Bioconcentration of Superlipophilic Persistent Chemicals

– Octachlorodibenzo-p-dioxin (OCDD) in Fish

Harald J. Geyer[1], Derek C. G. Muir[3], Irene Scheunert[2], Christian E. W. Steinberg[1], Antonius A. W. Kettrup[1]

GSF – Research Center for Environment and Health, Neuherberg, P.O. Box 1129, D-85758 Oberschleißheim, Germany
[1] GSF, Institute of Ecological Chemistry, [2] GSF, Institute of Soil Ecology
[3] Freshwater Institute, Canada Department of Fisheries and Oceans, 501 University Crescent, Winnipeg, Manitoba, Canada, R3T 2N6

Corresponding author: Harald J. Geyer

Dedicated to Prof. Dr. Friedhelm Korte on the occasion of his 70th birthday anniversary

Abstract

According to present understanding, persistent superlipophilic chemicals – such as octachlorodibenzo-*p*-dioxin, octachlorodibenzofuran, Mirex etc – with log K_{OW} > 6 and cross sections > 9.5 Å, bioconcentrate in aquatic organisms only little from ambient water. The most convincing argument against it is that in bioconcentration experiments with superlipophilic chemicals amounts applied exceeded water solubility by several orders of magnitude. This paper describes various methods for determining bioconcentration factors (BCF) of superlipophilic compounds. As exemplified with octachlorodibenzo-*p*-dioxin, BCF values evaluated by these methods match well with those calculated by QSARs for fish and mussels based on log K_{OW} and water solubility. As expected, these BCF values exceed previous values by several orders of magnitude.
For BCF evaluation of superlipophilic chemicals in aquatic organisms we recommend:
(i) flow-through systems, kinetic method (OECD guideline No. 305 E)
(ii) ambient concentrations < water solubility
(iii) during the uptake and especially during the elimination phase no toxic effects of the test organisms should occur.

1 Introduction and Definitions

Aquatic organisms may be contaminated by chemicals either by direct uptake through gills or skin or indirectly by ingestion of food or contaminated sediment particles. The **bioconcentration factor (BCF)** is defined as the ratio of the steady state concentration of chemicals in aquatic organisms (C_O) and the corresponding concentration in ambient water (C_W) (Bruggeman 1982; Spacie & Hamelink 1985):

$$BCF = \frac{C_O}{C_W} \qquad \frac{[ng/kg]}{[ng/L]} \qquad (1)$$

Because 1 L water is equal 1 kg, the BCF values are dimensionless.

[4] NTEQ: Nordic TCDD Toxic EQuivalents (Ahlborg et al. 1992)

The real BCF value of a persistent chemical is independent of the ambient concentration. When under nearly equal experimental conditions with fish of the same species, sex, age, body weight, and lipid content bioconcentration factors for the same chemicals differ by orders of magnitude, it is necessary to ask whether a "true" bioconcentration factor was found. Consequently, experimental conditions have to be re-examined.
We compiled and re-examined BCF values of octachlorodibenzo-p-dioxin (OCDD) and attempt explanations for the apparent dependence of the BCF values on ambient concentrations of these superlipophilic chemicals. We also present methods for estimating "true" bioconcentration factors.

Bioconcentration, i.e. the *direct uptake* of a chemical by an aquatic organism from ambient water, has to be distinguished from *indirect contamination,* such as **biomagnification, bioaccumulation,** and **ecological magnification** (Streit 1992; Ernst 1985).
The term **biomagnification** is used for the dietary uptake via contaminated food. The **biomagnification factor** (BMF) of a chemical is the ratio between the concentrations in fish and food at steady state (Sijm & Opperhuizen 1990). Steady state, however, is not easily to reach and BMF is difficult to measure (Sijm et al. 1992).
Bioaccumulation is defined as the uptake of substances *via* food and water.
Ecological magnification means increasing chemical concentrations in the food chain (Ernst 1985).
There are different opinions whether or not ecological magnification occurs. In a previous study, levels of polychlorinated dioxins and furans (PCDD/F) from different species of seal in the Baltic Sea and at background stations, such as Spitzbergen, were found to be very similar. PCDD/F levels were in the range of 14 – 300 pg NTEQ[4]/g lipid in grey, harbor and ringed seal. Baltic herring have PCDD/F levels of 30-420 pg NTEQ/g lipid, indicating that there is no biomagnification of PCDD/F in seals (Bignert et al. 1989).
Macek et al. (1979) reviewed data derived from simple experimental laboratory food chains and concluded that most chemicals are not biomagnified. This statement fits well the

common theory of the partitioning process of lipophilic chemicals, which considers animals as simple lipid aggregates (CONNELL 1986).

Others, however, suggest that biomagnification of hydrophobic chemicals does occur in the food chain (CLARK et al. 1988; CONNOLLY & PEDERSEN 1988; see also FLETCHER-ROSE & MCKAY 1993).

Recently, SIJM et al. (1993) presented a life-cycle biomagnification model for the accumulation of polychlorinated biphenyls (PCB) in fish. The model includes biotransformation, life stage, sex, and growth of the fish. Biomagnification of PCB was studied in the guppy *(Poecilia reticulata)*. Juvenile guppies (first generation) were fed PCB-contaminated food for 30 weeks. Thereafter, elimination was studied for 2 years. The biomagnification factors of PCB at 30 weeks of exposure ranged from 0.03 to 6. SIJM et al. (1993) concluded that biomagnification factors cannot be determined from the steady-state concentrations in fish and food. However, if the ratio between the concentrations in fish (on a lipid basis) and food after 30 weeks is calculated, the factors range from 0.035 to 1.38.

This paper deals with **bioconcentration** of superhydrophobic chemicals from water, since the substances studied (octachlorodibenzo-*p*-dioxin or Mirex), are bioconcentrated less than calculated from their *n*-octanol/water partitioning coefficient (K_{OW}) (BRUGGEMAN et al. 1984; OPPERHUIZEN et al. 1985; Muir et al. 1985, 1986).

Bioconcentration factors can be expressed as:

a) BCF_W: on a wet weight basis
b) BCF_L: on a lipid basis
c) BCF_D: on a dry weight basis.

The BCF_L value being the most important for comparisons can easily be calculated from the BCF_W value if the lipid content (% on a wet weight basis) of the organism is known:

$$BCF_L = \frac{BCF_W \cdot 100}{L\ (\%)} \qquad (2)$$

2 Bioconcentration Bioassay for Superliphophilic Chemicals

To measure steady-state bioconcentration factors of superlipophilic chemicals with log $K_{OW} > 6$, such as octachlorodibenzo-p-dioxin (OCDD), octachlorodibenzofuran (OCDF), decachlorobiphenyl, Mirex etc., it is essential to determine both their uptake rate constants (K_u) and elimination (depuration) rate constants (K_e) in flow-through systems:

$$BCF = \frac{K_u}{K_e} \qquad (3)$$

Such extremely hydrophobic compounds reach steady-state concentrations only after considerably long time periods (several months).

For theoretical considerations and the determination of bioconcentration factors by the kinetic method, see for instance OECD Revised Draft Guideline No. 305E (OECD 1992), NEELY (1979), MCCARTY et al. (1989), and BUTTE (1991). Since ambient chemical concentrations must be kept constant during the test, flow-through systems should be applied.

3 Current Notion on the Bioconcentration

Bioconcentration of chemicals in aquatic organisms can be considered to be the partitioning of a chemical between the lipid phase of the organisms and the ambient water at equilibrium. The wet weight bioconcentration factors of chemicals having log $K_{OW} < 6$, depend on K_{OW} as given in equation (4) (GOBAS et al. 1986; BIENERT et al. 1993):

$$\log BCF_W = a \log K_{OW} + b \qquad (4)$$

This linear regression should be applied to chemicals with log $K_{OW} < 6$. However, for chemicals with log $K_{OW} > 6.5$, "parabolic" or "bilinear" relationships between log BCF and log K_{OW} have been proposed (GOBAS et al. 1989; CONNELL & HAWKER 1988; MUIR et al. 1985; NENDZA 1991). Bioconcentration factors of such superlipophilic chemicals were much lower than predicted from their log K_{OW}, as evidenced with OCDD, OCDF, Mirex etc. The only exception is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), for which in laboratory studies MEHRLE et al. (1988), BRANSON et al. (1985), SCHMIEDER et al. (1993), and COOK et al. (1991) found relatively high bioconcentration factors in rainbow trout, fathead minnow, medaka, and carp.

Steric parameters, such as molecular size and shape or cross sections can influence the bioconcentration of hydrophobic chemicals in aquatic organisms. OPPERHUIZEN et al. (1985) proposed a lack of membrane permeability due to cross sections larger than 9.5 Å.

Recently, CHESSELLS et al. (1992) suggested that an important factor for the lower bioconcentration may be the relatively low solubility of superhydrophobic chemicals in lipids.

4 Arguments Against low Bioconcentration Potentials of Superlipophilic Chemicals

a) OCDD has been identified as the PCDD with the highest concentration in marine and freshwater biota, such as mussels, shrimps, and fish (MIYATA et al. 1988; OEHME et al. 1989; LUCKAS & OEHME 1990; PETREAS et al. 1992; FROMMBERGER 1991). However, this is not always the case (RAPPE 1991; ZACHAREWSKI et al. 1989). Relatively high OCDD concentrations in aquatic organisms do not necessarily reflect high bioconcentration potential of OCDD. The question is whether this concentration could be explained by the high ambient water concentration of OCDD or by the high concentration in the food.

Recent bioconcentration experiments by MUIR et al. (1986) and GOBAS & SCHRAP (1990) revealed elevated concentrations of OCDD in the organisms, even when the gastrointestinal tract and the gills were removed. This means that such superlipophilic chemicals can penetrate membranes and are bioconcentrated in aquatic organisms.

b) It is evident that BCF values of superlipophilic chemicals in fish, mussels, and other aquatic organisms must be relatively low when short exposure times are used and no steady state can be reached (MIYATA et al. 1989).

c) Various chemicals are metabolized and excreted faster than the original compounds. Therefore they are not bioconcentrated to the extend as predicted from their log K_{OW} (GOBAS & SCHRAP 1990; MUIR & YARECHEWSKI 1988; SIJM et al. 1989; DE WOLF 1992). Superlipophilic, highly chlorinated chemicals, however, are very resistant to metabolic transformation in aquatic organisms (OPPERHUIZEN & SIJM 1990; GOBAS & SCHRAP 1990; HUCKINS et al. 1982). Metabolism cannot serve as an explanation for the low bioconcentration factors of OCDD, Mirex, and other highly chlorinated superlipophilic substances.

d) The main argument against the relatively low experimentally determined bioconcentration potential of highly hydrophobic compounds in fish and mussels is the application of relatively high concentrations of these chemicals in the water. The concentrations exceeded the aqueous solubility by several orders of magnitude. Although the aqueous solubility of OCDD ranges between 74 and 400 pg/L (FRIESEN et al. 1990; GOBAS & SCHRAP 1990; SHIU et al. 1988; DOUCETTE & ANDREU 1988; FRIESEN & WEBSTER 1990), several authors carried out experiments with concentrations of > 10^3 pg/L (BRUGGEMAN et al. 1984; OPPERHUIZEN et al. 1985; MUIR et al. 1986; GOBAS & SCHRAP 1990). If only the "truly" dissolved chemical is able to be absorbed *via* gills (SERVOS & MUIR 1989; BLACK & MCCARTHY 1988), the use of supersaturated concentrations (dissolved plus sorbed) will clearly underestimate the BCF values (GOBAS & SCHRAP 1990). Low uptake of superlipophilic chemicals is caused by low bioavailability rather than by low bioconcentration potential (OPPERHUIZEN & SIJM 1990; LOONEN 1993).
In the studies of 2,3,7,8-TCDD, referred to above, BCF determinations were carried out with chemical concentrations below water solubility. As a consequence, experimentally achieved BCF values of TCDD match those predicted from the corresponding log K_{OW} value (GOBAS & SCHRAP 1990; SCHMIEDER et al. 1993).

5 Method for Predicting the BCF_L Value of OCDD

Wet weight bioconcentration factors (BCF_W) of OCDD in various fish species were compiled from recent papers (BRUGGEMAN et al. 1984; MUIR et al. 1985, 1986; GOBAS & SCHRAP 1990; LOONEN et al. 1993). Only steady-state BCF data obtained in flow-through systems were considered. For comparison, BCF_W values were transformed into BCF_L values (BRUGGEMAN et al. 1984; GEYER et al. 1985). **Table 1** contains body weights, lipid contents, BCF_L values, and corresponding ambient OCDD concentrations. To assess the most likely BCF_L (ambient OCDD concentrations < water solubility), experimental BCF_L data of OCDD were plotted against respective external OCDD on a log/log basis (→ *Fig. 1*). The most likely BCF_L value of OCDD in fish was obtained from an extrapolation of the linear relationship down to a water solubility of 74 pg/L.

6 Results and Discussion

Although only few BCF data of OCDD are available, it is obvious that the experimentally obtained BCF_L values in different fish species depend on ambient OCDD concentrations (→ *Table 1, Fig. 1*). This means that BCF_L values are increasing with decreasing external concentration in the experiments. Using only the three highest BCF_L values (*center of* → *Fig. 1*), experimentally determined under nearly identical conditions by MUIR et al. (1985, 1986), a linear regression was found (Equation 5):

$$\log BCF_L = 11.18 - 1.74 \cdot \log C_W \quad (5)$$

where C_W is the OCDD concentration [pg/L] in ambient water. At a water solubility of 74 pg/L, this regression reads $8.5 \cdot 10^7$. This BCF_L value – which is obviously the correct one – exceeds even the published maximum value by two orders of magnitude. The same applies, of course, to BCF_W values (GEYER et al. 1993).
The same procedure was applied by GEYER et al. (1992) to Mirex ($C_{10}Cl_{12}$, log K_{OW}: 6.89). We have used the experimental data from HUCKINS et al. (1982). The authors exposed fathead minnows (*Pimephales promelas*) in a flow-through system to 0.37, 3.8 and 33 μg/L Mirex for 56 days. In the fish the chemical was bioconcentrated 51,300, 12,400 and 3700 times of the concentration of Mirex in water. It is obvious that the bioconcentration factors (BCF_W) are increasing with decreasing concentration of Mirex in water. If the correlation between bioconcentration factors and ambient water concentrations is extrapolated to the water solubility of Mirex, a BCF_W value of 130,000 is obtained (GEYER et al. 1992). As expected, BCF_W values at ambient concentrations not exceeding water solubility lie clearly above the maximum values published thus far (HUCKINS et al. 1982): 130,000 rather than 51,300, 12,400 or 3700. However, these are no steady-state bioconcentration factors, because the uptake of Mirex in fathead minnows was determined after 56 days. VEITH et al. (1979) published a bioconcentration factor of 18,100 for Mirex in the same fish species. It is clear that this value is also too low, since after 32 days steady-state was not reached.
With mussels and fish, which are contaminated *via* sediment, Geyer et al. (1990, 1991, 1992) calculated BCF values using equation (6):

$$BCF = \frac{C_O}{C_W} = \frac{C_O \cdot K_{OC} \cdot \%OC}{C_S \cdot 100} \quad (6)$$

where % OC is the organic carbon content (%) of the sediment, K_{OC} the sorption coefficient, and C_S the sediment OCDD concentration (on a dry weight basis).
This *indirect method* revealed BCF values which are congruent with those obtained by the above extrapolation.

Table 1: Bioconcentration factors on a wet weight basis (BCF_W) and on a lipid basis (BCF_L) of OCDD in different fish species depending on OCDD concentrations in ambient water (C_W)

Fish species	Mean body weight (g)	Lipid content (%)	Ambient OCDD conc. (pg/L)	Bioconcentration Factor		Reference
				BCF_W	BCF_L	
Guppy (male)	0.1	3.5	$4.0 \cdot 10^6$	< 1050	$< 3 \cdot 10^4$	BRUGGEMAN et al. (1984)
Guppy (female)	0.079	7.5	$6.4 \cdot 10^5$	703	$9.4 \cdot 10^3$	GOBAS & SCHRAP (1990)
Rainbow trout	0.3	6.9	$4.15 \cdot 10^5$	34	$4.9 \cdot 10^2$	MUIR et al. (1986)
Rainbow trout	0.3	6.9	$2.0 \cdot 10^4$	136	$2.0 \cdot 10^3$	MUIR et al. (1986)
Fathead minnow	1.7	3.5	$9.0 \cdot 10^3$	2226	$6.4 \cdot 10^4$	MUIR et al. (1986)
Guppy (female)	0.91	9.7	$8.0 \cdot 10^2$	1308[a)]	$1.35 \cdot 10^4$[a)]	LOONEN et al. (1993)
Fathead minnow	1.7	3.5	$1.0 \cdot 10^3$	22,300	$6.4 \cdot 10^5$	MUIR et al. (1985)
Fish	0.73	5.0[b)]	$7.4 \cdot 10$[c)]	$4.3 \cdot 10^6$	$8.5 \cdot 10^7$[d)]	GEYER et al. (1992)

a) Exposure time: 21 days
b) Assumed average lipid content (% on wet weight basis) of fish
c) Water solubility of OCDD
d) Predicted by extrapolation from equation (5)
Source: Modified from GEYER et al.: Chemosphere 25, 1257–1264 (1992) with permission.

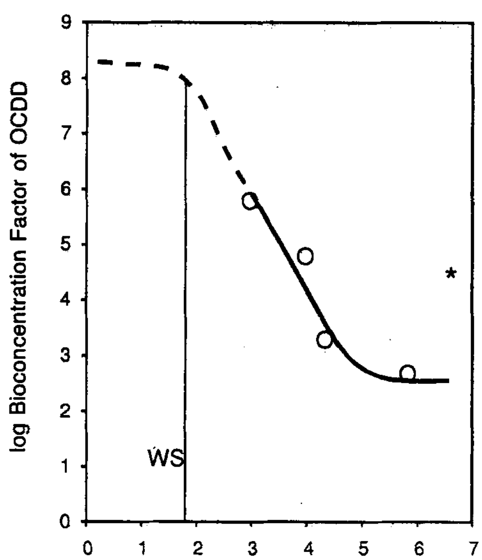

Fig. 1: Relationship between the bioconcentration factor on lipid basis (BCF_L) of octachlorodibenzo-*p*-dioxin (OCDD) in fish and the OCDD concentration in ambient water (WS: water solubility of OCDD = 74 pg/L; *: BCF_L value of BRUGGEMAN et al. 1984).

Another way calculating BCF values of OCDD is the application of two quantitative structure-activity relationships (QSAR) based on log K_{OW} of OCDD: 8.60 (BURKHARD & KUEHL 1986) *(→ equation 7)* as well as water solubility (OCDD: 74 pg/L) *(→ equation 8),* which was developed for mussels *(Mytilus edulis)* on a wet weight basis (GEYER et al. 1982):

$$\log BCF_W = 0.858 \cdot \log K_{OW} - 0.808 \quad (7)$$
$$\log BCF_W = 4.94 - 0.682 \cdot \log WS\ (\mu g/l) \quad (8)$$

These equations give an estimate for OCDD BCF_W of $3.7 \cdot 10^6$ and $5.7 \cdot 10^7$, respectively. Although both figures differ considerably, they indicate again that experimentally derived BCF values are too small by at least one or two orders of magnitude.

Applying a QSAR for fish from VEITH et al. (1979) *(→ equation 9)* and from MACKAY (1982) *(→ equation 10):*

$$\log BCF_W = 0.85 \cdot \log K_{OW} - 0.70 \quad (9)$$
$$\log BCF_W = 1.00 \cdot \log K_{OW} - 1.32 \quad (10)$$

for OCDD in fish, BCF_W values of $4.1 \cdot 10^6$ and $1.9 \cdot 10^7$, are obtained.

Using several different approaches, we have presented evidence that the thesis concerning bioconcentration of superlipophilic chemicals in aquatic animals such as fish and mussels from ambient water has to be revised – at least in parts. The thesis that chemicals with log $K_{OW} > 6$ are bioconcentrated to a significantly lower degree than predicted from their K_{OW} is generally no longer valid.

However, this statement may be still true for several superlipophilic chemicals with peculiar molecular size or shape, such as paraffins, organosilicon compounds with very long chain (OPPERHUIZEN et al. 1987) and organic colorants (MOSER & ANLIKER 1991). According to SIJM et al. (1993), the threshold value of membrane permeability is no longer valid: It is above the cross section of 9.5 Å and may be dependent on fish species and temperature.

With increasing lipophilicity the uptake velocity is clearly declining and steady-state conditions are not achieved within some days or few weeks, but in many instances only after many months. One consequence is that the BCF values of superlipophilic chemicals can be evaluated only under flow-through conditions using the "kinetic method" (OECD 1992). It appears self-evident that aquatic organisms should be exposed only to concentrations below water solubility. This is also valid for aquatic toxicity tests with these organisms. Otherwise these data are meaningless. However, to fulfil both experimental conditions with superlipophilic compounds, severe practical problems emerge. In the past all OCDD bioconcentration experiments failed these requirements resulting in BCF values which were much too low.

7 Recommendations

We recommend for BCF evaluations of superlipophilic chemicals in aquatic organisms such as fish, mussels, etc.:

1. flow-through systems according to the "kinetic method" (OECD guideline No. 305 E);
2. ambient concentrations < water solubility;
3. during the uptake and especially during the elimination phase no toxic effects of the test organisms should occur.

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