SECOND YEAR PROGRESS REPORT TO
THE DEPARTMENT OF ENERGY

DOE/ER/60809--2

Influence of Sorption/Desorption Processes on the
Bioavailability of Organic Contaminants

DE91 007360

William F. Guerin and Stephen A. Boyd

Introduction:

Many of the problem contaminants found in soils and groundwaters are non-ionic and relatively insoluble. Under appropriate conditions, many of these compounds are degradable by bacteria provided nutrients, electron acceptors and the compounds themselves are biologically available. However, non-ionic organic compounds (NOCs) bind tenaciously to soil particles potentially limiting their bioavailability. While the individual processes of sorption and biodegradation have received much attention in recent years, little is known about the interactions of these processes. The primary objective of our DOE-funded research project has been to elucidate the influences of sorption and desorption processes on the bioavailability of NOCs. Conflicting reports in the literature suggest that sorption may increase, decrease, or have no effect on bioavailability although the majority of published work has studied proteins, fatty acids, and other normal bacterial growth substrates as sorbates (van Loosdrecht, et al, 1990). Some of this variability arises because sorbed solutes interact with sorbents *via* different mechanisms including cation and anion exchange, adsorption, complexation with surface-associated metals and partitioning. Also, bacterial activities may be altered upon attachment of the cells to the sorbent surface. Clearly, resolution of this problem requires detailed knowledge of a system with multiple components. We are, therefore, approaching this problem on a fundamental level.

Background

Non-ionic organic compounds have been shown to bind to soil particulates to an extent which is dependent on the compound-specific octanol-water partition coefficient (K_{ow}) and the fractional carbon content of the soil. Such binding has been postulated to occur by a partition mechanism in which the soil organic matter behaves as an organic solvent phase. Such a mechanism accounts for the linear sorption isotherms and non-competitive binding observed for these compounds and predicts that as the soil:solution ratio or the organic carbon content of the soil increases, the amount of solute remaining in solution decreases. Partitioning is generally a very rapid process, although recent work has suggested that true equilibrium is attained only after extended soil-water contact periods. Similarly, desorption of sorbed compounds often exhibits hysteresis in which a fraction of the sorbed material desorbs slowly or appears to be irreversibly bound.

To explain such phenomena, several models of NOC binding to soils have been proposed. Two such models are the radial diffusion model (Steinberg et al, 1987; Wu and Gschwend, 1986) and the two-compartment (mass transfer) model (Karickhoff, 1984). In the former, the sorbent is envisioned to be a porous, spherical aggregate in which intraparticle diffusion is retarded by sorptive interactions along tortuous intra-aggregate micropores. Slow sorptive and desorptive equilibrium in such a model results from the retarded diffusion of solute from the interior of the aggregate to the external surface which is in equilibrium with the aqueous phase. Support for this model comes from studies in which the effective diffusion coefficients (Steinberg et al, 1987; Wu and Gschwend, 1986) and biodegradation rates (Rijnaarts et al, 1990) for sorbed solutes were found to decrease as the average aggregate diameter increased.

The two-compartment model is less explicit in the physical picture of solute-sorbent interaction which it describes, but also provides a reasonable explanation for experimental data (Karickhoff and Morris, 1985). Here, sorbed solute is partitioned into a labile and a non-labile phase, the proportions of which depend on the equilibration period and the partition coefficient of

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

MASTER

EB

the system. Rapid uptake of soluble contaminant into a labile compartment is followed by the slow sequestering of this sorbed material into a non-labile phase. Desorption kinetics show increased hysteresis as the contact period (and proportion of solute in the non-labile phase) increases (Pignatello, 1989). The non-labile sorbed material in this model may be the result of migration into the interior of aggregates, unspecified chemical interactions between the solute and sorbent, or physical entrapment of solute molecules within the sorbent matrix. The kinetics and extent of sorption and desorption thus depend in a complex and little understood manner on the organic and mineral composition of the sorbent, its particle size distribution, its intra-aggregate pore structure and the duration of the equilibration period.

It would be expected that bacteria would have full access to dissolved and readily desorbable solute. The availability of much of the sorbed material, however, would depend on the kinetics of desorption. Removal of solute in the aqueous phase surrounding soil particles (either through biodegradation or transport) acts to upset the sorptive equilibrium such that desorption occurs. According to Fick's first law;

$$dq/dt = -DA (dC/dx)$$

the diffusive flux (dq/dt) is directly proportional to the diffusion coefficient (D), the area of the diffusion barrier (A) and the concentration gradient across this barrier (dC/dx). Assuming D and A are constants, diffusion (or desorption from a soil particle) is seen to increase as the change in concentration (dC) across the barrier (particle surface) increases, or as the thickness (dx) of the diffusion barrier decreases. From a biological standpoint (Koch, 1990), the former can be effected by an increase in the affinity of the bacteria for the substrate as expressed by the organism-specific Michaelis-Menten (half saturation) constant. A decrease in the thickness of the diffusion barrier (the distance between source and sink) may result when bacteria attach directly to particles to which the solute is sorbed. Both desorption and biodegradation rates are enhanced by soil crushing which acts to increase A and decrease dx (Steinberg et al, 1987; Rijnaarts et al, 1990). These concepts are the foundation for much of the progress reported below.

Progress to Date:

For our studies, we have chosen naphthalene as a model compound since it is moderately soluble in water ($S = 31.7 \mu\text{g ml}^{-1}$), sorbs to soils to an extent which varies considerably over the range of soil organic carbon contents typical of surface and subsurface soils ($\log K_{oc} = 2.73$), and is readily degraded by a variety of soil bacteria. Four soils ranging in organic carbon content, cation exchange capacity and other characteristics were collected from field sites in Michigan and partially characterized. Two test organisms have been used in our work; *Pseudomonas putida* (ATCC 17484) and a non-motile, gram-negative naphthalene degrader isolated from a petroleum-contaminated soil and designated NP-Alk.

Soil Properties. Soils were characterized for their naphthalene-binding capacities by construction of sorption isotherms using batch equilibration methods. The organic carbon contents of the soils were determined by a private laboratory (Huffman Labs, Golden, CO). The cation exchange capacities were determined as the barium displaceable magnesium from the magnesium saturated soils (Rhoades, 1982). Soil humic acids were extracted and their aromatic carbon contents determined (Traina et al, 1990). Characteristics of the four soils studied are given in Table 1. All $\log K_{oc}$ values determined agree fairly well with the literature value of 2.73 (Abdul et al, 1987) although the non-log transformed K_{oc} values vary considerably. This variation will be discussed below as it relates to the kinetics of naphthalene desorption.

Characterization of Naphthalene Degraders. The test organisms, *P. putida* and NP-Alk, were characterized as to their concentration-dependent kinetics of naphthalene mineralization, their intermediary metabolism of the substrate, their susceptibility to the antibiotic, chloramphenicol, and their propensities for attachment to the soils under study. Knowledge of the attachment status of

cells in bioavailability assays is important from the standpoint that attached and free-living bacteria may exhibit different naphthalene mineralization efficiencies. Furthermore, as discussed above, attachment may act to shorten diffusion paths for sorbed naphthalene resulting in enhanced desorption and biodegradation rates.

Measuring the attachment of bacteria to the soils under study has presented a challenge. We have investigated several methods for determining the attachment of bacteria to soils, each with its own advantages and drawbacks. These have included:

1. Incubation of radiolabeled cells with soils for timed periods after which the soil particles are allowed to settle and the radioactivity in the cleared supernatant is measured (Ogram et al, 1985). Using this method, we have observed non-linear relationships between the degree of attachment and the soil:solution ratio in a manner which suggests that the concentration of dissolved organic carbon (DOC) released from the soil dictates the extent of attachment. Below a threshold DOC level, attachment is soil concentration-dependent. As the soil:solution ratio (and DOC concentration) increases, attachment is limited. Figure 1 shows that for all but the Oshtemo soil (0.76% OC), attachment is constant above a soil concentration of 100 mg/ml. This method suffers from the simultaneous settling of the bacteria with the soil and the extended periods required to obtain a cleared supernatant, during which detachment may occur.

2. Epifluorescence direct counting of bacteria in unseparated soil-bacteria slurries. This method suffers from the inability to observe stained cells beneath soil particles and the ambiguity of determining whether a cell associated with a soil particle is truly attached.

3. Flow cytometry. We have had some success in measuring attachment by this method which relies on the differential light scatter and fluorescence exhibited by soil particles and stained bacteria when excited by laser light. While still under investigation by us, this method suffers from the low scatter signal to noise ratio observed for particles of bacterial dimensions and the non-specific binding of fluorescent stains to abiotic particles as well as to bacteria.

4. Filtration of soil-bacterial suspensions through 3 μ m filters as a means of separating attached from free-living (plus attached to particles < 3 μ m dia.) bacteria. Using this method, we have observed a direct linear relationship between the soil:solution ratio and the extent of attachment. Entrapment of cells within the soil cake formed on the filter is the major drawback to this method.

5. Our current preferred method of measuring bacterial attachment to soils involves a low speed centrifugation step to remove sand and silt-size particles from suspension, followed by a 3 μ m filtration step. This method results in no filter clogging (all particles remaining in suspension pass through the filter) and examination of the filtrates by epifluorescence microscopy reveals that >95% of the bacteria are truly free-living. When compared to soil-free controls treated identically, plate counts of filtrates show a variable degree of attachment of the pseudomonad to the soils during the time frames of our bioavailability assays. Interestingly, the degree of attachment appears to be related to the DOC concentration in the slurries, indicating a behavioral (chemotactic) response to organic matter. Low DOC levels promoting attachment to the Oshtemo soil (Figure 1) could be counteracted by the addition of aqueous soil extract from the Colwood soil (Figure 2). Serial replacement of DOC with fresh PBS (phosphate-buffered saline) increased the extent of attachment of the pseudomonad to the Colwood soil. In contrast, NP-Alk does not exhibit DOC-modulated attachment and binds to Oshtemo and Capac soils equally despite their widely different organic carbon contents. Being non-motile, and therefore non-chemotactic, NP-Alk attaches to soils non-specifically.

During bioavailability assays, it is desirable for the substrate to be completely mineralized to CO₂ and H₂O without the accumulation of metabolites which would partition differently between the soil and aqueous phases than the parent compound. Using high performance liquid chromatography (HPLC), a wet chemical method for the analysis of polyphenolic compounds (Guerin and Jones, 1988), UV-visible spectrophotometry, and GC/MS, no significant accumulation of naphthalene metabolites was detected at any point during the batch culture of *P. putida* or NP-Alk on naphthalene. This permits the measure of ¹⁴CO₂ production from ¹⁴C-naphthalene to serve as an index of mineralization.

In some bioavailability assays, long incubation periods and/or high substrate concentrations present the potential problem of cell division (growth) which would cause $^{14}\text{CO}_2$ production to deviate from first-order kinetics to a process more accurately described by Monod or logistic models (Simkins and Alexander, 1984). To avoid this, we investigated the influence of chloramphenicol (an inhibitor of protein synthesis and cell division) on naphthalene metabolism by the pseudomonad. We found that at concentrations up to 34 ppm, naphthalene mineralization rates were unaffected by chloramphenicol although cell division was arrested. We have not found it necessary to incorporate chloramphenicol into our studies routinely since the concentration of naphthalene is generally insufficient to allow even a single cell doubling. In addition, we are interested in understanding the role that cell attachment plays in substrate bioavailability and chloramphenicol may inhibit this process as well.

We have noted little culture age-dependent differences in the ability of the pseudomonad to mineralize naphthalene. Cells remain fully viable and show only minor decreases in their specific naphthalene mineralization rates even 3 to 4 days into stationary culture (Figure 3). This simplifies protocols somewhat by not requiring a tightly regimented procedure for the preparation of these cells for use in bioavailability assays. NP-Alk exhibits an unusual, biphasic growth pattern when cultured in batch at high naphthalene concentrations (Figure 4). NP-Alk cells are induced for naphthalene mineralization during Phase I growth but are uninduced during Phase II (Figure 5). SDS-PAGE analysis of proteins in crude cell lysates shows several major differences in salicylate-grown or Phase I cells grown on naphthalene and those in Phase II growth. Filter-sterilized supernatant fluids from Phase I cultures supports the production of $>10^8$ cells of NP-Alk but no growth for any of five other naphthalene-degrading isolates. Our current hypothesis is that NP-Alk synthesizes a surfactant which facilitates the coordinate and complete mineralization of naphthalene during Phase I. Phase II growth is supported by the subsequent utilization of the surfactant as a carbon and energy source and results in the formation of large amounts of an unidentified intracellular storage product as visualized by transmission electron microscopy of thin-sectioned cells. NP-Alk displays many of the characteristics of an oligotrophic bacterium (Alexander, 1985; Rittman et al, 1986); 1) it reduces naphthalene to low ppb concentrations during Phase I growth, 2) it grows slowly on nutrient-rich agar media, and 3) it has a lower half-saturation constant (K_m) for naphthalene than the pseudomonad (see below). We routinely use overnight, early stationary phase (I) cultures which are fully induced for naphthalene mineralization in bioavailability assays.

Mineralization experiments are conducted in batch in 150 ml serum vials. Prior to inoculation with washed cell suspensions, sterile soils are pre-equilibrated with naphthalene solutions for periods of hours to months. For preserving aggregate structure, slurries are hand-shaken daily over the equilibration period; otherwise, samples are machine-shaken. At precisely timed intervals, duplicate aliquots of the inoculated slurries are withdrawn and injected into tubes containing 1 ml of 2N HCl. $^{14}\text{CO}_2$ is trapped on KOH-saturated filter paper contained in a cup suspended from the tube stopper. Working with soil slurries promotes the homogeneous distribution of naphthalene throughout the system and permits the sampling of representative aliquots.

Development of Bioavailability Assay. We have developed a simple, yet powerful kinetic method of assessing the bioavailability of sorbed naphthalene. Analysis of the kinetics of bacterial naphthalene mineralization involves the non-linear regression analysis (SYSTAT) of $^{14}\text{CO}_2$ production (P = percent initial activity as $^{14}\text{CO}_2$) over time (t) to obtain a first-order rate constant (k) according to the equation;

$$P = P_{\max} [1 - e^{-kt}]$$

where $P_{\max}$ represents the maximal percent mineralization and is treated as an unknown parameter to be estimated (Robinson, 1985). Rate constants (min^{-1}) are normalized to $P_{\max}$ (dimensionless)

and to the initial substrate concentrations (ng ml^{-1}) to obtain initial mineralization rates ($\text{ng ml}^{-1} \text{ min}^{-1}$). These can be compared directly within experiments where the cell density is constant or they can be normalized to cell density to obtain specific mineralization rates ($\text{ng cell}^{-1} \text{ min}^{-1}$) for inter-experiment comparisons. This analysis allows direct comparison of the rates as well as the extents of degradation.

The influence of substrate concentration (S) on reaction rate (V) is described for non-growing microbial cultures by the Michaelis-Menten equation:

$$V = V_{\max} [S / (K_m + S)]$$

where $V_{\max}$ is the maximal rate of reaction and K_m is the substrate concentration at which the rate is equal to one-half the maximal rate. When S is much greater than K_m , V is equal to $V_{\max}$. When S is much less than K_m , on the other hand, V is directly proportional to the substrate concentration, S. Mineralization rates were measured over a range of naphthalene concentrations to construct double reciprocal (Lineweaver-Burke) plots for the determination of K_m and $V_{\max}$ for NP-Alk and 17484. Table 2 shows that NP-Alk has a significantly higher affinity (lower K_m) for naphthalene than 17484, while the latter is capable of a higher maximal rate of naphthalene mineralization.

Using the constants obtained for 17484, a Michaelis-Menten plot of rate vs. concentration predicts that at the soil:solution ratios normally used in bioavailability assays, an initial naphthalene concentration of $10 \mu\text{g/ml}$ results in equilibrium aqueous phase naphthalene concentrations that would always support the maximum mineralization rate (Figure 6). This prediction was borne out in mineralization assays using 17484 and both the Capac and Colwood soils as sorbents (Figure 7). Figure 8 shows the relationship between equilibrium aqueous phase naphthalene concentrations and mineralization rates calculated from the data in Figure 7. Apart from one anomalous control value, all points are seen to fall on a horizontal line corresponding to $V_{\max}$ in Figure 6. Despite constant rates, the extent of mineralization ($P_{\max}$) decreased slightly with increasing soil:solution ratio in the Colwood slurries.

From Figure 6, it can also be predicted that at low initial naphthalene concentrations (below K_m), decreasing equilibrium aqueous phase naphthalene concentrations, resulting from increasing soil:solution ratios, will result in linear decreases in mineralization rates. If we assume that only dissolved naphthalene is available to microbial degraders, data for mineralization rate vs. equilibrium aqueous phase naphthalene concentrations obtained in soil slurries should fall on a theoretical line connecting a soil-free datum point with the origin. Deviations from this line would indicate that biologically available naphthalene concentrations are greater than (points above the line) or less than (points below the line) the concentrations of unsorbed naphthalene. A similar approach to assessing bioavailability of sediment-sorbed phenol and dodecyltrimethylammonium chloride was used by Slomp and Young (1988). This method has proven more useful in assessing the bioavailability of NOCs than the modelling approach of Ogram et al (1985), which we originally proposed using. By establishing experimental conditions (substrate concentrations below K_m) under which the hypothetical response (utilization of only dissolved substrate) results in a linear relationship between rates and dissolved concentrations, positive and negative rate deviations from the line can be readily interpreted. Our analysis also permits evaluation of the influence of sorption on the extent of mineralization by examination of estimates of the model parameter, $P_{\max}$. Inherent limitations in Ogram's models are the assumptions of continuous equilibrium between the sorbed and aqueous phases, and the ultimate attainment of complete mineralization of sorbed compound (i.e., constant $P_{\max}$). This requires instantaneous desorption from the sorbed state as the aqueous phase contaminant concentration is reduced through biodegradation. Although these assumptions may be valid for ionic compounds like 2,4-D or highly soluble NOCs like benzene, they are clearly not valid for lower solubility NOCs such as naphthalene or phenanthrene. Because bioavailability limitations are likely to be most evident with such low solubility compounds which are known to desorb more slowly, the models of Ogram were found to be inappropriate to this study.

Bioavailability Experiments. The bioavailability of sorbed naphthalene to 17484 with soil:solution ratios of 7.7 to 190 mg ml⁻¹ and an initial naphthalene concentration of 51 ng ml⁻¹ (ppb) are shown in Figure 9. Mineralization curves for both the Colwood and Capac soils deviated somewhat from first order kinetics. Nevertheless, first order mineralization rates were derived by fitting the data to the model and these are plotted against equilibrium aqueous phase naphthalene concentrations in Figure 10. With the Colwood soil as sorbent, mineralization rates were directly related to the equilibrium aqueous phase naphthalene concentrations falling on or near the theoretical line. Upward deviations in rates from the theoretical line with the Capac soil as sorbent, indicated available naphthalene concentrations in excess of the equilibrium aqueous phase concentrations. The positive linear slopes during the latter parts of some progress curves (Figure 9) may indicate a desorption rate limitation of mineralization at this stage of the assay. Mineralization of slowly desorbing naphthalene from a non-labile phase could explain the poor model fits observed in Figure 9. Reproducible inflections in the mineralization curves with an exponential phase followed by a linear phase during which mineralization is limited by the rate of desorption of non-labile sorbed naphthalene are characteristic of bioavailability assays employing 17484. Notably, deviations from ideal first order kinetics were more pronounced in the Colwood than in the Capac soil. According to the rule of thumb formulated by Karickhoff (1984), the time required to attain complete desorption is numerically equal to the K_{oc} of the system in minutes. A comparison of K_{oc} values in Table 1 indicates that desorption would be 1.5 times slower for the Colwood than for the Capac soil.

First order model estimates of P_{max} obtained from the data in Figure 9 show that despite the observed reductions in mineralization rates, sorption had little effect on the extent of mineralization by 17484. Figure 11 shows that P_{max} was constant at all soil:solution ratios and was equal to the soil-free control value. Thus, 17484 appears capable of facilitating the complete and rapid desorption of naphthalene bound to soil.

In contrast to 17484, sorbed naphthalene does not appear to be available to NP-Alk. Figure 12 shows mineralization time courses for naphthalene sorbed to Capac soil for both organisms. While data for 17484 closely resembled earlier results, dramatic decreases in both rate and extent of mineralization were observed at increasing soil:solution ratios for NP-Alk. Furthermore, excellent first-order model fits were obtained for the data.

Mineralization rates for 17484 and NP-Alk, derived from the data in Figure 12, are plotted against the equilibrium aqueous phase naphthalene concentrations in Figure 13. As observed previously, rates deviate upward from the theoretical line for 17484 indicating availability of sorbed substrate. Rates are seen to fall near but slightly below the theoretical line for NP-Alk clearly indicating that sorbed substrate is unavailable to this organism.

Figure 14 shows the relationship between P_{max} (derived from non-linear regression analysis of the data in Figures 12) and the equilibrium aqueous phase naphthalene concentration. It is seen that sorption has little effect on the extent of naphthalene mineralization by 17484. For NP-Alk the extent of naphthalene mineralization decreases linearly with increasing soil:solution ratio. Although the non-zero intercept obtained by linear regression analysis of the data indicates that some of the sorbed naphthalene becomes available to NP-Alk over the course of the assay, the data indicate that sorbed naphthalene is largely unavailable to this organism.

It is clear from these results, that organism-specific properties dictate whether and to what extent sorbed naphthalene is biologically available. Some of the relevant characteristics of the two organisms studied to date are shown in Table 2. Among these characteristics, we believe that motility and chemotaxis are of major significance. The ability of *P. putida* to sense and swim toward naphthalene bound to soils permits it to relocate on the particle surface after depletion of substrate in one location. In so doing, 17484 is able to facilitate the desorption of labile bound naphthalene. NP-Alk, lacking this ability, binds non-specifically to soils where only the naphthalene which diffuses to the cells is available. For NP-Alk, naphthalene biodegradation is strictly desorption rate-limited.

These organism-specific differences are clearly depicted in Figure 15. In a comparison of

the four soils at identical soil:solution ratios and an initial naphthalene concentration of 96 ng/ml, mineralization rates by 17484 were significantly correlated with equilibrium aqueous phase naphthalene concentrations but all fell above the theoretical line corresponding to the soil-free rate. Deviations from the theoretical line increased with decreasing K_p for naphthalene (Table 1). It has been found that polycyclic aromatic hydrocarbons partition increasingly into a non-labile sorbed phase with increasing K_p and that the sorbed fraction in the labile phase is proportional to the resultant equilibrium aqueous phase concentration (Karickhoff and Morris, 1985). It thus appears that labile sorbed naphthalene is biologically available to 17484. For NP-Alk, a linear relationship between rates and equilibrium aqueous phase naphthalene concentrations was also found, but here all points fell on the theoretical line, thus satisfying the hypothesis that only dissolved naphthalene is biologically available to this organism. The markedly different bioavailability of naphthalene sorbed to identical soils to the two test organisms is an unexpected and extremely interesting result. The characteristics of 17484 that may increase its access to sorbed material include its apparent chemotaxis, motility, and propensity and site of attachment to soil. The inability of NP-Alk to degrade sorbed naphthalene seems consistent with its non-specific attachment to soil, and its lack of motility and chemotactic behavior.

Aging Studies. In ongoing experiments, we are also evaluating the influence of prolonged soil-naphthalene contact in regulating the bioavailability of the sorbed material. Eight sets of experimental vials (two soil-free controls and two Colwood soil slurries per set) containing identical total naphthalene concentrations were set up in November, 1990. At intervals, vials from one set plus a freshly prepared control are inoculated with 17484 and mineralization data analyzed. The results of the first four sets of vials, representing aging periods up to 58 days, are shown in Figure 16. These data show that upon aging, the ratio of the initial mineralization rate in soils to that in controls decreases progressively and approaches the theoretical rate ratio (i.e., the ratio expected if only dissolved naphthalene were available in soil slurries). Aging has no effect, however, on the extent of mineralization (P_{max}) by 17484. These results are consistent with the hypothesis that 17484 has complete access to labile sorbed naphthalene. As aging acts to shift sorbed naphthalene from a labile to a non-labile sorbed compartment, reductions in initial mineralization rates result. By establishing steep concentration gradients at the sorbent surface, the desorption of non-labile naphthalene is facilitated such that no reductions in the extent of mineralization were observed over the time course of the assay.

With a workable bioavailability assay now in hand, we will focus more of our future efforts in elucidating the effects of aging on bioavailability in laboratory studies. We will also continue our efforts to obtain field aged soils to confirm laboratory results.

It is clear from results obtained to date, that generalizations about the bioavailability of sorbed NOCs are elusive. Organism-specific and sorbent-specific properties lead to variable results suggesting that sorbed naphthalene availability depends both on the organism and the fraction of sorbed naphthalene which resides in a labile phase. The rate at which sorbed material desorbs from the soils, the extent of attachment by bacteria to particles harboring sorbed compound, and the ability of the organisms to locate and utilize the sorbed compound, are questions for further study.

Bioavailability of Aged PCB Residues. We have conducted initial experiments to assess the bioavailability of aged PCB residues in the Glenn Fall drag-strip soil. This soil was contaminated with Arochlor 1242 about 20 years ago. Briefly, the results did suggest that the aged PCBs in the contaminated drag-strip soil were less available than PCBs added to clean sediment. This result is based on comparing the rate of degradation of a specific PCB congener in either the aged (Glenn Falls) or recently added (clean sediment) Arochlor 1242 mixture to that of a non-Arochlor PCB congener that was added to each sample. However, these results are complicated by the fact that overall, dechlorination activity in the drag-strip soil was higher than in the clean sediments. The comparatively high degree of availability of PCBs in the drag-strip soil makes it difficult to observe the effects of aging, and suggests that other environmental factors have also affected bioavailability in this sample. One such factor may be the presence of a residual petroleum oil (which was also

used on these soils) phase. We have recently shown that such anthropogenic oil phases act as powerful sorbent phases for PCBs and other organic contaminants (Boyd and Sun, 1989). PCBs held in such a phase may have significantly higher desorption rates and therefore be more readily available.

Because the results described above were somewhat inconclusive, we have initiated new experiments using sediments from a different PCB site, and we have revised our experimental design. The sediments are from the Saginaw River in Michigan and are contaminated with Arochlor 1242. Like the drag-strip soil, the Arochors (PCBs) are largely unaltered and are over 20 years old. However, preliminary experiments with these sediments have shown that the aged PCBs are resistant to anaerobic dechlorination by the Hudson River bacteria, suggesting that they may be biologically unavailable. To directly assess this hypothesis, we have added two PCB congeners that are not present in Arochlor 1242, to the sediment. The first congener is added at high concentrations, equivalent to the indigenous PCB concentration. This will ensure that there is a sufficient concentration of an available PCB to initiate dechlorination. The second (non Arochlor 1242) congener is added at a lower concentration, similar to concentrations of individual PCB congeners in the indigenous Arochlor 1242. The rate of dechlorination of the latter (added) congener will be compared to similar (eg., other pentachlorobiphenyls) indigenous PCBs to demonstrate differential bioavailability due to aging. As a control, clean Hudson River sediments will be spiked with Arochlor 1242 and inoculated with the Hudson River organisms. This sediment is known to support dechlorination and will establish the dechlorination activity of the inoculum.

Because of the protracted incubation times of 16 weeks, these experiments are still underway. If successful, they will provide a direct assessment of the bioavailability of aged PCBs in an actual field sample, and establish the role of aging in reducing bioavailability.

Research Plans:

During the remaining 5 months of our second year investigations, our objectives are:

1. To clarify the relationship between DOC, soil:solution ratio and attachment of cells to soils.
2. To further compare the relative influences of sorption on the bioavailability of naphthalene to the two organisms discussed above.
3. To relate attachment to the apparent utilization of labile sorbed naphthalene by 17484.
4. To complete bioavailability experiments using field weathered sediments containing aged PCB residues.
5. To compare manuscripts for publication of our results. We anticipate papers dealing with:
 - a. The influence of dissolved organic carbon on attachment of bacteria to soils.
 - b. A description of the novel naphthalene-degrading isolate, NP-Alk.
 - c. Two or more papers describing our results on the bioavailability of sorbed naphthalene to microbial degraders as a function of organism motility, substrate affinity and propensity for attachment, and as a function of soil-specific properties such as organic matter content, K_{oc} for naphthalene, and desorption kinetics.

Project Output:

In addition to providing training for one post-doctoral research associate (W.F. Guerin) and one Ph.D. graduate student (S. Sun), the following presentations have evolved from this project:

- Guerin, W.F. and S.A. Boyd. 1990. Influence of sorption on the biodegradation of naphthalene by soil bacteria. Soil Science Society of America Abstracts, p. 250.
- Guerin, W.F. and S.A. Boyd. 1991. Bioavailability of soil-sorbed naphthalene to *Pseudomonas putida*. American Society for Microbiology, Annual Meeting, Dallas, TX.

Summary of Accomplishments:

1. Developed a quantitative method for directly assessing the bioavailability of organic contaminants in soil-water systems.
2. Determined that the models of Ogram et al (1985) are inappropriate for describing the influences of sorption on biodegradation for poorly water soluble non-ionic organic contaminants.
3. Demonstrated that sorption to soil can render contaminants at least partially (or completely) unavailable for microbial degradation.
4. Initial experiments with aged PCB residues from field samples suggest lower availability than recently added PCBs. Sample identification and accessibility is a problem for these studies.
5. Contaminants held in the labile compartment of soil organic matter may be available to bacteria. The fraction of labile contaminant is related to:
 - a. Water solubility of the compound.
 - b. The sorption coefficient (K_p).
 - c. Contaminant aging.
 - d. Characteristics of the soil organic matter phase.
6. Bacteria-specific properties are important in determining availability of sorbed contaminants *via* access to the labile compartment. Properties of potential importance are:
 - a. Motility.
 - b. Attachment.
 - c. Chemotaxis.

References:

- Abdul, S.A., T.L. Gibson and D.N. Rai. 1987. Haz. Waste Haz. Materials. 4:211.
- Alexander, M. 1985. Biodegradation of organic chemicals. Environ. Sci. Technol. 18:106-111.
- Boyd, S.A. and S. Sun. 1989. Residual petroleum and PCB oils as sorptive phases for organic contaminants in soil. Environ. Sci. Technol. 24:142-144.
- Guerin, W.F. and G.E. Jones. 1988. Two-stage mineralization of phenanthrene by estuarine enrichment cultures. Appl. Environ. Microbiol. 54:929-936.
- Karickhoff, S.W. 1984. Organic pollutant sorption in aquatic systems. J. Hydraulic Engin. 110:707-735.
- Karickhoff, S.W. and K.R. Morris. 1985. Sorption dynamics of hydrophobic pollutants in sediment suspensions. Environ. Toxicol. Chem. 4:469-479.
- Kelly, F.X., K.J. Dapsis and D.A. Lauffenburger. 1988. Effect of bacterial chemotaxis on dynamics of microbial competition. Microb. Ecol. 16:115-131.
- Koch, A.L. 1990. Diffusion. The crucial process in many aspects of the biology of bacteria. Adv. Microbial Ecol. 11:37-70.
- Ogram, A.V., R.E. Jessup, L.T. Ou and P.S.C. Rao. 1985. Effects of sorption on biological degradation rates of (2,4-dichlorophenoxyacetic) acid in soils. Appl. Environ. Microbiol. 49:582-587.
- Pignatello, J.J. 1989. Sorption dynamics of organic compounds in soils and sediments. In, B.L. Sawhney and K. Brown (eds.), Reactions and Movement of Organic Chemicals in Soils. Soil Science Society of America, Madison, WI. pp. 45-80.
- Rhoades, J.D. 1982. Cation Exchange Capacity. In, A.L. Page (ed.), Methods of Soil Analysis, part 2. Soil Science Society of America, Madison, WI. pp. 149-157.
- Rittmann, B.E., L. Crawford, C.K. Tuck and E. Namkung. 1986. *In situ* determination of kinetic parameters for biofilms: isolation and characterization of oligotrophic biofilms. Biotechnol. Bioengineer. 28:1753-1760.
- Rijnaarts, H.H.M., A. Bachmann, J.C. Jumelet and A.J.B. Zehnder. 1990. Effect of desorption and intraparticle mass transfer on the aerobic biomineralization of hexachlorocyclohexane in a contaminated calcareous soil. Environ. Sci. Technol. 24:1349-1354.
- Robinson, J.A. 1985. Determining microbial kinetic parameters using nonlinear regression analysis. Adv. Microb. Ecol. Vol. 8, pp. 61-114.
- Shimp, R.J. and R.L. Young. 1988. Availability of organic chemicals for biodegradation in settled bottom sediments. Ecotoxicol. Environ. Safety. 15:31-45.
- Simkins, S. and M. Alexander. 1984. Models for mineralization kinetics with the variables of substrate concentration and population density. Appl. Environ. Microbiol. 47:1299-1306.
- Steinberg, S.J., J.J. Pignatello and B.L. Sawhney. 1987. Persistence of 1,2-dibromoethane in soils: Entrapment in intraparticle micropores. Environ. Sci. Technol. 21:1201-1208.
- Traina, S.J., J. Novak and N.E. Smeck. 1990. An ultraviolet absorbance method of estimating the percent aromatic carbon content of humic acids. J. Environ. Qual. 19:151-153.
- van Loosdrecht, M.C.M., J. Lyklema, W. Norde and A.J.B. Zehnder. 1990. Influence of interfaces on microbial activity. Microbiol. Rev. 54:75-87.
- Wu, S.-C. and P.M. Gschwend. 1986. Sorption kinetics of hydrophobic organic compounds to natural sediments and soils. Environ. Sci. Technol. 20:717-725.

TABLE 1

SOIL CHARACTERISTICS

SOIL	% OC ¹	CEC ²	K _p ³	K _{oc} ⁴	log K _{oc}	% AROMATIC C ⁵
CAPAC	3.46	12.2	12.51	362	2.56	28.6
COLWOOD	5.36	21.6	28.93	540	2.73	28.9
OSHTMO	0.76	5.6	4.56	600	2.78	28.0
SCHOOLCRAFT	1.84	8.0	8.07	440	2.64	

¹ percent organic carbon (dry weight)² cation exchange capacity (meq/100g)³ naphthalene partition coefficient⁴ organic carbon-normalized partition coefficient for naphthalene⁵ percent aromatic carbon of the soil humic acid fraction [Traina et al (1990)]

TABLE 2

ORGANISM COMPARISON

CHARACTERISTIC	<i>P. putida</i> (17484)	NP-Alk
Coordinate mineralization of naphthalene	yes	yes
K _m for naphthalene	224 ng ml ⁻¹	121 ng ml ⁻¹
V _{max} for naphthalene	7.8 ng cell ⁻¹ min ⁻¹ (x 10 ⁷)	6.4 ng cell ⁻¹ min ⁻¹ (x 10 ⁷)
Motile	yes	no
Chemotactic	yes?	no
Attachment	sensitive to DOC	insensitive to DOC
Growth on nutrient-rich media	rapid	slow
Surfactant production	no	yes?
Mineralizes sorbed naphthalene	yes?	no

Figure 1. Influence of soil concentration on attachment of *P. putida* (17484) as determined by settling and radioassay of labeled cells in cleared supernatant.

Figure 2. Influence of exogenous dissolved organic carbon on attachment of *P. putida* (17484) to Oshtemo soil. Total liquid volume of suspensions is 20 ml.

Figure 3. Effect of culture age on naphthalene mineralization kinetics by *P. putida* (ATCC 17484). Cell densities ($\times 10^8$): 22h, 0.94; 47 h, 1.0; 72 h, 1.0; 122 h, 1.46.

Figure 4. Growth curve for NP-Alk on Naphthalene (200 mg/l).

Figure 5. Mineralization kinetics for naphthalene by NP-Alk in Phase I and Phase II growth (see Figure 2).

Figure 6. Michaelis-Menten plot using experimentally-derived values of K_m and $V_{\max}$ for *P. putida* (17484). Shaded areas show the ranges of the resultant equilibrium aqueous phase naphthalene concentrations at the soil:solution ratios used and when initial naphthalene concentrations are either 10 ug/ml or 100 ng/ml.

Figure 7. Naphthalene mineralization time courses by *P.putida* (17484) at an initial naphthalene concentration of 10 ug/ml and with Capac and Colwood soils as sorbents at the indicated concentrations.

Figure 8. Relationship between the mineralization rates (calculated from the data in Figure 7) and equilibrium aqueous phase naphthalene concentration.

Figure 9. Naphthalene mineralization time courses by *P. putida* (17484) at an initial naphthalene concentration of 51 ng/ml and with Capac and Colwood soils as sorbents at the indicated concentrations.

Figure 10. Relationship between the mineralization rates (calculated from the data in Figure 9) and equilibrium aqueous phase naphthalene concentration.

Figure 11. Relationship between the extent of mineralization ($P_{\max}$, derived from the data in Figure 9) and equilibrium aqueous phase naphthalene concentration.

Figure 12. Naphthalene mineralization time courses for *P. putida* (17484) and NP-ALK at an initial naphthalene concentration of 38 ng/ml and with Capac soil as sorbent at the indicated concentrations.

Figure 13. Relationship between the mineralization rates (calculated from the data in Figure 12) and equilibrium aqueous phase naphthalene concentrations. Dashed lines are theoretical lines obtained for *P. putida* (17484) and NP-Alk in soil-free controls.

Figure 14. Relationship between the maximal percent mineralization (P_{max}) and equilibrium aqueous phase naphthalene concentration (calculated from the data in Figure 12) for *P. putida* (17484) and NP-Alk in Capac soil suspensions.

MINERALIZATION RATE AS A FUNCTION OF EQUILIBRIUM
[NAPHTHALENE]_{aq} FOR FOUR SOILS AT IDENTICAL SOIL:SOLUTION

Figure 15. Naphthalene mineralization rates as a function of equilibrium aqueous phase naphthalene concentrations for *P. putida* (17484) and NP-Alk in four soils at identical soil:solution ratios (200 mg/ml) and initial naphthalene concentrations (58.7 ng/ml). Theoretical relationships shown by dashed line in upper panel and solid line in lower panel.

Figure 16. Influence of aging in Colwood soil slurries on the rate and extent ($P_{\max}$) of naphthalene mineralization by *P. putida* (17484) expressed relative to the control (soil-free) values. The theoretical rate ratio (assuming availability of only dissolved naphthalene) is shown by the dashed line in the upper panel.

END

DATE FILMED

03 / 01 / 91