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Unmetabolized Compounds, Their Properties and Implications

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INTRODUCTION

In the absence of sufficient rates of metabolism or excretion, compounds once absorbed by the organisms will accumulate. This bioaccumulation process is known for different classes of chemicals, and in many species. For most hydrophobic organic compounds bioaccumulation is considered to be a physical/chemical partition process. In this approach active enzyme - mediated transfer across membranes is considered unimportant from the mechanistic as well as from the kinetic points of view. Storage of the accumulating compound occurs mainly in the fatty tissue of organisms. For this reason the general term "lipophilic compounds" is used for these chemicals. Good correlations are often found between accumulation parameters of the compounds, like bioaccumulation factor (K_b), rate of uptake (k_1) or rate of elimination (k_2), and lipophilicity parameters like K_{ow} (Könemann & Van Leeuwen 1980, Neeley et al. 1974, Veith et al. 1979). Although correlations are satisfactory for many classes of chemicals, they are not sufficient for several others. Especially in classes of extreme hydrophobicity (low aqueous solubility) correlations frequently fail (Bruggeman et al., 1984, a, Zitko & Hutzinger, 1976).

There are probably several reasons for this, which in general can be separated in two types:
- alterations of the behaviour of the compound in the phases which are involved in the accumulation process.
- alterations in the transfer mechanisms between the phaseswhich are involved.

In this paper some of the potential alterations will be discussed for extremely hydrophobic chemicals.

ACCUMULATION IN FISH OF HYDROPHOBIC COMPOUNDS

High concentrations of hydrophobic compounds in fish and other aquatic organisms can result from uptake either via food of directly from the ambient water. For most compounds the latter process (bioconcentration), is dominant (Bruggeman et al. 1981). Uptake from food (biomagnification) is important for only extremely hydrophobic compounds, because of the very low pollutant concentrations in the ambient water.

In the bioaccumulation-process, fish extract hydrophobic compounds from water via their gills. As a consequence high pollutant concentrations in body-lipids can be reached from equilibrium-partitioning between water and fish. For many organic chemicals a simple two compartment-model with first order rate constants gives a kinetic description of the bioconcentration-process (Branson et al. 1975). In addition a rapid estimation can also be made about the accumulation-behaviour of the compounds.

$$C_{water} \underset{k2}{\overset{k1}{\rightleftharpoons}} C_{fish}$$

$$\frac{d\,C_f}{dt} = k_1 C_w - k_2 C_w \qquad (1)$$

At steady-state levels, where $\frac{dC_f}{dt} = o$, a bioconcentration factor is represented by

$$K_c = \frac{C_f}{C_w} = \frac{k_1}{k_2} \qquad (2)$$

The biomagnification model is very similar to this, and a biomagnification factor (K_m) can be defined as

$$C_{food} \xrightarrow{k_1} C_{fish} \xrightarrow{k_2} C_{water}$$

with $\frac{dC_f}{dt} = o$ in steady state is

$$K_m = \frac{C_{fish}}{C_{food}} \qquad (3)$$

The rest of the paper is mainly concerned with the bioconcentration-process, because this is theoretically the best estimation of an ideal partioning-process.

Log K_{ow} - log K_c Relationship

According to the above stated equations, bioconcentration is regarded as a balance between two kinetic processes, expressed by first order rate constants of the uptake and elimination.

An alternative approach is to consider an organism as a combination of physical-chemical phases (MacKay 1982). For bioconcentrating compounds these phases in the organism can reach thermodynamic equilibrium with each other as well as with the surrounding medium. This can be defined mathematically by the chemical potential or the fugacity of the compounds in the different phases. For an organism with n phases, with volume fractions V_i, and activity coefficients the bioconcentrationfactor K_c, can be expressed by

$$K_c = \frac{C_f}{C_w} = \gamma_w V_w \left(\sum_i^n \frac{Y_i}{\gamma_i V_i} \right) \qquad (4)$$

γ_w = activity coefficient of the compound in water.

γ_i = activity coefficient of the compound in fish phase i.

V_w = molar volume of water phase.

V_i = molar volume of fish phase i.

Y_i = fraction volume

The above equation shows that the bioconcentration-process is the result of the ratio of the activity coefficient in the water phase (γ_w) and in the fish phase (γ_i) and volume of fish phase i (V_i).

In practice, for hydrophobic compounds, the lipid phase, with V_i, will be the most important site for bioconcentration. When other phases are ignored, and also the differences of the properties of various lipids, the equation is simplified to:

$$K_c = \frac{C_f}{C_w} = \frac{\gamma_w V_w}{\gamma_{lipid} V_{lipid}} Y_{lipid} \quad (K_c \text{ on wet weight base})$$

A similar expression can be stated for a partition-process of a compound in a n-octanol/water two phase system.

$$K_{ow} = \frac{C_{n\text{-octanol}}}{C_{water}} = \frac{\gamma_w V_w}{\gamma_o V_o} \qquad (6)$$

From the equations 5 and 6, it might be obvious that good relationships between K_c and K_{ow}, as discussed, are results of the fact that both processes are primarily controlled by the activity coefficient in the water phase.

The relationship of K_c and K_{ow} can be expressed by

$$\frac{K_c}{K_{ow}} = \left(\sum_i^n \frac{Y_i}{\gamma_i V_i} \right) (\gamma_o V_o) = Y_1 \frac{\gamma_o V_o}{\gamma_1 V_1} \qquad (7)$$

Equation 7 demonstrates that for organisms with similar Y_l and with similar phases (γ_l) the ratio γ_o/γ_l will be fairly constant, so that K_c can be correlated to K_{ow}. Here n-octanol can be regarded as a sufficient surrogate model-compound for the lipid-phase, because of its comparable balance between hydrophobic and lipophilic character.

In this way, lipophilicity is considered to be a molecular property that finds its expression in the tendency of partitioning between water and lipids. Because natural lipids cannot be defined, an artificial medium which can be standardized (n-octanol) is chosen. For many compounds with theoretical log K_{ow} values above 5-6, K_c-K_{ow} relationships no longer holds (Tulp & Hutzinger 1978). This may indicate that the above described bioaccumulation process is more than a simple partitioning process. For several chemicals this might be due to metabolism which would strongly favour elimination thus lowering K_c and K_m values (Opperhuizen et al., 1984, unpublished data). However, many non-metabolizable compounds also show a poor K_{ow} - K_c relationship. Some of the factors that can influence the accumulation process, which will be discussed in the rest of the paper, are: (I) Molecular properties of the accumulating compounds, other than those characterized by K_{ow}; and (II) Influence of body lipid composition of biota.

I) Molecular properties of the accumulating compounds, other than those characterized by K_{ow}

As described by Bruggeman et al. (1981) and Gunkel and Streit (1980), the process of bioaccumulation involves a number of more fundamental processes, such as:

1) transfer of the compound from the surrounding environment, across the gill membrane by diffusion
2) transport mediated by body fluids
3) concentration in lipophilic biological structures (membranes, liposomes).

It is obvious that transport across biomembranes is of great importance for the bioaccumulation process. The mechanism of permeation of hydrophobic compounds across membranes is regarded as a diffusion process (Stein, 1981). In this theory the rate of permeation (P) is governed by the lipophilicity of the compound, expressed in terms of the lipid water partitioning coefficient K.

$P_m = D_m d^{-1} K$

P_m: permeability coefficient for diffusion across but within the membrane phase (= rate of permeation)
D_m: diffusion coefficient for diffusion within the membrane
K : lipid-water partition coefficient
d : membrane thickness

The diffusion coefficient D_m appears to be inversely proportional to the molecular weight of the diffusing molecule according to:

$D_m = D_o M^{-Sm}$

thus

$P_m = D_o d^{-1} M^{-Sm_K} = P_o M^{-Sm_K}$

D_o: the calculated diffusion coefficient for a solute of unit molecular weight
M : molecular weight of the diffusant molecule
S_m: a parameter describing the mass selectivity of the permeant barrier.

In practice this relationship has been the guideline for most studies on nonelectrolyte permeability (Leib & Stein 1969, Stein 1981).

This relationship is based on the assumption that the rate limiting step in the permeation of a solute is diffusion in the membrane phase and that the mechanism of the diffusion of hydrophobic compounds is the same through cell membranes as through a bulk-lipid phase. Membrane permeation rate in this case may be very closely related to the bioaccumulation uptake rate constant k_1. Both quantities are largely a function of the hydrophobicity of the compound. For compounds with intermediate hydrophobicity k_1 and K_c are linearily correlated with K_{ow}. However, for some extremely hydrophobic compounds diffusion through the membrane interface (not through the membrane phase) may be rate limiting. In principle the membrane interface diffusion is also related to hydrophobicity. For several chemicals, however, the interfacial transfer can be disturbed. In these cases structural characteristics of the compounds and of the interface will be of major importance.

The unfavourable interaction (increase in free energy) of the permeating compound with the polar "heads" of the phospholipids governs the permeation process. This interaction increases when the effective area of the permeating compound increases. Here, the minimal internal diameter or the effective area of the chemical can be important. The effective area of a molecule is the product of its width and thickness, which can be calculated from interatomic distances and Van der Waal's radii (Mackay et al. 1980, Opperhuizen et al. 1984, unpublished data). When molecules become more hydrophobic, their volume increases and corresponding effective areas may in most cases (but definitely not in all) increase as well. As a result membrane permeation, k_1 and K_b may become independent of the compound's hydrophobicity. This means that increasing hydrophobicity does not lead to increasing permeation rates.

For some compounds, such as hexabromobenzene, there is no significant uptake in fish (Bruggeman et al., 1984, a, Zitko & Hutzinger 1976). However, a high K_b value would be expected on the basis of K_{ow} or other lipophilicity parameters. Obviously membrane-passage is blocked by the bulky nature of the compound. This may be expected to happen if membrane passage is a process where the permeating molecul

can only move in and out the membrane through "holes" (see fig. 1). The dynamic motion of the membrane constituents may result in the formation of transient "holes" in the membrane. These "holes" probably do not extend completely across the membrane, so that no pores are formed. When the size of these holes is too small in relation to the effective area of the molecule, no significant uptake takes place. Several authors have suggested that molecular weight may be a limiting factor for the bioaccumulationprocess. From a mechanistic point of view spatial dimensions (molecular volume or bore) rather than mass are the critical factorscontrolling membrane passage.

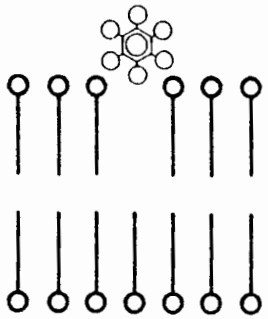

Fig. 1. SCHEMATIC REPRESENTATION OF BLOCKAGE OF MEMBRANE TRANSFER OF BULKY COMPOUNDS.

II. Influence of body-lipid composition of biota

All three processes, discussed in this section - diffusion in the membrane phase for small hydrophobic molecules, interfacial transfer for larger and more hydrophobic molecules and blockage of membrane passage - are affected by the packing order of the phospholipids in the membrane.

The packing order (area per chain) is the result of the amount of branched and unsaturated fatty acids in relation to saturated acids present in the membrane (Jain 1972). Because branched-chains and unsaturated fatty acids occupy more area than their saturated straight-chain homologs, packing will be less tight, when membranes contain more unsaturated or branched acids.

Unsaturation and branching of fatty acids are influenced by many external factors (Jain 1972). Thus factors which have an influence on the membrane-constitution, may also affect the bioaccumulation potential of a compound.

The linear relationship between log K_b and log K_{ow} is the result of the fact that at least within the same species the ratio of γ_o and γ_{fish}, or in practice γ_{lipid}, is constant. As indicated above the activity coefficient of a compound in water is the predominant

factor governing its bioaccumulation and octanol-water partitioni According to MacKay et al. (1980) γ_w, which is inversely proporti to the compound's solubility, is related to hydrophobicity, expre sed in terms of Total Surface Area (TSA) as follows:

$$\gamma_w = A\ (TSA) + B \qquad A \text{ and } B \text{ are constants}$$

γ_l is suggested to be independent of the hydrophobicity of the compound. This may be true for compounds with K_{ow} values up to 5; however serious deviation from the linear log K_b - log K_{ow} relationship (eg when log K_{ow} values > 5) may be caused by an increase in γ_l compared to γ_o. This is because the lipid phase is not a homogenous phase resulting from differences in lipid con stitution, due to a variety of external factors, and the rigid structure of biomembranes.

Lipid-lipid forces are short range Van der Waals forces, which are effectively proportional to surface contact area. Thus a bioaccumulating compound will have less area of close contact with branched or unsaturated fatty acids than with saturated fatty acid The ratio of unsaturated and branched to saturated fatty acids present in the total membrane system of an organism may have a considerable consequence to the γ_o - γ_{fish} relationship, resulting in an increase of γ_{fish} to γ_o when membrane systems become more unsaturated or brached. The amount of proteins and of organic pollutants (Packham et al. 1981) in the membrane-systems, may be expected to affect the γ_o - γ_{fish} relationship also. This might be due to perturbation of the molecular structure of the membrane and alteration of the phase properties of the lipids.
In a rigidly oriented membrane phase a branched molecule may have much less contact with hydrocarbon chains than in the correspondin bulk lipid or octanol phase. As a result γ_{fish} may increase in relation to γ_o, when branching of the molecules increases, resulti in a lower value for K_b than predicted from the octanol-water partition coefficient. For compounds of intermediate lipophilicit (log K_{ow} : 1-5) fat solubility and as a result γ_l may be more or less constant.
We expect that with further increasing lipophilicity fat solubilit will decrease, as a result of an increasing activity coefficient (Dobbs et al. 1983). The increase of the activity coefficient with lipophilicity may be greater for a rigidly organized lipid phase, such as membranes, than for the corresponding bulk lipid (or octan phase.
This would lead to the breakdown of the simple linear relationship between log K_{ow} and log K_b at log K_{ow} values >6 (Tulp, Hutzinger, 1978) (see also fig. 2.).

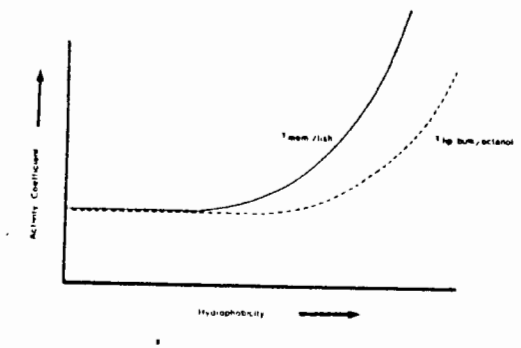

Fig. 2. SCHEMATIC REPRESENTATION OF THE RELATIONSHIP BETWEEN THE ACTIVITY COEFFICIENTS OF COMPOUNDS WITH INCREASING HYDROPHOBICITY, IN MEMBRANE/OR FISH (——) AND BULK LIPIDS/OR N-OCTANOL (--).

The membrane's thickness is another membrane characteristic, which is likely to affect the partition process. Accumulation experiments by Hardy et al. (1974), who fed fish feed containing a variety of n-alkanes showed that C_{26} was the length for maximum retention (absorption minus excretion). Fish bioaccumulation experiments with polydimethyl siloxanes showed lower accumulation with increasing number of silicon-units (Bruggeman et al., 1984, b).

In biomembranes hydrocarbon chains of the phospholipid molecules are arranged tail-to-tail and perpendicular to the plane of the membrane, although slight bending of the chains has sometimes been indicated. Hydrocarbon chains of natural lipids may vary between n = 14 to n = 18, resulting in biomembranes with a corresponding thickness of about 50-70 Å. (Jain 1972). For molecules such as the n-alkanes, with chain lengths approaching the phosphorus-to-phosphorus distance (50 Å for a membrane with a thickness of 70 Å) interaction with the hydrophilic parts of the phospholipids may contribute to the activity coefficients of that compound in the membrane phase. As a result such a compound may experience a higher activity coefficient in the membrane phase than in the corresponding lipid (or octanol) phase.

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