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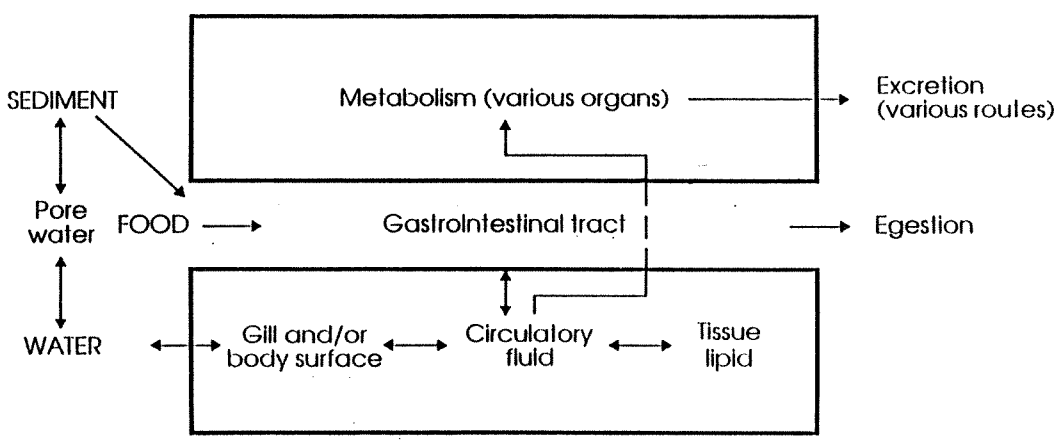
Accumulation and elimination of polycyclic aromatic hydrocarbons (PAH) and persistent organochlorines in gill-breathing marine animals.

A review



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Abstract:

A summary is given from recent literature as regards bioaccumulation, metabolism and release of PAH, PCB, DDT, PCDF/PCDD (polychlorinated dibenzofurans/dibenzo-p-dioxins), HCB, OCS (octachlorostyrene) and other persistent organochlorines in marine invertebrates and fish. Relative importance of the main principal exposure routes is heavily dependent on factors such as habitat, mode of life, size and trophic level. Except in the vicinity of point sources, food is probably the predominant exposure route in fish top predators. Biomagnification is primarily important for (biodegradation resistant) superhydrophobic substances (log K_{ow} = 5-7). Observed half-lives of DDT with metabolites and higher chlorinated PCBs in fish have been up to 1-3 years, whereas other micropollutants may have half-lives in weeks (e.g. PCDF /PCDD) or days (PAH).

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2. PAH
3. Bioakkumulering
4. Utskillelse

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Accumulation and elimination of polycyclic aromatic hydrocarbons (PAH) and persistent organochlorines in gill-breathing marine animals. A review

Oslo, 7 April 1992

Project coordinator: Jon Knutzen

Preface

The present report has in part been sponsored by the Norwegian Program for Marine Pollution and was written as a part of a more comprehensive document: Organochlorines and PAHs in the marine environment: State of the art and research needs (eds.: A. Molven and A. Goksøyr, Royal Norwegian Council for Scientific and Industrial Research, Oslo, 1992).

Oslo, 7 April 1992.

Jon Knutzen

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1. INTRODUCTION

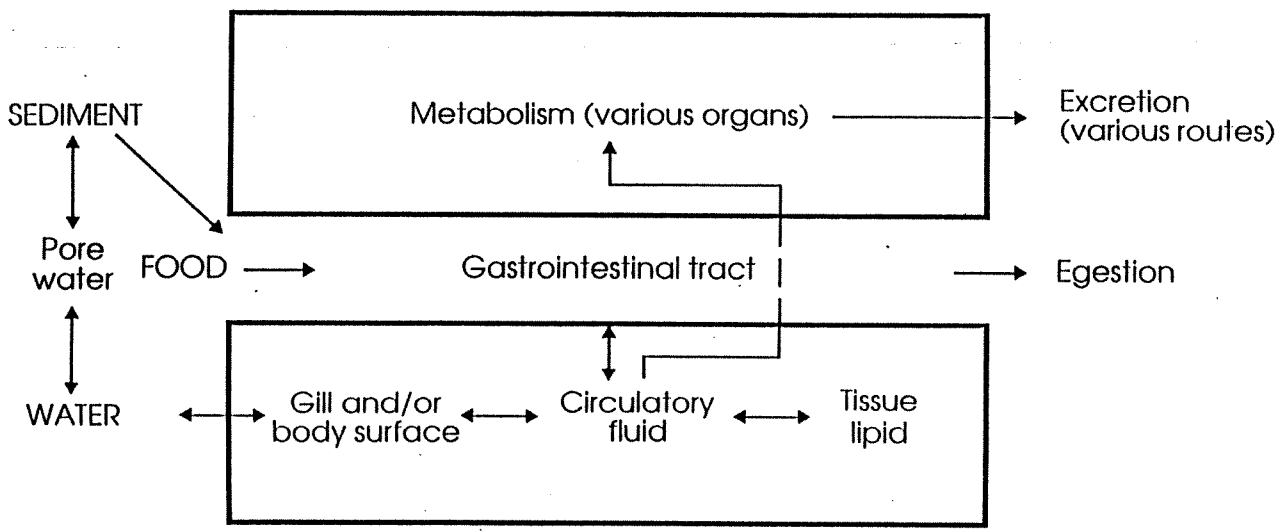
The following review is limited to PAH and well known and persistent chloroorganics like PCBs, DDT, HCB and substances with similar properties. Among the organohalogens not considered are the complex effluents from bleachery processes in the pulp industry (cf. Södergren et al., 1989), and chlorinated/brominated alkylbenzenes (Källqvist and Martinsen, 1987). Unknown halogenated substances with possibly hazardous properties are presently subject of numerous planned an ongoing studies (Håkansson et al., 1990, 1991). Some of these chemicals are synthesized naturally (Holm et al., 1990). It is important to identify possibly toxic substances within sum parameters like EPOCl/Br (extractable persistent organic chlorine/bromine), also because the sum parameters themselves do not appear to be suitable for bio-monitoring purposes (Knutzen et al., 1988, 1991).

2. BIOACCUMULATION - BIOCONCENTRATION - BIOMAGNIFICATION

The term **bioaccumulation** is commonly used in a broad sense, i.e. for the overall process of uptake from all sources (Clark et al., 1988; Connell, 1988).

Bioconcentration is mostly seen as the result of uptake from water alone (Veith et al., 1979; Chiou, 1985; Clark et al., 1988; Connell, 1988, 1990), excluding dietary routes (Barron, 1990).

Biomagnification implies uptake from food. The term may be used generally for food chain transfer (Connell,. 1988, 1989, 1990), or more specifically about the added concentration caused by contaminated food compared to bioconcentration solely from water (Clark et al., 1988; Ekelund, 1989); sometimes about increased concentration with higher trophic levels (Evans et al., 1991). If used in the latter sense, concentrations should be expressed on lipid basis for true biomagnification to be revealed (Ekelund, 1989). (Apparent biomagnification can result from higher fat content in predator than prey).



Figur 4.1. Principal routes of uptake and elimination in a generalized aquatic animal.

In accordance with the above terminology the bioconcentration factor (BCF) is defined as the ratio between concentration in organism and concentration in water, at an assumed steady state. Principal routes of exposure is schematically depicted in fig. 4.1. The relative importance of the potential uptake and elimination routes depend on several factors and vary considerably from one group of organisms to another (habitat, feeding, metabolic capacity, etc.).

In addition to ingestion and via pore water (fig. 4.1), direct contact is a third possibility of sediment related exposure. However, at equilibrium between interstitial water and sediment, fugacity theory (Clark et al., 1988) implies that this should not give additional exposure (Ekelund et al., 1987).

3. UPTAKE FROM WATER

Uptake from water takes place as a passive diffusion process at the body surface, in larger animals mainly via the gills. PAH, organichlorines and other lipophilic compounds dissolve in the lipoid cell membranes of the gill epithelium and is transported with blood to fatty tissue. Concentration or activity gradients, degree of resistance to metabolism and the organism's fat content regulate the stady state concentration in the organisms. Small species will reach equilibrium fastest, due to higher ratio of respiratory surface area to body volume than in large animals.

The transfer of pollutants into the organism depends on many factors, including temperature, water flow, gill morphology, gill or total respiratory surface area versus body size, blood flow from the gills, solubility and diffusional properties of the substance in question. For more detailed information about relevant factors and for examples of kinetic modeling of the gill uptake process, it is referred to Spigarelli et al. (1983), Mackay and Hughes (1984), Gobas et al. (1986), Gobas and Mackay (1987), Barber et al. (1988, 1991), Hawker and Connell (1986, 1988), Pärt (1989), Barron (1990), Hayton and Barron (1990), Erickson and McKim (1990a, b) and Clark et al. (1990).

The uptake from water may be regarded as a simple partition process between water and lipids within the organism, across the lipid membranes of the gill (body) surface and via the circulatory fluid (larger organisms). Support to this concept come from the relation which many authors have found between log BCF and log K_{ow} (partition coefficient octanol : water) in fish, mussels and crustaceans (see Connell, 1988, for review and in addition Sugiura et al., 1978, Ernst, 1980, Kenaga and Goring, 1980 with ref., Könemann and van Leeuwen, 1980, Gossett et al., 1983, Bruggeman et al., 1984, Esser, 1986, Opperhuizen et al., 1988, Niimi et al., 1989, Geyer et al., 1991 and Voogt et al., 1991). Further, for 38 substances with low water solubility, Chiou (1985) found K_{ow} to correlate well with partition coefficients in a model lipid (triolein) : water.

Ideally, and at equilibrium, log BCF = log K_{ow} , but in all the straight line relations in the above references correction factors are incorporated. Primarily, the correction factors express that octanol solibility is a less than perfect simulation of lipid solubility (Dobbs and Williams, 1983). Any significant metabolism or elimination via the feces during the experiment will also contribute to a weaker relationship.

At equilibrium BCF is also given by the ratio k_1/k_2, i.e. between the uptake and clearance rate constants.

From the log BCF/log K_{ow} relation it follows that equilibrium concentrations on wet weight basis generally will be highest in species with a high fat content. The relationsship established for one species should in theory be valid for others, provided that BCF is expressed on lipid weight basis.

In this way one should also be able to calculate the approximate concentration on fresh weight basis if the lipid content was known, with reservation for very low fat organisms (Ekelund, 1989).

According to Barron (1990), however, even on lipid basis there will still be interspecies variations about one half order of magnitude in the BCF values for a narrow range of K_{ow}. Besides, accumulation of any one xenobiotic substance can be influenced by several species dependent factors: lipid composition and distribution, differences in body transport, metabolic capacity, size and physiological factors in general (Barron, 1990 with references). The influence of species related factors on the accumulation of several organochlorines - independent of K_{ow} - is for instance evident from the results of Swackhamer and Hites (1988). Consequently, it is not to be expected that the lipid content should be the sole determinant of bioconcentration, and that the lipid based log BCF/log K_{ow} relation should be more than approximate.

To predict bioconcentration behaviour from K_{ow} has been shown by Schüürmann and Klein (1988) to give inaccurate results, unless the most related compounds are treated as separate groups, in this case chlorinated hydrocarbons and PAH. (Since PAH are rapidly metabolized by fish, and thus break the presupposition of non-degradability, it seems a paradox that these substances are included in log BCF/log K_{ow} relationships together with more persistent chemicals. Perhaps part of the explanation is that the relation is based too much on (short term?) accumulation tests, and less on data from nature. In a study by Connor (1984) fish sediment ratio of organochlorines were about 3 orders of magnitude higher than for PAH with similar log Kow. See further discussion by Connor and also Southworth et al. (1980, 1981)).

For the reliability of the log BCF/log K_{ow} relationship it is also of interest that determinations of water solubility and partition coefficients is difficult for substances with log $K_{ow} > 5.5$ (Tulp and Hutzinger, 1978, Brooke et al., 1986), resulting in significant variation in the figures given by different authors (Chessels et al., 1991). Another possible source of error is in determination of lipid concentrations, variation of 3.5 times resulting from different fat extraction methods (Randall et al., 1991, see also Ferraro et al., 1990). Schneider (1982) pointed out the importance of analyzing on comparable fat fractions. (See also discussion in Boon and Duinker (1986) on the effect on accumulation from variation in amount and type of lipid content).

For superhydrophobic substances with log $K_{ow} = 5 - 6$ several studies have revealed a breakdown of the linear log BCF/log K_{ow} relation, i.e. significantly lower BCF than expected (Sugiuara et al., 1978; Tulp and Hutzinger, 1978; Könemann and van Leuwen, 1980; Bruggeman et al., 1981, 1984; Muir et al., 1985a, b; Opperhuizen et al., 1985; Gobas et al., 1989a; Voogt et al., 1990; see also review of results in Opperhuizen and Sijm, 1990).

A possible practical reason for some of the discrepancies observed is that real BCFs have not been achieved due to the long time required to reach equilibrium in uptake experiments with superhydrophobic substances. Connell and Hawker (1988) indicated times to equilibrium of 3/4 - 1 year for compounds with log K_{ow} 6 - 7, thereafter decreasing with increasing K_{ow} .

Several physiochemical properties have been offered as explanations for the less than expected BCF for superhydrophobic substances. From uptake experiments with chorinated paraffines in fish Zitko (1974) suggested that molecular weight about 600 might be an upper limit for bioaccumulation (see also Zitko and Hutzinger, 1976 and Tulp and Hutzinger, 1978). Data reviewed by Brooke et al. (1986), however, show a few examples of considerable accumulation (log BCF $\approx$ 4) for chemicals with $600 < MW < 700$. Further, the importance of stereochemical factors (molecular volume, suface area, effective cross section), combined with physiochemical

properties of membrane barriers (pore size, solvent characteristics) has been focused on by several authors: Tulp and Hutzinger (1978), Shaw and Connell (1984), Bruggeman et al. (1984), Muir et al. (1985a, b), Opperhuitzen et al. (1985). The latter suggested that an effective molecular cross section of 0.95 nm was the upper limit of passive lipid membrane penetration, - see also Anliker and Mosel (1987). On the other hand, some bioconcentration has later been observed for slightly larger molecules (0.96 nm, Gobas et al., 1988, 1989a, see also Muir et al., 1986 and Gobas and Schrap, 1990).

Reduced lipid solubility of superhydrophobic substances may also cause a relatively lower degree of bioconcentration than predicted from the log BCF/log K_{ow} linear correlation (Gobas et al., 1987, Banerjee and Baughman, 1991). In addition to the observation of Dobbs and Williams (1983), that the solubility of several organochlorines in natural fats did not correlate well with their octanol solubilities, it has been pointed out that for voluminous molecules the dissolution process will be hampered by the highly structured form of lipids in organisms compared to a homogenous solvent like octanol (Gobas et al., 1987).

Thermodynamic consideration respecting bioconcentrations versus octanol : water partitioning lead Opperhuizen et al. (1988) to the conclusion that octanol is a poor model for biotic lipids (see also Magee, 1991).

The net result of the above factors comes to expression in a parabolic curve between log BCF and log K_{ow}; the straight line relationship being limited to log K_{ow} about 3 - 5, and log BCF reaching a maximum below 4.5 - 5. For substances with log K_{ow} above $\approx$ 6.2 - 6.5 BCF is generally decreasing (e.g. Muir et al., 1985b and Hawker, 1990a).

The most important external factors influencing uptake from water are concentrations of **particulate organic material** (POM) and **dissolved organic matter** (DOM, mostly humic and fulvic acids).

When uptake from water is the main accumulation route, commonly occurring concentrations of suspended organic solids can reduce the bioavailability radically for hydrophobic substances (log $K_{ow} > 5$) due to sorption onto the particles (Adams, 1987, Servos et al., 1989a, Schrap and Opperhuizen, 1990, Gobas and Russel, 1991), For less hydrophobic substance no such effect was found by Opperhuizen and Stokkel (1988).

In some instances also **inorganic suspended** material can sorb xenobiotics to the extent that decreased bioavailability results (Eaton et al., 1983).

In contrast with the above results it has been demonstrated for deposit feeders that addition of adsorbing material may enhance accumulation (Landrum and Scavia, 1983; Ekelund et al., 1987).

The review of Schrap (1991) on bioavailability is partly misleading in treating the effect of suspended solids while referring to studies mostly concerned with availability impact of dissolved macromolecules.

Association of PAH and organochlorines with humic acids (e.g. Gjessing and Berglind, 1981, 1982, Johnsen et al., 1989, McCarthy et al., 1989) has in several studies been shown to reduce bioavailability.

This has been observed for several PAH in small crustaceas (*Daphnia*) by Leversee et al. (1983), McCarthy (1983) and McCarthy et al. (1985), Kukkonen et al. (1989), Oikari and Kukkonen (1990) and Kukkonen and Oikari (1991). Several researchers have documented the same effect on

PAH availability to amphipods (Landrum et al., 1985, 1987) and fish (Spacie et al., 1983 (B(a)P, but not anthracene), McCarthy and Jimenez, 1985 (B(a)P, not naphtalene), Black and McCarthy, 1988, and Johnsen et al., 1989).

Black and McCarthy (1988) also found proportionality between decrease in uptake and the reduction in freely dissolved PAH caused by added dissolved organic macromolecules. Perhaps the observations of Weston (1990) also may be explained by less available free hydrocarbons in solution due to association with DOM.

Significantly reduced uptake of various organochlorines in the presence of dissolved humic acids has been observed in crustaceans by Servos and Muir (1989) and Kukkonen et al. (1990) and in fish by Carlberg et al. (1986), Kukkonen and Oikari (1987), Black and McCarthy (1988) and Servos et al. (1989b). In contrast to this, Muir et al. (1985b) found it probable that other factors than DOM were responsible for relatively low BCFs for hepta- and octachlorodibenzo-p-dioxin.

Varying results for uptake of Lindane from water with respectively springtime and autumn humus prompted Carlberg et al. (1986) to speculate on the effect of differing humic acid composition. This hypothesis later has been confirmed by observations of Oikari and Kukkonen (1990), Kukkonen et al. (1990) and Kukkonen and Oikari (1991), particularly pointing to the share of hydrophobic acids in DOM as important for the reduction in PAH availability.

On the basis of theoretical water chemistry considerations Gobas and Russel (1991) seem to conclude that under most prevailing concentrations of dissolved organic matter this factor should have little influence on bioavailability measured as concentrations of xenobiotics in fish. Nevertheless, several of the above studies have been conducted with natural water qualities (Carlberg et al., 1986; Servos and Muir, 1989; Kukkonen et al., 1989; Johnsen et al., 1989; Oikari and Kukkonen, 1990; Kukkonen and Oikari, 1991), or the added concentrations can be met with in waters with a moderate to high natural humus content (McCarthy et al., 1985; Black and McCarthy, 1988).

In his assessment of results so far, McCarthy (1989) concluded that the probable mechanism behind decreased availability was that the association of humic acid with superhydrophobic chemicals resulted in a complex more or less impenetrable to gill membranes.

4. SEDIMENT EXPOSURE

Accumulation from sediments may either be direct from ingestion or via pore water (fig. 1). References to cases of adsorption to the body surface from direct contact with contaminated particles, followed by absorption into body fluids, is also given by Knezovitch et al. (1987). As stated in the introduction, this possibility is not considered here on the grounds of fugacity theory. At equilibrium between sediment and interstitial water, which most often must be assumed to be realized, body contact with sediment should give noe additional exposure than already coming via pore water.

Provided that this holds true, the remaining questions are

- How available are organic micropollutants in pore water?
- What is the absorption efficiency in the gut for contaminants associated with particles?
- To what degree are sediments an indirect source of xenobiotics through food chains?

Obviously the answers to these questions will be highly variable, depending on solibility of the chemicals, type of sediment, habitat, mode of feeding and efficiency of gut absorption in the organisms considered (Nalepa and Landrum, 1988).

Most exposed will be organisms which burrow in the sediment and eat deposited material. Examples of contamination in such species are given by Fowler et al. (1978); McLeese et al. (1980); Eadie et al. (1982a, b, 1984); Augenfeld et al. (1982); Landrum and Scavia (1983); Rubinstein et al. (1983, 1984, 1990); Oliver (1984, 1987); Varanasi et al. (1985); Reichert et al. (1985); Karickoff and Morris (1985); Frank et al. (1986); Shaw and Connell (1987); Klump et al. (1987, 1991); McLeese and Burridge (1987); Connell et al. (1988); Landrum (1988, 1989); Landrum and Poore (1988); Knezovich and Harrison (1988); Evans and Landrum (1989); Schuytema et al. (1988, 1990); Gobas et al. (1989b); Boese et al. (1990); Fry and Fisher (1990); Mac et al. (1990); Ferraro et al. (1990); Lee et al. (1990); McElroy et al. (1990); Weston (1990); Evans et al. (1991); Landrum et al. (1991); cf. also review paper by McElroy et al. (1989).

In some of these studies it has been concluded that pore water appears to be the most important route of contamination (Eadie et al., 1984; Oliver, 1987; Shaw and Connell, 1987; Connell et al., 1988; Knezovich and Harrison, 1988; Schuytema et al., 1988, 1990); in others ingestion of sediments (Fowler et al., 1978; Landrum and Scavia, 1983; Klump et al., 1987, 1991; Landrum and Poore, 1988; Boese et al., 1990; Weston, 1990).

The differentiation betweeen the two ways of exposure is difficult and the conclusions often uncertain. The results of Weston (1990) with a marine polychaete indicated that for the more hydrophobic substances (log $K_{ow} > 5$) ingestion might be the main route, interstitial water for the relatively more water solube compounds (as also stated by Adams, 1987). The kinetic model of Gabric et al. (1990) for uptake in oligochaetes, using data from Oliver (1987), suggests pore water as the primary source. On the other hand, the results of Lee et al. (1990) and Klump et al. (1991) indicated an absorption efficiency as high as about 50% for organochlorines in the gut of a deposit-feeding clam and a mysid.

Weston (1990) found reduced bioavailability of B(a)P when the sediment organic carbon increased from 0.3 to 1% (for further references to the inverse relationship between organic carbon in sediments and bioavailability, see Knezovich et al., 1987). That the origin of the contaminant can influence bioavailability considerably has been observed by Farrington et al. (1983): Oil derived PAH were taken up in organism to a larger degree than PAH bound in soot particles, in accordance with the easier dissolution of the former into pore water (Socha and Carpenter, 1987). Contaminants in fine grained sediments may be less bioavailable (Augenfeld et al., 1982), and freshly added pollutants may be less tightly bound than in older deposits (Varanasi et al., 1985). The possible role of ageing is also mentioned by Landrum (1989).

Uptake of xenobiotic, hydrophobic substances from sediments in **epifauna** or **fish** has been observed by McLeese et al. (1980); Dillon (1982); Rubinstein et al. (1983, 1984, 1990); Varanasi et al. (1985, 1987); Larsson (1986); Krahn et al. (1987); Malins et al. (1987); Stein et al. (1987); Evans and Landrum (1989); Payne et al. (1988); Schuytema et al. (1988, 1990); Batterman et al. (1989); Mac et al. (1990); Klump et al. (1991); see also Kuehl et al. (1987).

The relative roles of alternative mechanisms behind uptake in epifauna and fish obviously require complicated test designs and have not been thrown much light upon. Rubinstein et al. (1984) observed uptake in fish by contact only, but believe that sediment contaminated prey is the main source in nature. The high content of PAH in the stomach of flounders from an area with PAH-contaminated sediments points in the same direction (Malins et al., 1987).

Further, in tests with flounder (*Platichthys flesus*) and the edible crab (*Cancer pagurus*) held for 3 months on fjord sediment heavily contaminated with polychlorinated dibenzofurans/dibenzo-p-dioxins (PCDF/PCDD), and without contaminated food, Berge and Knutzen (1991) observed apparent equilibrium concentrations of PCDF/PCDD about an order for magnitude below field levels. Kuehl et al. (1987), however, observed substantial accumulation of PCDF/PCDD from sediments in carp, and suggested that most of the accumulation came from ingested sediment.

Klump et al. (1991) observed sediment ingestion in *Mysis*, a planktonic crustacean which is benthic during the daytime, thus pointing at the potential of this species (Duyn-Henderson and Lasenby, 1986) and others with similar vertical migrations (e.g. shrimps and prawns) to mobilize contaminants from sediments into pelagic food chains.

5. DIETARY ROUTE

Food as an exposure route may be of importance by:

- increasing the doses of toxicants above the exposure from water.

- causing biomagnification in the sense of increasing concentrations with higher trophic levels, and thus endanger top predators in the sea, and in particular fish eating birds, mammals (e.g. Olsson, 1987) and humans.

That deposit feeders are exposed via food in variable extent is evident from the studies referred in the previous chapter. In plankton/seston feeding invertebrates varying importance of the food route has been shown by among others: Corner et al. (1976); Dobroski and Epifanio (1980); Harding et al. (1981); Trucco et al. (1983); Fortner and Sick (1985); Majewski and Scherer (1985); Pruell et al. (1987); Broman et al. (1990, 1992).

Whereas Fortner and Sick (1985) and Majewski and Scherer (1985), respectively in an oyster species and the common mussel (*Mytilus edulis*), found that uptake from water was the predominant source in laboratory tests with B(a)P, calculations based on field observations gave seston intake as the most important uptake route for PAHs and polychlorinated dibenzodioxins/dibenzofurans (PCDD/PCDF) in *Mytilus* from the Baltic (Broman et al., 1990, 1992). It is not clear, however, how much of the uptake via seston is from assimilation in the intestinal tract, respectively absorption from seston in contact with the gill surface (Broman et al., 1990a).

In a crab species Lee et al. (1976) found larger uptake of B(a)P from food than from water.

Not all particle bound PAH is available to filter feeders. Bender et al. (1987) observed that coal particles were ingested, but no or insignificant amounts of PAH were assimilated in the gut. The very high PAH burden in mussels from Norwegian fjord recipients for smelter effluents (Bjørseth et al., 1979; Knutzen, 1987, 1989 with references) are strong indications, though, that PAH in soot particles are readily available.

Biomagnification in the strict sense (see above) does not take place to any significant extent for PAH because of the biodegradation capacity of most aquatic organisms at higher trophic levels, in particular fish (several papers in Varanansi, 1989, Stegeman and Lech, 1991, with ref.).

Investigations with fish in which PAH uptake from food has been shown include: Solbakken et al. (1979, 1980, 1984); Fair and Sick (1983); Maccubin et al. (1985); Malins et al. (1985a, b, 1987); McCain et al. (1990) and McElroy et al. (1991).

There is, however, an uncertainty as to the degree of assimilation of PAH from the intestine. The results of Solbakken et al. (1979, 1984) demonstrated assimilation, and the same applies to the studies conducted by Balk et al. (1984); Malins et al. (1985a, b, 1987); McCain et al. (1990) and McElroy et al. (1991). In all these investigations PAH-metabolites have been extracted from liver, kidney or other tissues.

On the other hand, Whittle et al. (1977) found most of B(a)P left in the stomach of herring, and Niimi and Dookhran (1989) found that dietary accumulation did not take place for several PAHs; the cause being low degree of assimilation (9 - 14%) and rapid elimination (metabolization). Limited gut absorption has also been observed by Solbakken et al. (1980) and Schnitzer et al. (1987).

With regard to **persistent organochlorines** there has been av long-standing discussion about the extent to which biomagnification occurs (see Harding and Addison, 1986, and Ekelund, 1989).

Among experimental fish studies which have documented food mediated intake of chlorinated hydrocarbons and related substances are: Macek and Korn (1970); Järvinen and Tyo (1978); Zitko (1980); Bruggeman et al. (1981, 1984); Skaar et al. (1981); Sommer et al. (1982); Bengtsson and Ôfstad (1982); Hilton et al. (1983); Stehlik and Merriner (1983); Pizza and O'Connor (1983); Rhead and Perkins (1984); Södergren (1984); van Veld et al. (1984); Fisher et al. (1986); Kleeman et al. (1986a); Muir and Yarechewski (1988); Niimi and Oliver (1988); Opperhuizen and Schrap (1988); Batterman et al. (1989); Bergqvist et al. (1990); Gobas and Schrap (1990); Muir et al. (1990); Loonen et al. (1991); de Boer and Pieters (1991) and Clark and Mackay (1991). The experiments of Servos et al. (1989a) also made it likely that OCDD accumulated in fish by a detritus based food chain.

Assimilation efficiency from the gastrointstinal tract into the fish (ratio between amount of chemical taken up and amount fed) varies somewhat with class of compounds. For PCBs 30 - 60% or even higher are common figures (e.g. Bruggeman et al., 1981, 1984; Hilton et al., 1983; Gobas et al., 1988a with ref.; Niimi and Oliver, 1988 with ref.; Opperhuizen and Schrap, 1988; de Boer and Pieters, 1991; Clark and Mackay, 1991); somewhat lower (20 - 30%) for the fully chlorinatd decachlorobiphenyl (Gobas et al, 1988a; Gobas and Schrap, 1990).

Other examples of observed/calculated absorption efficiencies are:

DDT: 35 - 80% (Macek and Korn, 1970; Hilton et al., 1983; Muir and Yarechewski, 1988; Niimi and Oliver, 1988 with ref.).

HCB: 55 - 85% (Niimi and Oliver, 1988 with ref.; de Boer and Pieters, 1991).

Chlordane: 40 - 60% (Hilton et al., 1983; Gobas et al., 1988a with ref.; Niimi and Oliver, 1988).

Octachlorostyrene: 68% (Niimi and Oliver, 1988).

Chlorinated paraffins: 1 - 6% (Zitko, 1980; few studies, depending on chain length and degree of chlorination, cf. Zitko, 1974 and Bengtsson et al., 1979).

Others: Se references in Niimi and Oliver (1988) and Gobas et al., (1988a).

The group of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/PCDF) generally show lower assimilation efficiencies: < 3 - 40%; particularly low for compounds which do not have 2,3,7,8-substitution of chlorine and for the fully chlorinated congeners octachlorodibenzo-p-dioxin/dibenzofuran (OCDD/OCDF, cf. Niimi and Oliver, 1986; Muir et al., 1986; Muir et al., 1990; Gobas and Schrap, 1990; Clark and Mackay, 1991).

On the basis of data from uptake and elimination studies, Opperhuizen and Sijm (1990) tentatively concluded that PCDD/PCDF can be divided into three groups with different properties. The first group of compounds are easily taken up, but are also readily metabolized and consequently have little or noe tendency to accumulate. Examples mentioned by the authors are lower chlorinated congeners without the 2,3,7,8-substitution configuration plus 2,3,7,8-TCDF. (The latter is probably misplaced. Kuehl et al. (1987) observed considerable accumulation of this compound in contrast to non 2,3,7,8-PCDF. It also has been observed in high concentrations in fish from PCDF recipients (e.g. Oehme et al., 1989)). The second group consists of OCDD and OCDF, which show very slow uptake (if at all), problably caused by their large molecular volume or effective cross-section, with resulting hampered membrane permeation. Thirdly, congeners with the 2,3,7,8-configuration are rapidly taken up, and more slowly metabolized (probably 2,3,7,8-TCDF should also be included here).

Experimental **biomagnification**, in the sense of higher concentrations in fish than food, has been observed or indicated in the studies of Hilton et al. (1983, DDT, Chlordane, Dieldrin, PCB); Bruggeman et al. (1984, PCB congeners); Opperhuizen and Schrap (1988, PCB congeners); Muir and Yarechewski (1988, 2378-H_6CDD but not 2367-H_7CDD); Batterman et al. (1989, 2378-TCDD); Muir et al. (1990, 23478-PCDD); Clark and Mackay (1991, PCB congener, Mirex; probably not HCB).

A tentative conclusion drawn by Opperhuizen and Sijm (1990) is that the proposed limit of 0.95 nm molecular effective cross-section for gill uptake is not necessarily valid for uptake from the intestine (also cf. Bruggeman et al., 1984, and Niimi and Oliver, 1988, with regard to a limit on molecular volume). Membrane properties of the gut may differ from the gill in somewhat larger membrane "holes" because of a differing lipid composition or the occurrence of substances like sterols. Opperhuizen and Sijm (l.c.) also point to the possibility of differences between fish species in this respect.

Dietary fat can enhance uptake of hydrophobic substances (van Veld, 1990 with references).

Accoring to Ekelund (1989) active uptake via pinocytosis (and thereby circumventing the membrane barrier) is a possibility in invertebrates, but improbable in fish. Neither is this mechanism mentioned in the review by van Veld (1990).

The relative importance of food versus water exposure is difficult to assess on the basis of laboratory tests, and the relevance of the conclusions from simple experimental conditions to the complexity in nature may be dubious. One of the more obvious sources of error with regard to fish is that tests often are conducted with quite small individuals; another is the variation seen with the more or less arbitrarily chosen water concentration (e.g. Rhead and Perkins, 1984; Barber et al., 1991). Bruggeman et al. (1984) stated that conclusions as to the relative importance of different exposure routes require a complete bioenergetic model which includes size, fat content, feeding habit and rate, growth, reproduction and respiration in the species considered. Other factors to be taken into account are age (Larsson et al., 1991), seasonal variation (Reuthergårdh, 1988 with ref.) and migration (Gramme et al., 1984; Connolly et al., 1991).

Evidence or strong indications of organochlorine biomagnification under natural conditions in freshwater come from data referred in Borgmann and Whittle (1983); Connor (1984); Niimi (1985); Oliver and Niimi (1985, 1988); Oliver (1987b); Clark et al (1988); Connolly and Pedersen (1988); Swackhamer and Hites (1988); Thomann (1989); Gagnon and Dodson (1990); Rasmussen et al. (1990); Barber et al. (1991); Connolly et al. (1991) and Evans et al (1991). Similar indications from estuaries or open ocean studies appear to be more scarce: Tanabe et al. (1984); McCain et al. (1990); Connolly (1991) and Thomann et al. (1991).

In their overviews of organic micropollutant occurrence in biota of the North Sea biomagnification is not mentioned (Holden, 1988), or it is claimed probably to be of little significance (Duinker, 1988). Also Harms and Kerkhoff (1988) maintain that water is the main source, but their referred data from the southern North Sea show higher concentration of PCBs, DDT and (to a lesser extent) HCB on lipid basis in cod (liver) than herring. However, published results from systematic studies of contaminants in different trophic levels of selected food chains appear to be lacking.

In the Baltic there are long series of observation in herring and cod, two species which in a general sense may be taken as succeeding levels in a food chain (Broman et al., 1992). The data of Haahti and Perttilä (1988) from Pori and Hankö 1980 - 1985, show that the lipid based concentrations of PCB and DDT in cod liver systematically exceeds herring muscle content with a factor of 1.5 - 2. The more sparse data of Miettinen et al. (1985) is in accordance with this. However, 1983-data for cod and herring fillet from the southern Baltic, show the same tendency merely for PCB and Σ HCH, not for Σ DDT or HCB (Falandysz, 1986a, b). Sprat, possibly being somewhat lower in the food chain hierachy than herring, had lower concentration than cod also with regard to Σ DDT (Falandysz, 1985).

The studies of Broman et al. (1992) of PCDF/PCDD in herring and cod from the Baltic gave generally higher concentrations of the 2,3,7,8-congeners in herring, as did the introductory observations by de Wit et al. (1990).

From this cursory overview of data from the North Sea and Baltic no definite conclusion can be drawn as to the possible role of biomagnification. As indicated above, one may suspect that this will not be possible even if the data were looked at more thoroughly, and that more studies aiming at this particular question are required.

For freshwater and estuarine environment there are several models describing the theoretical basis for evaluating the role of food chain transfer: Borgmann and Whittle (1983); Thomann and Connolly (1984); Connolly and Pedersen (1988); Connell (1989); Thomann (1989); Rasmussen et al. (1990); Barber et al. (1988, 1991); McCain et al. (1991) and Thomann et al. (1991). Such models appear to be lacking for fjords, continental seas and the open ocean.

The degree of biomagnification varies for the different chemicals; thus this mechanism seems to be more important for Σ DDT/DDE than for PCB (Oliver and Niimi, 1986, 1988; Swackhamer and Hites, 1988; Evans et al., 1991). Few data are available for individual PCB congeners, and Oliver and Niimi (1988) so far saw no or only uncertain indications of variation in the biomagnification potential of specific compounds in fish in contrast to findings in marine mammals (e.g. Boon and Eijgenraam, 1988, and Norstrom and Muir, 1988). The octachlorostyrene tendency to biomagnification has been found about similar to PCB, whereas the tendency was markedly lower in toxaphene than in PCB, and somewhat inconsistent for HCB (Oliver and Niimi, 1988). Other compounds which possibly biomagnify include mirex, heptachlor and dieldrin (Oliver and Niimi, 1988; Connolly and Pedersen, 1988). For hexachlorocyclohexanes biomagnification tendency was weak compared to Σ DDT and PCB (Tanabe et al., 1984; Oliver and Niimi, 1988).

The biomagnification phenomenon, apparently being in conflict with fugacity theory of equilibration between fish and water, is explained by processes in the gastrointestinal tract. Digestion and absorption of lipids from the gut cause contaminant concentration on lipid basis to increase, a fugacity gradient between the gut and the surrounding fish tissue (fig. 4.1) is established, and the xenobiotics are transported into the fish. Consequently, for substances with a slow elimination rate, the net result will be higher fugacity in the predator than in its prey and in water (Connolly and Pedersen, 1988; Gobas et al., 1988a).

Slow elimination occurs particularly among recalcitrant substances with log K_{ow} > 5 (see below). From the model of Thomann (1989), based on efficiencies of uptake from water and food, together with elimination rates, it is concluded that biomagnification primarily will be important for chemicals with log K_{ow} 5 - 7.

Obviously, this effect will tend to be more manifest for each higher step in a food chain, and will be most evident in top predator among fish (and even more so among aquatic fish consumers, see for instance Tanabe et al., 1984). In the model of Rasmussen et al. (1990) it is estimated that the concentration on lipid basis will increase by a factor about 2 for each higher trophic level (of gill breathing species).

From this follows that the food transfer route tend to be dominant for the body burden in fish top predators, at least in some freshwater and estuarine localities (Oliver and Niimi, 1985, 1988; Batterman et al., 1989; Thomann, 1989; Rasmussen et al., 1990; Thomann et al., 1991). Estimated contribution via food has been 90% or even more (Thomann and Connolly, 1984; Thomann et al., 1991, although Barber et al. (1991) warns against underestimating uptake from water for lower chorinated PCBs). Possibly, general reservation should also be taken for extremely hydrophobic compounds (log K_{ow} > 6.5 - 7, (cf. Thomann, 1989). Within this group it is an increasing possibility of impeded assimilation caused by molecular dimensions. On the other hand, this restriction may affect gill uptake even more (e.g. discussion in Ekelund, 1989).

6. DEPOSITION AND ELIMINATION

Hydrophobic substances generally are distributed and deposited within organisms according to the fat content of different organs and tissues (Connell, 1989). Thus the distribution may differ considerably between species. Members of the cod family have their fat reserve in the liver, whereas salmonids have fat deposits assiciated with muscle tissue. Organic micropollutant are deposited accordingly (e.g. Ingebrigtsen et al., 1988, 1990 and Hektoen et al., 1992). The pattern of accumulation is also influenced by different fat fractions being unevenly represented in different tissues (e.g. Kamman et al., 1990) and variation between species (e.g. Ingebrigtsen et al., 1990, who found much of a PCB congener in the brain of cod, little in the brain of rainbow trout).

In the main, **elimination** is the combined process of **diffusive release** over respiratory surfaces, according to equilibration partitioning, and **excretion of metabolites** which are more water soluble than the mother substances, mostly through urine and gallbladder/feces. However, also spawning may represent a significant loss, eventually with transfer to the offsprings (e.g. Knickmeyer and Steinhart, 1989 with ref.).

There is a vast amount of literature about the metabolic capacity of various groups of organisms (fish, crustacea, polychaetes) with respect to biotransformation of PAH and more or less persistent organochlorines/organobromines by means of the cytochrome P450 dependent monooxygenase

enzyme system. However, only a few studies describe these enzymes in any molecular detail (for reviews, see Stegeman, 1989; Goksøyr and Förlin, 1992).

For biotransformation of PAH it can be referred to reviews by James (1989); Varanasi et al. (1989) and Stegeman and Lech (1991). The general picture from such studies is that enzyme-activity versus PAH is highest in fish, 1 - 2 orders of magnitude lower in mussels and snails, and with intermediate values in crustaceans and polychaetes (for references see Neff, 1985; Mix, 1986; Capuzzo, 1987, and Stegeman and Kloepper-Sams, 1987). There is some doubt about the in vivo capability of mussels to degrade PAH, but the change of PAH-profile in mussels vs their feed (seston) were interpreted by Broman et al. (1990) as indicative of metabolism.

Echinoderms are also capable of metabolizing PAH, whereas endeavours to detect the degradation enzymes in sponges, coeclenterates and Cnidaria so far have given negative results (see references in Knutzen, 1989). Worth noting is the problem possibly constituted by persistent PAH degradation products (Hinga and Pilson, 1987; Varanasi and Stein, 1991).

Metabolism in aquatic animals of the comparatively more persistent organochlorines are less known (Hamdy and Gooch, 1986; Stegeman and Lech, 1991), and few studies have given convincing evidence of metabolites (Moore and Walker, 1991), even if activation of the cytochrome P-450 enzyme system often has been demonstrated in a variety of organisms (Stegemann and Lech, 1991 with ref.)

Solbakken et al. (1984) refer several fish studies which have failed in detecting metabolites, Among the few studies with concrete evidence of PCB metabolism in fish are Sanborn et al. (1975); Melanchon and Lech (1976; Hinz and Matsumura (1977) and Stein et al. (1984, 1987). References to previous PCB metabolization studies in fish can also be found in Brown et al. (1987), including Metcalf et al. (1975) who demonstrated metabolites of lower chlorinated biphenyls both in fish and in some invertebrates. Kuiper and Hanstveit (1987) reported no metabolism of PCB by plankton communities (as contrasted with for instance chlorinated phenols) and referred to other negative results. Goerke and Ernst (1986), however, detected metabolism of a pentachlorobiphenyl in a polychaete species.

Metabolites of toxaphene (chlorinated camphenes) has been registered in various aqautic organisms (Saleh, 1991 with references). Biotransformation of DDT result in the more persistent DDE (and other metabolites), as revealed in trends of decreasing DDT/Σ DDT ratio (e.g. Olsson and Reutergårdh, 1986). The review on polychlorinated diphenyl ethers by Becker et al. (1991) has no reference to degradation studies with aquatic species. Hexachlorocyclopentadiene is reported to be metabolized by fish (Podowski et al., 1991). Remarkably few references to observations of *in vivo* biotransformation of the more persistent organochlorine pesticides are given in the review by Miyamoto et al. (1990).

In their overview papers about HCB Ernst (1986) and Bro-Rasmussen (1986) do not mention any studies which have indicated degradation of this substance in fish. However, Bauer et al. (1989a, b) found metabolites of both HCB and octachlorostyrene in the common mussel.

Evidence or indication of PCDD/PCDF metabolism in fish are found in several papers: Branson et al. (1985); Muir et al. (1985a, several non 2,3,7,8-TCDD and P_5CDD, more uncertain respecting 2,3,7,8-H_6CDD and -H_7CDD); Muir et al. (1986, 1,3,6,8-TCDD); Kleeman et al. (1986,a, b, 2,3,7,8-TCDD); Mehrle et al. (1988, 2,3,7,8-TCDF); Muir and Yarechewski (1988, non 2,3,7,8-TCDD and P_5CDD); Sijm and Opperhuizen (1988, 2,4-DCDD); Sijm et al. (1989, several TCDD, but not indicated for 2,3,7,8-TCDD); Gobas and Schrap (1990, various non 2,3,7,8-PCDD); Sijm

et al. (1990, non 2,3,7,8-P_4- and P_5CDD, but not 2,3,4,7,8-PCDF). Opperhuizen and Sijm (1990) suggest that the lower bioaccumulation than expected from log K_{ow} values for PCDD/PCDF is caused by a combination of relatively rapid metabolism and membrane permeation hindrance, these factors being of unlike importance for different subgroups, as referred above (ch. 4.3.4).

Elimination rates for PAH have been reviewed by Knutzen (1989 with ref., see also Niimi, 1987). For bivalves, which have little or no metabolic capacity, the time to **50% reduction** of body burden varies between less than 1 day for naphtalene and up to 40 days for the 4- and 5-rings PAH (in the main 5 - 20 days). Time to **90% reduction** has been up to 50 days in *Mytilus edulis* (one uncertain result indicating about 300 days in *Modiolus modiolus*, the horse mussel). In crustacea half-lives have been measured to < 1 - 20 (35) days (see also Little, 1985), and in whole fish mostly < 1 - 5 days, up to 30 (?) days.

Recent studies of PAH depuration in fish include Niimi and Dookhran (1989, half-lives 1- 4 days, except phenyl naphtalene : 25 days); Lemaire et al. (1990, half-lives in different organs), and Steward et al. (1990). For elimination rates in non edible species it is referred to NRC/Canada (1983) and Neff (1985).

Biological half-lives of organochlorines and other chemicals in fish have been reviewed by Niimi (1987). For persistent pesticides like DDT and metabolites, chlordane, dieldrin etc. reported values are in the order of < 10 - > 495 days, with the slowest elimination for DDT/DDE and mirex (see references in Niimi, 1987, several results with half-life more than 6 months). Toxaphene halv-lives have been observed to be 7 - 63 days.

Other examples are (days):

Lower (1 - 5) chlorobenzenes : 1 - 5 (47).
HCB: 12 - > 224 (Norheim and Roald (1985, 60 - 70).
Di- and trichlorobiphenyls: 2 - 196.
Tetrachlorobiphenyls: 2 - 890.
Penta - decachlorobiphenyls: 73 - > 1000.

In contrast to these in part very high figures, more easily degradable compounds like mono-to pentachlorphenols had half-lives of the order 1 - 2 (7) days (Niimi, 1987, with ref.).

The considerable variation within higher chlorinated PCBs (tetra- and above) presumably reflects that the metabolization rate is strongly dependent on number and particularly the placement of chlorine atoms on the phenyl rings (Sipes and Schnellman, 1987; Lutz and Dedrick, 1987). This difference is also seen in the variable persistence of these compounds in nature (Hansen, 1987). Duursma et al. (1991) give an example of very slow depuration of certain PCB congeners in fish (eel).

Of particular interest for the situation in two heavily contaminated Norwegian fjords is the rather slow depuration of octachlorostyrene; with estimated liver and whole fish half-lives of respectively about 140 and 90 - 120 days (Norheim and Roald, 1985). Even this may be optimistic in the light of recent monitoring results from the Frierfjord, showing no response in cod liver more than 3 months after a 95% reduction in discharge amounts (Knutzen and Green, 1991). There are indications that OCS is a more hazardous contaminant in food than HCB (unpublished evaluation from the Norwegian National Institute of Public Health).

Depuration rates for PCDD/PCDFs in fish generally have been found to be considerably shorter than other chlorinated substances with similar degree of hydrophobicity (cf. Opperhuizen and

Sijm, 1990), a strong indication in itself that this group is more subject to metabolization than for instance DDT and most highly chlorinated PCBs.

Further, when studied together, it has been found that non 2,3,7,8 PCDD/PCDFs are eliminated faster than the most toxic congeners (Muir et al., 1985b; Muir and Yarechewski, 1988). Substances without the 2,3,7,8-configuration generally have calculated half-lives of < 5 - 13 days (Muir et al., 1985b; Niimi and Oliver, 1986; Muir and Yarechewski, 1988; Gobas and Schrap, 1990; Sijm et al., 1990). Similar elimination half-lives have been observed for 2,3,7,8-TCDF (12/< 1-3 days, cf. Opperhuizen et al., 1986 and Merhle et al., 1988, respectively) and OCDD/OCDF (< 11 - 15 days, cf. Muir et al., 1985b, 1986; Nimi and Oliver, 1986; Gobas and Scrap, 1990).

Slowest depuration has been observed for 2,3,7,8-TCDD (15/105/≈ 300/15 - 48/≈ 60 - 120 days, cf. in the given order: Opperhuizen et al., 1986 (calculated here); Kleeman et al., (1986a); Kuehl et al., 1987; Mehrle et al., 1988; Batterman et al., 1989 (approximate, read from figures)). Half-lives of the other 2,3,7,8-congeners have been found in the range 10 - 110 days (Muir et al., 1985b, 1990; Opperhuizen et al., 1986; Muir and Yarechewski, 1988, - see also Kuehl et al., 1987).

It is seen that the calculated elimination rate may vary considerably for the same substance. Opperhuizen and Sijm (1990) point out the possible effect on the results from test conditions, in particular exposure concentration and size of test fish (possibly also route of exposure).

Generally, short or intermediate half-lives have been observed for organochlorines in **mussels**. Examples are: Böckman et al. (1976, 90% reduction of HCB in about 10 days, and similar results referred in Knutzen and Green, 1991); Solbakken et al., (1985, < 1 day for lindane); Russel and Gobas (1989, respectively 4 and 10 days for HCB and OCS); Calambokidis et al. (1979, 3 - 50 days for various PCBs, depending on degree of chlorination, 8 - 39 days for commercial PCB mixtures); Kannan et al. (1989, 5 - 26 days for selected PCBs, depending on number of chlorine substitutions and configuration); Berge and Knutzen (1991, 25/33/36 days for HCB/OCS/Deca-chlorobiphenyl).

Quite slow excretion of a short chain, highly chlorinated paraffins in the common mussel has been reported by Renberg et al. (1986). With the same species Berge and Knutzen (1991) observed half-lives of 2,3,7,8-PCDD/PCDFs mostly in the range 10 - 50 days, with extremes of 100 - 270 days for hepta- and octa-congeners.

7. CONCLUDING REMARKS - RESEARCH NEEDS

The log BCF/log K_{ow} relationship for uptake from water of persistent compounds is only approximate and of limited predictive value. Behind the considerable deviations revealed, particularly for superhydrophobic substances (log K_{ow} > 5 - 6), are molecular structure and dimensions in relation to membrane penetration, lipid solubility, variation between species and experimental difficulties (long time to equilibrium), problems with accurate determinations of partitioning coefficients. More refined structure - acitivity relations are needed to describe bioaccumulation potentials of xenobiotics adequately.

At equilibrium between concentrations (activities) of chemicals in sediment and interstitial water uptake in fish comes from ingestion or via pore water, not from direct contact of body surfaces with contaminated sediment.

Generally, uptake from food plays increasing relative role with increasing size of animals and for each higher trophic level. Consequently, food will usually be the predominant source of contaminants in fish top predators.

After reduction in direct discharges it is to be expected that sediment-based pelagic food chains will play a key role for contaminant levels in exploitable fish. It therefore is a need for more knowledge of such food chains: which species are involved, assessment of the importance of possible transfer routes and analysis of contaminant concentrations at different trophic levels.

Within the group of so-called persistent chemicals various pollutants differ very much with respect to depuration rate - observed/calculated half-lives in fish range from a few days to more than two years. It seems perhaps warranted to concentrate research relatively more on the group of really recalcitrant substances in order to characterize them adequately as regards chemical and ecotoxicological properties. Such knowledge will be necessary in assessing the hazard which long-term circulation of these chemicals in the biosphere represents, as well as the time scale for restoring recipients relieved of most direct discharges.

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