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Influence of Particle Surfaces on the Bioavailability to Different Species of 2,4-Dichlorophenol and Pentachlorophenol

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Studies on the bioavailability of contaminants that accumulate in sediments have been complicated by the chemical and structural variability of substrates and by the different biological properties of test organisms that are used by regulators. The purpose of this work was to overcome some of these difficulties by devising a test system that used artificial particles with known chemical surfaces. These were coated with 2,4-dichlorophenol or pentachlorophenol and fed to oligochete worms (*Lumbriculus variegatus*) and midge larvae (*Chironimus riparius*). The adsorption coefficient (K_d) of the particle surface was compared with the concentration of contaminant accumulated by the test organisms. There were major differences in bioaccumulation between the two species used despite identical particles and pollutants. This clearly reflects differences in the uptake and detoxification pathways between species. The particle surface and its interaction with the chlorophenols was a major factor in the accumulation of the contaminants in an organism. The techniques that are described provide a way of standardizing results between different natural sediments and different test organisms and provide some insights into the processes involved in bioaccumulation from particle surfaces.

Introduction

It has been known for many years that sediments are the ultimate sink for a large number of pollutants in the aquatic environment. There has, however, been considerable controversy about their bioavailability to benthic fauna (*1*). There have been three confounding aspects to this discussion. The first has raised the question of the route of uptake, particularly in relation to the relative importance of pore water versus sediment particle sources (*2*). The second relates to the heterogeneous nature of sediments in which the depth profile frequently reflects a change in the age, local redox conditions, degradation, and availability of xenobiotics (*3*). Finally, there are obvious biological factors that influence the uptake of pollutants, of which the most conspicuous is probably the feeding pattern of the organism being used. Most pollutants are bound to the surface layers of sediment particles so that animals that selectively ingest sediment fractions with relatively large surface area-to-mass ratios are likely to receive a larger dose than nonselective feeders (*4*). The subject has been reviewed in depth in relation to aqueous and particle-bound routes of uptake with the emerging view that direct uptake from sediments by ingestion is an important, and in some organisms the dominant, route of uptake (*5*, *6*). This has raised considerable concern that sediments may have toxic effects on the benthic fauna with the subsequent ecological consequences including the possibility of sedimentary pollutants reentering the food chain of aquatic organisms.

A number of different approaches have been taken to these problems. Animals collected from the natural environment can be analyzed on the basis that there is "equilibrium partitioning" between the sediment and the organism (*7*), or the rate of uptake can be measured to give a "flux" value (*8*). The variability of sediment composition has been more difficult to control with the organic carbon content generally being the main parameter that is used to standardize the results from different laboratories but with "dissolved organic carbon" being a further complicating factor (*9*, *10*).

To overcome some of these problems of interlaboratory standardization and to provide a better understanding of the processes involved in pollutant uptake, we have devised techniques for using commercial resin particles as "artificial sediments". These materials can be selected so as to provide anionic or cationic electrostatic sites or to supply surfaces with different degrees of hydrophobicity. Particles of these types can then be used to adsorb pollutants prior to their ingestion by various macroinvertebrates. The subsequent absorption of the pollutants by the organisms can then be determined at various time intervals. The advantages of this approach are (1) the particles can be obtained for specified size ranges, (2) the surface chemistry is defined and capable of being chosen according to the experimental parameters being studied, (3) the material is uniform, meeting quality assurance standards and without any other initial gradients of the types found in natural sediments, (4) the equilibrium between water and particle surface is easily determined, i.e., pore water is capable of being chemically defined, (5) there is no dissolved organic carbon fraction, and (6) if required these artificial materials can be used as standards against which it is possible to calibrate natural sediments.

The experiments described in this paper were undertaken using very low concentrations of pollutants at levels at which no mortality was detectable. They, therefore, relate to bioaccumulation processes without any identifiable dose−response effects due to metabolic disruptions. Since the results are for identical particle surface/pollutant interactions, any differences in uptake rates between species are due to differences in the absorptive mechanisms of organisms. For similar reasons any differences between pollutants when the same organism and the same particles are used will identify differences in the way that pollutants interact with different particle surfaces.

Materials and Methods

Chemicals. A variety of beads were obtained from manufacturers of high-performance liquid chromatography systems. These included those with surfaces containing quaternary ammonium (Dowex 1X8 400), sulfonyl (Toyopearl SP 650M), or phenyl (Toyopearl Phenyl 650M) groups. These represent the various types of cationic, anionic, and hydrophobic interactions that might be expected to occur in different sediments. In addition, a dimethylditallow-substituted montmorillonite clay was synthesized (*11*), and a 1-mm sieved natural sediment with 2% organic carbon (supplied by WRc, Medmenham, U.K.) was also used. All the materials

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except the natural sediment were in the 30−50-μm diameter size range. Artificial sediments were made by mixing 75% acid-washed sand, size 100−300 μm (BDH), with 25% washed test material (w/w).

^{14}C-Labeled 2,4-dichlorophenol (2,4-DCP, Sigma) and ^{14}C-labeled pentachlorophenol (PCP, Sigma) were used as suitable well-studied contaminants. The 2,4-DCP has a log K_{ow} of 3.23 and a pK_a of 7.85, while the PCP has a log K_{ow} of 5.24 and a pK_a of 4.75 (*12*), so that comparisons could be made between somewhat similar molecules which differ by several orders of magnitude in their octanol/water distribution and their degree of dissociation at the experimental pH. The distribution of the contaminant molecules was determined by liquid scintillation spectroscopy using a Packard model 2250 and Hionic Fluor scintillation fluid. Animal material was solubilized using Soluene-350 (Packard).

Organisms and Test Procedures. Two test organisms were maintained in culture using standard procedures. The oligochete *Lumbriculus variegatus* was obtained from a commercial source (Sciento, U.K.) and kept under standard conditions (*13*). The third instar larva of the midge *Chironimus riparius* was bred from a laboratory colony (WRc, Medmenham) using standard protocols (*14*). Both organisms were maintained in aerated dechlorinated tap water with the composition Ca^{2+}, 2.4 mmol/L; Na^{+}, 0.5 mmol/L; Mg^{2+}, 0.1 mmol/L; HCO_3^{-}, 4.3 mmol/L; Cl^{-}, 0.7 mmol/L; SO_4^{2-}, 0.2 mmol/L at pH 8.3 in a controlled temperature room at 20 °C on a 18/6-h light/dark regime. Both species were fed on commercial fish flake (Tetrafin) during culture and depuration.

Water spiked with 2,4-DCP or PCP was shaken with samples of the particles and analyzed to determine the time taken to reach equilibrium. The experiments were then repeated using a range of concentrations of 2,4-DCP and PCP. The concentrations in the two phases were normally determined by analyzing the system after 4 h, which was well in excess of the equilibration times. In the case of natural sediment where equilibration took much longer, the study period was extended to 5 days. The distribution coefficient K_d (mol kg^{-1} solid/mol kg^{-1} solution) was derived from adsorption isotherms by plotting the initial slopes for each type of particle with at least eight different concentrations of 2,4-DCP or PCP in the range of $(7.5 \times 10^{-8})-(7.5 \times 10^{-7})$ mol/L after shaking the samples over a 4-h period.

All artificial sediments were equilibrated with the contaminant for 24 h and left to stand for 48 h before use. The K_d value for each type of particle was used to calculate constant 2,4-DCP or PCP water concentrations so that all organisms were exposed to a standard "pore water" value of 10^{-10} mol/L. This was checked at the start and end of each experiment to ensure that there were no variations greater than 10%. Under the conditions of these experiments, the uptake of 2,4-DCP and PCP into the test organisms was proportional to the concentrations in the water so that it was possible to correct for any small changes during the period of the experiment (*15*). Five animals were included in each glass test vessel with 2 g of substrate and 20 mL of water in groups of 10 replicates. They were left for 48 h and then depurated by feeding on clean sand for a further 24 h. Preliminary experiments showed that superficial 2,4-DCP and PCP were washed off the body surface, and the gut contents of both organisms were voided within about 12 h of being placed in the clean substrate (*15*). To ensure that the body loads of 2,4-DCP and PCP in the test organisms did not include any residual adsorbed material that was not removed by washing, the procedures were repeated with freshly killed animals that were fixed with a brief treatment of 0.5-h exposure to formaldehyde. These dead animals were kept agitated with the test systems so as to simulate movement. Any small differences in the 2,4-DCP or PCP content of the water were normalized by expressing the results as a concentration factor (mol contaminant g^{-1} organism/mol contaminant g^{-1} water).

Samples of 2,4-DCP and PCP that had been exposed to sediment during the protocol test periods were extracted in sulfuric acid/acetonitrile (1:1000 v/v), sonicated, and filtered. Animals that had similarly been exposed to the chlorinated phenols were homogenized, acidified in 50% (v/v) sulfuric acid, neutralized with potassium carbonate, and extracted three times with an equal volume of *n*-hexane:ethyl ether (70:30) prior to evaporating and dissolving the extract in methanol. These samples were analyzed by HPLC using a Varian 9010 instrument linked to a Packard 500TR flow scintillation spectrometer to detect the presence of breakdown products that would indicate metabolism of the parent compounds.

TABLE 1. K_d Values for Adsorption of 2,4-DCP and PCP on Various Particles[a]

	2,4-DCP			PCP		
particle type	K_d	r^2	pH	K_d	r^2	pH
natural sediment	24	0.98	7.8	21	0.96	7.3
Toyopearl SP	45	0.97	7.5	7	0.85	7.5
Toyopearl phenyl	138	0.99	8.5	161	0.98	7.8
quat clay	1400	0.98	8.0	4908	0.98	7.8
Dowex 1X8	3745	0.96	7.5	575	0.97	7.2

[a] Values are based on linear regressions in the concentration range $(7.5 \times 10^{-8})-(7.5 \times 10^{-7})$ mol/L. pH values are for dosed particles exposed to water for 4 h.

TABLE 2. Concentration Factors of 2,4-DCP and PCP for Organisms Exposed at Equilibrium pH Values to Various Substrates for 48 h and Depurated for 24 h[a]

	L. variegatus		*C. riparius*	
substrate	dead	live	dead	live
	2,4-DCP			
water only	6.8 ± 1.8	14.4 ± 10.7	0.5 ± 0.6	6.7 ± 2.2
natural sediment	0	24.1 ± 4.4	0	5.5 ± 5.8
Toyopearl SP	2.6 ± 1.8	17.1 ± 3.2	1.6 ± 0.9	1.6 ± 0.9
Toyopearl phenyl	11.6 ± 2.0	101.2 ± 15.5	1.8 ± 0.4	1.9 ± 0.4
quat clay	3.7 ± 1.5	143.4 ± 9.3	2.2 ± 0.8	2.1 ± 0.8
Dowex 1X8	2.6 ± 0.7	4.6 ± 0.8	1.6 ± 0.6	1.7 ± 0.6
	PCP			
water only	29.1 ± 1.3	39.5 ± 2.6	7.7 ± 0.9	
natural sediment	29.0 ± 2.0	453.3 ± 44.2	11.1 ± 1.2	
Toyopearl SP	17.4 ± 3.5	305.0 ± 39.6	3.5 ± 0.3	
Toyopearl phenyl	35.7 ± 4.7	334.1 ± 36.1	6.1 ± 1.2	
quat clay	40.3 ± 6.2	4.4 ± 0.7	3.1 ± 0.5	
Dowex 1X8	14.0 ± 5.1	2.4 ± 1.4	5.1 ± 1.4	

[a] Values are means ± SE for $n = 10$. Data are given for live animals and dead animals maintained in similar but agitated systems.

Results

The 2,4-DCP and PCP distribution coefficient (K_d) values of the various particles are given in Table 1 together with the pH of the water containing these materials in the test system. The concentration of contaminants in the two species of test organisms after exposure to the various substrates is shown in Table 2 for both live and dead specimens. Both the body and water concentrations are expressed in 10^{-10} mol g^{-1} so that the normalized resultant is the increase in body load or concentration factor under these standardized conditions. Analysis by HPLC/scintillation spectrometry showed no significant (<5%) occurrence of metabolic products from either sediment microorganisms or test organism metabolism during the period of the experiments with the exception of

PCP in *C. riparius* which was extensively degraded. No data are reported for PCP in *C. riparius* because of this interaction.

The uptake into dead organisms was designed to measure passive uptake from the water into animals that were clearly not assimilating sediment. In the case of *C. riparius* washing the dead organisms for 24 h reduced the body load of 2,4-DCP to virtually zero. In the case of *L. variegatus* there was a slightly larger retention. It is clear, however, that the dead organisms are accumulating less than the living ones, even when they were both simply in water and not feeding. This is probably because the dead animals lack a blood circulation that would facilitate removal of material from the animal's epidermis.

It should be noted that the particle that binds both contaminants the strongest, i.e., Dowex 1X8 400, carries hundreds of times more pollutant into the animal's alimentary tract than most of the other materials. Despite this there was virtually zero absorption into either species from this particle. This confirms that the depuration procedure was effective and that it was the cationic surface properties of this particle binding the anionic contaminants that were responsible for the absence of absorption.

Discussion

The ability of organisms to assimilate material from ingested sediments is determined by the rates at which those materials are removed from the particle surfaces and absorbed by the gut mucosa. The adsorption coefficient K_d gives an indication of the variety of influences that will affect particle binding, while K_{ow} shows how partitioning between lipids and water will influence the uptake of a molecule into a cell. Plotting the body load or amount of a contaminant that is accumulated in the body against K_d shows that there is a roughly parabolic relationship in that both small and large values of K_d tend to result in a reduction in the amounts of 2,4-DCP and PCP being accumulated (Figures 1 and 2).

Parabolic curves of the type shown in Figure 1 occur in a number of assays for the uptake of pharmacologically active molecules into the body, and various explanations have been advanced to explain them in terms of absorption systems. The most obvious suggestion is that they arise from repeated partitioning between aqueous and lipid systems. Those materials with a low octanol/water coefficient become trapped in the aqueous phase and only penetrate into the cell membrane with difficulty. Molecules with a high K_{ow} become concentrated in a lipid phase from which it is hard to leave and enter an aqueous phase. Consequently the movement of molecules across a biological epithelium is optimal at intermediate K_{ow} values producing a parabolic relationship between uptake and the octanol/water partition coefficient (*16*). A similar interpretation can be made for the sediment assays obtained in the current work. Thus, if very little pollutant is bound to the sediment (low K_d), then very little will enter the animal and be absorbed. Alternatively if the contaminant partitions largely onto the particles (high K_d), then the contaminant is ingested but is likely to remain on the surface of the solid phase. This results in a parabolic curve, and the values between the two extremes effectively define the sediment properties that permit varying degrees of absorption and potential toxicity in the test organisms (Figure 1).

The parabolic curves shown in Figure 1 demonstrate the effect of the particle surfaces that were used in this study, and their value is 3-fold. First, such curves could be used to derive, from the K_d of the natural sediment, an approximate value for the uptake that would occur from exposing this material to the biota. This is possible since the properties of the natural sediment are not dissimilar from the curves for the resin particles. Second, these curves enable comparisons to be made between the different types of test organisms

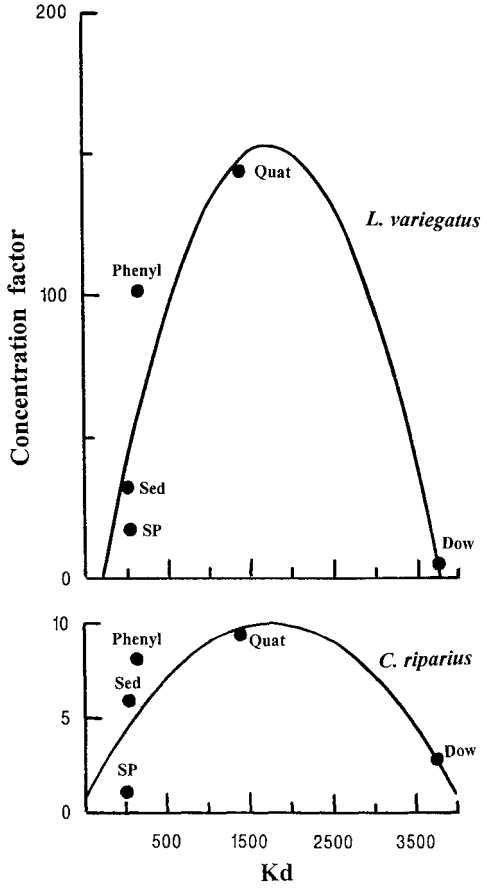

FIGURE 1. Plot of the organism concentration factors against particle K_d. The calculated parabolic curves show the effect of various particle surfaces on the absorption of 2,4-DCP by the test organisms *L. variegatus* (top) and *C. riparius* (bottom). Note the difference in the scales but that both species show the same sequence of interaction with the particles. SP, Toyopearl SP; Sed, natural sediment; Phenyl, Toyopearl phenyl; Quat, dimethylditallow ammonium substituted clay; Dow, Dowex 1X8 400.

used by regulators. Clearly the results obtained from *L. variegatus* and *C. riparius* demonstrate that different organisms give results that may differ from each other by several orders of magnitude, and the uptake into *C. riparius* is, in fact, so small that it is always less than the uptake from water alone. This suggests that these organisms are protected by the construction of a surrounding tube within which the water becomes depleted of contaminants. Third the curves shown in Figure 1 are for a single pollutant with a constant K_{ow} using the same series of particles with differing surface properties. The differences between these curves are, therefore, caused by differences in the biology of the test organisms since everything else is constant. Since the two species are of approximately the same size with a similar rate of intestinal throughput (*17*), the most likely explanation for this difference is the presence of a hydrophilic peritrophic membrane in the alimentary tract of the midge larvae that reduces the assimilation of hydrophobic molecules. Finally, the results demonstrate that if electrostatic forces are involved in the contaminant/particle association, as with chlorinated phenols binding to sulfonyl and quaternary ammonium surfaces, the energy of interaction may be much stronger than with hydrophobic interactions. This is an important feature to note when trying to standardize toxicity tests because natural sediments are often mixtures of a large number of components. Thus, the K_d of a natural sediment is a dubious concept since varying the proportions of the various components will produce equally variable K_d values. Because of this it is

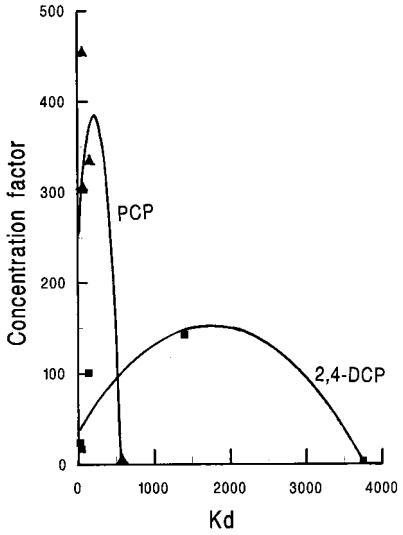

FIGURE 2. Concentration factors for PCP and 2,4-DCP in *L. variegatus.* Curves are fitted for comparison with Figure 1 and to illustrate the shift of the curve for PCP (▲) to the left and upward of that for 2,4-DCP (■). This shift corresponds with the larger $K_{o/w}$ value of PCP.

often difficult to predict how pollutants are likely to interact with natural particle surfaces and thus with the biota. The present work provides one way of trying to avoid such confusion by standardizing such measurements.

The technique of using well-characterized surface effects can also be exploited to demonstrate the factors likely to influence the bioavailability of different contaminants. In Figure 2 the same series of particle surfaces have been used to compare the accumulation of 2,4-DCP and PCP in a single species of worm. The raw data is given in Tables 1 and 2, but it has been plotted in Figure 2 to emphasize two features. The most conspicuous effect is that the curve for PCP has shifted dramatically toward lower K_d values and higher concentration factors. These changes are associated with increased K_{ow} (PCP, 5.24; 2,4-DCP, 3.23) making the PCP less strongly bound and more bioavailable. At the same time the decreased pK_a (PCP, 4.75; 2,4-DCP, 7.85) changes the relative affinity for the particle surfaces so that the anionic Toyopearl SP binds the PCP very weakly when compared with the 2,4-DCP. By way of contrast the quaternary ammonium tallow-substituted clay now binds the PCP so strongly that none of the contaminant is accumulated in the worms, while the same surface transmits the largest dose of 2,4-DCP to *L. variegatus.*

These experiments demonstrate that resin beads with controlled surface chemistry may be used as "artificial sediments" to investigate the relative uptake of pollutants by different benthic organisms. The same approach may also indicate how different contaminants may affect these processes and thus provide a more standardized way of comparing potentially toxic materials.

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Literature Cited

(1) Reynoldson, T. B. *Hydrobiologia* **1987**, *149*, 53.
(2) Landrum, P. F. *Environ. Sci. Technol.* **1989**, *23*, 588.
(3) Luoma, S. N. *Hydrobiologia* **1989,** *176/177*, 379.
(4) Kukkonen, J.; Landrum, P. F. *Aquat. Toxicol.* **1995**, *32*, 75.
(5) Baker, R. A.*Organic Substances and Sediments in Water*; Lewis: Chelsea, MI, 1991; 3 Vol.
(6) Burton, G. A. *Sediment Toxicity Assessment*; Lewis: Chelsea, MI, 1992.
(7) Di Torro, D. M.; Zarba, C. S.; Hansen, D.; Berry, W. J.; et al. *Environ. Toxicol. Chem.* **1991**, *10*, 1541.
(8) Lee, H. In *Sediment Toxicology Assessment*; Burton, G. A., Ed.; Lewis; Chelsea, MI, 1992; p 267.
(9) Karickhoff, S. W.; Brown, D. S.; Scott, T. A. *Water Res.* **1970**, *13*, 241.
(10) Landrum, P. F.; Robbins, J. A. In *Sediments: Chemistry & Toxicity of In-Place Pollutants*; Baudo, G., Giesy, J. P., Muntau, H., Eds.; Lewis: Chelsea, MI, 1991; p 237.
(11) Breakwell, I. K.; Homer, J.; Lawrence, M. A. M.; McWhinnie, W. R. *Polyhedron* **1995**, *14*, 2511.
(12) Escher, B. I.; Schwarzenbach, R. P. *Environ. Toxicol. Chem.* **1996**, *30*, 260.
(13) Phipps, G. L.; Ankley, G. T.; Benoit, D. A.; Mattson, V. R. *Environ. Toxicol. Chem.* **1993**, *12*, 269.
(14) *Am. Soc. Testing and Materials*; Section E1383; ASTM: Philadelphia, PA, 1994.
(15) Davies, N. A.; Edwards, P.; Lawrence, M. A. M.; Taylor, M. G.; Simkiss, K. *Environ. Toxicol. Chem.*, in press.
(16) Hansch, C.; Clayton, J. M. *J. Pharm. Sci.* **1973**, *62*, 1.
(17) Brooke, L. T.; Ankley, G. T.; Call, D. J.; Cook, P. M. *Environ. Toxicol. Chem.* **1996**, *15*, 223.

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