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Bioaccumulation of Phthalate Esters in Aquatic Food-Webs

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This chapter explores the bioaccumulation behavior of several phthalate esters in aquatic food-webs. It includes: (i) a compilation of bioconcentration data from reported laboratory studies in the literature, (ii) an overview and discussion of the results from a recently completed food-web bioaccumulation field study, and (iii) an analysis of the results of a bioaccumulation modeling study. The study concludes that laboratory and field studies indicate that phthalate esters do not biomagnify in aquatic food-webs. Higher molecular weight phthalate esters (DEHP, DnOP, and DnNP) show evidence of trophic dilution in aquatic food-webs, which is consistent with findings from laboratory and modeling studies which indicate that metabolic transformation is a key mitigating factor. Bioaccumulation patterns of DBP, DiBP, and BBP indicate no significant relationship with trophic position consistent with a lipid-water partitioning model. The lowest molecular weight phthalate esters (DMP and DEP) show bioaccumulation factors in laboratory and field studies that are greater than predicted from a lipid-water partitioning model. The considerable variability in the field-derived bioaccumulation factors (BAFs) for lower molecular weight phthalate esters across aquatic species suggests that species-specific differences in metabolic transformation can have a significant effect on observed bioaccumulation. With some exceptions discussed below, the bioconcentration and bioaccumulation factors of the phthalate esters discussed in this paper are below the UNEP bioaccumulation criterion of 5000. The low bioavailability of the high-molecular weight phthalate esters in natural waters is the main reason why the BAFs of the higher molecular weight phthalate esters are below the UNEP bioaccumulation criterion. Since the intention of the bioaccumulation criteria is to identify substances as being "bioaccumulative", if they (like PCBs) biomagnify in the food-web then current evidence supports the conclusion that phthalate esters do not appear to be "bioaccumulative".

Keywords. Bioaccumulation, Biomagnification, Phthalate Esters, Aquatic Food-Webs, Fish

Abbreviations

BAFs	Bioaccumulation Factors
BBP	Butylbenzyl Phthalate
BCFs	Bioconcentration Factors
BSAF	Biota-Sediment Accumulation Factor
DBP	Di-*n*-Butyl Phthalate
DEHP	Di-2(Ethylhexyl) Phthalate
DEP	Diethyl Phthalate
DiBP	Diisobutyl Phthalate
DMP	Dimethyl Phthalate
DnNP	Di-*n*-Nonyl Phthalate
DnOP	Di-*n*-Octyl Phthalate
LRTAP	Long-Range Transboundary Air Pollution Protocol
MS-MS detection	Mass Spectrometry-Mass Spectrometry detection
PCBs	Polychlorinated Biphenyls
POPs	Persistent Organic Pollutants
QA/QC	Quality Assurance/Quality Control
UNEP	United Nations Environmental Program

1 Introduction

Dialkyl phthalate esters are hydrophobic substances with octanol-water partition coefficients ranging between $10^{1.61}$ for dimethyl phthalate esters to values exceeding 10^8 for congeners like diundecyl phthalate ester and ditridecyl phthalate ester [1]. Due to their hydrophobicity, phthalate esters are often believed to have a high potential to bioconcentrate and bioaccumulate in aquatic organisms.

The degree of bioaccumulation and the mechanism by which phthalate esters are absorbed and retained by aquatic organisms is of considerable importance as the United Nations Environmental Program (UNEP) long-range transboundary air pollution protocol (LRTAP) on POPs as well as domestic legislation in Canada (Canadian Environmental Protection Act, 1999) and several countries [2] aim to eliminate substances from commerce that are bioaccumulative, persistent, and toxic. The bioaccumulation criterion identifies chemicals as "bioaccumula-

tive" if they exhibit bioaccumulation or bioconcentration factors (BAFs or BCFs) greater than 5000 in aquatic organisms. In absence of BAF or BCF data "bioaccumulative" substances are defined as compounds with octanol-water partition coefficients (K_{OW}s) greater than 10^5. The intent of these legislative efforts is to identify substances that biomagnify in aquatic food-webs. Biomagnification is the process in which the lipid-normalized concentration of the chemical increases with each step in the food-web. The significance of biomagnification is that organisms at the top of the food-web are exposed to chemical concentrations that are greater than those at lower trophic levels. The scientific rationale for the bioaccumulation criterion is based on findings for persistent organochlorines, such as PCBs and chlorobenzenes, which indicate that persistent substances biomagnify in aquatic food-webs if laboratory-derived bioconcentration factors exceed approximately 5000 or the octanol-water partition coefficient of the substance exceeds approximately 10^5. Several authors have suggested that the bioaccumulation behavior of phthalate esters is not comparable to that of persistent organochlorines such as PCBs. Laboratory studies, which in most cases involved one particular phthalate ester (i. e., diethylhexyl phthalate ester), have pointed out that the bioaccumulation factors of phthalate esters are typically less than expected from their lipid-water partitioning properties [3]. Metabolism and a reduced bioavailability of phthalate esters have been proposed to be the main factors causing the lower than expected bioaccumulation factors of phthalate esters [3 – 8]. However, field studies to confirm this hypothesis have not previously been reported.

In this chapter, we will explore the bioaccumulation behavior of several phthalate esters in aquatic food-webs. We will present a compilation of bioaccumulation data from reported laboratory studies in the literature and from a recently completed bioaccumulation field study that we conducted in a marine food-web. The objective of our analysis is to gain insights into the mechanisms of phthalate ester uptake, elimination, and bioaccumulation in aquatic food-webs. This information can be useful in assessing the bioaccumulative potential of this group of ubiquitous and widely used substances relative to other chemical classes.

2 Bioaccumulation Nomenclature

Bioconcentration is defined as the process in which an aquatic organism achieves a concentration level that exceeds that in the surrounding water as a result of exposure of the organism via the respiratory surface and the skin [9]. Bioconcentration refers to a condition, usually achieved under laboratory conditions, in which the organism is exposed to a chemical substance in the water, but not in its diet. The underlying mechanism of this process is the lipid-water partitioning property of the substance [10]. A number of depuration processes including egestion in fecal matter, deposition in eggs, growth, and metabolic transformation can interfere with the lipid-water partitioning behavior of the chemical substance, typically resulting in bioconcentration factors that are less than the corresponding lipid-water partition coefficient [9].

Bioaccumulation refers to the process by which the chemical concentration in an aquatic organism achieves a level that exceeds that in the water as a result of chemical uptake through all routes of chemical exposure (e. g., dietary absorption, transport across the respiratory surface, dermal absorption). Bioaccumulation takes place under field conditions. It is a combination of chemical bioconcentration and biomagnification [9].

Biomagnification refers to the process by which the chemical concentration in the predator exceeds that in the prey organisms it consumes. In this chapter biomagnification refers to field conditions where the predator and prey organisms are simultaneously exposed to chemical via both water and diet. Biomagnification has been observed in aquatic and terrestrial food-webs in the field [11, 12] and under laboratory conditions and mechanisms for this process have been postulated [13, 14].

Food-web bioaccumulation is the process by which the chemical concentration in organisms increases with increasing trophic level. It is the result of a sequential series of biomagnification events.

Trophic dilution is the process by which the chemical concentration in organisms drops with increasing trophic level. It occurs when the chemical concentration in the predator remains below the concentration in the prey, typically as a result of metabolic transformation of the chemical in the predator.

3 Phthalate Ester Nomenclature

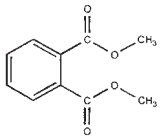
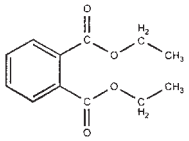
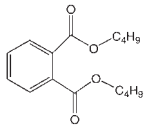
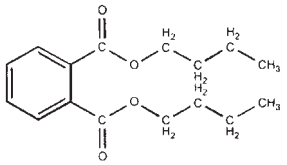
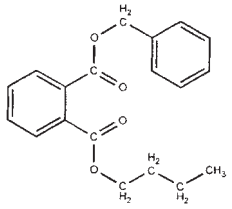
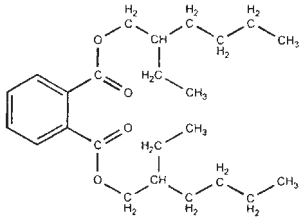
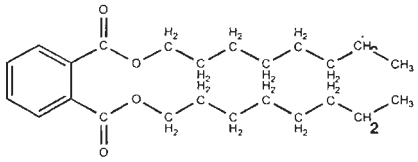
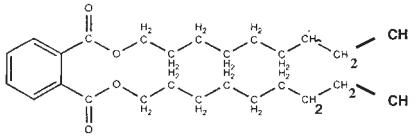
Phthalate esters include a large number of substances that share a common chemical core structure. The phthalate esters discussed in this document and their chemical structures and acronyms are listed in Table 1.

4 Bioconcentration Studies

Several laboratory studies have investigated the bioconcentration of phthalate esters in various fish species, algae, macrophytes, polychaetes, molluscs, crustacean, aquatic insects, and other organisms. The data reported in these studies have been compiled and reviewed in Staples et al. [3].

When evaluating the results from laboratory bioconcentration studies, it is important to recognize some of the characteristics and experimental artifacts of bioconcentration studies for phthalate esters. First, the majority of reported bioconcentration studies involve only one particular phthalate ester, that is, DEHP (Table 1). Bioconcentration data for other phthalate esters are scarce, causing much of the experimental evidence on the bioaccumulation of phthalate esters to rely on observations for a single congener. Secondly, the majority of the reported studies use radiolabeled phthalate ester congeners. Because phthalate esters can be subject to metabolic transformation in organisms, BCFs based on total radioactivity (i. e., radioactivity from the parent substance and its metabolites) can be greater than BCFs based on radioactivity of the parent (i. e., unmetabolized) compound alone. BCFs determined with the use of radioactive test sub-

Table 1. Chemical structure and acronyms of phthalate esters

Phthalate Ester	Abbreviation	Chemical Structure
Dimethyl Phthalate	DMP	
Diethyl Phthalate	DEP	
Di-iso-butyl Phthalate	DiBP	
Di-*n*-Butyl Phthalate	DBP	
Butyl Benzyl Phthalate	BBP	
Di(2-Ethylhexyl) Phthalate	DEHP	
Di-n-Octyl Phthalate	DnOP	
Di-n-Nonyl Phthalate	DnNP	

stances may therefore overestimate the BCF of the parent substance. Third, the aqueous solubility of especially the higher molecular weight phthalate esters is low and the water concentrations used in some of the bioconcentration tests significantly exceed the aqueous solubility. Results from these bioconcentration tests are difficult to interpret. On one hand, water concentrations above the aqueous solubility indicate that a considerable fraction of the total chemical concentration in the water is not available for uptake via the respiratory surface. On the other hand, phthalate esters can form emulsions when concentrations are in excess of the water solubility due to their surface-active properties. These emulsions can create micelles that may adhere to the outer surface of the organism and which may be also ingested by organisms. As a result, it is unclear to what degree BCFs determined at concentrations above the water solubility are representative of field conditions. Fourth, water concentrations of phthalate ester that are constant over the exposure duration are typically not achieved in the bioconcentration tests due to the low phthalate ester concentrations in the water, rapid absorption by fish, and degradation in the water phase. Ignoring this experimental artifact in deriving the BCF can lead to an underestimate of the BCF, especially when nominal or initial water concentrations are used to derive the BCF or uptake rate constants [15]. Fifth, the exposure duration in most bioconcentration tests is relatively short and typically much shorter than exposure conditions in the field. For example, a number of bioconcentration tests have been conducted over a period of one day or less while a 28 day period is generally recommended for bioconcentration studies [16]. Experiments that use short exposure times have a tendency to underestimate the actual BCF, since steady-state conditions may not be achieved.

To eliminate some of the experimental artifacts of laboratory bioconcentration tests in the analysis of reported bioconcentration data, we plotted BCFs in aquatic macrophytes, algae, benthic invertebrates, and fish (Fig. 1), and then eliminated BCF data determined under conditions in which (i) the water concentration exceeded the water solubility and (ii) the exposure time was less than three days. The remaining BCF data are presented as a function the chemical's octanol-water partition coefficient in Fig. 1. Figure 1 also shows the BCF expected if phthalate esters simply partition between the water and lipids of the organisms. A 5% lipid content is assumed. Figure 1 illustrates a number of characteristics of the bioaccumulation behavior of phthalate esters. First, despite the availability of a large number of experimental BCF data, there are few data that meet basic data quality criteria. This illustrates the experimental difficulties of measuring BCFs for phthalate esters. Secondly, reported BCFs for individual phthalate esters exhibit a large variability. This variability has also been noticed by other authors. For example, Karara and Hayton [17–18] report BCFs for DEHP in 1-5 g Sheepshead Minnow (*Cyprinodon variegatus*) of 6–637 L kg^{-1} wet weight within a temperature range of 10–23 °C. Thirdly, the reported BCFs of the higher molecular weight phthalate esters are below those expected from lipid-water partitioning. This has been explained by metabolic transformation of phthalate esters [4] and by a reduced bioavailability of phthalate esters in the bioconcentration tests [5–8, 20, 21]. Fourth, the BCFs for DMP and DEP are approximately an order of magnitude greater than expected from simple lipid-to-water partitioning.

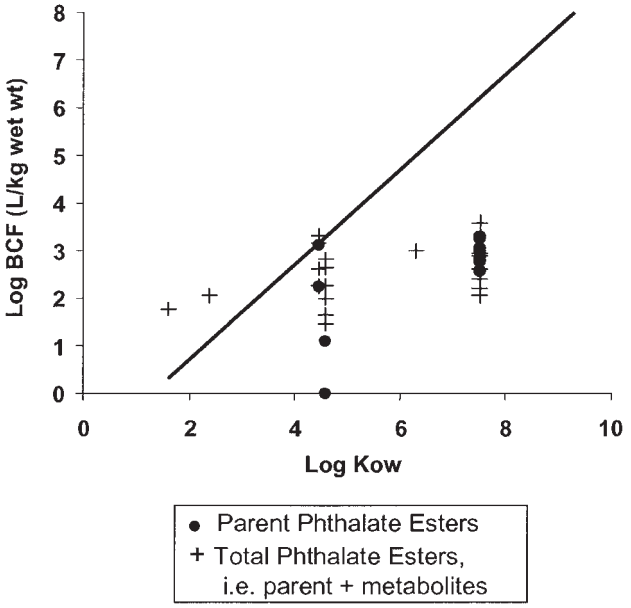


Fig. 1. Laboratory-derived bioconcentration factors (BCFs) of parent (●) and total (i.e., parent phthalate ester and metabolites), (+) phthalate esters in various aquatic organisms. The *solid line* represents the lipid-water partitioning assuming a 5% lipid content (i.e., $BCF = 0.05\ K_{OW}$)

Fifth, when bioconcentration factors of individual congeners are compared between different taxa, it appears that bioconcentration factors for the higher molecular weight phthalate esters in benthic organisms are greater than those in fish. The latter has also been observed by Staples et al. [3] and Wofford et al. [21] who explained these observations by inter-species differences in metabolic transformation rates. Finally, the bioconcentration factors are generally less than 5000.

5 Dietary Bioaccumulation Studies

While the bioconcentration of phthalate esters has been investigated in many studies, the dietary bioaccumulation of phthalate esters has received little attention. Macek et al. [23] examined the dietary transfer of DEHP from daphnids to bluegills (*Lepomis macrochirus*) and concluded that the contribution of the dietary route to the equilibrium body burden in the bluegill may be small. Gloss and Biddinger [24] investigated dietary transfer in daphnids that were feeding on dihexyl phthalate ester-contaminated algae. Perez et al. [25] suggested that dietary exposure was responsible for seasonal differences in the accumulation of DEHP in marine biota in microcosm studies. Staples et al. [3] conducted theoretical calculations to show that as much as 60% of the DEHP exposure in predators could be derived from the diet. They further argued that the general increase

in the rate of metabolic transformation with increasing trophic level may result in trophic dilution in which organisms at the top of the food-web contain lower concentrations than those in organisms at lower levels.

6 Bioaccumulation Studies from Sediments

Uptake and bioaccumulation of sediment-associated phthalate esters has been investigated by Woin and Larson [26] and Brown et al. [27] in dragon flies and chironomid larvae. Based on these data, Staples et al. [3] estimated that the lipid and organic carbon-normalized biota-sediment-accumulation factor (BSAF) for DEHP is 0.1 kg organic carbon (OC) kg^{-1} lipid in dragonflies and 0.5 kg organic carbon kg^{-1} lipid for DEHP and diisodecyl phthalate ester in chironomids. They concluded that these values are lower than the theoretical BSAFs based on an equilibrium partitioning value of 1.0 kg organic carbon kg^{-1} lipid [28], and suggested that metabolic transformation is a plausible explanation for this discrepancy.

7 Food-Web Bioaccumulation Studies

A field study to assess the food-web bioaccumulation of a range of phthalate esters was recently carried out by our research group. The details of the study can be found in Mackintosh [29]. The study involved the collection and subsequent chemical analysis of phthalate esters in water, sediments, algae, plankton, filter feeders (mussels), deposit feeders, forage fish, benthic feeding fish and predatory fish, and carnivorous water fowl in a marine embayment referred to as False Creek. Table 2 lists the species included in the study. With the exception of the dogfish, all species selected can be considered resident species. Environmental media were collected from three different stations in the embayment with a sampling frequency of three or four samples per site. Since inter-site variability in concentration was not a significant factor, concentration data were reported for all stations combined, representing a sample size of 12 for sediment and water and nine for the biota investigated. The trophic status of the organisms (Table 2) was identified by applying the trophic positioning model by Vander Zanden and Rasmussen [30] to dietary composition data from various studies [31 – 38]. A conceptual diagram of the food-web is presented in Fig. 2. The study focused on eight individual phthalate esters, that is, DMP, DEP, DiBP, DBP, BBP, DEHP, DnOP, and DnNP. Water concentration measurements identified dissolved and particulate-bound phthalate ester fractions in the water [29].

7.1 Biota-Sediment-Accumulation

Figure 3 shows the biota-sediment-accumulation factors (BSAFs) of phthalate esters in a range of benthic invertebrate species as a function of the seawater-corrected octanol-water partition coefficient. Octanol-water partition coeffi-

Table 2. Names of species and their trophic position included in the bioaccumulation field study

Species common name	Species Latin name	Trophic position
Green algae	Enteromorpha intestinalis	1.00
Brown algae	Nereocystis luetkeana & Fucus gardneri	1.00
Phytoplankton		1.00
Minnows		2.33
Shiner perch	Cymatogaster aggregata	
Pacific staghorn sculpin	Leptocottus armatus	
Cutthroat trout	Salmo clarki clarki	
Three spine stickleback	Gasterosteus aculeatus	
Whitespotted greenling	Hexogrammos stelleri	
Starry flounder	Platichthys stellatus	
Manila clams	Tapes philippinarum	2.40
Blue mussels	Mytilus edulis	2.48
Pacific oysters	Crassostrea gigas	2.48
Geoduck clams	Panope abrupta	2.53
Pile perch	Rhacochilus vacca	3.05
Striped seaperch	Embiotoca lateralis	
Forage fish		3.25
Pacific herring	Clupea harengus pallasi	
Surf smelt	Hypomesus pretiosos pretiosus	
Northern anchovy	Engraulis mordax mordax	
Purple starfish	Pisaster ochraccus	3.47
Surf scoters	Melanitta perspicilata	3.49
Pacific staghorn sculpin	Leptocottus armatus	3.51
Dungeness crabs	Cancer magister	3.55
Flatfish		3.64
English sole	Pleuronectes ventulus	
Starry flounder	Platichthys stellatus	
Whitespotted greenling	Hexogrammos stelleri	3.81
Spiny dogfish	Squalus Acanthias	4.07

cients of phthalate esters in seawater were derived from those measured in freshwater [1] following Xie [39]. The observed BSAFs are shown in relation to the BSAF based on simple organic carbon-lipid partitioning (i.e., BSAF = 1/0.35 = 2.86 kg OC kg^{-1} lipid) [28, 40–43]. It shows that among the various benthic species, the BSAFs in geoduck clams are the highest. The BSAFs in geoduck clams are the lowest for DMP and then appear to increase with increasing log K_{OW} to values that, with the exception of DEHP, are not statistically different from 2.86. BSAFs for the other benthic species are significantly lower than those in geoduck clams and show a parabolic relationship with K_{OW} with maximum BSAFs for DBP, DiBP, and BBP. Figure 3 illustrates that there is a substantial variability in the BSAFs among benthic organisms. Sediment burying invertebrates like the geoduck clams and Manila clams appear to exhibit higher BSAFs than the invertebrates (e.g., Dungeness crabs) inhabiting surficial sediment. Filter feeding benthic invertebrates such as the mussels and oysters exhibit intermediary BSAFs.

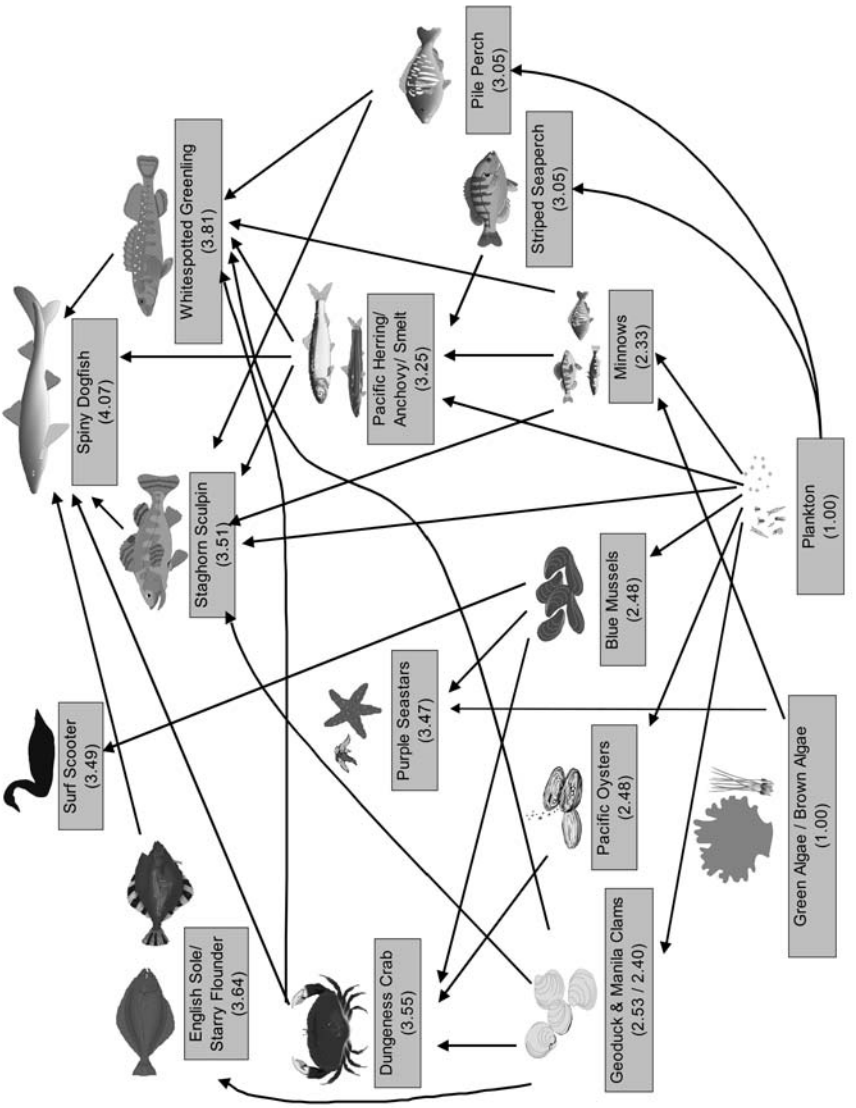


Fig. 2. A simplified conceptual diagram of the food-web interactions in the False Creek food-web

One of the contributing factors to the differences in the observed BSAFs between the species is the chemical disequilibrium that appears to exist between the sediments and the overlying water. This disequilibrium is illustrated in Fig. 4, which shows the observed sediment-water distribution coefficients (expressed in terms of L kg^{-1} organic carbon) in relation to the sediment-seawater partition coefficients (L kg^{-1} organic carbon), derived from the seawater corrected octanol-water partition coefficients according to Seth et al. [44]. A sediment-water disequilibrium occurs if the sediment-water distribution coefficient exceeds the chemical's sediment-water partition coefficient. It can be expressed by the degree

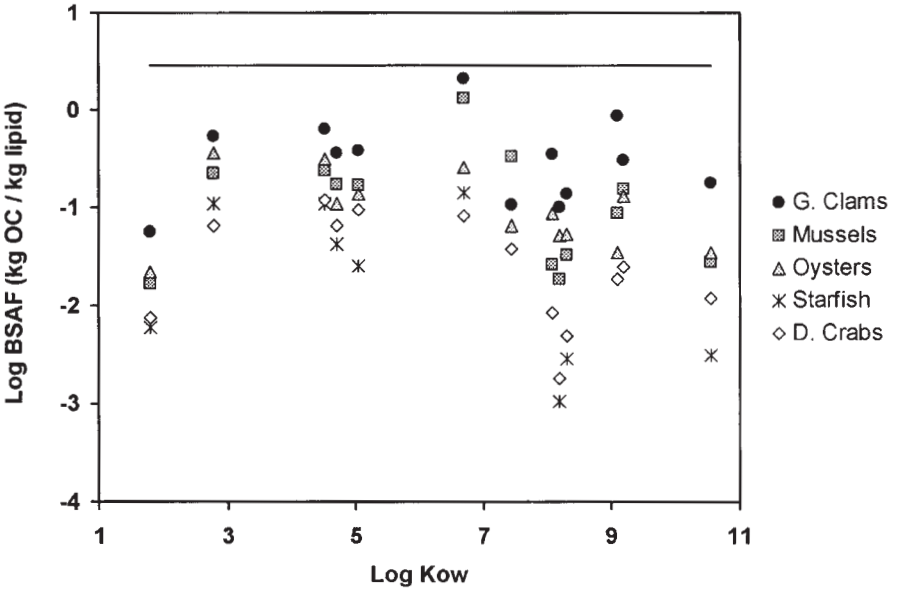


Fig. 3. Biota-sediment-accumulation factors (BSAFs) in units of kg organic carbon kg^{-1} lipid of phthalate esters in a range of benthic invertebrate species as a function of the octanol-seawater partition coefficient. The *solid line* represents the sediment-organism equilibrium partition coefficient (BSAF = 2.86)

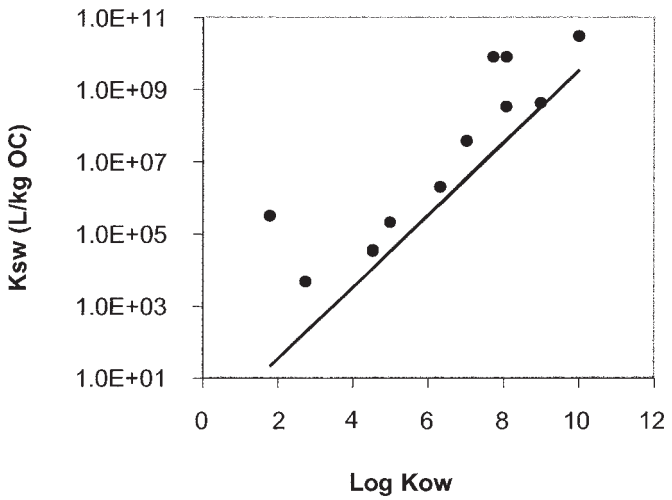


Fig. 4. Sediment-water distribution coefficients in units of L kg^{-1} organic carbon in relation to sediment-seawater partition coefficients (L kg^{-1} organic carbon), derived as 0.35 K_{OW} according to Seth et al. [44]

to which the observed sediment-water distribution coefficient (K_{SW}) exceeds the sediment-water equilibrium partition coefficient ($K_{SW, EQ}$), that is, $K_{SW}/K_{SW, EQ}$. It represents a situation in which the sediments are at a higher concentration than sediment-water partitioning thermodynamics dictates. Figure 4 illustrates that sediment-water disequilibria fall with increasing K_{OW} from values as high as 229,000 for DMP to 41 for DEP and reach a constant value between approximately two and ten for the higher molecular weight phthalate esters. A disequilibrium of ten indicates that the sediment pore water concentration is an order of magnitude greater than the concentration in the overlying water. A value above unity also suggests that the sediments are serving as an exposure source to the water column. From a bioaccumulation perspective, the significance of the apparent disequilibrium between sediments and overlying water is that the degree of direct exposure of the organism to sediments and associated interstitial water versus exposure to the overlying water will have a significant effect on the body burden of the exposed organism. A sediment burying invertebrate (such as geoduck clams) with greater contact to sediments is therefore expected to be exposed to a higher effective concentration than epibenthic organisms that inhabit the epilimnion (e. g., the Dungeness crab), where they are exposed to the overlying water. Differences in an organism's habitat utilization are therefore expected to be partly responsible for the differences in the BSAFs that are observed. Other factors, such as metabolic transformation, growth dilution, and low dietary uptake efficiencies of phthalate esters may also play a role.

7.2 Food-Web Bioaccumulation

Figure 5 illustrates the relationship between the lipid equivalent concentration (C_L in ng g^{-1} lipid) of phthalate esters and trophic position for the organisms in the False Creek food-web. For fish and shellfish, lipid equivalent concentrations were derived by dividing the wet weight-based concentration C_B (ng g^{-1} wet weight) by the lipid content L (kg lipid kg^{-1} organism or tissue on a wet weight basis):

$$C_L = C_B/L \quad (1)$$

For algae and plankton, the calculation of the lipid equivalent concentration was conducted as:

$$C_L = C_B^*/(L_B^* + 0.35\,\phi_{OC}) \quad (2)$$

where C_B^* is the chemical concentration on a dry weight basis, L_B^* is the lipid content on a dry weight basis (kg lipid kg^{-1} sample, dry weight), ϕ_{OC} is the organic carbon content (kg OC kg^{-1} sample, dry weight), and 0.35 is a proportionality constant reflecting the differences in the sorptive capacities between organic carbon and octanol [44]. The reason for the difference in the methodology for lipid normalization between algae, plankton, fish, and shellfish is that due to the low lipid content of algae and plankton (i. e., 0.1 – 0.4%) but high organic carbon content (i. e., ϕ_{OC} = 33 – 40%) lipids are not the main site for chemical accumulation [45]. The purpose of the lipid normalization is to remove the effect of differences

in lipid content among organisms of different trophic levels on phthalate ester concentrations.

Figure 5 illustrates that there are no statistically significant relationships between the lipid equivalent concentrations and trophic position for DMP, DEP, DiBP, DBP, and BBP. Analysis of covariance shows that lipid equivalent concentrations do not appear to increase or drop significantly (i.e., $P > 0.05$) between trophic levels. This indicates that these phthalate esters do not biomagnify in the food-web. Biomagnification in the food-web is defined as the increase in the lipid equivalent concentration with increasing trophic level. The apparent constancy of the lipid equivalent concentrations with increasing trophic position suggests that the accumulation of these phthalate esters is due to simple water-to-lipid partitioning, which produces approximately equal lipid equivalent concentrations in the various organisms of the food-web.

For the higher molecular weight phthalate esters (i.e., DEHP, DnOP, and DnNP) there appears to be a statistically significant drop (i.e., $P < 0.05$) in the lipid equivalent concentration with increasing trophic position. The latter indicates trophic dilution, in which lipid equivalent concentrations in organisms decline with increasing trophic level. The observations indicate that higher trophic level organisms are exposed to lower concentrations of these higher molecular weight phthalate esters than organisms of lower trophic levels.

7.3 BAFs

Figure 6 illustrates the observed relationships between the BAF (expressed in units of L kg^{-1} equivalent lipid) and K_{OW} for all the species included in the field bioaccumulation study. To simplify Fig. 6, the species are grouped into trophic guilds. For the purpose of this analysis, we distinguished between algae, plankton, benthic invertebrates, small forage fish, predatory fish, and aquatic birds. To provide a basis for comparison, Fig. 6 also presents the expected BAFs assuming that only simple lipid-water partitioning of the chemicals between the organisms and the water controls bioaccumulation [10], that is, BCF (L kg^{-1} equivalent lipid) = K_{OW}. This simple model ignores the potential role of dietary uptake, biomagnification, metabolism, growth dilution, and the reduction of the chemical bioavailability due to sorption in the water phase.

Figure 6 illustrates a number of characteristics of the bioaccumulation behavior of phthalate esters in the field. Firstly, BAFs of individual phthalate esters exhibit a considerable variability. The variability in BAFs ranges from a factor of approximately 30 for the lower molecular weight phthalate esters to a factor of 1000 for DEHP, DnOP, and DnNP. There appears to be no apparent relationship between the BAF and the trophic position of the organism for the lower molecular weight phthalate esters. This indicates that the observed variability in the BAF is not due to differences in trophic position among the organisms sampled. However, for the higher molecular weight phthalate esters, there appears to be a trend for the BAFs to drop with increasing trophic position. This trend is the main reason that the BAFs of the higher molecular weight phthalate esters show a greater variability than the BAFs of the lower molecular weight phthalate esters.

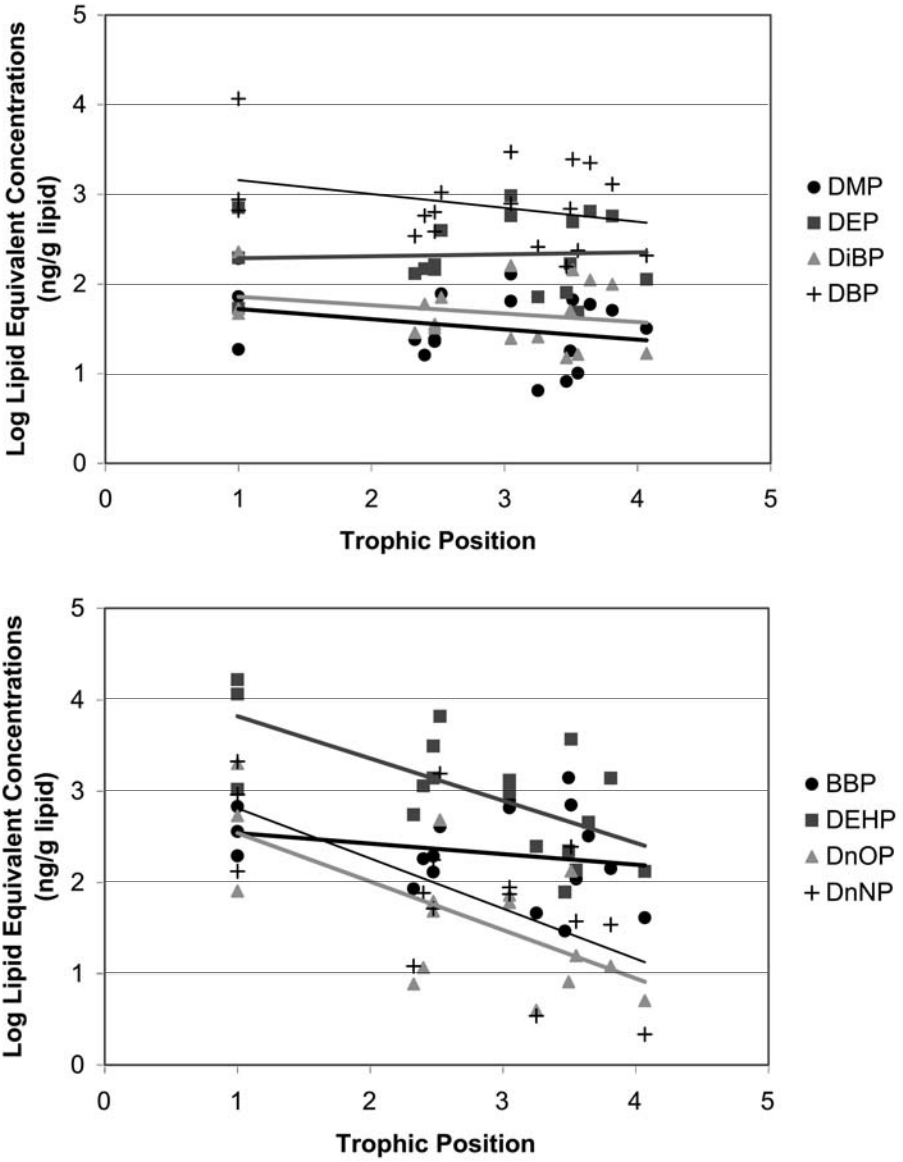


Fig. 5. Relationship between the lipid equivalent concentrations of phthalate esters and trophic position for a range of organisms in a marine food-web

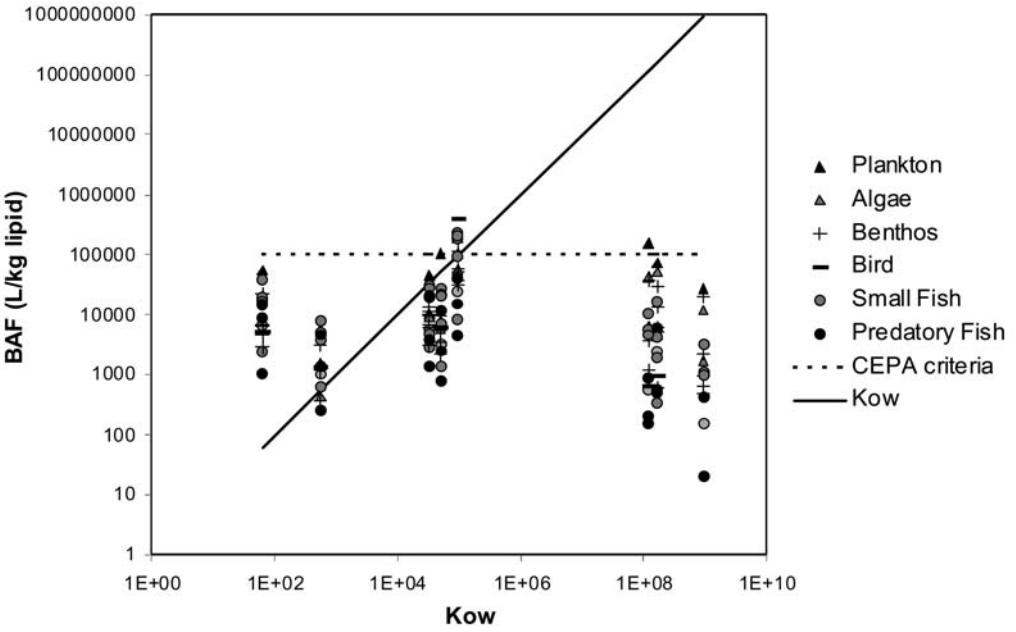


Fig. 6. Relationships between the observed BAF, expressed relative to the total concentration in the water, in units of L kg^{-1} equivalent lipid in algae, plankton, benthos, small fish, predatory fish, and birds, and the octanol-seawater partition coefficient for phthalate esters. The *dashed line* represents the Canadian Environmental Protection Act's bioaccumulation criterion expressed on a lipid-normalized basis assuming a 5% lipid content

Secondly, the comparison of the observed BAFs with the BCFs derived by simple lipid-water partitioning shows that the BAFs for DMP and DEP are greater than expected from simple lipid-to-water partitioning. In particular, the BAF of DMP is much greater (i.e., on average a factor of 100) than that expected based on lipid-water partitioning. Considering that (i) all biota and water concentration exceed the method detection limits by many fold, (ii) DMP showed high extraction recoveries, negligible degradation in and evaporative losses from the water samples (due to immediate analysis at low temperature), and (iii) positive MS-MS confirmation in water and biota samples, it is unlikely that the much higher than expected BAFs are due to analytical error. Also, due to the mass-specific analysis, metabolites can be ruled out as a factor. A possible explanation for the high BAFs for DMP is the large disequilibrium between sediments and overlying water. Contact of organisms with the sediments (e.g., sculpins burying in sediments) may elevate the body burden of DMP in organisms over that absorbed from the overlying water.

Thirdly, the BAFs of DBP, DiBP, and BBP are generally in reasonable agreement with the BCFs based on simple lipid-water partitioning. This suggests that the bioaccumulation of these substances is mainly the result of chemical exchange between the organism and the water via the respiratory surface of the organisms. Dietary uptake, metabolic transformation, and growth dilution appear to play a

secondary role, but may contribute to the variability in the observed BAFs among the different organisms. The BAFs of the higher molecular weight phthalate esters (i. e., DEHP, DOP, DnNP) are lower than anticipated based on lipid-water partitioning. The low bioavailability of the total water concentration is a key factor causing the BAFs of the higher molecular weight phthalate esters to fall below the lipid-water partition coefficients. For example, our study suggests that approximately 0.1% of the total water concentration of DEHP is freely dissolved and hence assumed to be available for uptake via the respiratory surface. The freely dissolved water concentration is believed to represent the phthalate ester concentration that can be absorbed by organisms via the respiratory surface area as a result of lipid-water partitioning. Figure 7 illustrates the BAF based on freely dissolved concentrations BAF_{fd} (L kg^{-1} lipid) normalized to the lipid-water partition coefficient, that is, BAF_{fd}/K_{OW}. It shows that the BAFs in plankton and green algae approach the lipid-water partition coefficients, that is, BAF_{fd}/K_{OW} is approximately 1.0. This result appears to be reasonable as algae can be expected to lack a metabolic transformation capability for lipid-like molecules such as

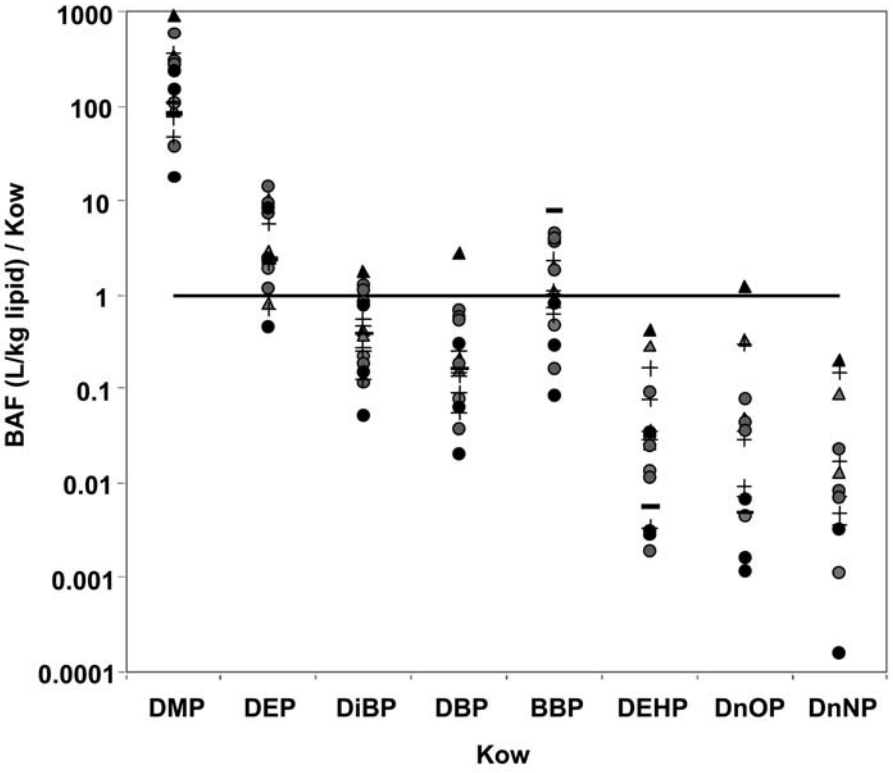


Fig. 7. Relationship between the observed BAFs, expressed in terms of the freely dissolved water concentration, in units of L kg^{-1} equivalent lipid, divided by the octanol-seawater partition coefficient (K_{OW}) in algae (*shaded triangles*), plankton (▲), benthos (+), small fish (*shaded circles*), predatory fish (●), and birds (–) for phthalate esters. The *solid line* represents the organism-water equilibrium partition coefficient ($BAF_L = K_{OW}$)

phthalate esters. The BAFs of the higher molecular weight phthalate esters in the majority of benthic invertebrates, forage fish, predatory fish, and birds are less than the lipid-water partition coefficients (i.e., $BAF_{fd}/K_{OW} < 1$), indicating that bioaccumulation factors for the freely dissolved phthalate ester are significantly less than their octanol-water partition coefficients. This suggests that processes other than lipid-water partitioning (e.g., metabolic transformation, growth, fecal excretion) have a significant effect on the BAF.

Fourthly, Fig. 6 further shows that when the BAFs are compared to the Canadian Environmental Protection Act's cut-off value of 5000 (if the BAF is expressed on a wet weight) or a 100,000 (if the BAF is expressed on a lipid weight basis), the BAFs of all the phthalate esters generally fall below the cut-off value. The only exceptions are the BAFs of BBP for green algae, plankton, geoduck clams, striped seaperch, pile perch, staghorn sculpins, and surfscoters.

8 Bioaccumulation Models

Bioaccumulation models can be useful tools in the investigation of the mechanism of phthalate ester bioaccumulation. The merit of such models is in investigating the role that uptake and elimination processes contribute to the observed BAFs. For example, these models can be used to estimate whether a chemical substance is predominantly absorbed by an organism from the water via the respiratory surface or through the dietary route. These models can also be applied to assess to what degree chemical substances can be expected to be eliminated via water or feces and to what degree metabolic transformation and growth affect tissue concentrations. Most importantly, the model can be used to test hypotheses regarding the mechanisms contributing to the bioaccumulation process. The hypothesis can be tested by comparing model predictions to observed data. In this section, we will discuss a general bioaccumulation model [46] for fish and test the model against the data from the field bioaccumulation study. The model esti-

Table 3. Model equations, parameters, and their units of the fish bioaccumulation model of Gobas [13]. $BAF = C_F/C_W = [k_1 \phi_{DW} + k_D C_D/C_W)]/(k_2 + k_E + k_M + k_G)$

Parameter	Units	Definition
ϕ_{DW}	fraction	Fraction of the water concentration that is freely dissolved
C_D	g kg^{-1} wet weight	Chemical concentration in diet
C_F	g kg^{-1} wet weight	Chemical concentration in fish
C_W	g L^{-1}	Total chemical concentration in overlying water
k_1	L water kg^{-1} organism wet weight d^{-1}	Uptake clearance rate from water
k_2	d^{-1}	Elimination rate constant from fish
k_D	kg food kg^{-1} organism wet weight d^{-1}	Dietary uptake clearance rate
k_E	d^{-1}	Fecal egestion elimination rate constant from fish
k_G	d^{-1}	Growth dilution rate constant
k_M	d^{-1}	Metabolic transformation rate constant from fish

Table 4. Bioaccumulation model input parameters and the results of the model calculations of the lipid-normalized BAF_L for several phthalate esters in a field exposed 0.1 kg staghorn sculpin (lipid content is 5.0%) and a 3 kg dogfish (lipid content is 15%) in relation to the observed BAFs [a]

Sculpins Phthalate ester	Log K_{OW}	C_W	C_D	ϕ_{DW} (%)	k_1	k_2	k_D	k_E	k_M	k_G	$BAF_{L,P}$	$BAF_{L,O}$
DMP	1.78	3.51	2000	100	83	27.7	0.0246	0.00616	0	0.0021	70.4	19,000
DEP	2.74	127	11,500	99	188	6.83	0.0246	0.00616	0	0.0021	552	3900
DBP	4.52	110	32,800	75.4	221	0.237	0.0246	0.00616	0	0.0021	14,200	22,000
DiBP	4.52	5.15	2750	75.4	221	0.134	0.0246	0.00615	0	0.0021	25,300	28,000
BBP	4.98	3.48	11,400	53.3	222	0.0464	0.0246	0.00614	0	0.0021	72,700	204,000
DEHP	8.08	228	131,000	0.11	222	0.0000369	0.00653	0.00163	0.0011	0.0021	16,000	16,000
DnOP	8.08	12.8	8700	0.11	222	0.0000369	0.00653	0.00163	0.0052	0.0021	10,000	10,000
DnNP	8.98	77.7	26,900	0.01	222	0.00000465	0.00107	0.000268	0.0001	0.0021	3100	3100
Dogfish Phthalate ester	Log K_{OW}	C_W	C_D	ϕ_{DW} (%)	k_1	k_2	k_D	k_E	k_M	k_G	$BAF_{L,P}$	$BAF_{L,O}$
DMP	1.78	3.51	2430	100	21.4	2.37	0.0148	0.00370	0	0.0011	89	9040
DEP	2.74	127	16,900	99	48.1	0.584	0.0148	0.00370	0	0.0011	560	891
DBP	4.52	110	3480	75.4	56.7	0.0114	0.0148	0.00367	0.17	0.0011	189	189
DiBP	4.52	5.15	82,700	75.4	56.7	0.0114	0.0148	0.00369	0.09	0.0011	3240	3240
BBP	4.98	3.48	12,800	53.3	56.8	0.00397	0.0147	0.00369	0.04	0.0011	11,800	11,800
DEHP	8.08	228	114,000	0.11	56.9	0.00000316	0.00392	0.000980	0.017	0.0011	580	580
DnOP	8.08	12.8	9800	0.11	56.9	0.00000316	0.00392	0.000980	0.052	0.0011	374	374
DnNP	8.98	77.7	15,100	0.01	56.9	0.000000397	0.000642	0.000161	0.03	0.0011	28	28

[a] The model input parameters are the octanol-seawater partition coefficient K_{OW}, the observed total water concentration C_W (ng L^{-1}), the dietary concentration C_D (ng kg^{-1} wet weight), the freely dissolved fraction of the water concentration ϕ_{DW} (%). The model output parameters are the gill uptake rate constant k_1 (L kg^{-1} wet weight), the gill elimination rate constant k_2 (day^{-1}), the dietary uptake rate constant k_D (kg food kg^{-1} wet weight day^{-1}), the fecal egestion rate constant (day^{-1}), the metabolic transformation rate constant k_M (day^{-1}), the growth rate constant k_G (day^{-1}), the model-predicted lipid equivalent bioaccumulation factor expressed based on the total water concentration $BAF_{L,P}$ (L kg^{-1} lipid) and the observed lipid equivalent bioaccumulation factor expressed based on the total water concentration $BAF_{L,O}$ (L kg^{-1} lipid).

mates a whole organism BAF in units of L kg^{-1} wet weight based on the total water concentration as:

$$BAF = C_F/C_W = [k_1 \phi_{DW} + k_D C_D/C_W]/(k_2 + k_E + k_M + k_G) \quad (3)$$

The corresponding lipid equivalent BAF_L (L kg^{-1} equivalent lipid) is BAF/L, where L is the lipid content of the fish (kg lipid kg^{-1} wet weight organism). The model parameters are explained in Table 3. The methods for the calculation of the uptake and elimination rate constants can be found in Gobas [46]. The model calculations are illustrated for several phthalate esters in a 0.1 kg staghorn sculpin (lipid content of 5.0%) and a 3 kg dogfish (lipid content is 15%) at a water temperature of 10 °C. The weight, lipid content, and temperature of the species correspond to the animals that were sampled as part of the field bioaccumulation study. The model input parameters and results are listed in Table 4 and a comparison of model-predicted and observed BAF_Ls are given in Fig. 8. The model calculations were conducted by using (i) a metabolic transformation rate constant k_M of 0 and (ii) a metabolic transformation rate constants that was fitted to produce a perfect agreement between model-predicted and field-observed BAF_Ls. The latter method is essentially an approximation of the potential magnitude of the metabolic transformation rate constant k_M.

The model calculations illustrate that the lower molecular weight phthalate esters (i. e., DMP, DEP, DiBP, DBP, and BBP) in sculpin and DMP, DEP, DiBP, and DBP in dogfish are almost exclusively absorbed from the water via the gills. Dietary uptake of these phthalate esters is small compared to the uptake from the water, and model-predicted biomagnification factors are less than 1.0, indicating that biomagnification is not expected to occur. The latter is supported by the results of the bioaccumulation field study, which shows that lipid equivalent concentrations as well as lipid equivalent BAFs do not increase with increasing trophic position. The model calculations further show that in sculpins the lower molecular weight phthalate esters are virtually completely depurated through gill elimination. Growth and fecal egestion do not have a significant effect on the BAF in sculpins. Metabolic transformation rate is not required in the model to explain the observed BAFs in sculpins in the field. This does not mean that metabolic transformation does not occur; only that its rate may be too low to have a significant effect on the BAF.

The model calculations show that with the exception of DMP and DEP, the lower molecular weight phthalate esters in dogfish require a substantial metabolic transformation rate to explain the observed BAFs. The model results suggest that metabolic transformation is the main route of depuration of the DBP, DiBP, and BBP in the upper-trophic level dogfish. As a result, the BAF_Ls of these substances are lower than their K_{OW}.

The model calculations show that the exposure of sculpins and dogfish to higher molecular weight phthalate esters (DEHP, DnOP, DnNP) is a combination of both direct exposure to the water and dietary uptake. Dietary uptake appears to be more important than direct uptake from the water. However, the uncertainty in the determination of the freely dissolved water concentrations prevents a more conclusive assessment of whether the diet or the water is the main source of uptake in these fish species. Staples et al. [3] predicted that as much as 60% of the

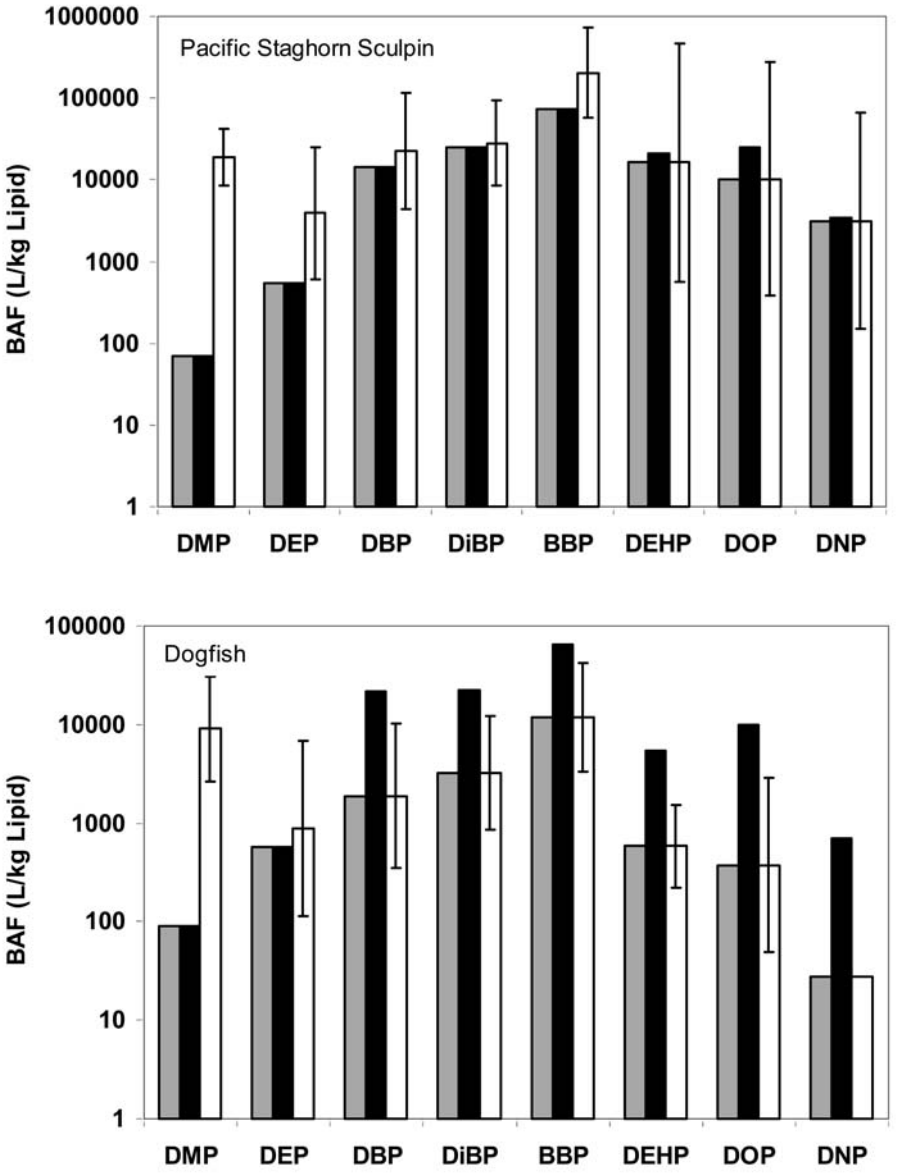


Fig. 8. Model-predicted lipid-normalized BAFs, expressed relative to the total concentration in the water, in units of L kg^{-1} lipid in relation to the observed values for staghorn sculpins and spiny dogfish. *Grey bars* represent model predictions assuming metabolism (see Table 4). *Black bars* represent model predictions assuming no ($k_M = 0$) metabolism. *White bars* represent the observed values

DEHP exposure in predators could be derived from the diet. The model shows that the depuration rates for the higher molecular weight phthalate esters are substantially smaller than those for the lower molecular weight phthalate esters. This means that even low rates of metabolic transformation can have a significant effect on the BAF. The model calculations show that for sculpins only small metabolic transformation rates need to be invoked to explain the observed BAFs in sculpins. Considering the error in the model parameterization and in the observed BAFs, it is unclear whether metabolic transformation has a significant effect on the BAF. Assuming no metabolic transformation, model predicted BAFs in sculpins are in good agreement with the observed values. In dogfish, however, high rates of metabolic transformation need to be used to explain the observed BAFs. The latter suggests that metabolic transformation is an important depuration process in dogfish causing the BAFs to be much lower than the lipid-water partition coefficients of these phthalate esters. Metabolic transformation rates of the higher molecular weight phthalate esters in dogfish may be between 0.02 and 0.05 d^{-1}. These rates are on average about an order of magnitude lower than depuration rates derived from laboratory bioconcentration studies with fish [3]. The lower rate of metabolic transformation may be due to (i) the larger size and higher lipid content of the dogfish compared to the fish used in the laboratory experiments and (ii) the much longer exposure duration in the field compared to that in laboratory tests. The high lipid content and size of the organism may generate a large lipid storage compartment for phthalate esters and reduce the fraction of phthalate in the fish that is available for metabolic transformation compared to that in smaller, less lipid-rich fish. The longer exposure duration in the field-exposed fish is likely to increase the fraction of the total amount of phthalate ester in less accessible "slow" storage compartments. The greater fraction of phthalate ester stored in these less accessible compartments (such as the lipids) may cause a smaller fraction of the total amount of phthalate ester in the fish to be available for metabolic transformation.

For DMP and DEP the model underestimates the observed BAFs, which may be explained by the apparent sediment-water disequilibria. The sediment-water disequilibria may cause the exposure concentration of this benthic fish species to exceed that measured in the overlying water. Use of the overlying water concentration can therefore be expected to underestimate the actual BAF in this fish species.

9 Conclusions

Currently, there exists considerable information on the bioaccumulation behavior of phthalate esters in aquatic systems. Laboratory experiments, field studies, and mathematical modeling studies have all been carried out. A number of conclusions can be drawn from the information available.

Firstly, there is no evidence from laboratory and field bioaccumulation studies to support the hypothesis that phthalate esters biomagnify in aquatic food-webs. Dietary bioaccumulation studies, sediment bioaccumulation studies in the lab and the field as well as the food-web bioaccumulation study discussed in this

chapter all indicate that food-web bioaccumulation (i. e., the increase of the lipid equivalent concentration with increasing trophic level) does not occur. This indicates that despite their high octanol-water partition coefficients, phthalate esters do not appear to biomagnify in aquatic food-webs.

Secondly, it is interesting that the lowest molecular weight phthalate esters DMP and DEP exhibit BAFs that are greater than their lipid-to-water partition coefficients. The results from laboratory bioconcentration experiments show similar results (Fig. 1). These observations suggest that DMP and DEP have a greater bioaccumulation and bioconcentration potential than indicated by their octanol-water partition coefficient. The laboratory observations may be explained by the possible formation of metabolic products of DMP and DEP that, due to the method of detection used, were indistinguishable from the parent compounds. However, due to the more specific detection methodology in the bioaccumulation field study (i. e., MS-MS detection), the formation of metabolic products of DMP and DEP is unlikely to explain the higher-than-expected BAFs. While analytical error of the water concentration of DMP and DEP is a likely cause for the higher-than-expected BAFs, the QA/QC procedures applied indicate that analytical error cannot explain the observations either. The model calculations indicate that DMP and DEP are almost exclusively absorbed from the water via the respiratory surface. The large apparent sediment-to-water disequilibria for DMP and DEP is the most likely explanation of the higher-than-expected BAFs in the field.

Thirdly, the lipid equivalent BAFs of DBP, DiBP, and BBP, appear to be fairly uniform among the organisms of the marine food-web investigated in this study (Fig. 5). This suggests that the organisms are exposed to a common source and that dietary uptake of phthalate esters has little effect on the BAF of these phthalate esters. Model calculations support this, by demonstrating that direct uptake of these phthalate esters from the water via the respiratory surface of the organisms can be expected to be the main exposure route and that dietary uptake is less important. The general agreement between lipid-normalized BAFs and lipid-water partition coefficients (Fig. 6) also support this conclusion and further indicates that the bioaccumulation of the lower molecular weight phthalate esters generally follows the lipid-water partitioning model. Laboratory bioconcentration studies suggest that the BCFs can reach values up to the lipid-water partition coefficients of these phthalate esters. However, several observed BCFs appear to be lower than the lipid-water partition coefficients. An interesting observation from the laboratory bioconcentration tests and the bioaccumulation field study is that there is a substantial variability in the observed bioconcentration and bioaccumulation factors. While experimental artifacts can be expected to be an important cause of the variability in the observed BCFs, they are an unlikely source of the variability in the BAFs. One potential cause of the observed variability is metabolic transformation. Several authors have implicated metabolic transformation as an important factor controlling the bioaccumulation factors of phthalate esters. The model calculations illustrate that for the metabolic transformation to have a significant effect on the BAFs of the lower molecular weight phthalate esters, the rates of metabolic transformation have to be relatively high. It is possible that metabolic transformation differ among or-

ganisms and that in certain organisms, metabolic transformation rates are sufficiently large to affect the BAF and cause some of the variability in the observed BAFs among organisms of the food-web. A second cause of the variability in BAFs may relate to the concentration gradients between sediment and water and differences among organisms in their interaction with the sediments and water. Water and sediment concentrations indicate that the sediments may provide higher phthalate esters exposure concentrations than the overlying water. Hence, the interaction of the organisms with the sediments and its pore water is likely to be responsible for some of the variability in the observed BAFs.

Fourthly, the BAFs of the higher molecular weight phthalate esters (i. e., DEHP, DnOP, and DnNP) show a tendency to decrease with increasing trophic position. This suggests that organisms at higher trophic levels are exposed to lower phthalate ester concentrations via prey. A similar apparent relationship between BCF and trophic status has been found in laboratory experiments in which BCFs were highest for algae and lowest for fish with invertebrates exhibiting intermediate values [3]. Assessment of the freely dissolved concentrations indicates that the higher molecular weight phthalate esters exhibit a very low bioavailability, that is, only a very small fraction of these phthalate esters in natural waters can be absorbed via the respiratory surface of aquatic organisms. When expressed relative to the freely dissolved concentration in the water, the BAFs in algae and plankton appear to be within an order of magnitude of the lipid-water partition coefficients. This suggests that partitioning is likely an important mechanism for bioaccumulation in algae and plankton. In higher trophic level organisms such as fish, model calculations indicate that dietary uptake is likely to be an important route of exposure as bioavailable concentrations in the water are expected to be very low. The inability of dietary uptake to cause biomagnification is therefore an interesting characteristic of the high-molecular weight phthalate esters in particular and phthalate esters in general. The model calculations provide two possible explanations for this phenomenon. First, it is possible that after these phthalate esters have been absorbed, the phthalate esters are metabolized in the fish. This explanation has been proposed by several authors and supported by the detection of some phthalate ester metabolites [3 – 8]. A greater rate of metabolic transformation has been suggested to explain the drop in BCFs with increasing trophic level. The other possible explanation is that phthalate esters ingested with the diet are very effectively metabolized in the gastro-intestinal tract even before they are absorbed (i. e., effectively decreasing k_D in Eq. (3)). This first-pass effect essentially prevents a significant rate of dietary uptake of the parent phthalate esters. The structural similarity between lipids and phthalate esters may favor such a process as pH and enzymatic conditions in the gastro-intestinal tract are tailor-made for the hydrolysis of lipids and perhaps phthalate esters. The uptake that would still occur is directly from the water. Model calculations illustrate that in the absence of dietary uptake the BAF can be expected to drop with increasing organism size (which correlates well with trophic level) as has been observed in the field study. This is due to the fact that with increasing organism size (and reducing area-to-volume ratio), the gill elimination and fecal egestion rates drop and become negligible compared to growth rates or even small metabolic transformation rates. This results in smaller BCFs for larger organisms. This second

hypothesis does not require the occurrence of a high rate of metabolism in the fish. The bioaccumulation behavior of the lower molecular weight phthalate esters is not consistent with a high rate of metabolism. It is therefore possible that phthalate esters are fairly slowly metabolized after they have been absorbed, but they are effectively metabolized in the gastro-intestinal tract before they are absorbed. We are currently carrying out laboratory experiments to distinguish between these two possible explanations. The toxicological significance of these different mechanisms is that metabolic transformation in organisms has the potential to create metabolic products, while an effective first-pass effect may prevent dietary uptake and the formation of potentially reactive metabolic products within the organism.

The majority of observed BAFs for phthalate esters did not exceed the bioaccumulation criterion of 5000 L kg^{-1} wet weight or 100,000 L kg^{-1} lipid if expressed on a lipid equivalent basis. Only BAFs of BBP in green algae, plankton, geoduck clams, striped seaperch, pile perch, staghorn sculpins, and surfscoters exceeded the bioaccumulation criterion The results of the field study also confirmed the hypothesis that these substances do not appear to biomagnify in the food-web.

Since the intention of the bioaccumulation criteria is to identify substances that, like PCBs, exhibit biomagnification, current evidence in the literature and from our study support the conclusion that phthalate esters do not appear to be bioaccumulative.

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