MINI-REVIEW

BIOAVAILABILITY OF ORGANOCHLORINES IN FISH

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Abstract—1. An overview is presented of the processs of chemical sorption and membrane permeation that control the bioavailability of organochlorines in natural waters.

2. The mechanisms of membrane permeation and chemical sorption to various types of dissolved and particulate matter is discussed and simple models are presented that can be used to estimate the bioavailability of organic chemicals in natural waters.

INTRODUCTION

In any assessment of the uptake, bioconcentration and toxic effects in fish of organic chemicals in natural waters, the first, and most fundamental step, is to determine the concentration of the chemical in the water that can be absorbed by the fish via the gill surface. This concentration of absorbable or bioavailable chemical is not necessarily equal to the total chemical concentration in the water. The fraction of the total chemical concentration in the water that can be taken up via the gills is usually referred to as the Bioavailability Solute Fraction (BSF), or simply the bioavailability.

There are two processes that are known to determine the bioavailability of organic chemicals in natural waters: first, interaction of the chemical with particulate and/or dissolved matter in the water can result in the formation of chemical aggregates and complexes that are too large to permeate through the gill membrane. It is generally believed that only chemicals in true solution (i.e. each solute molecule is surrounded by water molecules only) are bioavailable. If this hypothesis is correct, it is possible to define the bioavailability or BSF as the ratio of the truly dissolved chemical concentration in the water C_b (g/ml) and the total chemical concentration in the water C_T (g/ml), i.e.

$$BSF = C_D/C_T. \qquad (1)$$

This hypothesis has been supported by several studies, which demonstrate that chemical concentrations and uptake rates in fish are lower when particulate or dissolved matter is present in the water (Black and McCarthy, 1988; Landrum *et al.*, 1985; McCarthy and Jimenez, 1985). The particulate or dissolved organic matter tends to "bind" the chemical, thus dropping the concentration of truly dissolved or bioavailable chemical in the water column.

The second process determining the bioavailability of a chemical to fish is the chemical's ability to permeate through the gill membrane. It has been

suggested that molecules with particular size configurations are simply "too big" to pass through the gill membrane and are thus unavailable for uptake from water by fish (Opperhuizen *et al.*, 1985; Anliker and Moser, 1987).

It thus transpires that to determine the bioavailability of organic substances in water it is important to know how the chemical concentration in the water is broken down to the proportions which are truly dissolved, and those which are associated with other "matter" present in the water. Equally important is the ability of molecules to permeate through biological membranes. We will provide a brief overview of the current knowledge regarding the processes that control the bioavailability of organic chemicals in aqueous systems.

SORPTION INTO PARTICULATE ORGANIC MATTER

Of particular relevance in the case of sorption of organic chemicals to organic matter in the water column are humic and fulvic acids and humins. These substances consist of a variety of organic materials, mainly of plant origin, with variable aromatic and alkane character and containing acidic and other functional groups. These materials vary considerably in molecular weight and may be present in dissolved and the particulate form, the relative amounts being dependent on pH.

Discrimination between dissolved organic matter (DOM) and particulate organic matter (POM) in the water column can be done by filtration and by centrifugation. In research studies, methods such as dialysis, selective absorption or head-space analysis are being used. Filtration is the most common method of providing an operational definition between "dissolved" and "suspended". However, much suspended organic matter is present with particle sizes of approx. 0.1 μm, which is operationally defined as dissolved, while in reality it is not in true solution. Such particles, although small, are still very large when compared to molecular dimensions of solute molecules, which are typically 0.2 nm or perhaps

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1/1000th of the size of the smallest particles which can be filtered out of solution.

Perhaps the best available simple phenomenological explanation of sorption into particulate organic matter is that an organic molecule, such as a PCB, causes considerable distortion of the surrounding local water matrix, forcing the water to establish a structure which is energetically unfavourable. Any process which minimizes this distortion by reducing the contact-area between the organic molecule and the water is highly favoured. An organic molecule can achieve this objective by dissolving in another phase (sorption), or by associating with the surface of organic matter (adsorption), thus displacing water from that surface.

It is generally believed that, for organic contaminants, the primary phases of sorptive capacity are the organic carbon-containing materials (Karickhoff, 1984). However, there remains controversy about the state of the chemical when sorbed to the organic matter. The most generally held current belief is that the chemical is dissolved in a solid solution form in, or on, a sponge-like matrix of organic matter. Sorption is therefore often viewed as a partitioning process of the chemical between the water and the organic content of particulate matter or simply the particulate organic matter (POM):

Dissolved chemical + POM

$$\Leftrightarrow Chemical - POM \quad (2)$$
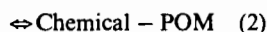
which can be expressed by a partition coefficient K_{OC} (ml/g):

$$K_{OC} = C_B/(C_D - [POM]) \quad (3)$$

where C_C (g/ml) is the concentration of "bound" or "sorbed" chemical, i.e. $(C_T - C_D)$, C_D is the concentration of dissolved chemical (g/ml) and [POM] is the concentration of particulate organic matter in the water (g/ml).

Different organic chemicals display different sorptive tendencies, largely because they vary in molecular size, in the presence of functional groups, such as hydroxyl or carbonyl groups and in aromaticity (Malcolm and MacCarthy, 1986; Gauthier et al., 1987). Molecular shape may also play an important role. Several correlations have been proposed between K_{OW} and the organic carbon–water sorption coefficients, K_{OC}, suggesting that K_{OC} is proportional to 40–60% of K_{OW} (Karickhoff, 1981). DiToro (1985) has suggested that, within experimental error, K_{OC} equals K_{OW}. The general concept is that the organic carbon phases are similar in properties to octanol, causing a chemical to partition between these phases and water with a ratio similar to that between octanol and water. This has proved to be a reliable and useful working hypothesis. Equation 3 can thus be rewritten to express the truly dissolved chemical fraction C_D/C_T, which is hypothesized to be equal to the bioavailable solute fraction (BSF), i.e.

$$BSF = 1/(1 + K_{OW} \cdot [POM]). \quad (4)$$

Under most natural conditions equation 4 may give a reasonable estimate of the extent of sorption and bioavailability. However, there are indications

Table 1. Estimates of the bioavailability BSF (%) of organic substances of different K_{OW} in natural waters, containing a concentration of respectively POM and DOM of 10^{-6} g/ml

Log K_{OW}	[POM] = 10^{-6}	[DOM] = 10^{-6}
2	99.99	99.999
3	99.9	99.999
4	99.0	99.999
5	90.9	99.999
6	50.0	99.999
7	9.09	99.998
8	0.99	99.998

that when the concentrations of particulate matter are very high, such as in the nepheloid layer in rivers and lakes or during dredging, the sorption coefficient, K_{OC}, tends to fall as the concentration of particulate matter increases (O'Connor and Connolly, 1980). This observation has been the subject of considerable discussion and controversy because it apparently contravenes the laws of thermodynamics, and no entirely satisfactory mechanism to explain it has been proposed (Gschwend and Wu, 1985; DiToro, 1985).

Table 1 lists some estimates of the bioavailability of organic substances in natural waters, where particulate organic matter concentrations are typically in the range of 10^{-7} to 10^{-5} g/ml. Table 1 shows that if the [POM] is 10^{-6} g/ml, a chemical with a log K_{OW} of 5 can be estimated to have a bioavailability of 90.9%. If K_{OW} increases, the bioavailability drops sharply to values as low as 0.99%, for chemicals with a log K_{OW} of 8.

INTERACTION WITH DISSOLVED ORGANIC CARBON

The mechanism by which dissolved organic matter affects the solubility and bioavailability of organic chemicals in water is presently not entirely clear. It has been suggested that dissolved organic matter (DOM) can act as a co-solvent or solubilizing agent which increases the solubility of the chemical by modifying the water structure to become more acceptable to the organic chemical. DOM will thus decrease the activity or fugacity of the chemical in the water, and thus reduce the partition coefficient of the chemical between the "water" and the fish lipids. On the other hand, it is possible that DOM increases the solubility of organic chemicals by forming aggregates or molecular complexes, which enhance the apparent solubility by forming "supersaturated" solutions and which cannot be absorbed via the gills of fish. In this case, the bioavailability of the chemical is reduced by DOM. Studies of the effect of co-solvents on chemical solubility have shown that the logarithm of the solubility of a chemicals S (mol/mol) is often a linear function of the volume fraction of co-solvent F (ml/ml), extending from the solubility of the solute in pure water S_w (mol/mol) to the solubility in the pure co-solvent S_C (mol/mol):

$$\log S = \log S_w + F \cdot (\log S_C - \log S_w). \quad (5)$$

This relationship appears to be maintained for many, but not all, solutes and various co-solvents (Yalkowsky, 1981; Yalkowsky and Rubino, 1985).

We can invoke this theory to estimate the effect of DOM on the bioavailability of the chemical in the water column. Considering the absence of information regarding the nature of DOM, it is reasonable to assume DOM has similar phase characteristics as 1-octanol, such that S_C equals the solubility in 1-octanol S_O (mol/mol) and equation 5 can be rewritten to

$$\log S = \log S_w + F \cdot (\log S_O - \log S_w). \qquad (6)$$

If the dissolved organic matter changes the water properties, causing a greater solubility of the chemical in the water, then the bioavailability remains the same but the octanol–water partition coefficient and bioconcentration factor will drop from S_O/S_w or K_{OW} to S_O/S, i.e. a drop by a factor S/S_w, which, when expressed on a logarithmic basis (equation 6) as $\log S/S_w$, corresponds to $F \cdot (\log S_O - \log S_w)$ or $F \cdot \log K_{OW}$.

If the co-solvent enhances the chemical's solubility by forming a co-solvent–solute complex, which cannot be absorbed by fish, the 1-octanol–water partition coefficient remains the same, i.e. S_O/S_w or K_{OW}, but the bioavailability drops to a BSF of S_w/S, which, according to equation 6, corresponds to $F \cdot (\log S_w - \log S_O)$ or $\log S_w/S$ (i.e. $\log$ BSF) when expressed on a logarithmic basis.

In both cases, the effect of the co-solvent is to drop the apparent bioconcentration factor, i.e. the measured ratio of the total chemical concentrations in the fish and the water. Assuming that the chemical's solubility in DOM equals that in octanol, it is possible to estimate the reduction in bioavailability or bioconcentration factor due to DOM in the water by replacing S_O/S_w in equation 6 by K_{OW}, resulting in

$$\log S/S_w = \log (1/\text{BSF}) = F \cdot \log K_{OW}. \qquad (7)$$

In natural waters, the volume fraction of dissolved organic carbon is usually in the range of 10^{-5} to 10^{-7}. For example, if F is 10^{-6}, i.e. approx. 1 mg of organic carbon per litre water, and $\log K_{OW}$ is 6, then $\log (S/S_w)$ is 6.10^{-6}, and the BSF or bioconcentration factor has fallen to 99.999%. Table 1 illustrates some other examples and demonstrates that, in general, the drop in BSF or apparent bioconcentration factor is very small and would not be detectable.

INTERACTION WITH ELECTROLYTES

Dissolved electrolytes do not sorb organic molecules. Rather, they exert a salting-out effect by increasing the activity of fugacity or the chemical. This can cause organic chemicals to sorb onto organic matter to a greater extent than in the absence of dissolved electrolytes. The effect of salt solutions on properties of dissolved organics has been well-documented by Aquan-Yuen (1979). The effect of salt solutions on the sorption of organic chemicals to dissolved and particulate matter becomes relevant only when electrolyte concentrations are very high, typically in the range of 30 g/l, as occurs in salt water. Fresh waters are normally at very much lower concentrations, such that the effect of electrolytes can often be ignored.

SORPTION ONTO MINERAL MATTER

It appears that wet mineral surfaces, such as clays, carbonates, and silicates, play a relatively minor role in sorbing organic chemicals in comparison to organic matter. It should be noted that dry mineral surfaces may have a high sorptive capacity, but this is often irrelevant in aquatic systems. It is possible that, as a result of microbial action, most mineral surfaces in the aquatic environment become covered with layers of living and dead microorganisms, thus, the mineral surface is essentially shielded from the water by an organic layer.

MEMBRANE PERMEATION

To explain the apparent lack of bioconcentration of octachloronaphthalene, hexabromobenzene and octachlorodibenzo-p-dioxin (OCDD) in the guppy (*Poecilia reticulata*) Opperhuizen *et al.* (1985) suggested that molecules with minimal internal cross-sections exceeding 0.95 nm cannot permeate through biological membranes and bioconcentrate in fish. Bioconcentration experiments with hydrophobic dyes, which are molecules with very large molecular dimensions, support this hypothesis (Anliker and Moser, 1987). However, Gobas *et al.* (1989) observed high bioconcentration factors in the guppy for some brominated biphenyls with minimal internal cross-sections, similar to that of hexabromobenzene, i.e. 0.96 nm. Muir *et al.* (1986) observed small amounts of OCDD and a metabolic product of OCDD in the rainbow trout and fathead minnow, which suggests uptake of the large molecular size octachlorodibenzo-p-dioxin in fish. A small but significant bioconcentration of OCDD in fish was also observed by Gobas and Schrap (1990). In membrane permeation studies with membrane vesicles, blockage of membrane permeation of large molecular size molecules, such as OCDD and hexabromobenzene, was not observed (Gobas *et al.*, 1988). However, the results of these experiments did indicate that the capacity of membrane vesicles to accommodate or store chemicals drops sharply with increasing molecular size.

Although, from a mechanistic point of view, it is conceivable that a "cut-off" diameter exists for passive membrane diffusion, the limited amount of data, the lack of consistent data and the confounding effects of other factors such as sorption make it difficult to determine at present if such a cut-off dimension exists and what its value is.

CONCLUSION

It can be concluded that if the cosolvent theory applies, the interactions of hydrophobic organic chemicals with dissolved organic matter in the water column have a negligible effect on the bioavailability and bioconcentration of organic chemicals in fish under normal environmental conditions. Solubilizing or activity-reducing effects, causing a drop in bioavailability, are likely to be due to particulate organic carbon. This suggests that for the assessment of the bioavailability of organic substances under environmental conditions, it is crucial to determine the concentration of particulate organic matter,

rather than the concentration of total organic matter, which contains both dissolved and particulate organic matter.

Continued efforts are required to determine if certain molecular configurations are unable to pass through the gill membranes of fish and other aquatic organisms. This process is of particular importance since it can provide a means to manufacture chemical substances that may have no impact on aquatic life due their non-bioavailability.

Finally, it is stressed that the basic hypothesis of bioavailability, namely that only truly dissolved chemical is bioavailable for uptake in fish, has been poorly addressed. This is largely due to the lack of water analysis techniques that can distinguish between chemical in sorbed and dissolved state.

Recently developed techniques such as gas-sparging (Sproule et al., 1990), may provide the necessary tools to address the basic hypothesis of bioavailability.

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