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Bioavailability and uptake of hydrophobic organic contaminants in bivalve filter-feeders

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The degree to which aquatic organisms accumulate organic contaminants depends upon rate processes involved in the uptake, distribution and elimination of the contaminant. These processes may be influenced by both abiotic and biotic factors, and may thus differ between locations and organisms. In this paper, environmental and physiological aspects of bioavailability and uptake of hydrophobic organic contaminants in bivalve filter-feeders are reviewed. This includes environmental distribution of contaminants; the relative importance of freely dissolved versus particle-associated contaminant uptake; and the role of physiological regulation of processes involved in contaminant uptake. It appears that more emphasis on considering food as a source of uptake together with particle concentration dependent physiological processes such as water-pumping, filtration and ingestion is important. Thus, contaminant accumulation in filter-feeding bivalves may be better predicted by bioenergetic rather than thermodynamic models.

1. Introduction

Traditionally, aquatic bioaccumulation studies have addressed the uptake of chemicals freely dissolved in the water phase and less attention has been paid to other uptake routes. In the real world, however, organisms are likely to be exposed to multiple sources of contaminants, due to contaminant distribution among different environmental compartments. Therefore, in any model of contaminant accumulation, the bioavailability and relative contribution of different routes of contaminant uptake must be considered.

Accumulation of hydrophobic organic contaminants (HOC) in filter-feeding bivalves is, as for other aquatic organisms, dependent on the concentration, bioavailability, and physicochemical properties of the contaminant (Farrington 1991). However, since filter-feeders pump water to obtain particulate organic matter (POM) utilised as food, particle-associated HOC may significantly contribute to the overall exposure of contaminants. Further, bivalves regulate pumping, filtration and ingestion rates in response to changes in particle concentration, i.e. the functional response (see review by Winter 1978), which will likely influence contaminant accumulation as well. Thus, POM may affect HOC accumulation in mussels by modifying bioavailability and route of exposure due to particle sorption (c.f. Schrap & Opperhuizen 1990), and/or by influencing physiological processes involved in contaminant accumulation. Filter-feeding bivalves are of great ecological and commercial importance and are extensively used in toxicity, bioaccumulation and biomonitoring studies throughout the world. Moreover, the possible role of dense populations of filter-feeding bivalves in contaminant cycling, adds additional emphasis to this topic.

In this paper, factors governing bioavailability and uptake of HOC, e.g. PAHs and PCBs, in bivalve filter-feeders are reviewed. The role of environmental partitioning of hydrophobic organic contaminants affecting the bioavailability and route of exposure to filter-feeding bivalves is discussed, together with factors influencing physiological regulation of pumping, filtration and ingestion rates of the organism itself. Main questions of interest are particle concentration dependent processes, if there are indications of a significant uptake of particle-associated organic contaminants, and if this uptake contributes additively to higher steady-state tissue burden than expected from water exposure alone? Data from both field and laboratory studies are used to explore these questions. Finally, some aspects of different approaches to experimentally assess the relative importance of freely-dissolved versus particle-associated contaminant uptake in bivalve filter-feeders, is discussed.

2. Environmental distribution of hydrophobic organic contaminants; implications for filter-feeders

Due to their physicochemical properties, HOC will distribute among the different compartments, forexample, between water, particulate organic matter, and dissolved organic matter, in the aquatic environment. Environmental factors such as temperature, salinity, and the amount and composition of organic material will influence this distribution (Mackay & Powers 1987, Jaffé 1991, Baxter et al. 1992, Koelmans & Lijklema 1992, Larsson et al. 1992, Bergen et al. 1993b, MacDonald et al. 1993, Millard et al. 1993). The tendency of hydrophobic contaminants to escape water, i.e. the hydrophobicity, highly favours partitioning to compartments rich in organic material. This partitioning distribution among compartments will normally reach apparent equilibrium within weeks, independent of the local variations in concentrations (Duursma et al. 1989).

POM has a substantial capacity to sorb HOC (Herbes 1977). Thus, hydrophobic contaminants will end up at orders of magnitude higher concentrations in algae (Lederman & Rhee 1982, Swackhamer & Skoglund 1993), and seston (Broman et al. 1991, Koelmans & Lijklema 1992), compared with the concentration of freely-dissolved contaminants in water. Further, the contaminant partitioning behaviour between water and particulate matter is apparently particle-concentration dependent (Lederman & Rhee 1982, Mackay & Powers 1987, Millard et al. 1993) in such a way that the partitioning coefficient decreases with increasing particle concentrations, which could be thought of as a dilution of the contaminant among the particles. Despite this dilution, however, the total fraction of particle-associated contaminants in a system increases with increasing particle concentrations (Richer & Peters 1993).

Filter-feeding bivalves remove particulate matter at high rates from the water column. The filtered particles are either retained for ingestion or egested as pseudofaeces. Of the ingested material, some is converted into biomass and a portion is excreted as faeces and dissolved waste products (Kautsky & Evans 1987, Dame & Dankers 1988, Dame et al. 1991). Thus, dietary exposure to particle-associated organic contaminants may present a significant source of uptake in filter-feeding bivalves, effectively retaining large amounts of highly contaminant-enriched particulate matter (c.f. Landrum et al. 1991).

The filtration rate, which to a large extent is particle-concentration dependent, dictates the rate of exposure to both dissolved and particle-associated contaminants. Most filter-feeders have a maximum filtration rate at low food concentrations (Griffiths et al. 1980, Navarro & Winter 1982, Sprung & Rose 1988, Riisgård 1991). The decrease in filtration rates at high food concentrations correlates with a reduced valve gap (Riisgård 1991) and changes in pumping activity, with more frequent interruptions, or a lower level of water transport (Sprung & Rose 1988). Also, the organic matter retained is not necessarily consumed. Ingestion rates do increase slightly with an increase in food concentrations

(Navarro & Winter 1982, Sprung & Rose 1988); this is counterbalanced by a decrease in dietary assimilation efficiency such that assimilation rates finally are nearly constant and independent of the food concentration (Navarro & Winter 1982). In addition, an ability to sort out particles rich in organic material has been reported for blue mussels (Kiørboe et al. 1980), and zebra mussels (Ten Winkel & Davids 1982).

The contaminant uptake in filter-feeding bivalves is also likely to be species and size related, due to interspecific differences in habitat, pumping activity, ingestion, assimilation, lipid content, and metabolic demand, (c.f. Capuzzo et al. 1989, Muncaster et al. 1990, Widdows & Donkin 1992). In experiments with zebra mussels, *Dreissena polymorpha*, smaller individuals or individuals with higher lipid content had both higher uptake rates and greater accumulation of contaminants (Bruner et al. 1994a). Moreover, contaminant uptake has been shown to increase with temperature (Fisher et al. 1993). Bruner et al. (1994b), have shown that particle-associated contaminants are available for dietary uptake in filter-feeding bivalves. Contaminant assimilation efficiencies will, however, vary widely depending on the properties of the contaminant and the particulate matter (c.f. Farrington 1989, Widdows & Donkin 1992, Bruner et al. 1994b).

Thus, complex interactions of biotic and abiotic factors will govern bioavailability and uptake of HOC in bivalve filter-feeders. In particular, the food-concentration dependent filtration and assimilation processes described above, together with contaminant partitioning behaviour, which also is particle-concentration dependent, will likely regulate bioavailability and uptake of organic contaminants to filter-feeding bivalves, possibly altering not only the total contaminant burden, but also the relative importance of different uptake routes.

3. Bioavailability and uptake of organic contaminants in bivalve filter-feeders

3.1. A conceptual model of contaminant uptake

The above-mentioned mechanisms may be summarised in a conceptual model of the relative importance of freely-dissolved versus particle-associated contaminant uptake in bivalve filter-feeders:

— Uptake of freely-dissolved contaminants. At low particle concentrations, the major exposure pathway of HOC is likely via the gills, i.e. the dissolved fraction in the water filtered by the organisms. The reasons for this are: a high filtration rate, i.e. a large volume of water pumped over the gills, and a low fraction of contaminants associated to particulate matter, both increasing the exposure to dissolved contaminants available for gill uptake. Concurrently, a low feeding rate results in a low exposure to particle-associated contaminants available for gut uptake.

— Uptake of particle-associated contaminants. At high particle concentrations, the pumping rate is lower and the fraction of particle-associated contaminants higher, implying a lower exposure to dissolved contaminants available for gill uptake. On the other hand, the feeding rate is higher, increasing the exposure to particle-associated contaminants available for gut uptake. Thus, the exposure is shifted towards a higher relative importance of particle-associated contaminant uptake as the particle concentration increases. Of course, this model is only applicable within a certain particle concentration, range. At very low particle-concentrations bivalves may cease to filter for long periods, and at high particle concentrations, some of the particulate matter cleared is rejected as pseudofaeces.

In the following sections, bioavailability and uptake of freely-dissolved versus particle-associated contaminants in bivalve filter feeders are discussed in more detail.

3.2. Bioavailability and uptake of freely-dissolved contaminants

The primary assumption underlying bioconcentration studies is that the compound is available for uptake directly from the water phase. The equilibrium partitioning concept, first proposed by Hamelink et al. (1971), has been applied in many bioaccumulation studies, and found to be applicable to contaminant bioaccumulation from water. It is widely accepted that freely-dissolved HOC with a molecular weight below approximately 600 g/mole can readily diffuse across biological membranes (e.g. Spacie and Hamelink 1985), and thus, are readily available for gill uptake in filter-feeding bivalves (Swinehart & Cheney 1987). The contaminant lipophilicity measured as the n-octanol/water partition coefficient, K_{ow}, is often used as an indicator of the tendency of organic chemicals to bioaccumulate, (e.g. Neely et al. 1974, Geyer et al. 1982, Mackay 1982, Geyer et al. 1991). However, this has usually been tested in simple laboratory systems containing the dissolved compound, and with the assumption that accumulated compounds are taken up from the dissolved phase only.

Indeed, many laboratory and field studies with filter-feeding bivalves have suggested that organic contaminants in the water phase is the predominant source of uptake; (e.g. Stegeman & Teal 1973, Fossato & Canzonier 1976, Burns & Smith 1981, Boryslawsky et al. 1985, Pruell et al. 1986, Muncaster et al. 1990, Kauss & Hamdy 1991, Bergen et al. 1993a), and that organic contaminants are accumulated by simple equilibrium partitioning between water and organism lipids. None of these studies, however, have been performed under such conditions that the relative importance of freely-dissolved contaminant uptake could be compared to particle-associated contaminant uptake.

Further, the distribution pattern of contaminant congeners with different physico–chemical properties was suggested to be an indicator of the exposure route. For example, lower-chlorinated PCB congeners (penta- and hexa-CBs) appeared to dominate in filter-feeding bivalves suggesting that direct water partitioning would predominate in mussels with the preferential uptake of less-chlorinated congeners, which are more water soluble and thus will more readily be in a freely-dissolved phase available for gill uptake (Porte & Albaigés 1993). A selective accumulation of low-chlorinated PCBs has also been reported by others, (e.g. Langston et al. 1978, Pruell et al. 1986, Tanabe et al. 1987, Bergen et al. 1993a). A consistent relationship has been observed between dissolved PCB concentrations and tissue concentrations in filter-feeding blue mussels, *Mytilus edulis*. However, when comparing the relative congener distribution, Bergen et al. (1993a) found that mussels had higher similarity to the PCB-congener distribution in the particulate phase compared with that in the dissolved phase, whereas Pruell et al. (1986) found a higher congener similarity to that detected in the dissolved phase. Further, Calambokidis et al. (1979) suggested that a selective retention of higher-chlorinated biphenyls appeared to be the result of the lower water solubility of these components, which is also supported by Fisher et al. (1993) who found that uptake clearance of organic contaminants in zebra mussels was positively correlated with K_{ow}. Therefore, the congener distribution pattern is not likely to be a useful tool in distinguishing between different routes of uptake, as it probably reflects the congener bioavailability and partitioning as much as differences in uptake routes.

3.3. Bioavailability and uptake of particle-associated contaminants

Sorption to POM generally reduces the biological availability of hydrophobic organic contaminants to gill-breathing aquatic organisms (e.g. Schrap & Opperhuizen 1990). The proposed mechanism is that the steady-state level of the contaminant, to a large extent, is determined by equilibrium partitioning via the gills, and sorption of organic contaminants to particles reduces the bioavailability by reducing the amount of freely-dissolved compounds available for accumulation across the gills (cf. Pärt 1989).

If the POM represents a food source for the organism, as in the case of filter-feeding bivalves, particle-associated contaminants may still be available via assimilation in the gastrointestinal tract. In fact, dietary-associated organic contaminants may be readily absorbed in the gut of aquatic organisms due to a passive diffusion driven by a higher fugacity in the gastrointestinal tract as a result of food digestion (Clark & Mackay 1991, Gobas et al. 1993).

In studies conducted using deposit-feeding bivalves (e.g. Ekelund et al. 1987, Boese et al. 1990), an increased uptake of contaminants were found in the presence of sediments. For example, particle-associated hydrophobic contaminants largely contributed to the total tissue burden in the deposit-feeding tellinid clam, *Macoma nasuta*, and the uptake of HCB via the gut from ingested solids was estimated as the single most important route of uptake, accounting for 63–84% of the total uptake (Boese et al. 1990).

However, few studies have addressed the question of bioavailability and uptake of particle-associated organic contaminants in filter-feeding bivalves. Bruner et al. (1994b) demonstrated in experiments with zebra mussels, *Dreissena polymorpha*, that particle-associated organic contaminants are available through dietary uptake, with a higher contaminant assimilation efficiency from algae than from resuspended sediment. Further, Widdows et al. (1982) found that both alkane and aromatic hydrocarbon concentrations were consistently higher in the tissue of blue mussels, *Mytilus edulis*, simultaneously exposed to the water-accommodated fraction of North Sea crude oil and algal food. High doses of chlorinated hydrocarbons in particulate food of zebra mussels, *Dreissena polymorpha*, resulted in initial non-equilibrium peak concentrations well in excess of that noted for water uptake alone (Brieger & Hunter 1993), with the uptake rate of PCB 77 following the order: sediment > food > water. Moreover, the uptake of benzo(a)pyrene adsorbed in sediment was more than 5 times higher in *Mytilus galloprovincialis* when sediment particles were suspended by stirring compared, with non-stirring conditions (Narbonne et al. 1992). In limnocorral studies with dibenzo dioxins/furans, Muir et al. (1992) found high lipid/organic carbon standardised bioavailability indices for the fresh-water mussel *Anodonta grandis* filtering organic particles, indicating food as a significant source of uptake. In a field experiment, Prest et al. (1992) simultaneously exposed freshwater clams, *Corbicula fluminea*, and a semi-permeable membrane sampling device (SPMD with triolein) at three sites on the Sacramento and San Joaquin Rivers. On a lipid-normalised basis, levels of organochlorine compounds were approximately 10 to 100 times higher in clams than in the SPMDs. Further, the clams had higher relative concentrations of higher-chlorinated PCB congeners. A possible explanation is that a large portion of the contaminants, particularly the higher chlorinated PCBs, are particle-associated and thus may not readily diffuse into the SPMD, whereas particle-associated contaminants may be retained and assimilated in the gut by the filter-feeder.

Contrary to the findings above, Fisher et al. (1993) found that uptake of hexachlorobiphenyl from water by the zebra mussel, *Dreissena polymorpha*, was impeded by sorption to uncontaminated algae, and lower uptake rates and higher elimination rates were found when organic contaminants where assimilated from algae compared with direct uptake from water. However, since assimilation from contaminated food is thought to be slow, it is likely that assimilation from algae did not contribute significantly to the body burden of zebra mussels during the short exposure time (6 hr).

3.4. Relative importance of freely-dissolved and particle-associated contaminant uptake

To conclude, many papers suggest that the predominant contaminant uptake is from the water phase, without comparing the relative uptake of freely-dissolved with that of particle-associated contaminants. The few studies that have addressed the question of bioavailability and uptake of particle-associated organic contaminants in filter-feeding bivalves show that particle-associated organic contaminants can be efficiently taken up from the diet. Assimilation efficiency will vary depending on the organism concerned, the properties of the contaminant, and the properties of the particulate matter. Both enhanced and reduced uptake of organic contaminants have been reported in the presence of particulate matter, and the significance of particle-associated contaminants in overall contaminant uptake remains unclear. Nevertheless, Broman et al. (1990) suggest that the diet is the predominant route of contaminant exposure to blue mussels in the Baltic Sea, and a predominant uptake of algae-associated contaminants has been indicated in a model of Lake Erie zebra mussels (Bruner et al. 1994b).

The presence of POM will affect the distribution of contaminants, and may alter the relative importance of different exposure routes by reducing the gill uptake and increasing gastrointestinal exposure. If the contaminants are readily available for dietary assimilation, and in cases where the elimination rate is low, the dietary exposure may become significant and contribute to a higher contaminant burden in the organism than expected from water exposure alone.

However, it is not an easy task to determine the relative contribution of different routes of uptake in multiple sources of contaminant exposure. To accurately distinguish between different routes, for example, dietary uptake with that taken up from water, even if the food is contaminated prior to addition to the test medium, the contaminant will strive to equilibrate between food and water confounding the routes. This problem is pronounced in studies with filter-feeders as particulate food is used, and sorption processes between POM and water are often very rapid, with an apparent equilibrium established within hours (Lederman & Rhee 1982, Autenrieth & DePinto 1991, Koelmans & Lijklema

1992, Koelmans et al. 1993, Richer & Peters 1993), even though desorption has been reported to be somewhat slower (Lederman & Rhee 1982, Autenrieth & DePinto 1991). At the same time, the duration of the experiments must be sufficient to allow complete food digestion and intestinal passage. Contaminants must have sufficient time to desorb from the particulate matrix before diffusion across the gut lining can take place and to allow for an increase of fugacity, due to food digestion, driving the diffusion of contaminants across the gastrointestinal membranes (Clark & Mackay 1991, Gobas et al. 1993). Moreover, ecologically relevant food and contaminant concentrations must be used in dietary uptake experiments because: (1) filtration and assimilation rates will differ due to differences in particle concentrations (Winter et al. 1977, Griffiths & King 1979, Griffiths et al. 1980, Riisgård 1991), (2) the use of unnaturally high algae concentrations will lead to sub optimal conditions for the filter-feeders (Riisgård 1991), and (3) the contaminant partition behaviour between water and particulate matter is particle-concentration dependent (Lederman & Rhee 1982, Mackay & Powers 1987, Millard et al. 1993).

Bioenergetic-based bioaccumulation models appear to be a promising tool for assessing the relative importance of different uptake routes. The advantage with these models is the incorporation of organism behaviour, such as filtration, assimilation and egestion. In contrast, equilibrium-partitioning models only correlate with contaminant concentrations (and lipid content) of the organism. To more strictly distinguish between water- and particle-associated uptake, a dual-tracer technique with ^{14}C- and tritium-labelled contaminants may be useful. To my knowledge, a dual-tracer technique has so far only been used to distinguish between cadmium uptake via food and seawater in *Mytilus edulis*, by labelling algae with ^{109}Cd and sea water with ^{115}Cd (e.g. Borchardt 1983).

4. Conclusions

Despite the large ecological role of filter-feeding bivalves, and their extensive use in toxicity testing, bioaccumulation studies, and environmental monitoring programs, remarkably little information concerning bioavailability, and different uptake routes of organic contaminants to these organisms were found in the literature. Several studies have suggested contaminants in the water phase as the dominant source of uptake. However, in almost all experimental designs reviewed, it was not possible to distinguish uptake of freely-dissolved from that of particle-associated contaminants, or to assess the relative importance of different uptake routes to the total contaminant burden.

It has been shown that particle-associated organic contaminants are available for dietary uptake and that the contaminant-assimilation efficiency can be high, but will vary depending on the organism concerned, the properties of the contaminant and the properties of the particulate matter. Both enhanced and reduced uptake of organic contaminants have been reported in the presence of particulate matter, and the significance of particle-associated contaminants in the overall contaminant uptake is not fully clear.

It is obvious that more attention should be paid to particle-associated contaminants as a source of exposure, and that particle-concentration dependent processes such as filtration and ingestion must be considered in the assessment of contaminant uptake by filter-feeding bivalves. In order to more accurately predict bioaccumulation in mussels, bioenergetic models incorporating functional response should be used instead of simple equilibrium partitioning models.

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