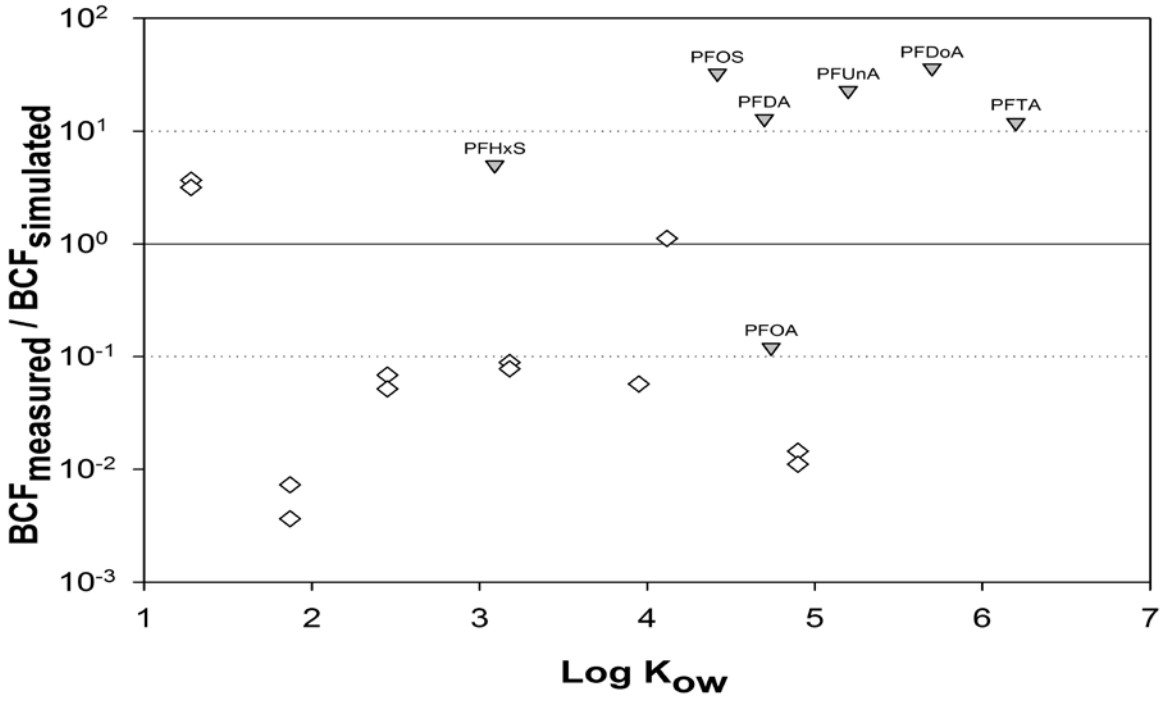




In contrast to whole body and liver simulations, the predictive power of the model was low for blood generating either overestimated or, as in the case of PFOS, PFDA, PFTA, PFUnA and PFDoA, underestimated

BCFs by more than one order of magnitude (Figure 65). Interestingly, measured bioconcentration of PFOA in blood was predicted well. For the four pharmaceuticals carbamazepine, naproxene, ibuprofen and bisoprolol, measured BCFs were overpredicted by up to a factor of nearly 300. Similar results were obtained for the surfactant dioctyl sodium sulfosuccinate (DSS; predicted: 60.8, measured: 3.47).

Figure 65: Relationship of measured BCF over simulated vs. logarithmized octanol/water partition coefficients for seven perfluorinated acids (measured data from Martin et al. 2003) and selected literature data points for blood in rainbow trout (*Oncorhynchus mykiss*). Solid and dashed/dotted lines represent 1:1 agreement and ± 1 log unit, respectively.



3.3.4.2 Measured and simulated uptake in whole body and liver

For the majority of included compounds, predicted and simulated uptake rate constants (k_1) in whole body were generally found to correlate well (Figure 66). An exception was seen for compounds with low (< 0), respectively high (>7) log K_{ow} values for which BCF values were overestimated. For eight polar compounds with log K_{ow} values between 4 and 6 (2,3,4-trichloroanisole, musk xylene, 2,5-dichlorobiphenyl, pentachlorobenzene, 2,4,6-tri-tert-butylphenol, chlorpyrifos, p-diisopropylbenzene and hexachlorobenzene), uptake in whole body was underestimated by more than one order of magnitude. Uptake rate constants for PFAS in whole body were relatively close to model predictions except for PFHxS and PFOA for which the uptake rates were overestimated by more than one and two orders of magnitude, respectively (PFHxS, predicted: 19.6, measured: 0.62; PFOA, predicted: 122.5, measured: 0.53). Similar results were obtained for liver (Figure 67), where k_1 values of PFHxS and PFOA were overpredicted by more than one order of magnitude (PFHxS; predicted: 104.5, measured: 5.80; PFOA; predicted: 62.16, measured: 1.40).

3.3.4.3 Measured and simulated depuration in whole body and liver

Rate constants for depuration (k_2) in whole body and liver were generally predicted well within one order of magnitude (Figure 68, Figure 69). An exception was seen for four hydrophilic compounds (log K_{ow} < 0) for which an overestimated depuration rate in whole body was observed (Anonymized compounds; Schmallenberg). Overestimated whole body depuration was also observed for three polar compounds; pentachlorobenzene, 2,4,6-tri-tert-butylphenol and 2,5-dichlorobiphenyl, for which k_2 values were overpredicted by more than one order of magnitude. In contrast, whole body depuration rates of pentachlorophenol were underesti-

mated by up to a factor of 33. Simulated and measured depuration rates of PFAs were generally found to correlate well for both whole body and liver. An exception was seen for PFHxS, for which the predictive power in liver was low (predicted: 2.13 day-1, measured: 0.058 day-1).

Figure 66: Relationship of measured k_1 over simulated vs. logarithmized octanol/water partition coefficients for twenty-nine anonymized compounds (circles), seven perfluorinated acids (triangles; measured data from Martin et al. 2003) and selected literature data points (diamonds) for whole body of rainbow trout (*Oncorhynchus mykiss*). Solid and dashed/dotted lines represent 1:1 agreement and ± 1 log unit, respectively.

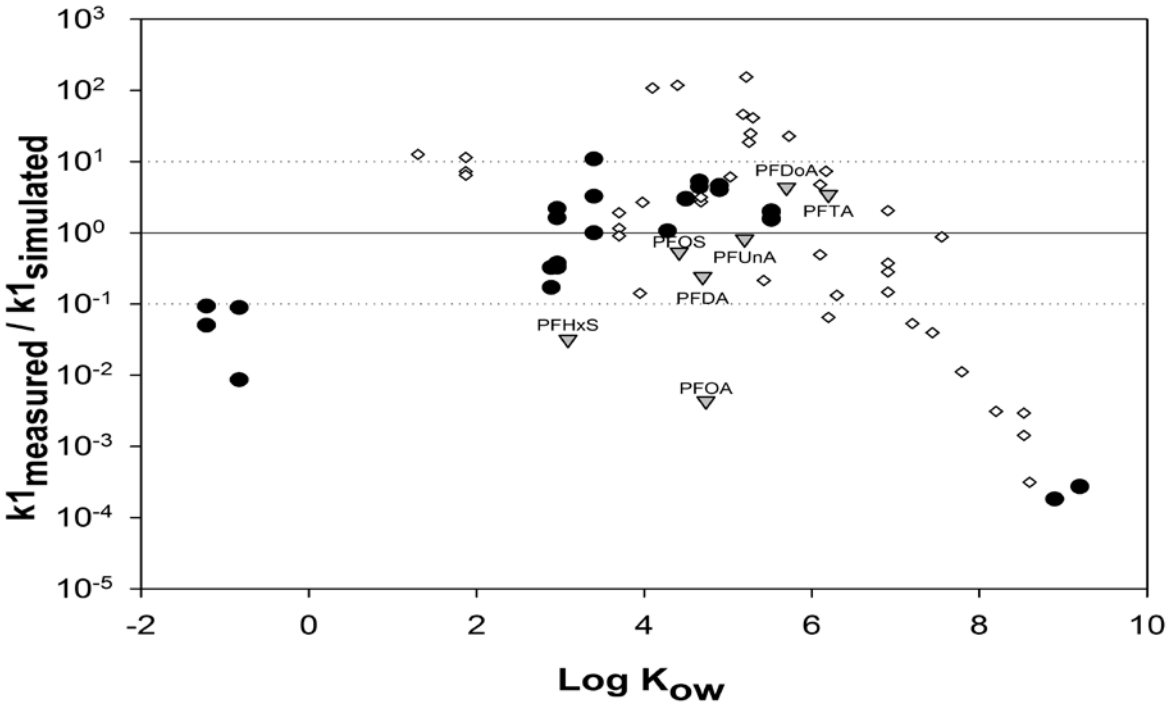


Figure 67: Relationship of measured k_1 over simulated vs. logarithmized octanol/water partition coefficients for seven perfluorinated acids for liver in rainbow trout (*Oncorhynchus mykiss*; measured data from Martin et al. 2003). Solid and dashed/dotted lines represent 1:1 agreement and ± 1 log unit, respectively.

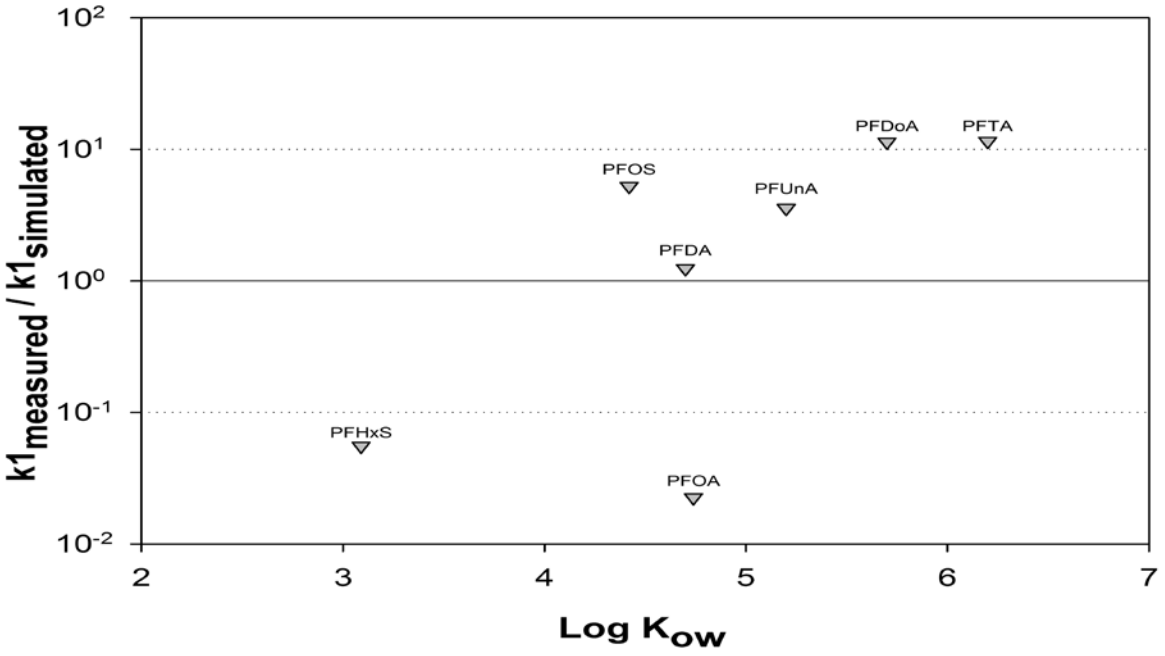


Figure 68: Relationship of measured k_2 over simulated vs. logarithmized octanol/water partition coefficients for twenty-nine anonymized compounds (circles) and seven perfluorinated acids (triangles; measured data from Martin et al. 2003) and selected literature data points (diamonds) for whole body of rainbow trout (*Oncorhynchus mykiss*). Solid and dashed/dotted lines represent 1:1 agreement and ± 1 log unit, respectively.

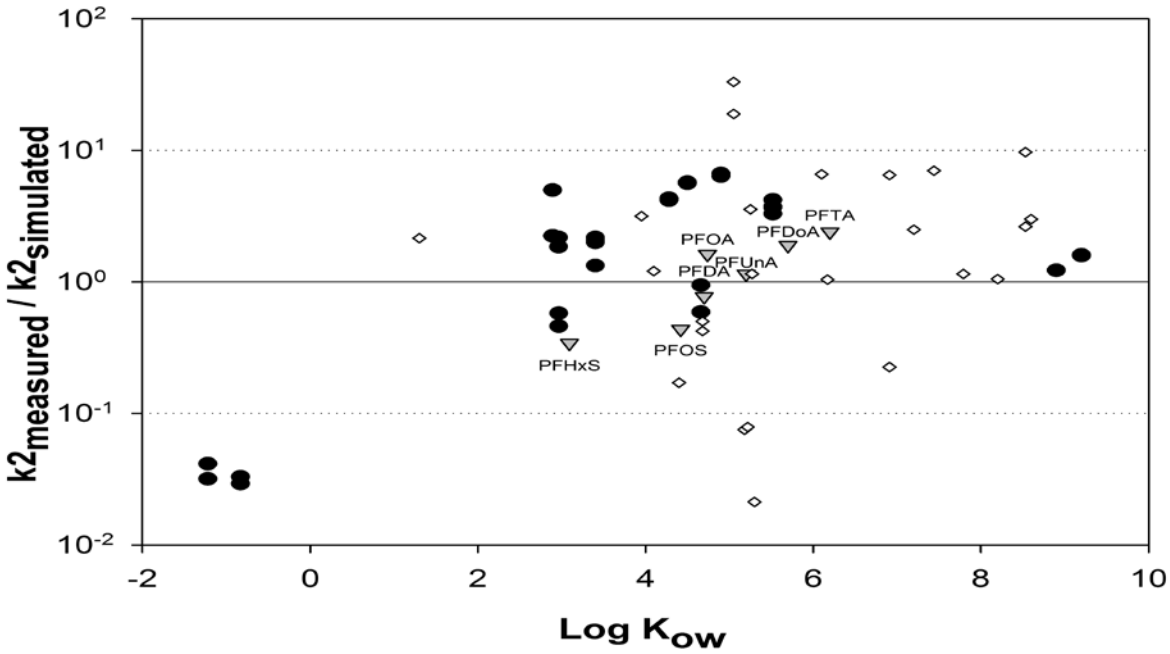
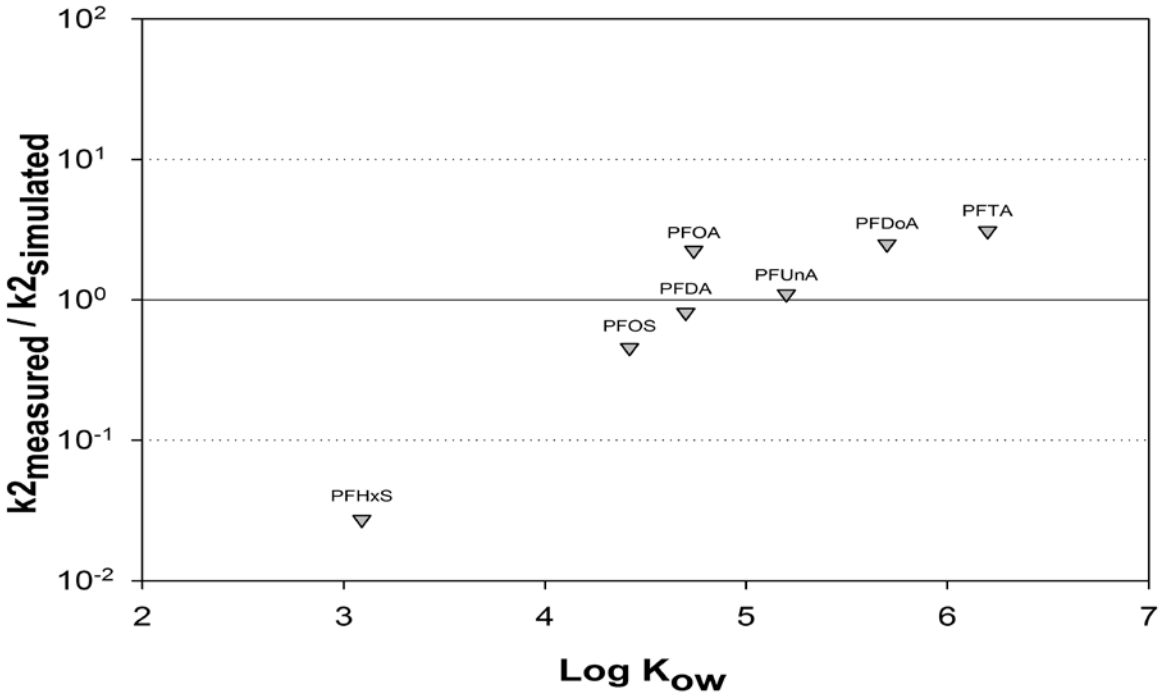


Figure 69: Relationship of measured k2 over simulated vs. logarithmized octanol/water partition coefficients for seven perfluorinated acids for liver in rainbow trout (Oncorhynchus mykiss; measured data from Martin et al. 2003). Solid and dashed/dotted lines represent 1:1 agreement and ± 1 log unit, respectively.



3.4 Discussion

A general distribution pattern for lipophilic compounds could not be found after investigating the available literature. One aspect that almost all studies had in common was that the muscle tissues usually had a very low, if not the lowest DF. As for the highest DF, fat, liver, bile and the GI tract were the compartments for which it could be calculated, depending on the organs/tissues investigated by the authors. It was noticeable, however, that liver was found to have the highest or among the highest DF in amost studies. It could be discussed whether metabolism was an important factor here, since changes in lipophilicity could be possible (Phase I, Phase II metabolism).

For Law et al. (1991) showing DFs for Pyrene, values were different from the model results and higher than simulated. DF rankings were different as the liver showed a higher DF than the kidney. Comparability was limited, however, as the study did not include a fat compartment and in addition concentrations in gill, gut and blood were considered.

Similar to the study above, a higher Pyrene DF was calculated for liver than for the kidney (Kennedy & Law 1990), which was different from modeling results. However, the model predicted the fat tissue to have the highest DF, which is also shown by these experimental results.

In case of Benzo[a]pyrene and Nonylphenol, the PBTK model did not include all compartments presented in the study (Seubert & Kennedy 2000, Lewis & Lech 1996), but had shown a substantially lower DF for liver / kidney and a higher DF for fat. Since dissection took place after 48 hours, it could be discussed if an equilibrium state was reached at the time of sampling. Modeling results indicate steady state conditions at 48 hours, however.

Bisphenol A comparison was very limited as the author only investigated liver, muscles and plasma, however, the DFs were similar and just slightly higher in the experiment than in the model.

4-tert-octylphenol was very difficult to compare with modeling results because the majority of investigated organs were not included in the model. A very high DF for bile was calculated and could probably be explained by metabolism. Nevertheless, the ranking fat > liver > kidney > muscle was not too different from model predictions although the model showed a DF of 12 for fat compared to a DF of 2.5 in the study.

Rotenone DF patterns were different from the model as the highest DFs were found for intestines and the heart and only low DFs of around 1 for liver, fat, kidneys and white muscle.

Oxytetracycline showed similar DFs for muscle and kidney compared to the model but a substantially higher DF for liver of around 20. Unfortunately, fat tissues were not considered in the experiment. Again, metabolism was not considered and implemented in the model and might be essential here as the liver is the principal organ for xenobiotic metabolism.

3.4.1 Predictions of bioconcentration in whole body, blood and liver

In general, the PBTK model was able to predict measured whole body BCFs. A decrease in predictive power was seen for extremely lipophilic compounds (log K_{ow} > 6) for which measured BCFs were overestimated with up to four orders of magnitude. The obtained results are in line with previous studies demonstrating the inadequacy of simple partitioning models to predict bioconcentration of compounds possessing log K_{ow} > 5.5 and 6.5, respectively (Connell and Hawker, 1988; Dimitrov et al., 2002). The discrepancy between bioconcentration and octanol-water partition coefficient seen for extremely lipophilic compounds has been related to influencing factors such as molecular size, lipid solubility and time period required to attain equilibrium (Hawker and Connell, 1985; Connell and Hawker, 1988; Dimitrov et al., 2002). Moreover, it has generally been stated that the main route of exposure for compounds possessing log K_{ow} above 5 is dietary uptake (Bruggeman et al., 1984), hence for extremely lipophilic compounds, models simulating direct uptake via water may not be appropriate.

Other chemicals for which the model failed to adequately predict measured whole body BCF were musk xylene, pentachlorobenzene, 2,5-dichlorobiphenyl and 2,4,6-tri-tert-butylphenol (Adolfsson-Erici et al. 2012). We found that measured BCFs were underestimated by a factor of up to 26, results which are consistent with a recent study by Stadnicka et al. (2012), where the same PBTK model was shown to generate underestimated internal concentrations of two polar organic compounds in fish; phenol and 2,4,5-trichlorophenol. As discussed by Stadnicka and coworkers, a putative reason could be that chemical behavior of polar compounds does not correlate well with the corresponding octanol/water partitioning coefficient (Ramos et al. 1997). This explanation does however not hold for the additional seven polar compounds investigated by Adolfsson-Erici et al. (2012) for which the measured BCFs were accurately predicted by our model. Understanding the mechanism of bioconcentration for these groups of chemicals clearly needs further experiments and will enlarge our overall understanding of bioconcentration and bioaccumulation.

Given the amphiphilic properties of PFAS, K_{ow} based models are generally not considered suitable for predictions of environmental fate (Houde et al. 2006). The fine agreement between simulated and measured whole body BCFs for PFOS, PFHxS, PFDA, PFTA, PFDoA and PFUnA (Martin et al. 2003) obtained in the present study was therefore rather unexpected. Of the PFAS, only PFOA was identified as an “outlier” as reflected by a clear overestimation of the measured BCF. In contrary to PFOS, PFOA has repeatedly been associated with a low bioconcentration potential in fish (reviewed by Fujii et al. 2007; Inoue et al. 2012). A possible explanation to these observations is related to the high water solubility of PFOA (3.4 to 9.5 g/L; U.S. EPA, 2005) which compared to PFOS (519 to 680 mg/L; OECD, 2002), allows for an effective excretion via gill permeation (Vierke et al. 2012).

In contrast to the successful predictions of measured whole body BCFs, bioconcentration in blood was for all PFAS, but PFOA and PFHxS, underestimated by more than one order of magnitude. Unlike other persistent organic pollutants, PFAS do not accumulate in lipids and adipose tissue, but are rather distributed in body compartments with high protein content (MacManus-Spencer et al. 2010). In a study by Vanden Heuvel et al. (1992), PFAS were shown to covalently bind proteins in plasma of rats following in vivo administration.

Particularly plasma albumin has been recognized as an important sink for PFAS (references in Ng and Hungerbühler 2013). Given the fact that albumin-like proteins have been identified in rainbow trout (Manera and Britti, 2006), the strong underestimation of measured blood BCFs observed in the present study is most likely related to the effective sorption of PFAS to blood proteins (Luebker et al. 2002; Jones et al. 2003). In contrast to the PFAS, blood BCF of another surfactant, dioctyl sodium sulfosuccinate (DSS), was slightly overestimated by our model. The reason behind this overprediction might be related to DSS metabolism, as confirmed by the authors (Goodrich et al., 1991). Overpredicted blood BCFs were furthermore seen for four pharmaceuticals; naproxen, ibuprofen, carbamazepine and bisoprolol. Rapid conjugation and elimination via the liver/bile route as reported in Ferreira-Leach and Hill (2001) and Lahiti et al. (2011) is assumed to play a major role.

With respect to the high protein content of the liver, the high agreement between simulated and measured liver BCFs for the majority of investigated PFASs was surprising because model predictions are based on the lipophilicity of the chemical substances. Bioaccumulation of PFAS however has been shown to be greatly influenced by specific interactions such as protein binding. (EFSA, 2008). Consistent with a study by Mortensen et al. (2011), measured accumulation of PFOS in liver was found to be slightly higher than predicted. As previously discussed for blood, underestimation of measured PFOS BCF can most likely be related to protein sorption. Contrary to PFOS and the other compounds, the bioconcentration potential of PFOA was clearly overpredicted by nearly two orders of magnitude. Predictions of kinetic constants in whole body and liver

Even if the model was able to predict whole body BCFs satisfactorily, the prediction of constants were much more scattered. The most prominent difference was the reversed relationship between model performance and octanol-water partition coefficient observed for the kinetic depuration rate. Whereas whole body BCF and kinetic uptake rate were poorly predicted for extremely lipophilic compounds ($\log K_{ow} > 6$), simulated and measured whole body elimination showed good agreement for all compounds but for those with high water solubility ($\log K_{ow} < 0$). As previously stated, chemical kinetics in fish are strongly affected by metabolizing, secretory and active transport systems (Nichols et al., 1990) so by the physiology of the organism and passing of organs and membrans (e.g. gills). The physiology of the organisms is much less understood or not as adequate and generic measurable as the lipid content of organs which is the main factor affecting the BCF in this model. Consistent with the observed underestimation of whole body BCFs for pentachlorobenzene, 2,4,6-tri-tert-butylphenol and 2,5-dichlorobiphenyl, predicted elimination rates for these polar compounds were overestimated by more than one order of magnitude. In the case of pentachlorophenol, the underestimated elimination can most likely be explained by metabolic transformation as previously confirmed for pentachlorophenol both in vitro and in vivo (Stehly and Hayton, 1989; Cravedi et al. 1999).

For the majority of PFAS, the model proved to be a suitable tool for predictions of uptake and elimination in both whole body and liver of rainbow trout. PFHxS and PFOA were identified as “outliers” as measured k_1 were overpredicted by more than one order of magnitude in both compartments.

3.5 Summary

The aim of this workpackage was to elucidate the possibility to use a PBTK model for identifying possible non lipid triggered organ distribution of compounds from an OECD 305 experiment in which only full body concentrations are measured. The assumption behind is that a PBTK model is a good representative of bioconcentration and bioaccumulation for fish and predicts the organ distribution correctly. Since organ distribution in a PBTK model is lipid based, non lipid distribution should show different organ distribution patterns and this difference in organ distribution also result in differences of bioconcentration factors and kinetic constants measured in OECD 305 experiments.

A literature search was conducted to find studies in which organ distribution of rainbow trouts were investigated. Eight studies were found and investigated in more detail. A general distribution pattern for lipophilic compounds could not be found as very different results were presented in the literature. One aspect that al-

most all studies had in common was that the muscle tissues usually had a very low, if not the lowest DF. It was noticeable, however, that liver was found to have the highest or among the highest DF in a couple of publications.

A PBTK model was implemented in order to predict uptake and distribution of chemical substances based on their lipophilicity. Uptake, elimination and distribution of chemicals in the fish are triggered by the lipid content of the organs and the K_{ow} of the compound. So the model assumes lipid based organ distribution only. In general, DF values were the highest for fat, followed by the kidney. The liver and richly perfused compartment had practically identical values and was ranked behind kidney, showing higher DFs than the muscles, which had the lowest values. In general the model was not able to predict the organ distribution found for most compounds.

In conclusion, this study demonstrates that the PBTK model is an effective tool for successful predictions of bioconcentration and kinetic rate constants of a wide range of organic compounds in rainbow trout. The highest predictive power was seen for simulations of moderately lipophilic compounds in whole body followed by liver whereas simulations in blood generally led to over- or underestimations of measured data. Despite assumptions of organ-specific accumulation, bioconcentration of investigated PFAS was reasonably well estimated, indicating a lipid triggered distribution of most PFAS in liver and whole body. In order to achieve further optimization of the PBTK model, particular emphasize should be placed on protein sorption, which would serve to increase the predictive power regarding blood simulations. Future challenges also include successful predictions of very polar compounds.

4 Definitions

The assimilation efficiency (α) is a measure of the relative amount of substance absorbed from the gut into the organism (α is unitless, but it is often expressed as a percentage rather than a fraction).

Bioaccumulation is generally referred to as a process in which the chemical concentration in an organism achieves a level that exceeds that in the respiratory medium (e.g., water for a fish or air for a mammal), the diet, or both.

Bioconcentration is the increase in concentration of the test substance in or on an organism (or specified tissues thereof) relative to the concentration of test substance in the surrounding medium.

The bioconcentration factor(BCF) at any time during the uptake phase of this accumulation test is the concentration of test substance in/on the fish or specified tissues thereof (C_f as mg/kg) divided by the concentration of the chemical in the surrounding medium (C_w as mg/L). BCF is expressed in $L \bullet kg^{-1}$. Please note that corrections for growth and/or a standard lipid content are not accounted for.

Biomagnification is the increase in concentration of the test substance in or on an organism (or specified tissues thereof) relative to the concentration of test substance in the food.

The biomagnification factor (BMF) is the concentration of a substance in a predator relative to the concentration in the predator's prey (or food) at steady-state. In the method described in this Guideline, exposure via the aqueous phase is carefully avoided and thus a BMF value from this test method cannot directly be compared to a BMF value from a field study (in which both water and dietary exposure may be combined).

The dietary biomagnification factor (dietary BMF) is the term used in this guideline to describe the result of dietary exposure test, in which exposure via the aqueous phase is carefully avoided and thus the dietary BMF from this test method cannot directly be compared to a BMF value from a field study (in which both water and dietary exposure may be combined).

The depuration or post-exposure (loss) phase is the time, following the transfer of the test fish from a medium containing test substance to a medium free of that substance, during which the depuration (or the net loss) of the substance from the test fish (or specified tissue thereof) is studied.

The depuration (loss) rate constant (k_2) is the numerical value defining the rate of reduction in the concentration of the test substance in the test fish (or specified tissues thereof) following the transfer of the test fish from a medium containing the test substance to a medium free of that substance (k_2 is expressed in day^{-1}).

The exposure or uptake phase is the time during which the fish are exposed to the test chemical.

The food ingestion rate (I) is the average amount of food eaten by each fish each day, relative to the estimated average fish whole body weight (expressed in terms of g food/g fish/day).

The kinetic bioconcentration factor (BCF_k) is the ratio of the uptake rate constant, k_1, to the depuration rate constant, k_2 (i.e.k_1/k_2). In principle the value should be comparable to the BCF_{ss}(see definition above), but deviations may occur if steady-state was uncertain or if corrections for growth have been applied to the kinetic BCF.

The lipid normalised kinetic bioconcentration factor (BCF_{kL}) is normalised to a fish with a 5% lipid content.

The lipid normalised, growth corrected kinetic bioconcentration factor (BCF_{kgL}) is normalised to a fish with a 5% lipid content and corrected for growth during the study period.

The lipid normalised steady-state bioconcentration factor(BCF_{ssL}) is normalised to a fish with 5% lipid content.

The octanol-water partition coefficient (K_{ow}) is the ratio of a chemical's solubility in n-octanol and water at equilibrium (OECD Guidelines 107 (2), 117 (3), 123 (4)); also expressed as P_{ow} The logarithm of K_{ow} is used as an indication of a chemical's potential for bioconcentration by aquatic organisms.

A steady-state is reached in the plot of test substance in fish (C_f) against time when the curve becomes parallel to the time axis and three successive analyses of C_f made on samples taken at intervals of at least two days are within ± 20% of each other, and there is no significant increase of C_f in time between the first and-last successive analysis. When pooled samples are analysed at least four successive analyses are required. For test substances which are taken up slowly the intervals would more appropriately be seven days.

The steady-state bioconcentration factor (BCF_{ss}) does not change significantly over a prolonged period of time, the concentration of the test substance in the surrounding medium being constant during this period of time (cf. Definition of steady-state).

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6 Annex 1: Data quality assessment sheets

Data sheets are ordered by alphabetical order of references.

Table 4: Klimisch Criteria*

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

* Klimisch, Andreae et al. (1997)

**OECD (1998)

6.1 Bioconcentration papers

Reference	Adolfsson-Erici et al. (2012)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight:45 g
Information on the test design	
Methodology used	OECD305 (1996)
Test substance	Organic chemicals
Test concentrations used	Mixture of chemicals
Number of replicates per test concentration	1
Number of organisms per replicate	Not stated
Number of fish per sampling	4
Test conditions	Flow-through
Duration of study	Uptake phase: 24 days Depuration phase: 56 days
Observation intervals	Day 2, 4, 8, 13, 24 during uptake and 10h, day 1, 2, 4, 10, 16, 30, 57 during depuration
Feeding	1% of body weight/day
Measurement of the exposure concentrations	Yes, 10-1100 ng/L depending on substance.
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, but stability during exposure and reaching a plateau is not given for all substances.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Stability of test substance in water during uptake dependent on substance and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Al-Ansari et al. (2013)
Information on the test species	
Test species used	Goldfish (*Carassius auratus)*
Life stage of the test species used	Adult, weight: 24.8 ± 5.9 g (uptake), 17.6 ± 2.19 g (depuration)
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	17α-ethinylestradiol
Test concentrations used	Control, 150 ng/L
Number of replicates per test concentration	1
Number of organisms per replicate	63
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 3 days Depuration phase: 3 days
Observation intervals	Hour 0, 1, 3, 6, 12, 24, 48, 72 during uptake and hour 0, 3, 6, 12, 24, 48, 72 during depuration
Feeding	Not stated
Measurement of the exposure concentrations	Yes, 141.1 ± 15.75 ng/L
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, but no information on mortality of fish.
Water quality criteria satisfied	Yes. The goldfish is not recommended as a test species, therefore no range is suggested.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish and oxygen saturation, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Andreu-Sánchez et al. (2012)
Information on the test species	
Test species used	Zebrafish (*Danio rerio*)
Life stage of the test species used	Adult, weight: 0.75 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Tebuconazole
Test concentrations used	Control, 0.2 mg/L
Number of replicates per test concentration	3
Number of organisms per replicate	125
Number of fish per sampling	2*2-3 from each replicate
Test conditions	Static
Duration of study	Uptake phase: 60 days No depuration phase
Observation intervals	10 samplings during uptake
Feeding	3 times/week
Measurement of the exposure concentrations	Yes, 0.23 mg/Le
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	No and no information on mortality of fish is available.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	Dissipation rate in water included in calculations (static exposure).
Study conducted to GLP	No
Overall comment on quality	Concentration of test substance in water during uptake decreased. No depuration experiment.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Belden et al. (2005)
Information on the test species	
Test species used	Channel catfish (*Ictalurus punctatus*)
Life stage of the test species used	Juvenile, weight:0.084 ± 0.013 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Hexahydro-1,3,5-trinitro-1,3,5-triazine
Test concentrations used	Control, 2 mg/L
Number of replicates per test concentration	44
Number of organisms per replicate	2
Number of fish per sampling	8
Test conditions	Semi-static
Duration of study	Uptake phase: 9 hours Depuration phase: 4 hours
Observation intervals	Hour 0.25, 0.50, 1, 2, 4, 9 during uptake and hour 0.25, 0.50, 1, 2, 4 during depuration
Feeding	Not stated
Measurement of the exposure concentrations	Yes, 2.1 mg/L.
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Yes, except that no plateau was reached.
Water quality criteria satisfied	Not stated
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	The study is of good quality. All validity criteria are satisfied, but a plateau was not reached within the exposure time.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Bishop & Maki (1980)
Information on the test species	
Test species used	Blugill (*Lepomis macrochirus*)
Life stage of the test species used	Juvenile, weight:0.49 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Surfactants and DDT
Test concentrations used	Control, two mixtures of chemicals: low (0.00003-0.1 mg/L) and high (0.0003-1.0 mg/L) treatment depending on substance
Number of replicates per test concentration	1
Number of organisms per replicate	Not stated
Number of fish per sampling	4
Test conditions	Flow-through
Duration of study	Uptake phase: 5 days Depuration phase: 3-30 days depending on substance
Observation intervals	Hour 0, 1, 3, 6, 8, 12, ,24 48, 120 during uptake and hour 8, 24, 48, 72, 96, 144, 168, 192, 216, 268, 288, 360, 384, 552, 720 during depuration
Feeding	2% of body weight/fish/day
Measurement of the exposure concentrations	Yes, 0.000026-0.08 mg/L in low treatment and 0.00023-0.76 mg/L in high treatment depending on substance
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish and a plateau was not reached in all substances.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According toOECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Bradbury et al. (1993)
Information on the test species	
Test species used	Medaka (*Oryzias latipes*)
Life stage of the test species used	Adult, weight: 0.2-0.5 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Aniline, 4-Chloroaniline
Test concentrations used	Mixture of both substances
Number of replicates per test concentration	3
Number of organisms per replicate	3
Number of fish per sampling	9
Test conditions	Static
Duration of study	Uptake phase: 320 minutes Depuration phase: 160 minutes
Observation intervals	Minute 0, 10, 20, 40, 80, 160, 320 (4-chloroaniline) during uptake and minute 0, 10, 20, 60, 160 during depuration
Feeding	Not stated
Measurement of the exposure concentrations	Yes, 11.0 ± 0.14 µg/L (aniline) and 7.61 ± 0.15 µg/L (4-chloroaniline)
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, but no information on mortality of fish and no plateau was reached.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD 305 (2012), a two-compartment model was fitted to data.
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish and oxygen saturation and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Bradford et al. (2006)
Information on the test species	
Test species used	Eastern mosquitofish (*Gambusia holbrooki*)
Life stage of the test species used	Adult, weight: 0.84 ± 0.34 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Sodium perchlorate
Test concentrations used	Control, 100, mg/L
Number of replicates per test concentration	5
Number of organisms per replicate	15
Number of fish per sampling	5-7
Test conditions	Semi-static
Duration of study	Uptake phase: 30 days Depuration phase: 20 days
Observation intervals	Day 0.5, 1, 2,10, 30 during uptake and day 0, 1, 2, 5, 10, 20 during depuration.
Feeding	Yes, ad libiltum 2 times/day.
Measurement of the exposure concentrations	Yes
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish.
Water quality criteria satisfied	Yes. The Eastern mosquitofish is not recommended as a test species, therefore no range is suggested.
Calculation of kinetic parameters	According to OECD 305 (2012)OECD305 (2012), k_1 alternatively calculated.
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish, but other validity criteria satisfied. Alternative calculation method for k_1.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Branson et al. (1975)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 8-10 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	2,2‘,4,4‘-tetrachlorobiphenyl
Test concentrations used	Control, 1, 10 µg/L
Number of replicates per test concentration	1
Number of organisms per replicate	40
Number of fish per sampling	4
Test conditions	Flow-through
Duration of study	Uptake phase: 5 days Depuration phase: 28 days
Observation intervals	Hour 6, 12, 24, 48, 120 during uptake and day 2, 11, 18, 28 during depuration
Feeding	Yes
Measurement of the exposure concentrations	Yes, 1.6 ± 0.2 µg/L and 9.0 ± 2.0 µg/L
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish and no plateau was reached.
Water quality criteria satisfied	No
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish and other validity criteria and water quality parameters not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Burke et al. (1991)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 16 ± 4 g, 20 ± 7 g (4°C)
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	2-phenyldodecane
Test concentrations used	Control, 0.323 µg/L; two experiments at 15°C and 4°C
Number of replicates per test concentration	1
Number of organisms per replicate	148 (control:12); 102 (control:9) (4°C)
Number of fish per sampling	6 (control: 1)
Test conditions	Flow-through
Duration of study	Uptake phase: 28 days Depuration phase: 28 days; 47 days (4°C)
Observation intervals	Day 6h, 1, 3, 5, 6, 10, 11, 17, 19, 21, 26, 28 during uptake and day 2, 4, 7, 14, 21, 28 during depuration; Day 1, 3, 7, 10, 14, 18, 21, 24, 28 during uptake and day 1, 3, 7, 14, 18, 25, 32, 47 during depuration
Feeding	3% of body weight/day
Measurement of the exposure concentrations	Yes, 0.34 µg/L (15°C) and 0.26 µg/L (4°C)
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	No, no plateau was reached and stability of substance in 15°C experiment was more variable than stated by the guideline. No information on mortality of fish.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According toOECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Concentration of test substance in water during uptake varied (>20%) and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Butte et al. (1991)
Information on the test species	
Test species used	Zebrafish (*Danio rerio*)
Life stage of the test species used	Adult, weight: 0.213 ± 0.041 g
Information on the test design	
Methodology used	OECD305 (1981)
Test substance	Isomeric hexachlorocyclohexanes
Test concentrations used	Mixture of substances: 10 µg/L of each substance
Number of replicates per test concentration	Not stated
Number of organisms per replicate	Not stated
Number of fish per sampling	Not stated
Test conditions	Flow-through
Duration of study	Uptake phase: 80 hours Depuration phase: 140 hours
Observation intervals	Not stated
Feeding	Not stated
Measurement of the exposure concentrations	Yes, 9.4-10.4 µg/L depending on compound
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	No
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Concentrations of test substance in water not stated and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Call et al. (1980)
Information on the test species	
Test species used	Fathead minnow (*Pimephales promelas*)
Life stage of the test species used	Juvenile, weight: 0.10-0.15 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Phenols
Test concentrations used	Control, 5, 50 µg/L of each substance
Number of replicates per test concentration	1
Number of organisms per replicate	80
Number of fish per sampling	5
Test conditions	Flow-through
Duration of study	Uptake phase: 28 days Depuration phase: 28 days
Observation intervals	Day 1, 2, 4, 7, 14, 21, 28 during uptake and day 1, 2, 4, 7, 14, 21, 28 during depuration
Feeding	Yes
Measurement of the exposure concentrations	Yes, 2.5-4.8 and 32.7-49.3 µg/L
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	The study is of good quality having been conducted according to a standardized methodology. Fish used in test system are not within the recommended weight range.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Chaisuksant et al. (1997)
Information on the test species	
Test species used	Mosquito fish (*Gambusia affinis*)
Life stage of the test species used	Adult, weight: 0.0019 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Bromo- and chlorobenzenes
Test concentrations used	Mixture of eight chemicals in 3 concentrations: 5-200 µg/L, 2.5-100 µg/L, 1.25-50 µg/L
Number of replicates per test concentration	5
Number of organisms per replicate	10
Number of fish per sampling	5
Test conditions	Semi-static
Duration of study	Uptake phase: 4 days Depuration phase: 4 days
Observation intervals	Hour 12, 24, 36, 48, 96 during uptake and hour 6, 12, 24, 48, 96 during depuration
Feeding	No, during elimination once daily.
Measurement of the exposure concentrations	Yes, 3.7-233 µg/L, 2.1-101 µg/L, 1.2-57 µg/L
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish and not all substances reached a plateau or were stable in water during uptake.
Water quality criteria satisfied	Yes. The Mosquito fish is not recommended as a test species, therefore no range is suggested.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Stability of test substance in water during uptake dependent on substance and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Debruijn & Hermens (1991)
Information on the test species	
Test species used	Guppy (*Poecilia reticulata*)
Life stage of the test species used	Juvenile, weight: 0.04-0.28 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	Organophosphorus pesticides
Test concentrations used	2 mixtures of 6 pesticides
Number of replicates per test concentration	1
Number of organisms per replicate	70
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 11 days Depuration phase: 17.5 days
Observation intervals	Hour 0, 0.5, 1, 2, 4, 8, 24, 72, 144, 240, 264 during uptake and hour 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 144, 216, 319, 420 during depuration
Feeding	Yes
Measurement of the exposure concentrations	Yes, 9-275 µg/L depending on the substance
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	The study is of good quality having been conducted to a standardized methodology. Slight differences from guideline: no control run in parallel and fish per sampling too low.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	De Voogt et al. (1991)
Information on the test species	
Test species used	Guppy (*Poecilia reticulata*)
Life stage of the test species used	Juvenile, weight: 0.135 g
Information on the test design	
Methodology used	The methodology is not described in detail in the paper.
Test substance	Fluorene, anthracene, pyrene
Test concentrations used	Control, mixture of substances
Number of replicates per test concentration	1
Number of organisms per replicate	6)
Number of fish per sampling	2
Test conditions	Static
Duration of study	Uptake phase: 2-4 days Depuration phase: 6-7 days
Observation intervals	No measurement of fish during uptake and 4 samplings during depuration
Feeding	Not stated
Measurement of the exposure concentrations	Yes, 40-883 µg/L at initiation
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	No, since no fish material was analyzed during uptake, no plateau could be determined. The test substance concentrations during uptake decreased. No information on mortality in fish.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD 305 (2012) only k_2 of interest.
Study conducted to GLP	No
Overall comment on quality	Concentration of test substance in water during uptake decreased.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Dewolf et al. (1993)
Information on the test species	
Test species used	Guppy (*Poecilia reticulata*)
Life stage of the test species used	Juvenile, weight: 0.337 ± 0.166 g
Information on the test design	
Methodology used	The methodology is not described in detail in the paper.
Test substance	Trichloroanilines
Test concentrations used	Mixture of test substances: 173-1768 nmol/L
Number of replicates per test concentration	1
Number of organisms per replicate	Not stated
Number of fish per sampling	2-3
Test conditions	Flow-through
Duration of study	Uptake phase: 7 days Depuration phase: 4-5 days
Observation intervals	14 samplings during uptake and 7 samplings during depuration
Feeding	2% of body weight every second day
Measurement of the exposure concentrations	Yes, 117-1125 nmol/L
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD 305 (2012), k_1 calculated alternatively.
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish, but other validity criteria satisfied. Alternative calculation method for k_1.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Deneer (1993)
Information on the test species	
Test species used	Guppy (*Poecilia reticulata*)
Life stage of the test species used	Juvenile, weight: 0.046-0.141 g
Information on the test design	
Methodology used	The methodology is not described in detail in the paper.
Test substance	Chlorpyrifos
Test concentrations used	0.9-37 µg/L
Number of replicates per test concentration	1
Number of organisms per replicate	40
Number of fish per sampling	2
Test conditions	Flow-through
Duration of study	Uptake phase: 20 days Depuration phase: 4 days
Observation intervals	14 samplings during uptake and day 1, 2, 3, 4 during depuration
Feeding	Not stated
Measurement of the exposure concentrations	Yes
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish and test substance was not stable during uptake.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Concentration of test substance in water during uptake varied (>20%) and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Deneer (1994)
Information on the test species	
Test species used	Three-spined stickleback (*Gasterosteus aculeatus)*
Life stage of the test species used	Juvenile, weight: 0.322 ± 0.122 g at end of experiment
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	Chlorpyrifos
Test concentrations used	0.25 µg/L
Number of replicates per test concentration	1
Number of organisms per replicate	70
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 21 days Depuration phase: 9 days
Observation intervals	Day 1, 2 3,, 4, 5, 6, 7, 9, 11, 14, 16, 20, 21 during uptake and day 1, 2, 3, 5, 6, 7, 8, 9 during depuration
Feeding	Not stated
Measurement of the exposure concentrations	Yes, 0.19 ± 0.03 µg/L
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish.
Water quality criteria satisfied	Yes, but temperature is slightly above the range recommended by the guideline.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish and temperature slightly below recommended range, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Ensenbach & Nagel (1991)
Information on the test species	
Test species used	Zebrafish (*Danio rerio*)
Life stage of the test species used	Juvenile, weight: 0.360 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	Lindane, 4-nitrophenol, phenol, 3,4-dichloroaniline
Test concentrations used	Mixture of chemicals
Number of replicates per test concentration	1
Number of organisms per replicate	Not stated
Number of fish per sampling	At least 3
Test conditions	Static
Duration of study	Uptake phase: 1-2 days Depuration phase: 2-5 days
Observation intervals	6-9 samplings during uptake and 9-10 samplings during depuration
Feeding	No
Measurement of the exposure concentrations	Yes, 6.2-610 nmol/L
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish and lindane was not stable during uptake.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012), a two-compartment model was fitted to data.
Study conducted to GLP	No
Overall comment on quality	Stability of test substance in water during uptake dependent on substance and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Ensenbach et al. (1996)
Information on the test species	
Test species used	Golden die (*Leuciscus idus melanotus*) and rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 1.46 g (L.m.) and 1.045 g (O.m.)
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	3,4-dichloroaniline
Test concentrations used	90nmol/L (L.m.) and 40nmol/L (O.m.)
Number of replicates per test concentration	1
Number of organisms per replicate	60 of each species
Number of fish per sampling	At least 3
Test conditions	Static
Duration of study	Uptake phase: 8-24 hours Depuration phase: 48 hours
Observation intervals	7-8 samplings during uptake and 8-9 samplings during depuration
Feeding	No
Measurement of the exposure concentrations	Yes, but not stated
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish.
Water quality criteria satisfied	Yes. The golden ide is not recommended as a test species, therefore no range is suggested.
Calculation of kinetic parameters	According to OECD305 (2012), a two-compartment model was fitted to data.
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Fox et al. (1994)
Information on the test species	
Test species used	Zebrafish (*Danio rerio*)
Life stage of the test species used	Juvenile, weight: 0.243 ± 0.048g
Information on the test design	
Methodology used	OECD305 (1981)
Test substance	Polychlorinated biphenyls (PCB)
Test concentrations used	Not stated
Number of replicates per test concentration	Not stated
Number of organisms per replicate	Not stated
Number of fish per sampling	Not stated
Test conditions	Flow-through
Duration of study	Uptake phase: 30 days Depuration phase: 30 days
Observation intervals	13 samplings during uptake and 17 samplings during depuration
Feeding	Not stated
Measurement of the exposure concentrations	Yes, 0.0048-2.6 µg/L depending on substance
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish and stability of test substance in water during uptake.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Concentrations of test substance in water not stated and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Gobas & Schrap (1990)
Information on the test species	
Test species used	Guppy (*Poecilia reticulata*)
Life stage of the test species used	Juvenile, weight: 0.079 ± 0.02 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	Polychlorinated dibenzo-p-dioxins and octochlorodibenzofuran
Test concentrations used	Mixture of chemicals
Number of replicates per test concentration	1
Number of organisms per replicate	170
Number of fish per sampling	10
Test conditions	Static (Chromosorb)
Duration of study	Uptake phase: 8 days Depuration phase: 2 days
Observation intervals	Day 6, 7, 8 during uptake and hour 2.5, 5.5, 8, 14, 24, 32, 40 during depuration
Feeding	Twice during uptake
Measurement of the exposure concentrations	Yes, 2.0-11 µg/L depending on the substance
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no all substances reached steady-state.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	The study is of good quality. All validity criteria are satisfied, but a plateau was not reached for all substances within the exposure time.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Gobas et al. (1989)
Information on the test species	
Test species used	Guppy (*Poecilia reticulata*)
Life stage of the test species used	Adult, weight: 0.098 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	Polybrominated biphenyls, brominated benzenes, mirex, polychlorinated biphenyls
Test concentrations used	Mixture of substances
Number of replicates per test concentration	1
Number of organisms per replicate	117
Number of fish per sampling	3-4
Test conditions	Static (Chromosorb)
Duration of study	Uptake phase: 31 days Depuration phase: 198 days
Observation intervals	Day 2, 5, 10, 12, 16, 20, 24, 26, 28, 30, 31 during uptake and day 2, 6, 14, 30, 42, 66, 98, 124, 160, 198 during depuration
Feeding	20 mg/fish/day during 16 days of uptake and depuration
Measurement of the exposure concentrations	Yes
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but not all substances reached a plateau and concentration in water decreased.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012), but elimination into feces considered.
Study conducted to GLP	No
Overall comment on quality	Concentration of test substance in water during uptake decreased.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Görge & Nagel (1990)
Information on the test species	
Test species used	Zebrafish (Danio rerio)
Life stage of the test species used	Juvenile (4 weeks), weight: not stated
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	Lindane and atrazine
Test concentrations used	17 µg/L (lindane), 135 µg/L (atrazine)
Number of replicates per test concentration	1
Number of organisms per replicate	150
Number of fish per sampling	2
Test conditions	Static
Duration of study	Uptake phase: 1 days Depuration phase: 1 days
Observation intervals	17 samplings during uptake and depuration
Feeding	No
Measurement of the exposure concentrations	Yes
Measurement of water quality parameters	Yes (temperature, O2)
Test validity criteria satisfied	Yes, but no information on mortality of fish.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012), a two-compartment model was fitted to data.
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Hoang et al. (2011)
Information on the test species	
Test species used	Mosquito fish (*Gambusia affinis*)
Life stage of the test species used	Juvenile, weight: not stated
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	Endolsulfan sulphate
Test concentrations used	Control, 0.25 µg/L
Number of replicates per test concentration	3
Number of organisms per replicate	40
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 35 days Depuration phase: 21 days
Observation intervals	Day 0, 7, 14, 21, 28, 35 during uptake and day 1, 3, 7, 14, 21 during depuration
Feeding	Daily
Measurement of the exposure concentrations	Yes, 0.26 ± 0.05 µg/L
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish.
Water quality criteria satisfied	Yes. The mosquito fish is not recommended as a test species, therefore no range is suggested.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Kalsch et al. (1991)
Information on the test species	
Test species used	Zebrafish (*Danio rerio*)
Life stage of the test species used	Adult, weight: 0.15-0.45 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	Anilines
Test concentrations used	Control, Mixture of anilines
Number of replicates per test concentration	1
Number of organisms per replicate	60
Number of fish per sampling	3
Test conditions	Static
Duration of study	Uptake phase: 10-100 hours Depuration phase: At least 48 hours
Observation intervals	10 samplings during uptake and 5-10 samplings during depuration
Feeding	No
Measurement of the exposure concentrations	Yes, concentrations were in the range of 0.13-0.21 µmol/L.
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish.
Water quality criteria satisfied	Yes, but oxygen saturation was not stated.
Calculation of kinetic parameters	According to OECD305 (2012), a two-compartment model was fitted to data.
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish and oxygen saturation, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Keizer et al. (1991)
Information on the test species	
Test species used	Guppy (*Poecilia reticulata*) and zebrafish (*Danio rerio*)
Life stage of the test species used	Adult, weight: 0.6 ± 0.15 g (*P.r.*) and 0.4 ± 0.1 g (*D.r.*)
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	Diazinon
Test concentrations used	Control, 0.1, 0.4 mg/L
Number of replicates per test concentration	1
Number of organisms per replicate	100
Number of fish per sampling	2*2-4
Test conditions	Semi-static
Duration of study	Uptake phase: 2 days Depuration phase: 1 days
Observation intervals	8 samplings during uptake and 2-4 samplings during depuration
Feeding	Not stated
Measurement of the exposure concentrations	Yes, 0.07 ± 0.01 and 0.31 ± 0.03 mg/L in guppy and 0.09 ± 0.01 and 0.38 ± 0.01 mg/L in zebrafish.
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Yes, but no information on mortality of fish.
Water quality criteria satisfied	Not stated
Calculation of kinetic parameters	According to OECD305 (2012), but modified to take into account the effects of metabolism.
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish and water quality parameters, but other validity criteria satisfied. Alternative calculation method.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Kikuchi et al. (1980)
Information on the test species	
Test species used	Common carp (*Cyprinus carpio*)
Life stage of the test species used	Juvenile, weight: 14.4 ± 2.9 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	Sodium alkylpoly(oxyethylene) sulfates
Test concentrations used	Control, 0.33, 0.34 mg/L for each substance separate experiment
Number of replicates per test concentration	1
Number of organisms per replicate	45
Number of fish per sampling	5
Test conditions	Flow-through
Duration of study	Uptake phase: 3 days Depuration phase: 5 days
Observation intervals	Hour 0.5, 2, 8, 24, 72 during uptake and hour 8, 24, 72, 120 during depuration
Feeding	No
Measurement of the exposure concentrations	Yes, 0.33 and 0.34 mg/L.
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish.
Water quality criteria satisfied	Yes, but oxygen saturation not stated.
Calculation of kinetic parameters	Alternative calculation method
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish, but other validity criteria satisfied. Alternative calculation method for k_1.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Kimerle et al. (1981)
Information on the test species	
Test species used	Bluegill (*Lepomis macrochirus*)
Life stage of the test species used	Juvenile, weight: 4 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	Linear alkylbenzene sulfonate
Test concentrations used	Control, 0.5 mg/L
Number of replicates per test concentration	1
Number of organisms per replicate	375 (100 in control)
Number of fish per sampling	4
Test conditions	Flow-through
Duration of study	Uptake phase: 21 days Depuration phase: 14 days
Observation intervals	Day 1, 3, 7, 11, 15, 21 during uptake and day 1, 2, 3, 5, 7, 9, 11, 14 during depuration
Feeding	2% of body weight/day
Measurement of the exposure concentrations	Yes, 0.46 ± 0.05 mg/L
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish.
Water quality criteria satisfied	Yes, but temperature is out of the range recommended by the guideline.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish and temperature slightly below recommended range, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Könemann & van Leeuwen (1980)
Information on the test species	
Test species used	Guppy (*Poecilia reticulata*)
Life stage of the test species used	Juvenile, weight: 0.62 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	Chlorobenzenes
Test concentrations used	Control, Mixture of chemicals (4-160 µg/L depending on substance)
Number of replicates per test concentration	1
Number of organisms per replicate	120
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 19 days Depuration phase: 63 days
Observation intervals	10 samplings during uptake and 5-12 samplings during depuration
Feeding	Yes
Measurement of the exposure concentrations	Yes, 0.3-116 µg/L depending on substance
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish.
Water quality criteria satisfied	Yes, but oxygen saturation is below the threshold stated by the guideline.
Calculation of kinetic parameters	According to OECD305 (2012), a two-compartment model was fitted to data. Kinetic parameters recalculated by Gobas et al. (1989)
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish and oxygen saturation slightly belowrecommended range, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Lin et al. (1997)
Information on the test species	
Test species used	Black silver carp (*Aristichthys nobilis*)
Life stage of the test species used	Juvenile, weight: 3-5 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	Butachlor, Thiobencarb, Chlomethoxyfen
Test concentrations used	Control, low level, high level
Number of replicates per test concentration	1
Number of organisms per replicate	85
Number of fish per sampling	2
Test conditions	Flow-through
Duration of study	Uptake phase: 14 days Depuration phase: 25 days
Observation intervals	12 samplings during uptake and 14 samplings during depuration
Feeding	Yes
Measurement of the exposure concentrations	Yes, 0.62-2.13 µg/L in low level and 2.81-17.9 µg/L in high level exposure depending on substance.
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish and the plateau phase was not reached for all tested substances.
Water quality criteria satisfied	Yes. The black silver carp is not recommended as a test species, therefore no range is suggested.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Linder et al. (1985)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 10 ± 4 g (mean during study)
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	Anthracene
Test concentrations used	Control, 50 µg/L, 50 µg/L and complex mixture of other chemicals as oil shale retort water
Number of replicates per test concentration	2
Number of organisms per replicate	15
Number of fish per sampling	2
Test conditions	Semi-static
Duration of study	Uptake phase: 3 days Depuration phase: 6 days
Observation intervals	Hour 0, 24, 48, 72 during uptake and day 2, 4, 6 during depuration
Feeding	No
Measurement of the exposure concentrations	Yes
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	No, concentration of test substance during uptake phase decreased.
Water quality criteria satisfied	Yes, but oxygen saturation is not stated.
Calculation of kinetic parameters	According to OECD305 (2012), but decreasing exposure concentration considered.
Study conducted to GLP	No
Overall comment on quality	Concentration of test substance in water during uptake decreased.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Loonen et al. (1994)
Information on the test species	
Test species used	Guppy (*Poecilia reticulata*)
Life stage of the test species used	Adult, weight: 0.91 ± 0.21 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (from fly ash)
Test concentrations used	200 µl fly-ash extract (chemicals extracted from 190 g fly-ash)
Number of replicates per test concentration	3
Number of organisms per replicate	25
Number of fish per sampling	2
Test conditions	Static (Chromosorb)
Duration of study	Uptake phase: 21 days No depuration phase
Observation intervals	Day 0, 1, 2, 4, 8, 14, 21 during uptake
Feeding	Not stated
Measurement of the exposure concentrations	Yes, not detected to 2.08 ng/L depending on substance.
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no all substances reached a plateau and stability during uptak e was not given for all substances.
Water quality criteria satisfied	Yes. Oxygen saturation not stated.
Calculation of kinetic parameters	According to OECD305 (2012), k_2 estimated since no elimination experiment conducted.
Study conducted to GLP	No
Overall comment on quality	Stability of test substance in water during uptake dependent on substance and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Martin et al. (2003a)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 5-10 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	Perfluorinated acids
Test concentrations used	Control, mixture of chemicals
Number of replicates per test concentration	1
Number of organisms per replicate	Not stated
Number of fish per sampling	3 (1 from control)
Test conditions	Flow-through
Duration of study	Uptake phase: 12 days Depuration phase: 33 days
Observation intervals	Hour 4.5, 9, 18, 36, 72, 144, 288 during uptake and hour 4.5, 9, 18, 36, 72, 144, 288, 456, 792 during depuration
Feeding	1.5% of body weight/day
Measurement of the exposure concentrations	Yes, 0.014-1.7 µg/L depending on substance
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, but stability of test substance during uptake phase not given depending on substance and no plateau was reached.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Stability of test substance in water during uptake dependent on substance and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	McLeese et al. (1981)
Information on the test species	
Test species used	Atlantic salmon (*Salmo salar*)
Life stage of the test species used	Juvenile, weight: Not stated
Information on the test design	
Methodology used	The methodology is not described in detail in the paper.
Test substance	Alkylphenols
Test concentrations used	Control, one concentration for each alkylphenol
Number of replicates per test concentration	5
Number of organisms per replicate	3
Number of fish per sampling	2
Test conditions	Static
Duration of study	Uptake phase: 4 days Depuration phase: 4 days
Observation intervals	Day 1, 2, 4 during uptake and day 1, 2, 4during depuration
Feeding	No
Measurement of the exposure concentrations	Yes, 0.07-0.83 mg/L depending on substance
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	No
Water quality criteria satisfied	The Atlantic salmon is not recommended as a test species, therefore no range is suggested. The oxygen saturation is not stated.
Calculation of kinetic parameters	According to OECD305 (2012), but decreasing concentration of substance during uptake phase considered.
Study conducted to GLP	No
Overall comment on quality	Concentration of test substance in water during uptake decreased.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Mehrle et al. (1988)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 0.38 ± 0.09 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	2,3,7,8-Tetrachlorodibenzodioxin, -furan
Test concentrations used	0, 115, 231, 463, 925, 1850 pg/L (TCDD) 0, 1,3, 2,7, 5,3 10.6, 21.3 ng/L (TCDF)
Number of replicates per test concentration	2
Number of organisms per replicate	50
Number of fish per sampling	Not stated
Test conditions	Flow-through
Duration of study	Uptake phase: 28 days Depuration phase: 28 days
Observation intervals	Day 7, 14, 21, 28 during uptake and day 28 during depuration
Feeding	Yes, ad libitum.
Measurement of the exposure concentrations	Yes, 1.1-789 pg/L (TCDD) and <0.02-8.78 ng/L (TCDF)
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	No
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Concentrations of test substance in water not stated and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Memmert et al. (2013)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 1.1-1.2 g
Information on the test design	
Methodology used	OECD305 (1996)
Test substance	Diclofenac
Test concentrations used	Control, 2, 20 µg/L
Number of replicates per test concentration	1
Number of organisms per replicate	70 (45 in control)
Number of fish per sampling	4
Test conditions	Flow-through
Duration of study	Uptake phase: 14 days Depuration phase: 14 days
Observation intervals	Day 4, 7, 10,12,14 during uptake and day 17, 20, 24, 28 during depuration
Feeding	2-3% of body weight/day
Measurement of the exposure concentrations	Yes, 2.1 and 18.7 µg/L
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (1996)
Study conducted to GLP	No
Overall comment on quality	The study is of good quality having been conducted to a standardized methodology.
Reliablity of study	Reliable
Klimisch Code	1

Reference	Min & Cha (2000)
Information on the test species	
Test species used	Zebrafish (Danio rerio)
Life stage of the test species used	Adult, weight: 0.2-0.4 g
Information on the test design	
Methodology used	OECD305 (1996)
Test substance	Phosphamidon, profenofos
Test concentrations used	20, 100 mg/L (phosphamidon) and 0.4 and 2 mg/L (profenofos)
Number of replicates per test concentration	1
Number of organisms per replicate	250
Number of fish per sampling	20
Test conditions	Flow-through
Duration of study	Uptake phase: 7 days Depuration phase: 12 hours
Observation intervals	Hour 6, 12, 24, 48, 72, 120, 144, 168 during uptake and hour 2, 4, 8, 12 during depuration
Feeding	1% of body weight/day
Measurement of the exposure concentrations	Yes, 198-995 µg/L (phosphamidon) and 4-20 µg/L (profenofos)
Measurement of water quality parameters	Yes (temperature, O2)
Test validity criteria satisfied	Yes, but no information on mortality of fish.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	Not stated
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish, but other validity criteria satisfied. Calculation method not stated, but test was conducted according to the guideline.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Muir et al. (1983)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*), fathead minnow (*Pimephales promelas*)
Life stage of the test species used	Juvenile, weight: 0.75 g (O.m.), 2.5 g (*P.p.*)
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	Triaryl phosphates
Test concentrations used	5, 50 µg/L of each substance
Number of replicates per test concentration	1
Number of organisms per replicate	Not stated
Number of fish per sampling	3
Test conditions	Static
Duration of study	Uptake phase: 1 days Depuration phase: 18 days
Observation intervals	Hour 1, 3, 6, 12, 24 during uptake and hour 12, 24, 48, 96, 144, 240, 432 during depuration
Feeding	Not stated
Measurement of the exposure concentrations	Yes, initial concentrations: 3.6-6.2 µg/L in low and 34.9-55-0 µg/L in high treatment depending on substance
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	No
Water quality criteria satisfied	Not stated
Calculation of kinetic parameters	According to OECD305 (2012), but k_1 was estimated using simple linear regression.
Study conducted to GLP	No
Overall comment on quality	Concentration of test substance in water during uptake decreased.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Muir et al. (1985)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*), fathead minnow (*Pimephales promelas*)
Life stage of the test species used	Juvenile, weight: 0.1-0.3 g (O.m.),1.0-2.5 g (*P.p.*)
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	Chlorinated dioxins
Test concentrations used	Mixture of chemicals in low and high concentration treatment
Number of replicates per test concentration	1
Number of organisms per replicate	33
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 4-5 days Depuration phase: 24-48 days
Observation intervals	Day 0.5, 1, 2, 3, 4, 5 during uptake and 4-5 samplings during depuration
Feeding	Yes, during elimination phase (1% of body weight/day)
Measurement of the exposure concentrations	Yes, 0.01-0.10 ng/L depending on substance
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	No, the exposure concentration in water decreased for some substances during uptake, a plateau was not reached for all substances. No information on mortality of fish.
Water quality criteria satisfied	Yes. Temperature during test was not stated.
Calculation of kinetic parameters	According to OECD305 (2012), but decreasing exposure concentration considered.
Study conducted to GLP	No
Overall comment on quality	Concentration of test substance in water during uptake decreased.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Muir et al. (1986)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*), fathead minnow (*Pimephales promelas*)
Life stage of the test species used	Juvenile, weight: 0.1-0.5 g (O.m.),1.5- 2.5 g (*P.p.*)
Information on the test design	
Methodology used	The methodology is not described in detail in the paper.
Test substance	1,3,6,8-tetrachlorodibenzi-*p*-dioxin, octachloro-dibenzo-*p*-dioxin
Test concentrations used	1-3 concentrations for each substance
Number of replicates per test concentration	1
Number of organisms per replicate	Not stated
Number of fish per sampling	3-6
Test conditions	Flow-through
Duration of study	Uptake phase: 4-5 days Depuration phase: 24-48 days
Observation intervals	6 samplings during uptake and 4-5 samplings during depuration
Feeding	Not stated
Measurement of the exposure concentrations	Yes
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Yes, but the exposure concentration in water decreased for some substances during uptake and a plateau was not reached for all substances.
Water quality criteria satisfied	Not stated
Calculation of kinetic parameters	According to OECD305 (2012), but decreasing exposure concentration considered.
Study conducted to GLP	No
Overall comment on quality	Concentration of test substance in water during uptake decreased.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Muir et al. (1994)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 1-2 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	Pyrethroids and DDT
Test concentrations used	Each chemical separately tested in Aldrich humic acid solution filtered through a 0.45µm filter
Number of replicates per test concentration	1
Number of organisms per replicate	15
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 4 days Depuration phase: 192 hours
Observation intervals	Hour 12, 24, 48, 72, 96 during uptake andhour 0, 24, 48, 96, 192 during depuration
Feeding	Yes, during elimination (0.015 g/body weight/day
Measurement of the exposure concentrations	Yes, 57-80 ng/L (dissolved)
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Yes, but stability during uptake is not given for all test substances and a plateau was not reached for all substances. No information on mortality of fish.
Water quality criteria satisfied	Not stated
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Stability of test substance in water during uptake dependent on substance and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Oliver & Niimi (1985)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 1-2 g
Information on the test design	
Methodology used	The methodology is not described in detail in the paper.
Test substance	Halogenated organics
Test concentrations used	Three mixtures of substances
Number of replicates per test concentration	1
Number of organisms per replicate	32
Number of fish per sampling	6
Test conditions	Flow-through
Duration of study	Uptake phase: 4 days No depuration phase
Observation intervals	Day 7, 21, 35, 50, 75, 96 during uptake
Feeding	Not stated
Measurement of the exposure concentrations	Yes, 0.6-210 ng/L depending on substance
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, but stability during uptake is not given for all test substances and a plateau was not reached for all substances. No information on mortality of fish.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Stability of test substance in water during uptake dependent on substance and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Opperhuizen & Voors (1987a)
Information on the test species	
Test species used	Guppy (*Poecilia reticulata*)
Life stage of the test species used	Juvenile, weight: 0.215 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Chlorobiphenyls, chlorodiphenylether
Test concentrations used	Mixture of chemicals
Number of replicates per test concentration	1
Number of organisms per replicate	60
Number of fish per sampling	2-4
Test conditions	Static (Chromosorb)
Duration of study	Uptake phase: 8 days Depuration phase: 12-56 days
Observation intervals	7 samplings during uptake and 4-9 samplings during depuration
Feeding	20 mg/g fish/day
Measurement of the exposure concentrations	Yes, 14-35 µg/L
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but exposure concentration of substance during uptake decreased.
Water quality criteria satisfied	Yes. Oxygen saturation was not stated.
Calculation of kinetic parameters	According to OECD305 (2012), but rate of metabolism was included.
Study conducted to GLP	No
Overall comment on quality	Concentration of test substance in water during uptake decreased.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Opperhuizen & Voors (1987b)
Information on the test species	
Test species used	Guppy (*Poecilia reticulata*)
Life stage of the test species used	Juvenile, weight: 0.098 g
Information on the test design	
Methodology used	The methodology is not described in detail in the paper.
Test substance	Polychlorinated aromatic ethers
Test concentrations used	Mixture of chemicals
Number of replicates per test concentration	1
Number of organisms per replicate	96
Number of fish per sampling	Not stated
Test conditions	Static (Chromosorb)
Duration of study	Uptake phase: 7 days Depuration phase: Not stated
Observation intervals	5-8 samplings during uptake and samplings during depuration not stated.
Feeding	Not stated
Measurement of the exposure concentrations	Yes, 209-2595 µg/30L depending on substance
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Yes, but exposure concentration of substance during uptake decreased. No information on mortality of fish.
Water quality criteria satisfied	Not stated
Calculation of kinetic parameters	Not stated
Study conducted to GLP	No
Overall comment on quality	Concentration of test substance in water during uptake decreased.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Ownby et al. (2005)
Information on the test species	
Test species used	Channel catfish (*Ictalurus punctatus*)
Life stage of the test species used	Juvenile, weight: 2.69 ± 0.69 g during experimentation
Information on the test design	
Methodology used	The methodology is not described in detail in the paper.
Test substance	Trinitrotoluene
Test concentrations used	7.7 µg/L
Number of replicates per test concentration	3
Number of organisms per replicate	6
Number of fish per sampling	4
Test conditions	Not stated
Duration of study	Uptake phase: 8 hours No depuration phase
Observation intervals	Hour 1, 2, 4, 8 during uptake
Feeding	Not stated
Measurement of the exposure concentrations	Yes, 7.5 µg/L
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Yes, but no information on mortality of fish and stability of substance during uptake.
Water quality criteria satisfied	Not stated
Calculation of kinetic parameters	According to OECD305 (2012), but k_1 calculatied using linear regression. k_2 was estimated from k_1 and BCF_{SS}.
Study conducted to GLP	No
Overall comment on quality	Concentrations of test substance in water not stated and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Paterson & Metcalfe (2008)
Information on the test species	
Test species used	Medaka (Oryzias latipes)
Life stage of the test species used	Adult, weight: 0.36 ± 0.02 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Fluoxetine
Test concentrations used	Control, 0.64 µg/L
Number of replicates per test concentration	1
Number of organisms per replicate	23
Number of fish per sampling	4-7
Test conditions	Semi-static
Duration of study	Uptake phase: 7 days Depuration phase: 21 days
Observation intervals	Day 0.2, 3, 7 during uptake and day 7, 14, 21 during depuration
Feeding	Yes, twice daily.
Measurement of the exposure concentrations	Yes, 0.55 µg/L
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, except for stability of exposure concentration during uptake.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Concentrations of test substance in water during uptake varied (>20%), but all other validity criteria are satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Rodgers et al. (1983)
Information on the test species	
Test species used	Bluegill (*Lepomis macrochirus*)
Life stage of the test species used	Juvenile, weight: 11.3 g (naphthalene), 7.8 g (lindane)
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Lindane, naphthalene
Test concentrations used	Control, 20, 200 µg/L (naphthalene), 0, 3, 30 µg/L (lindane)
Number of replicates per test concentration	1
Number of organisms per replicate	40
Number of fish per sampling	4
Test conditions	Flow-through
Duration of study	Uptake phase: 4-6 days Depuration phase: 5-12 days
Observation intervals	4 samplings during uptake and five samplings during depuration
Feeding	No
Measurement of the exposure concentrations	Yes, 9.5 and 111.6 µg/L (naphthalene), 2.77 and 31.57 µg/L (lindane)
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but stability during uptake is not given for all test substances. No information on mortality of fish.
Water quality criteria satisfied	Not stated
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Stability of test substance in water during uptake dependent on substance and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Schettgen (2000)
Information on the test species	
Test species used	Zebrafish (*Danio rerio*)
Life stage of the test species used	Adult, weight: 0.1-0.25 g
Information on the test design	
Methodology used	OECD305 (1996)
Test substance	Triclosan
Test concentrations used	Triclosan in water with different pH (6-9)
Number of replicates per test concentration	Not stated
Number of organisms per replicate	94-147
Number of fish per sampling	3-4*2 fish
Test conditions	Flow-through
Duration of study	Uptake phase: 3-13 days depending on pH Depuration phase: 6-13 days
Observation intervals	9-11 samplings during uptake and 7-14 samplings during depuration
Feeding	Yes, daily.
Measurement of the exposure concentrations	Yes, 36-49 µg/L.
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Stability during uptake and plateau dependent on substance and pH (Triclosan).
Water quality criteria satisfied	Not stated
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Concentrations of test substance in water not stated and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Schettgen (2000)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 1.5-3.5 g
Information on the test design	
Methodology used	OECD305 (1996)
Test substance	Pyrethroids
Test concentrations used	0.5-5 µg/L depending on substance
Number of replicates per test concentration	1
Number of organisms per replicate	Not stated
Number of fish per sampling	Not stated
Test conditions	Flow-through
Duration of study	Uptake phase: 4 days Depuration phase: 4-9 days
Observation intervals	8-9 samplings during uptake and depuration
Feeding	Yes, daily.
Measurement of the exposure concentrations	Yes, 0.4-4.0 µg/L depending on substance
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Stability during uptake and plateau dependent on substance and pH (Triclosan).
Water quality criteria satisfied	Not stated
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Stability of test substance in water during uptake dependent on substance and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Schmieder et al. (1995)
Information on the test species	
Test species used	Medaka (*Oryzias latipes*)
Life stage of the test species used	Juvenile, weight: 0.175 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	2,3,7,8-Tetrachlorodibenzodioxin
Test concentrations used	Control, 180 pg/L
Number of replicates per test concentration	1
Number of organisms per replicate	85
Number of fish per sampling	5-10 (2-3 from control)
Test conditions	Flow-through
Duration of study	Uptake phase: 12 days Depuration phase: 175 days
Observation intervals	Day 2, 4, 6, 10, 12 during uptake and day 28 during depuration.
Feeding	Yes, twice daily.
Measurement of the exposure concentrations	Yes, 101 ± 26 pg/L.
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, except for stability of substance during uptake (>20%).
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Concentrations of test substance in water during uptake varied (>20%), but all other validity criteria are satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Seo et al. (2002)
Information on the test species	
Test species used	Common carp (*Cyprinus carpio*)
Life stage of the test species used	Juvenile, weight: 0.5-1.5 g
Information on the test design	
Methodology used	OECD305 (1996)
Test substance	Pyribenzoxim
Test concentrations used	Control, 0.1, 1mg/L
Number of replicates per test concentration	1
Number of organisms per replicate	64
Number of fish per sampling	4
Test conditions	Flow-through
Duration of study	Uptake phase: 28 days Depuration phase: 14 days
Observation intervals	11 samplings during uptake and 3 samplings during depuration
Feeding	Twice/day
Measurement of the exposure concentrations	Yes, 0.089 and 0.685 mg/L
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (1996)
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Sijm & van der Linde (1995)
Information on the test species	
Test species used	Fathead minnow (*Pimephales promelas*)
Life stage of the test species used	Different age classes, weight:0.045-1.17 g
Information on the test design	
Methodology used	The methodology is not described in detail in the paper.
Test substance	Polychlorinated benzenes and biphenyls
Test concentrations used	0.046-27 µg/L depending on substance and age class used
Number of replicates per test concentration	1
Number of organisms per replicate	24-41
Number of fish per sampling	1-3
Test conditions	Static
Duration of study	Uptake phase: 5 days Depuration phase: 6 months
Observation intervals	Hours 2, 4, 6, 8, 24, 48, 72, 96, 120 during up-take and days 1, 2, 3, 4, 8, 16, 31, 64, 106, 202 during depuration
Feeding	Not stated
Measurement of the exposure concentrations	Yes
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012) but evaporation loss considered.
Study conducted to GLP	No
Overall comment on quality	Concentrations of test substance in water not stated and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Sijm et al. (1993a)
Information on the test species	
Test species used	Guppy (*Poecilia reticulata*) and fathead minnow (*Pimephales promelas*)
Life stage of the test species used	Juvenile, weight: 0.18 ± 0.05 g (*P.r.*), 0.39 ± 0.12 g (*P.p.*)
Information on the test design	
Methodology used	The methodology is not described in detail in the paper.
Test substance	Halogenated benzenes
Test concentrations used	Not stated
Number of replicates per test concentration	1
Number of organisms per replicate	4 (uptake experiment), 8-16 (elimination experiment)
Number of fish per sampling	Not stated
Test conditions	Semi-static
Duration of study	Uptake phase: 30 min Depuration phase: 4 days
Observation intervals	Minutes 5, 10, 30 during uptake and not stated during depuration
Feeding	Not stated
Measurement of the exposure concentrations	Yes
Measurement of water quality parameters	Yes (O_2)
Test validity criteria satisfied	No
Water quality criteria satisfied	No
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Concentration of test substance in water during uptake decreased.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Sijm et al. (1993b)
Information on the test species	
Test species used	Goldfish (*Carassius auratus*)
Life stage of the test species used	Not stated
Information on the test design	
Methodology used	The methodology is not described in detail in the paper.
Test substance	Polychlorinated dibenzo-p-dioxins and dibenzo-furans in fly ash
Test concentrations used	Control, one aquarium containing piperonylbut-oxide (5 mg/L) and contaminated water, the other containing contaminated water only.
Number of replicates per test concentration	1
Number of organisms per replicate	8
Number of fish per sampling	1-2
Test conditions	Not stated
Duration of study	Uptake phase: 5 days Depuration phase: 17 days
Observation intervals	Hour 11, 120 during uptake and day 1.7, 6, 17 during depuration
Feeding	Yes, in elimination phase.
Measurement of the exposure concentrations	Yes
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Yes, but no information on stability of sub-stance during uptake and not all substances reached a plateau.
Water quality criteria satisfied	Not stated
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Concentrations of test substance in water not stated and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Smith et al. (1990)
Information on the test species	
Test species used	American flagfish (*Jordanella floridae*)
Life stage of the test species used	Juvenile, age: 4 – 6months
Information on the test design	
Methodology used	ASTM (1978).
Test substance	Chlorobenzenes and chlorophenols
Test concentrations used	Control, 5 µg/L
Number of replicates per test concentration	2 per exposure and 1 control
Number of organisms per replicate	48
Number of fish per sampling	3 per replicate
Test conditions	Flow-through
Duration of study	Uptake phase: 28 days Depuration phase: 14 days
Observation intervals	Day 1, 3, 7, 14, 21, 28 during uptake and day 1, 2, 3, 4, 5, 7, 10, 14 during depuration
Feeding	Yes
Measurement of the exposure concentrations	Yes, 2.7 – 4.1 µg/L depending on substance
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, but no information on mortality.
Water quality criteria satisfied	The American flagfish is not recommended as a test species, therefore no range is suggested.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish and oxygen saturation, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Southworth et al. (1979)
Information on the test species	
Test species used	Fathead minnow (*Pimephales promelas*)
Life stage of the test species used	Adult, weight: 0.609 ± 0.231 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Acridine
Test concentrations used	Control, 100 µg/L
Number of replicates per test concentration	1
Number of organisms per replicate	100
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 17 days Depuration phase: 3 days
Observation intervals	14 samplings during uptake and 8 samplings during depuration
Feeding	3% of body weight/day
Measurement of the exposure concentrations	Yes, 100 µg/L
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Souhworth et al. (1980)
Information on the test species	
Test species used	Fathead minnow (*Pimephales promelas*)
Life stage of the test species used	Juvenile, weight: 0.075 ± 0.019 g
Information on the test design	
Methodology used	The methodology is not described in detail in the paper.
Test substance	Dibenz[*a,h*]acridine
Test concentrations used	8.8 µg/L
Number of replicates per test concentration	1
Number of organisms per replicate	16
Number of fish per sampling	2
Test conditions	Static
Duration of study	Uptake phase: 4 days Depuration phase: Not stated
Observation intervals	Not stated
Feeding	Not stated
Measurement of the exposure concentrations	Yes
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, but no information on mortality of fish and concentration in water decreased.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012), but modified by including a metabolic elimination rate and decline of test substance in water.
Study conducted to GLP	No
Overall comment on quality	Concentration of test substance in water during uptake decreased.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Southworth et al. (1981)
Information on the test species	
Test species used	Fathead minnow (*Pimephales promelas*)
Life stage of the test species used	Juvenile, weight: 0.090 - 0.210 g
Information on the test design	
Methodology used	The methodology is not described in detail in the paper.
Test substance	Benz(*a*)acridine
Test concentrations used	Control, 42, 78µg/L
Number of replicates per test concentration	1
Number of organisms per replicate	20
Number of fish per sampling	2
Test conditions	Semi-static
Duration of study	Uptake phase: 7-9 days Depuration phase: Not stated
Observation intervals	Not stated
Feeding	2% of body weight/day
Measurement of the exposure concentrations	Yes
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, but no information on mortality of fish and concentration in water decreased.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012), but modified by including a metabolic elimination rate and decline of test substance in water.
Study conducted to GLP	No
Overall comment on quality	Concentration of test substance in water during uptake decreased.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Spacie et al. (1983)
Information on the test species	
Test species used	Bluegill (*Lepomis macrochirus*)
Life stage of the test species used	Juvenile, weight: 0.1-0.6 g
Information on the test design	
Methodology used	The methodology is not described in detail in the paper.
Test substance	Anthracene and Benzo[*a*]pyrene
Test concentrations used	0.7-1.0 µg/L depending on substance
Number of replicates per test concentration	Not stated
Number of organisms per replicate	Not stated
Number of fish per sampling	Not stated
Test conditions	Static
Duration of study	Uptake phase: 4 hours Depuration phase: 5 days
Observation intervals	3 samplings during uptake and 5-7 samplings during depuration
Feeding	No
Measurement of the exposure concentrations	Yes
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, but no information on stability of test substance during uptake and a plateau was not reached.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Concentrations of test substance in water not stated and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Tolls & Sijm (1999)
Information on the test species	
Test species used	Fathead minnow (*Pimephales promelas*)
Life stage of the test species used	Juvenile, weight: 0.66 0.21 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Octaethylene glycol monotridecyl ether
Test concentrations used	0.2 mg/L
Number of replicates per test concentration	1
Number of organisms per replicate	52
Number of fish per sampling	4
Test conditions	Flow-through
Duration of study	Uptake phase: 33 hours Depuration phase: 32 hours
Observation intervals	Hour 1, 2, 4, 8, 14, 20, 25, 30, 33 during uptake and hour 3, 7, 17, 32 during depuration
Feeding	Yes, in elimination phase.
Measurement of the exposure concentrations	Yes, 0.20 ± 0.02 mg/L
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	The study is of good quality offering information and fulfilling validity criteria. Slight differences from guideline: no control run in parallel.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Tolls et al. (2000)
Information on the test species	
Test species used	Fathead minnow (*Pimephales promelas*)
Life stage of the test species used	Juvenile, weight: 0.5-1 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Alcohol ethoxylates
Test concentrations used	Two mixtures of 3 substances
Number of replicates per test concentration	1
Number of organisms per replicate	Not stated
Number of fish per sampling	3-4
Test conditions	Flow-through
Duration of study	Uptake phase: 54-72 hours Depuration phase: 9 days
Observation intervals	9 samplings during uptake and hour 2, 4, 6, 24 during depuration
Feeding	Yes, in elimination phase.
Measurement of the exposure concentrations	Yes, 0.021-0.195 µM depending on substance
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, but no information on stability of substance during uptake and reaching of plateau.
Water quality criteria satisfied	Yes. No information on oxygen saturation.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Concentrations of test substance in water not stated and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Tolls et al. (1997)
Information on the test species	
Test species used	Fathead minnow (*Pimephales promelas*)
Life stage of the test species used	Juvenile, weight: 0.5-1 g
Information on the test design	
Methodology used	OECD305 (1981)
Test substance	Linear alkylbenzenesulfonates
Test concentrations used	Experiment A-D
Number of replicates per test concentration	1
Number of organisms per replicate	Not stated
Number of fish per sampling	2-4
Test conditions	Flow-through
Duration of study	Uptake phase: 2 (A), 7-8 days (B-D) Depuration phase: not stated
Observation intervals	2-8 samplings during uptake and not stated during depuration
Feeding	1% of body weight/day (except for experiment A)
Measurement of the exposure concentrations	Yes
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes. No information on oxygen saturation.
Calculation of kinetic parameters	According to OECD305 (2012), for estimation of k_1 a linear regression was used.
Study conducted to GLP	No
Overall comment on quality	The study is of good quality having been conducted to a standardized methodology. All validity criteria are satisfied, but an alternative calculation method was applied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Tsuda et al. (1988)
Information on the test species	
Test species used	Willow shiner (*Gnathopogon caerulescens*)
Life stage of the test species used	Adult, weight: 1.8-2.9 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Benthiocarb, simetryne
Test concentrations used	10 µg/L of each substance
Number of replicates per test concentration	1
Number of organisms per replicate	60
Number of fish per sampling	6
Test conditions	Flow-through
Duration of study	Uptake phase: 14 days Depuration phase: 7 days
Observation intervals	Day 0, 1, 3, 7, 10, 14 during uptake and day 1, 2, 3, 7 during depuration
Feeding	No
Measurement of the exposure concentrations	Yes, 2.8 µg/L (benthiocarb) and 8.1 µg/L (simetryne)
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, but stability of test substance during uptake was >20% for benthiocarb.
Water quality criteria satisfied	The Willow shiner is not recommended as a test species, therefore no range is suggested.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Stability of test substance in water during uptake dependent on substance, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Tsuda et al. (1989b)
Information on the test species	
Test species used	Willow shiner (*Gnathopogon caerulescens*)
Life stage of the test species used	Adult, weight: 1.8-2.5 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Diazinon, IBP, malathion, fenitrothion
Test concentrations used	Two mixtures of two chemicals: 10 µg/L of each substance
Number of replicates per test concentration	1
Number of organisms per replicate	45
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 7 days Depuration phase: 7 days
Observation intervals	Hour 0, 12, 24, 48, 72, 168 during uptake and hour 6, 12, 24, 48, 72, 168 during depuration
Feeding	Yes, 50 mg/fish/day
Measurement of the exposure concentrations	Yes, 2.4-6.5 µg/L depending on the substance.
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes
Water quality criteria satisfied	The Willow shiner is not recommended as a test species, therefore no range is suggested.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Tsuda et al. (1990b)
Information on the test species	
Test species used	Willow shiner (*Gnathopogon caerulescens*)
Life stage of the test species used	Adult, weight: 1.7-2.4 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Oxadiazon, CNP, Chlomethoxynil
Test concentrations used	5 µg/L of each substance
Number of replicates per test concentration	1
Number of organisms per replicate	30
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 14 days Depuration phase: 14 days
Observation intervals	Day 0, 1, 3, 7, 10, 14 during uptake and day 1, 3, 7, 14 during depuration
Feeding	Yes, 50 mg/fish/day
Measurement of the exposure concentrations	Yes, 1.8-2.4 µg/L depending on substance.
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes. The Willow shiner is not recommended as a test species, therefore no range is suggested.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Tsuda al. (1992a)
Information on the test species	
Test species used	Common carp (*Cyprinus carpio*) and willow shiner (*Gnathopogon caerulescens*)
Life stage of the test species used	Juvenile, weight: 14-22 g (*C.c.*); adult, weight: 0.93-1.43 g (*G.c.*)
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Simazine, chlorothalonil, captan
Test concentrations used	3-8 µg/L depending on the substance
Number of replicates per test concentration	1
Number of organisms per replicate	30
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 14 days Depuration phase: 3-7 days
Observation intervals	Day 0, 1, 3, 7, 10, 14 during uptake and hour 3, 6, 12, 24,72, 168 (*C.c.*) during depuration
Feeding	Yes, 0.3 g/fish/day (*C.c.*) ; 20 mg/fish/day (*G.c.*)
Measurement of the exposure concentrations	Yes, 0.16-7.7 µg/L depending on substance.
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but in carp not for all substances, stability during the uptake is given.
Water quality criteria satisfied	Yes. The Willow shiner is not recommended as a test species, therefore no range is suggested.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	The study is of good quality. All validity criteria are satisfied, but a plateau was not reached for all substances within the exposure time.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Tsuda et al. (1992b)
Information on the test species	
Test species used	Willow shiner (*Gnathopogon caerulescens*)
Life stage of the test species used	Adult, weight: 0.98-1.52 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Organophosphorus pesticides
Test concentrations used	Two mixtures of two chemicals: 5 µg/L of each substance
Number of replicates per test concentration	1
Number of organisms per replicate	30
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 7 days Depuration phase: 3 days
Observation intervals	Hour 0, 24, 72, 120, 168 during uptake and hour 6, 12, 24, 48, 72 during depuration
Feeding	Yes,20 mg/fish/day
Measurement of the exposure concentrations	Yes, 0.6-5.1 µg/L depending on the substance.
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, but not all substances reached a plateau during uptake.
Water quality criteria satisfied	The Willow shiner is not recommended as a test species, therefore no range is suggested.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Tsuda et al. (1993a)
Information on the test species	
Test species used	Common carp (*Cyprinus carpio*)
Life stage of the test species used	Juvenile, weight: 14-21 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Organophosphorus pestcides
Test concentrations used	Two mixtures of four chemicals: 3.3 µg/L of each substance
Number of replicates per test concentration	1
Number of organisms per replicate	25
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 7 days Depuration phase: 3 days
Observation intervals	Hour 0, 24, 72, 120, 168 during uptake and hour 6, 12, 24,72 during depuration
Feeding	Yes, 0.2 g/fish/day
Measurement of the exposure concentrations	Yes, 0.3-3.4 µg/L depending on substance.
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but not for all substances, stability during the uptake and reaching a plateau in carp is given.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Stability of test substance in water during uptake dependent on substance and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Tsuda et al. (1993b)
Information on the test species	
Test species used	Common carp (*Cyprinus carpio*)
Life stage of the test species used	Juvenile, weight: 16-20 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Chloroanilines
Test concentrations used	20, 2 µg/L of each substance
Number of replicates per test concentration	1
Number of organisms per replicate	27
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 14 days Depuration phase: 3 days
Observation intervals	Hour 0, 24, 72, 168, 240, 336 during uptake and hour 6, 12, 24,72 during depuration
Feeding	Yes, 0.2 g/fish/day
Measurement of the exposure concentrations	Yes, 10.4-16.1 µg/L in high concentration and 0.3-0.8 µg/L in low concentration depending on substance.
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but not for all substances, stability during the uptake is given.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Stability of test substance in water during uptake dependent on substance, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Tsuda et al. (1995)
Information on the test species	
Test species used	Killifish (*Oryzias latipes*)
Life stage of the test species used	Juvenile, weight: 0.10-0.15 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Diazinon, fenthion, fenitrothion
Test concentrations used	8-24 µg/L depending on the substance (each tested separately), one mixture of the three substances with the same concentrations
Number of replicates per test concentration	1
Number of organisms per replicate	250
Number of fish per sampling	30
Test conditions	Flow-through
Duration of study	Uptake phase: 3 days Depuration phase: 1 days
Observation intervals	Hour 0, 12, 24, 48, 72 during uptake and hour 6, 12, 24 during depuration
Feeding	No
Measurement of the exposure concentrations	Yes, 2.5-4.5 µg/L depending on substance
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but stability of substance during uptake is not given for all substances.
Water quality criteria satisfied	Yes, but temperature is below the range recommended by the guideline.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Stability of test substance in water during uptake dependent on substance, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Tsuda et al. (1996)
Information on the test species	
Test species used	Killifish (*Oryzias latipes*)
Life stage of the test species used	Juvenile, weight: 0.21-0.33 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Fenthion, fenthion sulfoxide, fenthion sulfone
Test concentrations used	10-50 µg/L depending on the substance (each tested separately), fenthion: low (10 µg/L) and high (50 µg/L) concentration tested
Number of replicates per test concentration	1
Number of organisms per replicate	250
Number of fish per sampling	20
Test conditions	Flow-through
Duration of study	Uptake phase: 6 days Depuration phase: 2 days
Observation intervals	Hour 0, 3, 6, 12, 24, 48, 72, 144 during uptake and hour 6, 12, 24, 48 during depuration
Feeding	2.5 mg/fish/day
Measurement of the exposure concentrations	Yes, 1.1-9.5 µg/L depending on substance
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but stability of substance during uptake is not given for all substances.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Stability of test substance in water during uptake dependent on substance, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Tsuda et al. (1997a)
Information on the test species	
Test species used	Killifish (*Oryzias latipes*)
Life stage of the test species used	Juvenile, weight: 0.18-0.25 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Diazinon, malathion,fenitrothion, EPN and their oxidation products (oxons)
Test concentrations used	Two mixtures of substances: 13.3 µg/L of each substance
Number of replicates per test concentration	1
Number of organisms per replicate	250
Number of fish per sampling	20
Test conditions	Flow-through
Duration of study	Uptake phase: 7 days Depuration phase: 2 days
Observation intervals	Hour 0, 12, 24, 48, 72, 120, 168 during uptake and hour 3, 6, 12, 24, 48 during depuration
Feeding	2 mg/fish/day
Measurement of the exposure concentrations	Yes, 4.4-12.8 µg/L depending on substance
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but stability of substance during uptake is not given for all substances.
Water quality criteria satisfied	Yes, but temperature is below the range recommended by the guideline.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Stability of test substance in water during uptake dependent on substance, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Tsuda et al. (1997b)
Information on the test species	
Test species used	Killifish (*Oryzias latipes*)
Life stage of the test species used	Juvenile, weight: 0.12-0.20 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Isoprothiolane and its degradation products
Test concentrations used	Each substance tested separately in concentrations from 8-16 µg/L, isoprothiolane was tested in two concentrations: 8 and 40 µg/L.
Number of replicates per test concentration	1
Number of organisms per replicate	250
Number of fish per sampling	20
Test conditions	Flow-through
Duration of study	Uptake phase: 7 days Depuration phase: 12 hours
Observation intervals	Hour 0, 12, 24, 48, 72, 120, 168 during uptake and hour 1.5, 3, 6, 12 during depuration
Feeding	2.5 mg/fish/day
Measurement of the exposure concentrations	Yes, 2.5-40.1 µg/L depending on substance
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but stability of substance during uptake is not given for all substances.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Stability of test substance in water during uptake dependent on substance, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Tsuda et al. (2001)
Information on the test species	
Test species used	Killifish (*Oryzias latipes*)
Life stage of the test species used	Juvenile, weight: 0.16-0.24 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	4-nonylphenols, 4-tert-octylphenol
Test concentrations used	8 µg/L of each substance tested in separate aquaria.
Number of replicates per test concentration	1
Number of organisms per replicate	250
Number of fish per sampling	20
Test conditions	Flow-through
Duration of study	Uptake phase: 7 days Depuration phase: 1 day
Observation intervals	Hour 0, 6, 12, 24, 48, 72, 120, 168 during uptake and hour 3, 6, 12, 24 during depuration
Feeding	2 mg/fish/day
Measurement of the exposure concentrations	Yes, 3.6-4.7 µg/L depending on substance
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but stability of substance during uptake is not given for all substances.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Stability of test substance in water during uptake dependent on substance, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Van den Heuvel et al. (1996)
Information on the test species	
Test species used	Bluegill sunfish (*Lepomis macrochirus*)
Life stage of the test species used	Juvenile, weight: 6.2 ± 3.1g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Avermectin B_{1a}
Test concentrations used	Control, 0.1 µg/L/
Number of replicates per test concentration	1
Number of organisms per replicate	110
Number of fish per sampling	Not stated
Test conditions	Flow-through
Duration of study	Uptake phase: 28 days Depuration phase: 14 days
Observation intervals	Day 0, 1, 3, 7, 10, 14, 21, 28 during uptake and day 1, 3, 7, 10, 14 during depuration
Feeding	3% of body weight/day
Measurement of the exposure concentrations	Yes, 0.099 ± 0.019 µg/L
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Van Eck et al. (1997)
Information on the test species	
Test species used	Guppy (*Poecilia reticulata*)
Life stage of the test species used	Adult, weight: 0.48 ± 0.26 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	1,2,4-Trichlorobenzene
Test concentrations used	200 µg/L
Number of replicates per test concentration	1
Number of organisms per replicate	44
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 16.9 days Depuration phase: 5 days
Observation intervals	Day 0, 1, 2, 6, 9, 13, 14, 15, 16 during uptake and day 17, 18, 19, 20, 21, 22 during depuration
Feeding	Yes
Measurement of the exposure concentrations	Yes, 136 ± 74 µg/L
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish and stability of test substance in water was >20%.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Concentration of test substance in water during uptake varied (>20%) and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Van Hoogen & Opperhuizen (1988)
Information on the test species	
Test species used	Guppy (*Poecilia reticulata*)
Life stage of the test species used	Adult, weight: 0.09-0.13 g
Information on the test design	
Methodology used	The methodology is not described in detail in the paper.
Test substance	Chlorobenzenes
Test concentrations used	Every test chemical: 0.1 of LC50 (96h)
Number of replicates per test concentration	1
Number of organisms per replicate	30
Number of fish per sampling	3
Test conditions	Static (Chromosorb)
Duration of study	Uptake phase: 5 days Depuration phase: 21 days
Observation intervals	Day 1, 2, 3, 4, 5 during uptake and at regular intervals during depuration
Feeding	2% of body weight/day only during elimination
Measurement of the exposure concentrations	Yes
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	No
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Concentrations of test substance in water not stated and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Versteeg & Shorter (1992)
Information on the test species	
Test species used	Fathead minnow (*Pimephales promelas*)
Life stage of the test species used	Juvenile, weight: 0.338 g
Information on the test design	
Methodology used	The methodology is not described in detail in the paper.
Test substance	Quarternary ammonium compounds
Test concentrations used	Test compound in presence and absence of humic material
Number of replicates per test concentration	1
Number of organisms per replicate	Not stated
Number of fish per sampling	8
Test conditions	Flow-through
Duration of study	Uptake phase: 1 days Depuration phase: 3 days
Observation intervals	Hour 0, 0.5, 1, 2, 4, 8, 24 during uptake and hour 24, 72 during depuration
Feeding	No
Measurement of the exposure concentrations	Yes
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Concentrations of test substance in water not stated and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Wakabayashi et al. (1987)
Information on the test species	
Test species used	Common carp (*Cyprinus carpio*)
Life stage of the test species used	Juvenile, weight: 6-9 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Alcohol ethoxylates
Test concentrations used	°Two exposure groups
Number of replicates per test concentration	1
Number of organisms per replicate	50-55
Number of fish per sampling	5
Test conditions	Flow-through
Duration of study	Uptake phase: 3 days Depuration phase: 5-7 days
Observation intervals	Hour 0.5, 2, 8, 24, 48, 72 during uptake and hour 8, 24, 72, 120 (168) during depuration
Feeding	No
Measurement of the exposure concentrations	Yes, 0.24-0.25 mg/L
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish and plateau is not reached in all substances.
Water quality criteria satisfied	Yes, but oxygen saturation not stated.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Test concentrations stable. No information on mortality of fish and oxygen saturation and other validity criteria not satisfied.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Welling & De Vries (1992)
Information on the test species	
Test species used	Guppy (*Poecilia reticulata*)
Life stage of the test species used	Juvenile, weight: 0.06-0.16 g
Information on the test design	
Methodology used	The methodology is not described in detail in the paper.
Test substance	Chlorpyrifos
Test concentrations used	°Control, 10 µg/L
Number of replicates per test concentration	4
Number of organisms per replicate	27 (uptake), 4 (depuration)
Number of fish per sampling	Not stated for uptake, 4 in depuration
Test conditions	Static
Duration of study	Uptake phase: 14 days Depuration phase: 2-21 days (separate experiment)
Observation intervals	Day 0, 10, 24, 48, 72, 120, 360, 502 during depuration
Feeding	No
Measurement of the exposure concentrations	Yes
Measurement of water quality parameters	Yes (O_2)
Test validity criteria satisfied	No
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Concentration of test substance in water during uptake decreased.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Werner & Kimerle (1982)
Information on the test species	
Test species used	Bluegill (*Lepomis macrochirus)*
Life stage of the test species used	Juvenile, weight: 6 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper
Test substance	C12-Alkylbenzenes
Test concentrations used	°Solvent control, 0.1 mg/L
Number of replicates per test concentration	1
Number of organisms per replicate	150
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 4 days Depuration phase: 4 days
Observation intervals	Hour 2, 4, 8, 12, 24, 48, 72, 96 during uptake and hour 2, 4, 8, 24, 48, 72, 96 during depuration
Feeding	3% of body weight/day
Measurement of the exposure concentrations	Yes, 0.092 mg/L
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	Yes
Overall comment on quality	Test concentrations stable. No information on mortality of fish, but other validity criteria satisfied.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Zitko (1980)
Information on the test species	
Test species used	Atlanic salmon (*Salmo salar*)
Life stage of the test species used	Juvenile, weight not stated
Information on the test design	
Methodology used	The methodology is not described in detail in the paper.
Test substance	Mirex and dechloranes
Test concentrations used	5-76 µg/L depending on substance
Number of replicates per test concentration	1
Number of organisms per replicate	3
Number of fish per sampling	Not stated
Test conditions	Not stated
Duration of study	Uptake phase: 4 days Depuration phase: 8 days
Observation intervals	Hour 12, 24, 48, 96 during uptake and hour 25.5, 102, 192 during depuration.
Feeding	Not stated
Measurement of the exposure concentrations	Yes, 0.36-6.06 µg/L depending on substance
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	No, concentrations of test substance in water decreased during uptake and plateau was not reached for all substances. No information on mortality of fish available.
Water quality criteria satisfied	The Atlantic salmon is not recommended as a test species, therefore no range is suggested. Oxygen saturation is not stated.
Calculation of kinetic parameters	Not stated
Study conducted to GLP	No
Overall comment on quality	Concentration of test substance in water during uptake decreased.
Reliablity of study	Not assignable
Klimisch Code	4

6.2 Biomagnification studies

Reference	Brown et al. (2002)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 2-5 g
Information on the test design	
Methodology used	The methodology used is not described in detail in the paper.
Test substance	°PCB 126
Test concentrations used	°Control, 12.4, 126 µg/kg
Number of replicates per test concentration	1
Number of organisms per replicate	Not stated
Number of fish per sampling	3
Test conditions	Not stated
Duration of study	Uptake phase: 30 days Depuration phase: 160 days
Observation intervals	Day 0, 5, 10, 20, 30 during uptake and day 5, 10, 20, 40, 80, 160 during depuration
Feeding	1.5% of body weight/day
Measurement of the exposure concentrations	Yes, 12.4 ± 0.175 and 126 ± 0.739 µg/kg
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Yes, but no information on stability of test substance during study and substance in control food.
Water quality criteria satisfied	Not stated
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Limited data on test methodology is available.
Reliablity of study	Reliable
Klimisch Code	2 or 4

Reference	Bruggeman et al. (1984)
Information on the test species	
Test species used	Guppy (*Poecilia reticulata*)
Life stage of the test species used	Adult, weight: 0.1 - 0.35 g
Information on the test design	
Methodology used	The methodology used is not described in detail in the paper.
Test substance	PCBs and pentachlorobenzene
Test concentrations used	°Control, 50 mg/kg of each compound
Number of replicates per test concentration	1
Number of organisms per replicate	130
Number of fish per sampling	6
Test conditions	Re-circulating, carbon-filtered water
Duration of study	Uptake phase: 70 days Depuration phase: 84 days
Observation intervals	9 sampling days during uptake and 4 sampling days during depuration
Feeding	2.0% of body weight/day
Measurement of the exposure concentrations	Yes, but are not stated.
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on stability of substance during study, homogeneity of spiked food and substance in food and fish control.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012) except for α.
Study conducted to GLP	No
Overall comment on quality	Limited data on test methodology is available and measured exposure concentrations are not stated.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Buckman et al. (2004)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 5-10 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Aroclor, PCB 202 and 209 and cytochrome P450-inducing mixture (containing congeners 77, 126, 169)
Test concentrations used	Control, high-dose treatment (10 mg/kg of aroclor mixtures and 0.5 mg/kg) and high-dose treatment with CYP-inducers (10 µg/kg)
Number of replicates per test concentration	1
Number of organisms per replicate	45
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 30 days Depuration phase: 160 days
Observation intervals	Day 0, 5, 10, 30 during uptake and day 0, 5, 10, 20, 40, 80, 160 during depuration
Feeding	1.5% of body weight/day
Measurement of the exposure concentrations	Yes
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, but no information on stability of test substance during study. Concentrations of substance in control food and fish were detected and included in calculations.
Water quality criteria satisfied	No, temperature (8°C) was outside of temperature range recommended for rainbow trout.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	The study is of good quality, but test temperature was too low and control food and fish were contaminated with test substance.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Dabrowska et al. (1999)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*) and yellow perch (*Perca flavescens*)
Life stage of the test species used	Juvenile, weight: 9-11 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	PCB 153
Test concentrations used	°Control, 5 µg/kg and 50 µg/kg applied in treatments with different fat levels of fish and food
Number of replicates per test concentration	1
Number of organisms per replicate	24
Number of fish per sampling	4
Test conditions	Flow-through
Duration of study	Uptake phase: 32 days Depuration phase: 27 (*O. mykiss*) and 34 (*P. flavescens*) days
Observation intervals	3 sampling days during uptake and 2-3 sampling days during depuration
Feeding	1.65 (*O. mykiss*) and 0.5-0.6 (*P. flavescens*)% of body weight/day
Measurement of the exposure concentrations	Yes, 5.2-5.4 and 50.9-51.5 µg/kg
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, but no information on stability of test substance during the study period, homogeneity of spiked food and substance in food and fish control.
Water quality criteria satisfied	No, temperature was slightly above range recommended for rainbow trout. The yellow perch is not recommended as a test species, therefore no range is suggested.
Calculation of kinetic parameters	According to OECD305 (2012) except for α.
Study conducted to GLP	No
Overall comment on quality	Limited data on validity criteria available.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Fisk et al. (1996)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 2-7 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	C12- and C16-chlorinated alkanes (2 of each)
Test concentrations used	°Control, low and high concentration of each of the four alkanes
Number of replicates per test concentration	1
Number of organisms per replicate	36-50
Number of fish per sampling	3
Test conditions	Not stated
Duration of study	Uptake phase: 40days Depuration phase: 160-173 days
Observation intervals	Day 5, 10, 20, 30, 40 during uptake and day 5, 10, 20, 40, 80, 160 (or 173) during depuration
Feeding	1.5% of body weight/day
Measurement of the exposure concentrations	Yes, 21-29 µg/kg in low concentration and 198-296 µg/kg in high concentration depending on substance. Very high concentration of C16H21Cl13: 2000 µg/kg
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Yes, but no information on stability of test substance during the study period, homogeneity of spiked food and substance in food and fish control.
Water quality criteria satisfied	Not stated
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Limited data on validity criteria available.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Fisk et al. (1997)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 5-9 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD)
Test concentrations used	°Control, 40, 190, 410 ng/kg
Number of replicates per test concentration	1
Number of organisms per replicate	38
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 30days Depuration phase: 180 days
Observation intervals	Day 5, 10, 20, 30 during uptake and day 5, 10, 20, 40, 80, 160 (or 173) during depuration
Feeding	1.5% of body weight/day
Measurement of the exposure concentrations	Yes, 40, 192, 413 ng/kg
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, mortality acceptable in lowest concentration (40 ng/kg). No information on stability of test substance during the study period, homogeneity of spiked food and substance in food and fish control.
Water quality criteria satisfied	No, temperature was slightly below range recommended for rainbow trout.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Limited data on validity criteria available.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Fisk et al. (1998a)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 2-7 g
Information on the test design	
Methodology used	The methodology used is not described in detail in the paper
Test substance	Polychlorinated alkanes
Test concentrations used	°Not stated
Number of replicates per test concentration	1
Number of organisms per replicate	Not stated
Number of fish per sampling	3
Test conditions	Not stated
Duration of study	Uptake phase: 40 days Depuration phase: 80 days
Observation intervals	Day 5, 10, 20, 30, 40 during uptake and day 5, 10, 20, 40, 80 during depuration
Feeding	Not stated
Measurement of the exposure concentrations	Yes, 40-1754 µg/kg depending on substance
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Yes, but no information on stability of test substance during the study period, homogeneity of spiked food and substance in food and fish control.
Water quality criteria satisfied	Not stated
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Limited data on methodology and fulfillment of validity criteria available.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Fisk et al. (1998b)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 2-4 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Organochlorine compounds
Test concentrations used	°Control, low and high treatment
Number of replicates per test concentration	1
Number of organisms per replicate	39
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 30 days Depuration phase: 160 days
Observation intervals	Day 5, 10, 20, 30 during uptake and day 5, 10, 20, 40, 80, 160 during depuration
Feeding	1.5% of body weight/day
Measurement of the exposure concentrations	Yes, 8-62 µg/kg in low and 99-688 µg/kg in high treatment depending on substance
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, but no information on stability of test substance during the study period. Concentrations of substance in control food and fish were detected and included in calculations.
Water quality criteria satisfied	No, temperature was slightly below range recommended for rainbow trout.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	The study is of good quality habing been conducted to a standardised methodology under GLP.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Fisk et al. (2000)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 1-5 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Polychlorinated alkanes (C10, C14, C18)
Test concentrations used	°Control, low and high concentration of each of the three alkanes
Number of replicates per test concentration	1
Number of organisms per replicate	36
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 40 days Depuration phase: 160 days
Observation intervals	Day 5, 10, 20, 30, 40 during uptake and day 5, 10, 20, 40, 80, 160 during depuration
Feeding	1.5% of body weight/day
Measurement of the exposure concentrations	Yes, 1.3-1.6 mg/kg in low concentration and 13-15 mg/kg in high concentration depending on substance
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Yes, but no information on stability of test substance during the study period and substance in food and fish control.
Water quality criteria satisfied	Not stated
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	The study is of good quality habing been conducted to a standardised methodology under GLP.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Goeritz et al. (2013)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Adult, weight: 314 ± 21 g
Information on the test design	
Methodology used	OECD305 (2012)
Test substance	Perfluoroalkyl substances
Test concentrations used	°Control, 500 µg/kg dry weight of each substance
Number of replicates per test concentration	1
Number of organisms per replicate	35
Number of fish per sampling	4-5
Test conditions	Flow-through
Duration of study	Uptake phase: 28 days Depuration phase: 28 days
Observation intervals	Day 7, 14, 28 during uptake and day 3, 7, 14, 28 during depuration
Feeding	2.6% of body weight/day
Measurement of the exposure concentrations	Yes, 172-347 µg/kg depending on substance
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	The study is of good quality having been conducted to a standardized methodology. Would be Klimisch Code 1, if juvenile fish were used.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Konwick et al. (2006a)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 17.6 ± 1.2 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Triazole fungicides
Test concentrations used	°Control and mixture of all chemicals
Number of replicates per test concentration	1
Number of organisms per replicate	45
Number of fish per sampling	3
Test conditions	Re-circulating, carbon-filtered water
Duration of study	Uptake phase: 8 days Depuration phase: 16 days
Observation intervals	Day 1, 2, 4, 8 during uptake and 6h, 12h, 18h, 24h, 36h, day 2, 4, 8, 16 during depuration
Feeding	1.5% of body weight/day
Measurement of the exposure concentrations	Yes, 24-36 mg/kg wet weight depending on substance
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, but no information on stability of test substance.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	The study is of good quality, but was not conducted to a standardized methodology offering information on all validity criteria.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Konwick et al. (2006b)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 10.2 ± 0.5 g.
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Organochlorine compounds
Test concentrations used	°Control, fipronil and fipronil mixed with other organochlorine compounds
Number of replicates per test concentration	1
Number of organisms per replicate	45
Number of fish per sampling	3
Test conditions	Re-circulating, carbon-filtered water
Duration of study	Uptake phase: 32 days Depuration phase: 96 days
Observation intervals	Day 2, 4, 8, 16, 32 during uptake and day 2, 4, 8, 16, 32, 96 during depuration
Feeding	1.5% of body weight/day
Measurement of the exposure concentrations	Yes, 0.2-12.3 mg/kg depending on substance
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, but no information on stability of test substance.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	The study is of good quality, but was not conducted to a standardized methodology offering information on all validity criteria.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Martin et al. (2003b)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 2-5 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Perfluorinated acids
Test concentrations used	°Control, mixture of perfluoroalkyl carboxylates and mixture of perfluoroalkyl sulfonates
Number of replicates per test concentration	1
Number of organisms per replicate	Not stated
Number of fish per sampling	3-6 (control: 1-2)
Test conditions	Flow-through
Duration of study	Uptake phase: 34 days Depuration phase: 41 days
Observation intervals	Day 7, 14, 21, 28, 34 during uptake and day 7, 14, 21, 28, 34, 41 during depuration
Feeding	1.5% of body weight/day
Measurement of the exposure concentrations	Yes, 0.3-1.2 mg/kg depending on substance
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, but no information on stability of test substance during the study period, homogeneity of spiked food and substance in food and fish control available.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	The study is of good quality, but information on validity criteria is missing and no standardized method was followed.
Reliablity of study	Reliable
Klimisch Code	4

Reference	Muir & Yarechewski (1988)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*) and fat-head minnow (*Pimephales promelas*)
Life stage of the test species used	Juvenile, weight: 0.5-1 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Polychlorinated dibenzo-*p*-dioxins
Test concentrations used	°Control, 100 µg/kg
Number of replicates per test concentration	1
Number of organisms per replicate	Not stated
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 30 days Depuration phase: 30-75 days
Observation intervals	Day 5, 10, 15, 20, 25, 30 during uptake and 5-8 samplings during depuration
Feeding	1.5% of body weight/day
Measurement of the exposure concentrations	Yes, 105-129 µg/kg dry weight depending on substance
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Missing information on all validity criteria.
Water quality criteria satisfied	No, temperature was slightly below range recommended for rainbow trout.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Only limited information on all validity criteria is available.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Muir et al. (1986)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 3 g (OCDD) and 17.4 g (TCDD)
Information on the test design	
Methodology used	The methodology is not described in detail in the paper.
Test substance	1,3,6,8-Tetrachlorodibenzo-p-dioxin and octa-chlorodibenzo-p-dioxin
Test concentrations used	°Control, 100 µg/kg dry weight
Number of replicates per test concentration	Not stated
Number of organisms per replicate	Not stated
Number of fish per sampling	Not stated
Test conditions	Flow-through
Duration of study	Uptake phase: 30 days Depuration phase: 20 days
Observation intervals	Day 5, 10, 15, 20, 25, 30 during uptake and day 2, 10, 20, 42, 60, 100, 140, 180 during depura-tion
Feeding	1% of body weight/day
Measurement of the exposure concentrations	Yes, 867 µg/kg (TCDD) and 721 µg/kg (OCDD) on a lipid-weight basis
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Missing information on all validity criteria.
Water quality criteria satisfied	Not stated
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Only limited information on all validity criteria is available.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Muir et al. (1990)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 2-3 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	2,3,4,7,8-Pentachlorodibenzofuran
Test concentrations used	°Control, 1, 10 µg/kg dry weight
Number of replicates per test concentration	1
Number of organisms per replicate	
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 31 days Depuration phase: 180 days
Observation intervals	Day 5, 10, 20, 31 during uptake and day 2, 10, 20, 42, 60, 100, 140, 180 during depuration
Feeding	2% (1.5% during depuration) of body weight/day
Measurement of the exposure concentrations	Yes, 0.82 and 9 µg/kg
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Missing information on all validity criteria.
Water quality criteria satisfied	Not stated
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Only limited information on all validity criteria is available.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Muir et al. (1992)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	°Juvenile, weight: 1-2 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	°2,3,7,8- Tetrachlorodibenzofuran
Test concentrations used	°Control, 0.361, 2.90, 3.60, 7.20, 42.8 µg/kg
Number of replicates per test concentration	1
Number of organisms per replicate	38
Number of fish per sampling	3
Test conditions	Flow-through
Duration of study	Uptake phase: 30 days Depuration phase: 140-180 days
Observation intervals	Day 5, 10, 20, 30 during uptake and 7-8 samplings during depuration
Feeding	2 % of body weight/day
Measurement of the exposure concentrations	Yes
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	°Yes, but no information on stability of test substance during the study period, homogeneity of spiked food and substance in food and fish control available.
Water quality criteria satisfied	No, temperature was slightly below range recommended for rainbow trout.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	The study is of good quality, but information on validity criteria is missing and no standardized method was followed.
Reliablity of study	Reliable
Klimisch Code	4

Reference	Nyholm et al. (2009)
Information on the test species	
Test species used	Zebrafish (*Danio rerio*)
Life stage of the test species used	Adult, weight: 0.5 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Brominated flame retardants
Test concentrations used	°Control, low-dose and high-dose treatments
Number of replicates per test concentration	1
Number of organisms per replicate	46
Number of fish per sampling	2
Test conditions	Not stated
Duration of study	Uptake phase: 42 days Depuration phase: 14 days
Observation intervals	Day 0, 3, 7, 14, 28, 35, 42 during uptake and day 7 and 14 during depuration
Feeding	2% of body weight/day
Measurement of the exposure concentrations	Yes, 0.3-0.9 nmol/g in the low-dose and 30-120 nmol/g in the high-dose treatment depending on the substance
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Missing information on all validity criteria.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Only limited information on all validity criteria is available.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	OECD (2012)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*) and Common carp (*Cyprinus carpio*)
Life stage of the test species used	Juvenile, weight: 0.7-8.4 g
Information on the test design	
Methodology used	OECD305 (2012).
Test substance	Hexachlorobenzene, Musk xylene, *o*-Terphenyl, Mehoxychlor, Benzo(*a*)pyrene
Test concentrations used	°Control, 25-150 mg/kg depending on substance
Number of replicates per test concentration	1 (test carried out by 8 laboratories)
Number of organisms per replicate	90
Number of fish per sampling	5
Test conditions	Flow-through
Duration of study	Uptake phase: 13 days Depuration phase: 28 days
Observation intervals	Day 3 during uptake and day 1, 3, 7, 14, 21, 28 during depuration
Feeding	3% of body weight /day
Measurement of the exposure concentrations	Yes, 22-779 mg/kg depending on substance
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	The study is of good quality having been conducted to a standardized methodology.
Reliablity of study	Reliable
Klimisch Code	1

Reference	Stapleton et al. (2004)
Information on the test species	
Test species used	Common carp (*Cyprinus carpio*)
Life stage of the test species used	Juvenile, length: 100 mm
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Polybrominated diphenyl ethers (BDE 28, 47, 99, 153) and polychlorinated biphenyls (52, 153, 180)
Test concentrations used	°Control, 100 µg/kg wet weight of each congener
Number of replicates per test concentration	3
Number of organisms per replicate	12
Number of fish per sampling	1
Test conditions	Flow-through
Duration of study	Uptake phase: 60 days Depuration phase: 40 days
Observation intervals	Day 0, 5, 10, 20, 30, 45, 60 during uptake and day 9, 25, 40 during depuration
Feeding	1 g/day/fish
Measurement of the exposure concentrations	Yes, 58-174 ng/d (BDE 99:470 ± 38 ng/d)
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on stability of substance in food during study and test substance in control food: In control fish, low concentrations of substances were detected (<3 µg/kg).
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	Alternative calculation method for kinetic parameters
Study conducted to GLP	No
Overall comment on quality	The study is of good quality with detailed information on methodology but differed in calculation method and did not follow a standardized guideline.
Reliablity of study	Reliable with restrictions
Klimisch Code	2

Reference	Tomy et al. (2004)
Information on the test species	
Test species used	Lake trout (*Salvelinus namaycush*)
Life stage of the test species used	Juvenile, weight: 55 ± 5 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Brominated diphenyl ethers
Test concentrations used	°Control, 2.5, 25 µg/kg
Number of replicates per test concentration	1
Number of organisms per replicate	70
Number of fish per sampling	5
Test conditions	Flow-through
Duration of study	Uptake phase: 56 days Depuration phase: 112 days
Observation intervals	Day 0, 7, 14, 28, 56 during uptake and day 7, 14, 28, 56, 112 during depuration
Feeding	1.5% of body weight/day
Measurement of the exposure concentrations	Yes, 0.9-3.4 µg/kg and 6.0-27.5 µg/kg depending on substance
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, but no information on stability of test substance during the study period, homogeneity of spiked food and substance in control food. Low concentrations of test substances in control fish were detected and included in calculations.
Water quality criteria satisfied	The lake trout is not recommended as a test species, therefore no range is suggested.
Calculation of kinetic parameters	According to OECD305 (2012) except for α.
Study conducted to GLP	No
Overall comment on quality	Only limited data on validity criteria is available and calculation method for α differs from the guideline.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Tomy et al. (2008)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 50 ± 5 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Syn- and anti-dechlorane plus
Test concentrations used	°Control, 1 mg/kg lipid weight
Number of replicates per test concentration	1
Number of organisms per replicate	60
Number of fish per sampling	4
Test conditions	Flow-through
Duration of study	Uptake phase: 49 days Depuration phase: 112 days
Observation intervals	Day 0, 7, 14, 21, 35, 49 during uptake and day 7, 22, 35, 49, 70, 112 during depuration
Feeding	1.0% of body weight/day
Measurement of the exposure concentrations	Yes,0.79 ± 0.03 mg/kg (syn-isomer) and 1.17 ± 0.12 mg/kg (anti-isomer)
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, small amounts of substance in control food were detected and included in calculations.
Water quality criteria satisfied	Yes
Calculation of kinetic parameters	According to OECD305 (2012) except for α.
Study conducted to GLP	No
Overall comment on quality	The study is of good quality but differed in calculation method for α from the guideline.
Reliablity of study	Reliable with restriction
Klimisch Code	2

Reference	Wong et al. (2002)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Immature, weight: 30-60 g
Information on the test design	
Methodology used	The methodology used is reasonably well described in the paper.
Test substance	Organochlorine compounds
Test concentrations used	°Control, treatment with chemical mixture
Number of replicates per test concentration	1
Number of organisms per replicate	54
Number of fish per sampling	2
Test conditions	Flow-through
Duration of study	Uptake phase: 40 days Depuration phase: 238 days
Observation intervals	Day 0, 13, 20, 27, 34, 40 during uptake and day 8, 14, 50, 86, 113, 141, 238 during depuration
Feeding	1.7% of mean lipid weight of fish/day
Measurement of the exposure concentrations	Yes, 1.9-3.7 mg/kg wet weight depending on substance
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Yes, but no information on stability of test substance during the study period. Homogeneity of spiked food was not within validity limits and substance in control food and fish was detected.
Water quality criteria satisfied	No, temperature was outside of range recommended for rainbow trout.
Calculation of kinetic parameters	According to OECD305 (2012)
Study conducted to GLP	No
Overall comment on quality	Most validity criteria not fulfilled or not stated.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Woodburn et al. (2008)
Information on the test species	
Test species used	Channel catfish (*Ictalurus punctatus*)
Life stage of the test species used	Juvenile, weight: 4 g
Information on the test design	
Methodology used	U.S.EPA (1996)
Test substance	Hexachlorobenzene
Test concentrations used	°Control, 340 µg/kg
Number of replicates per test concentration	1
Number of organisms per replicate	85
Number of fish per sampling	5
Test conditions	Flow-through
Duration of study	Uptake phase: 28 days Depuration phase: 14 days
Observation intervals	Day 0, 3, 5, 7, 10, 14, 21, 28 during uptake and day 3, 7, 10, 14 during depuration
Feeding	2% of body weight/day
Measurement of the exposure concentrations	Yes, 327 µg/kg (96% of nominal)
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on mortality of fish, stability of substance in food during study, homogeneity of spiked food and test substance in control food or fish.
Water quality criteria satisfied	The channel catfish is not recommended as a test species, therefore no range is suggested. Oxygen saturation is not stated.
Calculation of kinetic parameters	Alternative method for calculation of kinetic parameters
Study conducted to GLP	Yes
Overall comment on quality	Only limited information on all validity criteria is available and alternative calculation method used.
Reliablity of study	Not assignable
Klimisch Code	4

Reference	Woodburn et al. (2013)
Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Life stage of the test species used	Juvenile, weight: 1.1-1.4 g
Information on the test design	
Methodology used	U.S.EPA (1996), OECD305 (1996)
Test substance	Octamethylcyclotetrasiloxane and decamethyl-cyclopentasiloxane
Test concentrations used	°Control, 500 mg/kg
Number of replicates per test concentration	2
Number of organisms per replicate	70
Number of fish per sampling	3 from each replicate
Test conditions	Flow-through
Duration of study	Uptake phase: 35 days Depuration phase: 42 days
Observation intervals	Day 1, 3, 7, 10, 14, 21, 28, 35 during uptake and day 1, 2, 4, 7, 14, 28, 42 during depuration
Feeding	3% of body weight/day
Measurement of the exposure concentrations	Yes, 457-458 mg/kg depending on substance
Measurement of water quality parameters	Yes (temperature, O_2)
Test validity criteria satisfied	Yes, but no information on test substance in control food or fish
Water quality criteria satisfied	Yes, but oxygen saturation is not stated.
Calculation of kinetic parameters	Alternative method for calculation of kinetic parameters
Study conducted to GLP	No
Overall comment on quality	The study is of good quality having been conducted to a standardized methodology, but calculation method differs from guideline recommendations.
Reliablity of study	Reliable with restriction
Klimisch Code	2

Reference	Zitko (1980)
Information on the test species	
Test species used	Atlanic salmon (*Salmo salar*)
Life stage of the test species used	Juvenile, weight not stated
Information on the test design	
Methodology used	The methodology is not described in detail in the paper.
Test substance	Mirex and dechloranes
Test concentrations used	0.60-9.12 mg/kg depending on substance
Number of replicates per test concentration	1
Number of organisms per replicate	Not stated
Number of fish per sampling	Not stated
Test conditions	Flow-through
Duration of study	Uptake phase: 42 days Depuration phase: 71 days
Observation intervals	Day 15, 28, 42 during uptake and day 16, 32, 49, 71 during depuration.
Feeding	Not stated
Measurement of the exposure concentrations	Yes, 0.59-8.88 mg/kg depending on substance
Measurement of water quality parameters	Yes (temperature)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	The Atlantic salmon is not recommended as a test species, therefore no range is suggested. Oxygen saturation is not stated.
Calculation of kinetic parameters	Not stated
Study conducted to GLP	No
Overall comment on quality	Only limited information on all validity criteria and methodology is available.
Reliablity of study	Not assignable
Klimisch Code	4