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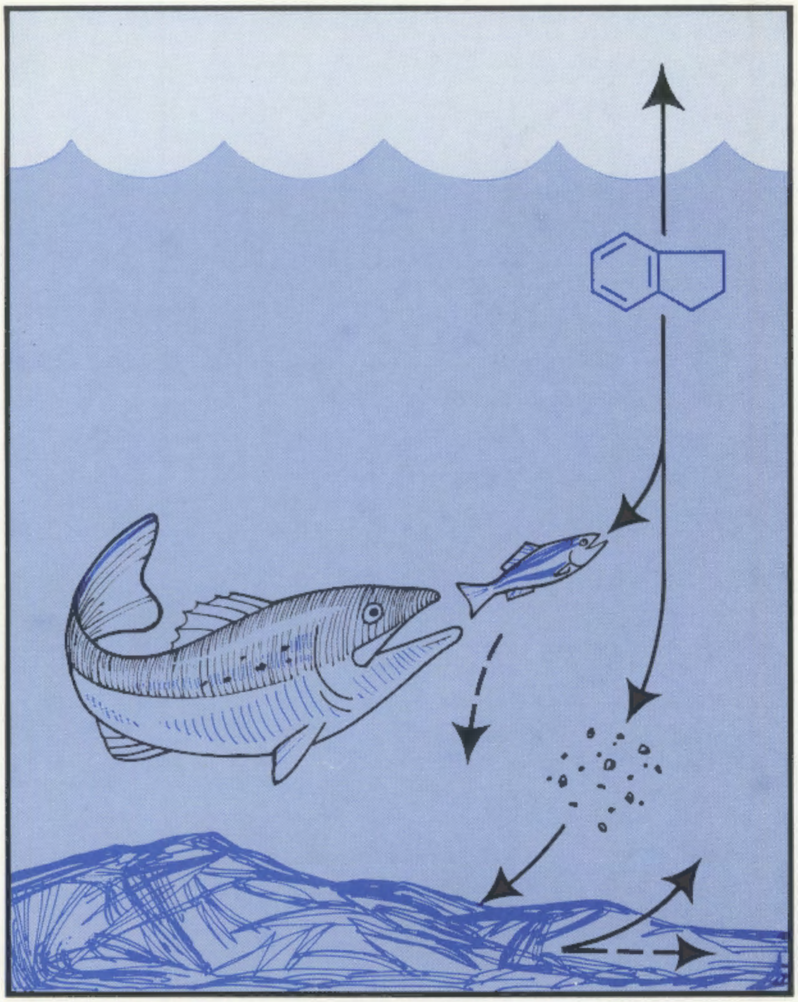
Behavior of Complex Mixtures in Aquatic Environments

A Synthesis of PNL Ecological Research



June 1984

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BEHAVIOR OF COMPLEX MIXTURES IN AQUATIC ENVIRONMENTS: A SYNTHESIS OF PNL ECOLOGICAL RESEARCH

D. H. Fickeisen
B. E. Vaughan, editors

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FOREWORD

The environmental behavior of complex mixtures of organic compounds and xenobiotic compounds in general represents possibly one of the most important research topics of the decade. This area has led to several studies by the National Research Council (NAS/NRC 1972a, 1972b, 1981) and a continuing symposium series by Battelle Press,* 1978-1984.

The term "complex mixture" has been recently applied to energy-related process streams, products and wastes that typically contain hundreds or thousands of individual organic compounds, like petroleum or synthetic fuel oils; but it is more generally applicable. Impetus for the present research originated in PNL's investigations on the toxicity of petroleum, coal liquids, and other organic wastes. During the past six years, the scientific basis for understanding these and similar materials has been so deficient that often not even the experimental paradigms for toxicological investigations could be adequately specified. Extensive development in the areas of chemical characterization, organism responses, ecosystems-level determinations and metabolism was needed because the state-of-the-art was not at the outset adequate to answer many environmental questions raised by possible release of these unique materials. Questions, for example, about long-term environmental degradation or chronic effects on ecosystems have been particularly difficult to deal with because quantitative methodology in these areas is limited.

PNL ecological research on complex mixtures has been directed at these several key topics. This report and a current critical review (Vaughan, 1984) synthesize results to date, relate these results to the current ecological research program, of DOE, and describe advances in scientifically understanding the responses of aquatic ecosystems to such materials.

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*Battelle Press; 505 King Ave., Columbus, OH 43201.

EXECUTIVE SUMMARY

A six-year program of ecological research has focused on four areas important to understanding the environmental behavior of complex mixtures: physicochemical variables, individual organism responses, ecosystems-level determinations, and metabolism. Of these areas, physicochemical variables and organism responses were intensively studied; system-level determinations and metabolism represent more recent directions of the program which have not yet received the same level of effort. Chemical characterization was integrated throughout all areas of the program, and state-of-the-art methods were applied.

Chemical Variables

The most important factors influencing the determination of biological response to oil-like materials were found to be partitioning from the oil to the water column and losses from the water column by a combination of "weathering" processes. The latter processes included partial microbial degradation, volatilization, water solubilization, and sorption to suspended particles. Weathering was demonstrable in both beaker and semi-natural outdoor exposures to water soluble fractions (WSF) of coal liquid, where shifts from predominantly phenolic to predominantly aromatic hydrocarbon composition could be demonstrated over a few hours for many of the exposure situations investigated. Of particular interest was the consistent observation that weathered WSFs were several times more toxic than fresh material based on equivalent concentrations of total carbon. Higher molecular weight residuals, of an evidently toxic nature, were demonstrable in the weathered materials. These residuals included phenols of C_6 and greater chain length and higher molecular weight aromatic hydrocarbons.

Detailed aromatic hydrocarbon and phenol analyses were carried out on both weathered and fresh materials, and on tissues, using mass spectrographic and gas chromatographic (MS/GC) procedures. The papers cited throughout this report should be consulted for details. In selected cases aniline-type and heterocyclic compounds were also determined. A focus was maintained on WSF chemistry because PNL's experience in petroleum research had verified that this was the most important route for chronic ecosystem effects (except for catastrophic spills). No scientific consensus currently exists on appropriate reference compound classes to be measured, but selection of the compounds examined was based on considerations of long-term persistence, inherent toxicity, and usefulness for delineating metabolic pathways.

Partitioning from oil to water was a particular problem in determining biological responses. The mortality response of daphnids, for example, could be varied at will within two orders of magnitude depending on kinetic energy of mixing and other preparation variables. For these reasons, specialized devices were developed for controlled exposures of fish and other organisms. Staff effort was also allocated to committees of the

American Society for Testing and Materials (ASTM) in order to help achieve some scientific consensus on the appropriate handling of oil-like materials. Out of these efforts a standardized daphnid bioassay (Daphnia magna) was developed for range-finding applicable to unknown or new materials. Because of widespread interest in octanol and oil/water partitioning coefficients, these approaches were also studied. Although octanol/water partitioning (k_{ow}) has been proposed to estimate bioaccumulation potential of new compounds, polarity of specific molecules can lead to significant bias. Metabolism may be a more important consideration to be investigated.

Organism Responses

In examining biological responses to a toxic material, good practice now requires multistaged, or "tiered," procedures in which several responses are determined, ranging from acute mortality to effects on growth, reproduction, behavior and ecosystems-level functioning. The so-called "sublethal" responses--impaired growth or reproduction--are generally considered to be more sensitive indicators of biological effect, and they may have greater relevance to ecosystem function.

Pacific Northwest Laboratory (PNL) implemented tiered determinations on two fish species, two aquatic insect species, a zooplankter, and a freshwater unicellular algal species, to examine ecosystem components from the major trophic levels. Procedures were implemented selectively because, like reproduction or behavior, time and effort levels rapidly escalated with the higher tiers of response.

Both growth and reproductive responses proved to be sensitive indicators of exposure to the coal liquid WSF for the three invertebrates examined (D. magna, Tanytarsus dissimilis, and Chironomus tentans). In addition, good chemical characterization and control were maintained over the longest period of exposure (7 days). These sublethal responses were at least six times more sensitive than corresponding mortality responses, and they were demonstrable at total organic carbon concentrations of about 1 mg/L for weathered WSF exposures. For all three organisms, and the alga mentioned below, weathered WSF fractions proved to be several times more toxic than fresh WSF (based on total organic carbon). Relative sensitivities were detected as daphnids > insects > algae. Phenolic compounds were principal constituents, but an aromatic hydrocarbon fraction also contributed significantly to the observed toxicity. Growth stimulation of algae at sublethal concentrations appeared to be a secondary consequence of the degradation of phenolic compounds by microbial organisms unavoidably present in the exposure systems.

In fish also, growth and reproduction were highly sensitive indicators of toxicity. Because of the longer time necessary to maintain chronic fish exposures (21 to 42 days) during sublethal determinations, in comparison to invertebrates, special exposure approaches were developed. In the fathead minnow (Pimephales promelas), growth was inhibited at 0.3 mg/L (phenolics) and spawning was inhibited at 0.6 mg/L (phenolics). Rainbow trout (Salmo gairdneri) were even more sensitive than the minnow and were readily killed at these low concentrations. Although phenolic compounds constituted about 95% of the organic carbon, the toxic responses observed probably cannot be attributed to any single compound class. Additionally, phenolics that were present as a complex mixture in the WSF were more toxic than the specific phenolic compounds tested individually.

Behavioral avoidance determinations are considered by some to be highly sensitive indicators of abnormal water conditions. They proved to be less sensitive than were the determinations of effects on growth and reproduction. This was true both for fathead minnow and for one of the three invertebrates studied. The fathead minnow, for example, consistently avoided acutely toxic concentrations of total phenolics when exposed to graded concentrations of WSF in a preference chamber of unique configuration. Confidence intervals (95%) were established to determine an AC_{50} of 1.5 mg/L total phenolics, based on a logistic model of concentration versus response, and the fit was excellent ($\alpha = 0.005$). Nevertheless, the minnow did not avoid lower concentrations known to adversely affect reproduction, for example 0.3 mg/L (phenolics). Rainbow trout were killed at the 1.5 mg/L concentration that minnows avoided, and an avoidance response in the trout could not be reliably established at lower concentrations. On the contrary, the trout were apparently attracted to concentrations of 2 mg/L (phenolics), a concentration sufficient to cause severe growth and reproductive abnormality. Among the invertebrates examined, avoidance response could not be demonstrated satisfactorily by ovipositioning in T. dissimilus. In the clam, however, valve closure was shown at concentrations known to be acutely toxic to insect larvae.

Systems Determinations

Ecosystems-level experimentation was not feasible for the mutagenic materials used in the present studies, so efforts were directed to community-level investigations that could be maintained under partial control of the laboratory. Between the highly controlled tank "microcosms" used by some research scientists and perturbations of the natural ecosystem attempted by others, certain trade-offs had to be considered: reliable sampling, minimum boundary effects, ease of replication, and realism with respect to the outdoor situation. An outdoor artificial stream system was developed, complete with natural sediments and colonized by natural processes (river water). This design was considered optimal with respect to trade-offs. In the artificial streams, five standard attributes of community function were measured during and following graded oil exposures: biomass, chlorophyll a, phaeophyton, community structure (i.e., algal and invertebrate species counts), and net photosynthesis. Phenolic and aromatic hydrocarbon chemistry was also determined in sediments and water during these exposures. In developing the artificial stream system, maintenance of normal sediment microbiotic activity (via allochthonous carbon sources in the flowing river water) was considered necessary. Indeed, microbial biosynthesis was found to contribute significantly, for example, to the higher molecular weight phenolic compounds (e.g., >C_6-phenolics) found at the conclusion of the experiments compared to those found in the initially oiled sediment. In the same experiments aromatic hydrocarbons remained constant throughout the exposure period.

Changes in algal biomass and community structure (i.e., algal and invertebrate species counts) following exposure to oiled sediments were in some cases visible to the eye. However, they were not determinable with quantitative reliability, at least for practicable levels of replication and sampling effort. When the source of contamination was shifted from the sediment to the water column, using a specially baffled system, algal biomass determination again proved to be an insensitive measurement. However, for the same level of effort, photosynthesis and respiration proved to be both sensitive and statistically reliable in detecting changes. For example, exposure of the colonized system to graded concentrations of total organic carbon, e.g., 0, 6, and 16 mg/L, caused a dose dependent inhibition of both photosynthesis and respiration, compared to higher concentrations reported in the literature.

Statistical analysis of these data sets took into serious consideration power and possibility of type II error. In general, the coefficients of variation were acceptable for this type of experiment (i.e., lower than those reported for field experimentation but higher than those reported for highly controlled microcosms using artificial sediments). It is clear that additional development will be required to perfect the state-of-the-art of measuring community responses to contaminants. Also, because of the largest cost considerations attached to the statistical replication requirement, additional work should focus on identifying new, more sensitive indicies of community function. Until new methods become available, respiration and photosynthesis seem to be the most useful measures, and they tend to integrate across species and trophic level.

Re-entrainment of chemical compounds from sediment to biota has not been intensively reported on in the literature. In estuarine and saltwater situations with clams and burrowing polychaetes (Macoma inquinata, Abarenicola pacifica), PNL experiments have established that the principal pathway for uptake of polycyclic aromatic hydrocarbons (PAH) from contaminated sediments is via interstitial water and not directly from the sediment.

Also, in the artificial stream experiments, modeling efforts were developed for predicting the disappearance in sediments of C_4-phenolics, and these predictions agreed well with the chemical determinations. However, the model appears to be of limited usefulness considering that variations in the mixture of constituent compounds might differentially influence microbial activities in other oil-like mixtures. Such an inference is supported both by the available literature and by the demonstration in present experiments that microbial biosynthesis of higher molecular weight phenolics proceeded concurrently with biodegradation of lower molecular weight phenolics in stream sediments. Clearly, microbial metabolism was an important factor that determined how compound distribution in the sediments changed over time, but its rate and direction are difficult to predict. Volatilization has been suggested as another factor to account for sediment disappearance of oil constituents. The literature indicates that volatilization is unimportant for 2- to 4-ring PAHs and polychlorinated biphenyls.

Metabolism and Fate

Metabolism of specific aromatic and phenolic compounds proved to be major concerns in models dealing with the hazard implications of coal liquids. These concerns are also applicable to petroleum and other organic wastes. In the context of complex mixtures, metabolism has been generally overlooked; for example, potentiation (as well as blocking) of mutagenic response was demonstrated in recent PNL research where crude fractions were added back to specific mutagenic compounds. Actually, metabolism and its by-products need to be evaluated at two ecosystem levels: in the sedimentary microbial system, and in the higher life forms like fish. PNL research in these areas has only recently commenced on selective topics.

Currently, state-of-the-art safety assessment models assume, incorrectly, that sedimentary microbiota metabolize most organic compounds completely to CO_2 and water, thus removing them from the ecological system. While in some cases complete biodegradation can be demonstrated under artificial conditions, both the laboratory data in present studies and the literature on other laboratory and field experiments show clearly that metabolic degradation is normally partial (i.e., when allochthonous carbon sources are present). Definite biosynthesis of phenolics in the $>C_6$ range was demonstrable, for example, in the present stream experiments. The possible biological re-entrainment of metabolic by-products from sediments to higher life forms cannot yet be definitively established, but the enzyme systems necessary for metabolism of most polycyclic organic compounds seem to be present in all life forms.

Metabolism at higher phylogenetic levels, e.g., in fish and shellfish, presents a similar problem in evaluating hazard implications. Several documented instances show the production of metabolic by-products that are more retentive, more toxic, or both, compared to the parent compound, and the hazard assessment models are not yet competent to deal with such observations.

Metabolic products of quinoline, identified in rainbow trout tissue after exposure via the water column, indicated that a rapid transfer from gills to liver took place, followed by excretion into the bile. The largest fractions, identified by GC/MS, were segregated in the gall bladder as at least seven hydroxyquinolines. These metabolites were shown by thin layer chromatography to exist in conjugated form, but not in glucuronic acid or sulfate conjugates as might be expected. Fractions remaining in gill or muscle tissue were surprisingly low, and only two thirds of the metabolites retained after the depuration interval (24 hr) were accounted for in the gall bladder.

Uptake kinetics for phenol, aniline, and quinoline were examined in rainbow trout and daphnids, with and without the presence of a complete WSF from crude coal liquid. Results were similar for both organisms; about 35% less radioactivity was accumulated in tissues when the WSF was present than when the pure compound alone was present (^{14}C-phenol). These results indicate that other lipophilic components compete with phenol for tissue absorption. Uptake kinetics in alga (Selenastrum quadracauda) were comparatively rapid for phenol and aniline. The results are indicative of surface sorption rather than metabolism.

The metabolic data obtained herein, or from literature sources to date, are not complete enough to warrant drawing firm conclusions about ecosystem-level implications. Although the enzyme systems for metabolizing ring compounds are ubiquitous among all life forms, differences in their physiological efficiency for metabolizing these compounds are likely to be substantial. There appear to be significant differences, for example, in how fish and crustaceans consumed as food handle ring compounds. Field observations suggest that inducibility of mixed function oxidases (MFO) may enable some aquatic ecosystems to adapt to oil without selective loss of organisms, but the data are fragmentary.

PNL ecological research on complex mixtures has been directed at these key topics. This report and a current critical review (Vaughan, 1984) synthesize results to date, relate these results to DOE's current ecological research program, and describe advances in scientifically understanding the responses of aquatic ecosystems to such materials.

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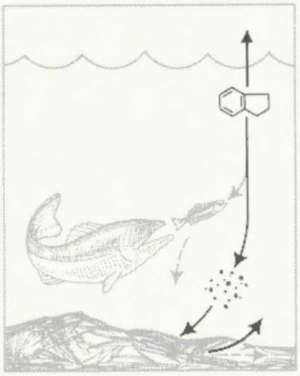

1.0 Variables Affecting the Determination of Toxicity

1.0 VARIABLES AFFECTING THE DETERMINATION OF TOXICITY

It is not an easy matter to determine the exposure of fish or other organisms to complex mixtures. Complex mixtures are characteristic of many organic contaminants. In their environmental behavior, they show new and categorically different features unlike simple solutes and most radioelement contaminants. Petroleum typifies the measurement problem in dealing with mixtures of many hundred thousands of organic compounds, each of which shows different water solubility, oil-water partitioning, particle-water partitioning, emulsifying, toxicological and other properties (Neff, 1979). Coal synthetic fuels, shale oil, and the organic fraction of industrial wastewaters or raw river water present similar problems (Andelman and Snodgrass, 1972; Andelman and Suess, 1970; Harrison et al., 1975; Moghissi and Foley, 1982; Stuermer et al., 1982a).

Liquid fossil fuels provide a most useful example for the study of complex mixtures. These liquids differ markedly in composition because they are derived from a variety of sources and because they have been subjected to different processing conditions (Felix et al., 1982; Later et al., 1981). Petroleum experience has shown that the water soluble fraction (WSF) is the major means whereby fish or other organisms may be contaminated (Anderson, 1982). However, between the parent oil and the water column, many interactions take place that potentially alter the distribution of constituent compounds presented for uptake, for example, to a fish.

Composition of WSFs derived from oils, partitioning between non-aqueous and aqueous phases, and the environmental persistence of constituent compounds are major factors to consider. In interpreting either laboratory or field environmental data on toxic compounds, these three factors may be more important than intrinsic biological toxicity. Previous to the current program, methods to deal with these three factors had not been developed systematically. In the synfuels program, PNL developed approaches for properly controlling the laboratory exposure of fish and other organisms to these new materials. Practicable approaches focused on partitioning, and preparation methods were developed that controlled partitioning in a realistic way.

Estimating environmental persistence proved to be more problematic, but PNL was able to devise methods to study at least those compounds which would more slowly enter the water column. These compounds are often toxic and high in molecular weight. In aquatic ecosystems, toxic compounds of low molecular weight will be rapidly dispersed hydrologically. Heavier compounds sorbed to particulate matter typically sink to the sediment where they may be acted upon by a variety of biological, physical and chemical processes (Jordan and Payne, 1980). There is currently no consensus on selection of reference compounds required in the study of complex mixtures (Vaughan, 1984), although a selection rationale has been recently proposed by PNL (Felice et al., 1984).

1.1 COMPOSITION OF WATER SOLUBLE FRACTIONS AND THEIR PREPARATION

While considered to be water insoluble, liquid fossil fuels form aqueous suspensions, and/or they partially dissolve in water. Often concentrations result that can be acutely toxic to aquatic organisms. The composition of water soluble fractions (WSFs), like the parent oil, may consist of thousands of individual compounds (see Figure 1). As a result, the WSF resulting from aqueous extraction of a liquid fuel is a unique mixture of components, and to interpret its toxicological properties the mixture must be chemically characterized.

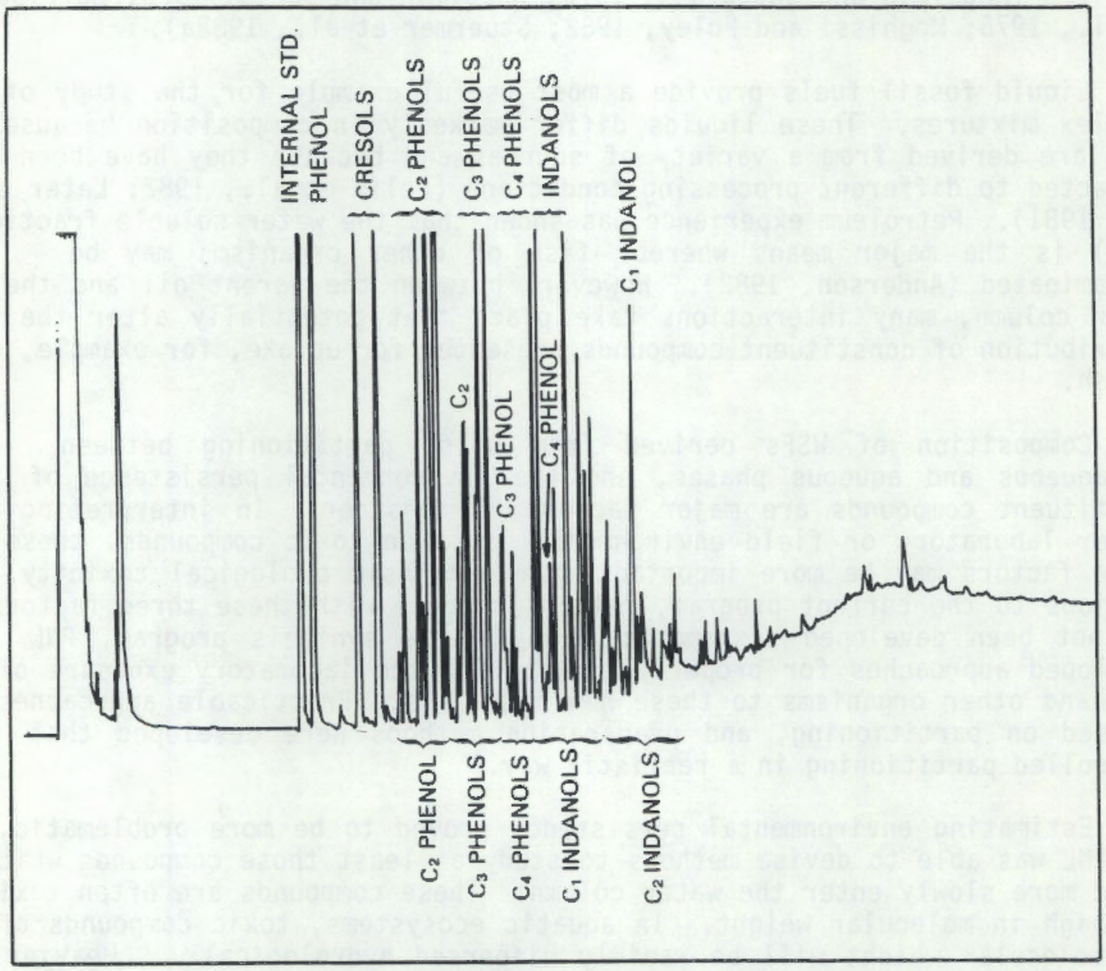

FIGURE 1. Complexity of fossil fuel WSF composition is illustrated by a capillary chromatogram of acetate derivatives of the phenols from a WSF of a liquified coal (SRC-II, 2.9:1 middle:heavy distillate blend).

Analytical procedures for determining the constituents of WSFs must be sufficiently detailed to obtain an adequate characterization, and must be rapid to provide adequate support for toxicological experiments. Therefore, we developed procedures capable of determining individual components in water at concentrations less than 0.01 mg/L by application of capillary gas chromatography. Methods to determine saturate and aromatic hydrocarbons (Bean et al., 1980; Riley and Bean, 1979, and to determine carboxylic acids (Riley et al., 1979) were established during the course of earlier DOE programs. Methods were developed recently for phenols (Bean et al., 1981a) and nitrogen bases (Felice, 1982) in synthetic fuels.

When we analyzed four different fossil fuels (coal liquids derived from an SRC-II process, shale oil from an in-situ process, Prudhoe Bay crude oil, and a No. 2 fuel oil) large differences in composition of the WSFs were demonstrated through application of the procedures cited above (Thomas, 1984). The coal-derived liquid resulted in a WSF composed of phenolic compounds which were shown to constitute 90% of the organic carbon (Figure 2). Nitrogen compounds, the next most abundant compound type in the coal WSF, consisted of less than 2% of the organic carbon.

In contrast to coal liquid WSFs, shale oil WSFs show a carboxylic acid concentration ten times higher, even though the coal liquid WSF contained more than 10 times as much organic carbon. Although these fuels are almost exclusively hydrocarbon in composition, the higher water solubility of the phenolic constituents results in their disproportionate representation in aqueous extracts.

Fossil fuels from different sources can generate WSFs radically different in component composition, and the WSF composition can be surprisingly different from the composition of the original oil. Furthermore, the very method of preparing a WSF of a particular fuel can lead to widely varying apparent toxicities, as will be discussed below. For these reasons every new application requires a chemical evaluation before either environmental behavior or toxicology can be inferred from experimental data.

1.2 PARTITIONING: EFFECTS OF PREPARATION METHOD ON COMPOSITION AND TOXICITY

In laboratory determinations of oil toxicity, the partitioning of compounds to oil, water, and atmosphere is difficult to control, because partitioning varies with time and affects an exceedingly large number of constituent compounds. Also in the relevant literature, many different methods of exposure can be found that, because of this difficulty, do not permit ready comparability of data for toxicological purposes (Bean et al.,

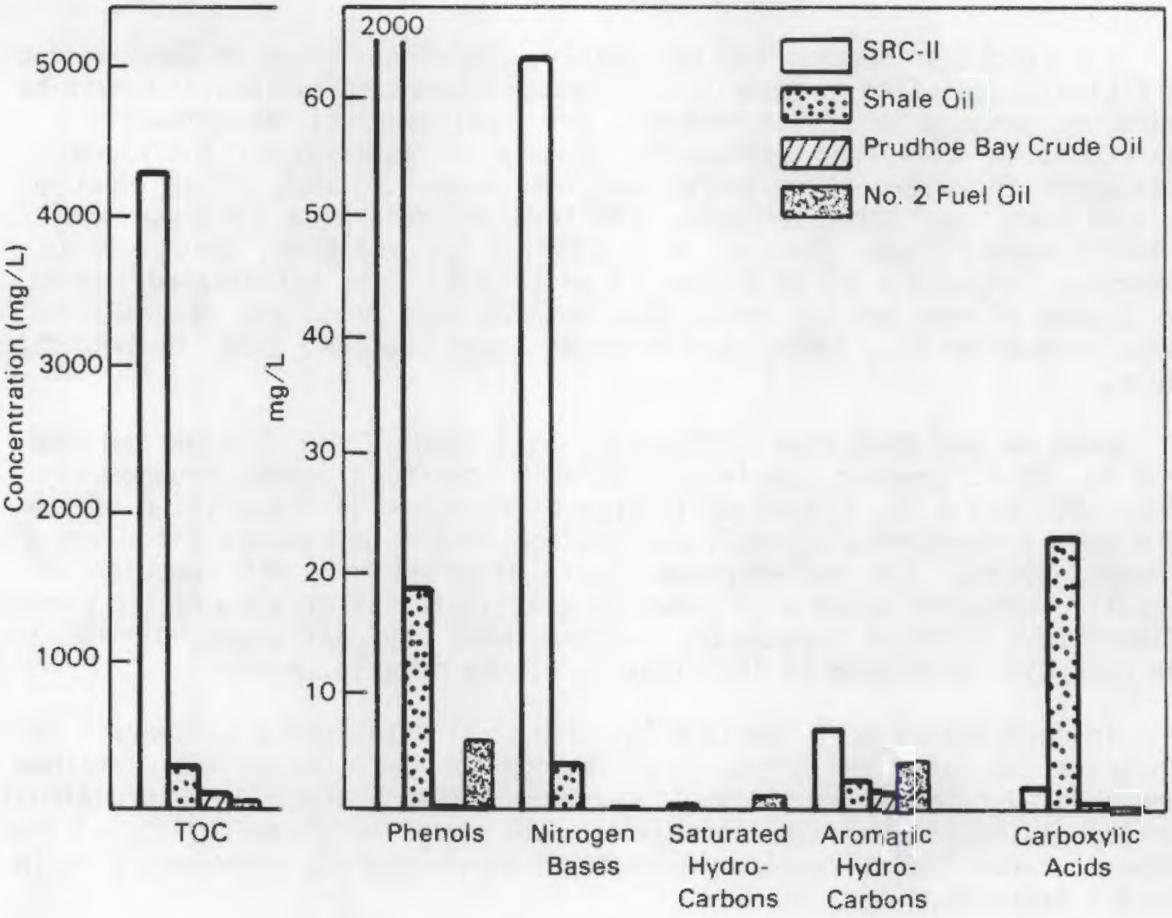

FIGURE 2. Chemical composition of WSFs of different fossil fuels shows a wide range. For SRC-II, phenols comprise over 90% of the total carbon in the WSF, while carboxylic acids are the major constituent of WSF of shale oil (each WSF was derived from a 1:10 oil to water ratio (v:v) except for phenols, which were a 1:100 oil to water ratio.

1974; Michael and Brown, 1978). PNL thus undertook to develop exposure methods that could be standardized and compared across other laboratories.*

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*This involved the continuing efforts of several staff members to guide the development of national standards by the American Society for Testing and Materials (ASTM) Committee E-47 (Biological Effects and Environmental Fate. PNL participated on the following subcommittees of E-47:

E 47.01	Aquatic Toxicology - D. D. Dauble, R. H. Gray and T. M. Poston
E 47.06	Environmental Fate of Chemical Substances.

Variations in the method of preparation were examined specifically to determine if the mixing energy, filtration, or water quality affected toxicity determinations. For example, standard mixes were prepared in 30-liter batches using a 3 to 1 blend of middle and heavy distillate cuts of a coal liquid (Bean et al., 1981b). The blend was representative of possible boiler fuels that might be manufactured. Three hundred milliliters of oil were added to 30 liters of water and mechanically mixed for 4 hr. Following mixing, the solution was allowed to stand. The water phase, which contained the water soluble fraction of the oil constituents, was then withdrawn from the solution. The WSF contained typically 1000 ppm total organic carbon comprised primarily of the lower molecular weight, polar constituents (e.g., phenols) as well as polycyclic aromatic compounds and heteroatomic polycycles.

When the standardized method of preparing batches was varied by changing the mixing energy, by filtering the water phase, or by allowing the batch to stand for long periods after initial mixing, marked differences in composition and toxicity were noted. The daphnid bioassay procedure was used for these determinations (Dauble et al., 1982b). Higher mixing energy (fast mix) resulted in an increased toxicity, much of which was removed by either 0.45 μ filtration or aeration (Figure 3). Allowing the mixture to stand for over 40 hr also reduced toxicity, but not as dramatically as filtration alone. This indicated that much of the toxicity in the fast-mix was due to suspended droplets of oil-phase material that could be removed either by filtration or settling. After 43 hr of settling, the composition of fast mix stock solution showed a significant reduction of toxic polycyclic aromatic hydrocarbons over time (Figure 4). About half of the remaining toxicity was associated with volatile components. In contrast, slow mix resulted in a somewhat less toxic WSF, but little of the toxicity was removed by filtration or aeration (Figure 3) suggesting that a larger relative proportion of polycyclic aromatic hydrocarbons were present, or at least that a larger proportion of higher MW compounds had entered the WSF.

Variations in pH hardness of the exposure water were examined also, but they had little effect on toxicity, at least within the range of organism tolerance to changes in water quality (Becker et al., 1983). Efforts were also made to ensure interlaboratory reproducibility of the daphnid bioassay (Strand and Vaughan, 1981).

1.2.1 Partitioning: The Effect of Compound Type on Oil/Water Distribution

Oil/water distribution coefficients can be used to determine the maximum concentrations that oil components might reach in aqueous solution. The data are useful for experimental design and for modeling the movement of chemicals through an aquatic ecosystem (Aaberg et al., 1983b). As compared to octanol/water partitioning coefficients (discussed later in this report), which serve another purpose, bituminous oil/water

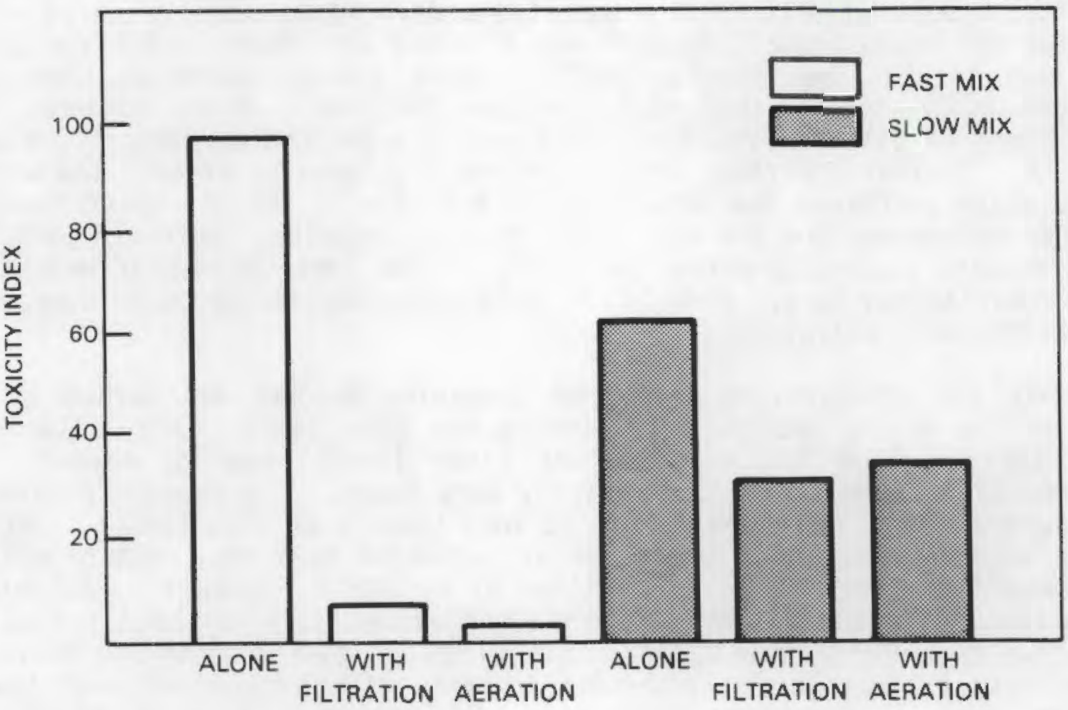


FIGURE 3. Comparison of toxicity by two different methods of batch mix preparation (Based on Bean et al., 1981).

partitioning coefficients provide information on water solubility where complex interactions may occur in the parent oil.

We developed methods for determining oil/water distribution coefficients for a variety of organic compounds. We used aqueous solutions prepared by equilibrating different oil samples with water (Thomas, 1984). The oils chosen for study included a crude petroleum oil (Prudhoe Bay Crude Oil), a petroleum distillate (No. 2 fuel oil), a coal liquefaction product (SRC-II), and a shale oil. Distribution coefficients were determined for various phenols, nitrogen bases, saturated and aromatic hydrocarbons, sulfur-containing aromatics, and carboxylic acids. As would be expected, differences were seen in the distribution coefficients of specific compounds that reflected the differing compositional makeup of the four oils (Figure 5).

When these differences were examined in more detail, information was gained as to how oil/water partitioning differed with compound type.

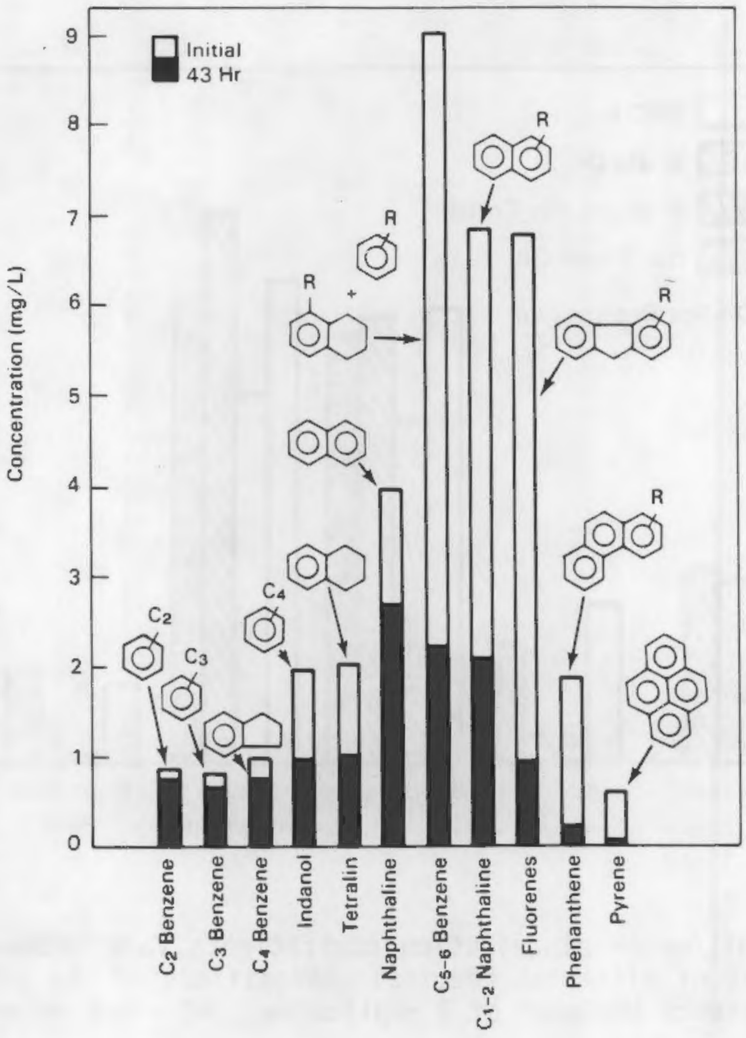


FIGURE 4. Concentrations of hydrocarbons in SRC/water suspension.

For compounds of comparable molecular weight, alkyl substituted phenols and the hydrophilic acids were found to be in highest relative concentration in the water phase (i.e., low oil/water distribution coefficient) followed by nitrogen bases, aromatic hydrocarbons, and saturated hydrocarbons. The trend from low oil/water distribution coefficients for acids to high coefficients for saturated hydrocarbons was the result of decreasing polarity and increasing lipophilicity. However, molecular clusters or aggregates complicated the oil/water determination of saturated hydrocarbons.

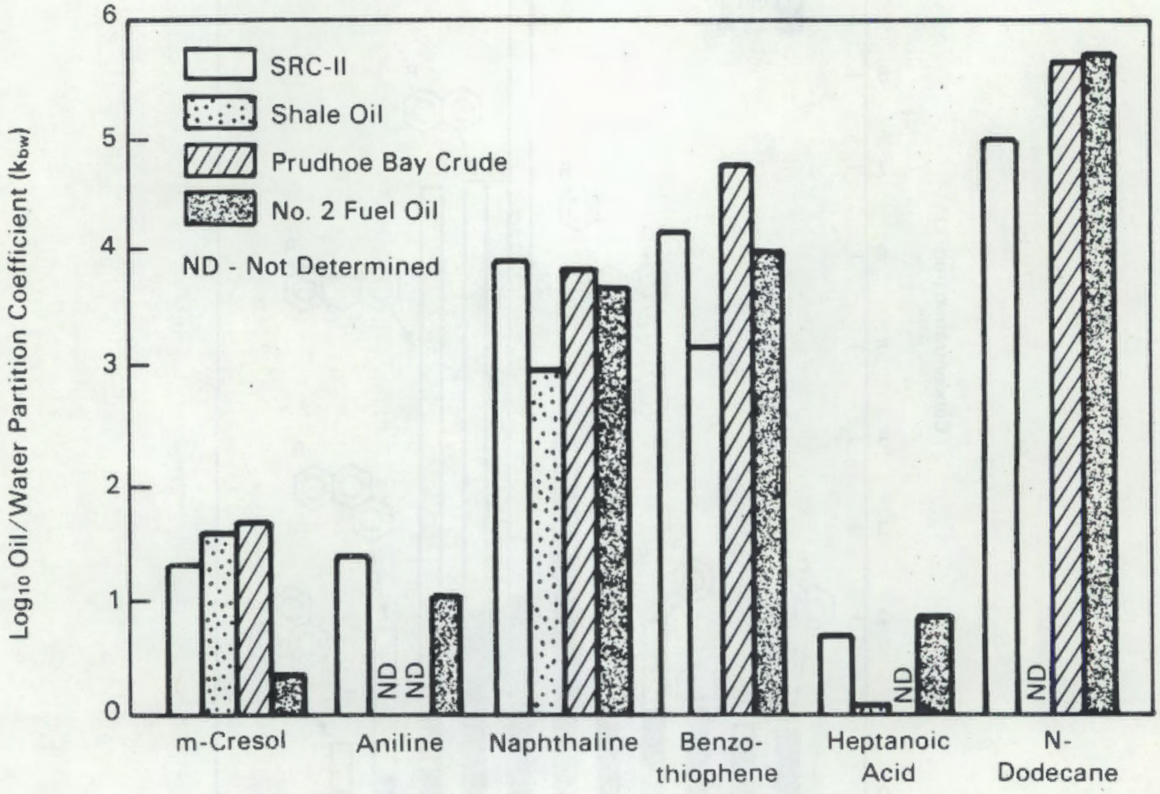


FIGURE 5. Log oil/water distribution coefficients vary between oils as a result of different chemical compositions of the oils. Each bar represents the mean of 3 replicates. ND = not determined.

For a given compound type, increasing alkyl substitution gave higher oil/water distribution coefficients. Addition of each methyl group increased the molecular weight and the hydrophobic character of the molecule, which decreased the water solubility, as indicated by the higher oil/water distribution coefficients. Regression of log oil/water, k_{bw}, on degree of alkyl substitution for hydrophilic acids, alkyl substituted phenols, and nitrogen bases gave straight lines with progressive divergence from linearity at the higher degrees of substitution (Figure 6).

1.2.2 Partitioning: Estimating Bioaccumulation Potential from Octanol/Water Distributions

A considerable body of research has shown that octanol/water distribution coefficients (k_{ow}), which are based on materials of consistent

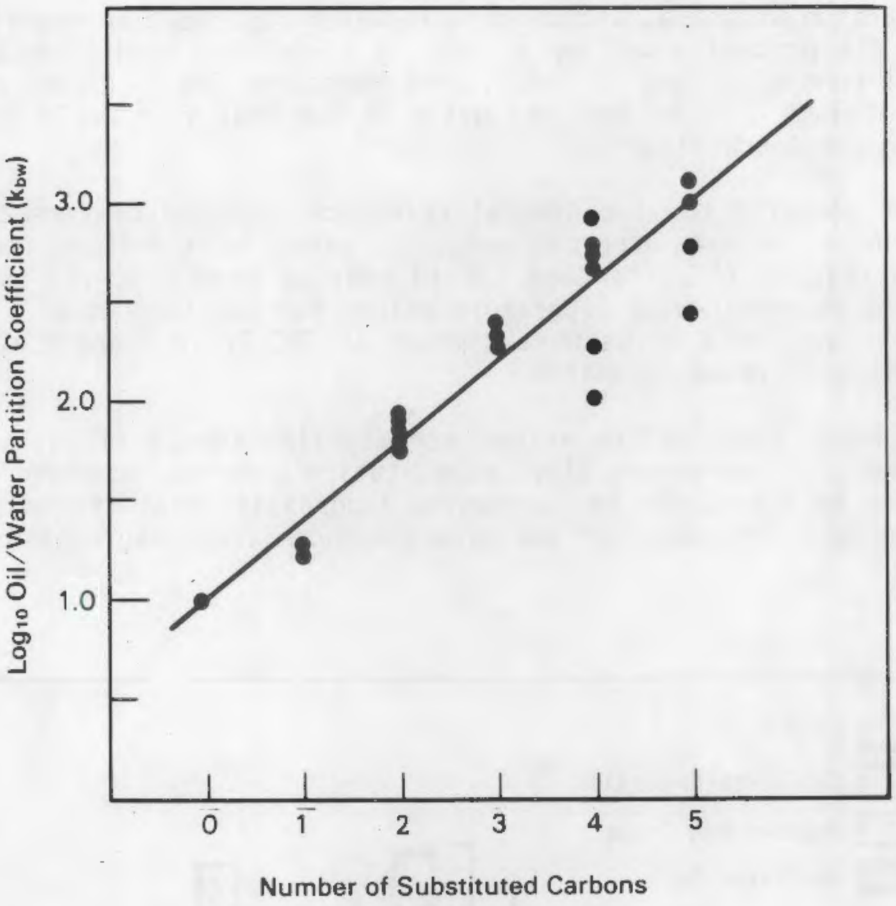


FIGURE 6. Increasing alkyl substitution of phenol resulted in decreased water solubility - higher oil/water distribution coefficients ($Y = 0.97 + 0.40X$; $r^2 = 0.84$). Data for phenols from a coal liquid.

composition, correlate well with bioconcentration factors (BCF) (Mackay, 1982; Southworth et al., 1980; Veith et al., 1980). Such data are thought to be related to toxicity through food chains (Schultz et al., 1980), although this point may be arguable (Vaughan, 1984). They are also useful for determining sorption on sediments and soils (Karickhoff et al., 1978; Brown and Flagg, 1981). Unfortunately, literature k_{ow} values are not available for many energy-related organic components, and those that are available present both methodological inconsistencies and variations of more than one order of magnitude. For these reasons, PNL undertook an intensive study to develop for modeling purposes a data base for k_{ow}, with emphasis on differences in compound types and appropriate methodology (Thomas, 1984). Major features of this study are summarized below.

In comparison with kow, bituminous oil/water distribution coefficients described in the preceding section are mainly useful for predicting maximum environmental concentrations of constituent compounds likely to be present in the water column. It is from the water column that fish would take up or accumulate these constituents.

We first compared kow for several reference compound classes across each of four oils, or more properly speaking, across WSFs derived from each of four oils (Figure 7). The coal liquid that we used (SRC-II) showed generally good agreement with literature values for kow (Leo et al., 1971). The other oils available to us were similar to SRC-II in respect to the reference compound classes examined.

Octanol/water distribution values were similar across all oils, but they increased with increasing alkyl substitution within a compound class. This trend can be attributed to increasing lipophilicity as the molecular weight and nonpolar character of the molecule increases. Regression line

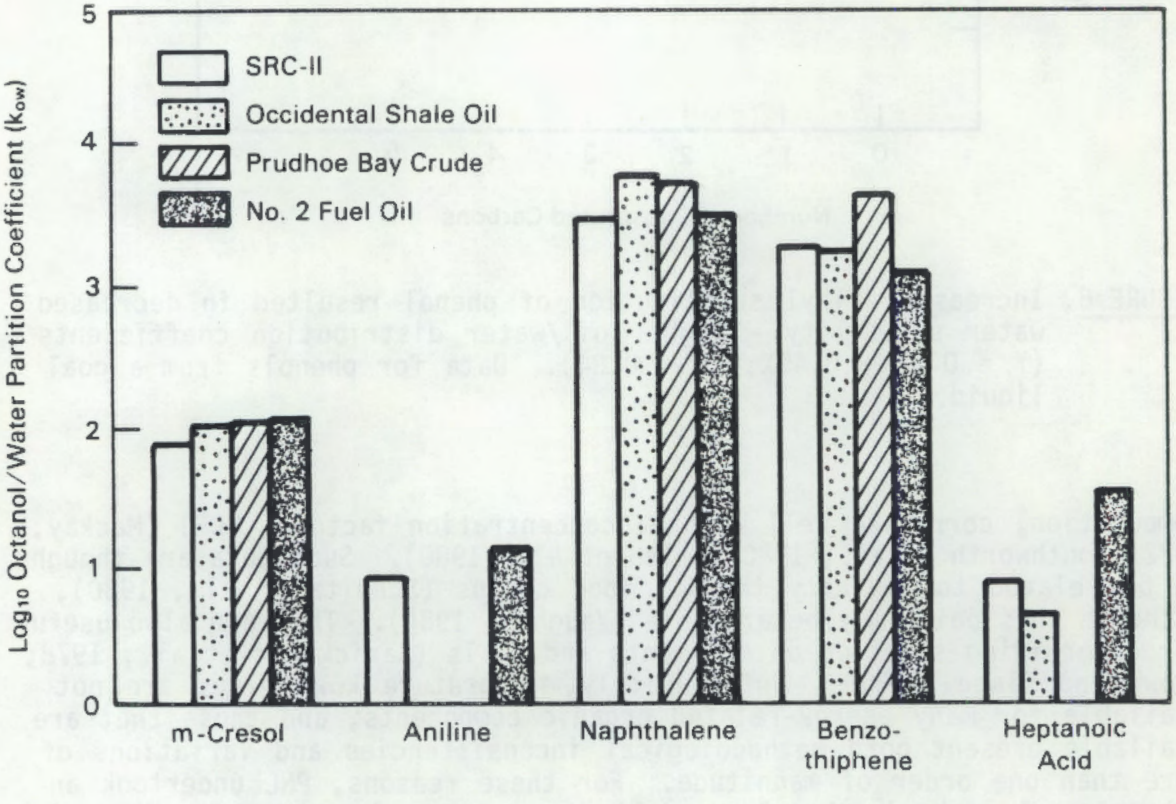


FIGURE 7. Log_{10} octanol/water partion coefficient for selected components of different oils. Each bar represents average of three replicates.

slopes are similar for aromatic components, but intercepts varied widely (Figure 8).

Compounds with similar kow values would be expected to be bioaccumulated in lipid tissues of organisms to nearly the same extent if no elimination mechanisms, such as metabolism, were available to the organisms. Because metabolism of various compounds differs, kow values provide data useful only for estimating the highest possible concentration a compound might reach in a biological system. Compounds such as aromatic hydrocarbons can be expected to bioaccumulate to much greater

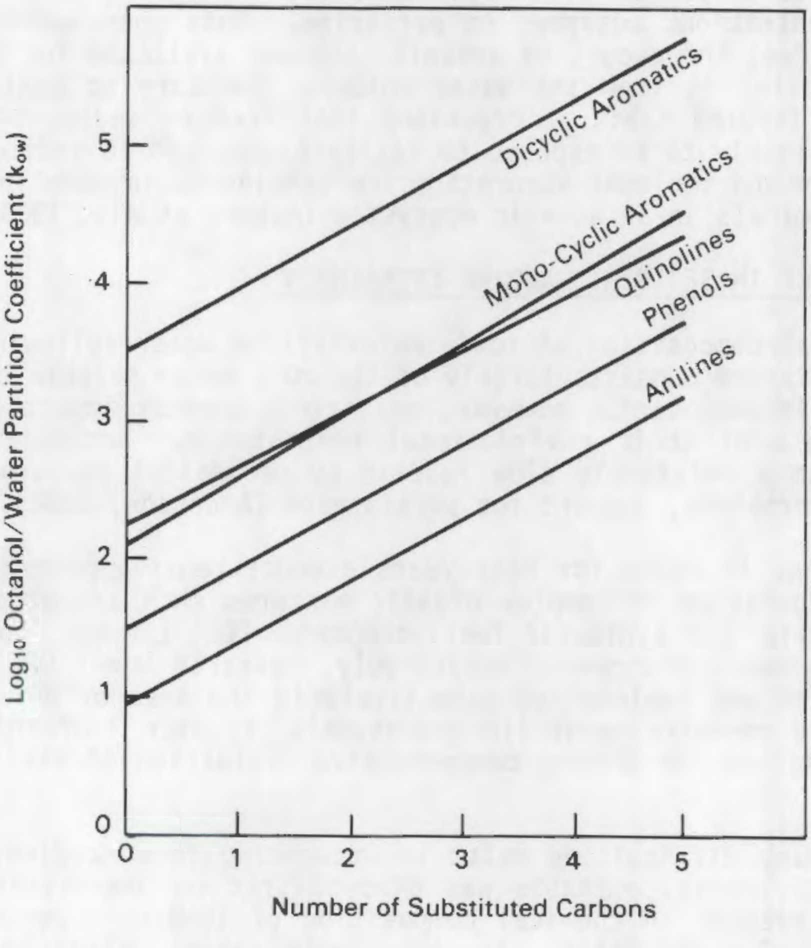


FIGURE 8. As alkyl-substitution increases, Log_{10} kow increases, indicating an increased toxicity and bioaccumulation potential. Linear regression lines for compounds from SRC-II (except carboxylic acids from occidental shale oil).

concentrations than low molecular weight acids, phenols, and nitrogen bases, which all have smaller k_{ow} values (Southworth et al., 1978).

It should be noted that sulfur or nitrogen heterocyclic analogs of neutral polyaromatic hydrocarbons show greater polarity. Consequently they depart significantly from the correlations of BCF and k_{ow} established for neutral compounds of similar configuration (Vassilaros et al., 1982; Southworth et al., 1981a).

Octanol/water distribution coefficients are also useful in predicting sediment adsorption of organic compounds. Carboxylic acids, phenols, and nitrogen bases would not be expected to adsorb on to sediment particles to very high concentrations relative to concentrations in the water column, whereas aromatic compounds with high k_{ow} values would be found in large relative concentrations adsorbed on particles. This phenomenon may, in some cases, affect the amount of organic compound available for biological uptake by removing it from the water column. Exposure to toxicant is influenced by feeding habits. Organisms that feed on sediment-dwelling prey are more likely to be exposed to toxicant adsorbed to sediment. Both bioaccumulation and sediment adsorption are considered in modeling the fate of organic chemicals in an aquatic ecosystem (Aaberg et al., 1983b).

1.3 PERSISTENCE IN SEDIMENTS AND RE-ENTRAINMENT

The initial composition of toxic materials in water following a spill of a complex mixture consists largely of the most water soluble components. The less soluble components, however, may have a greater impact on chronic toxicity because of their environmental persistence. Accumulation in sediments, with a relatively slow release by mechanical disturbance and activity of microbiota, account for persistence (Anderson, 1982).

PNL has had in place for many years a comprehensive program on the environmental behavior of complex organic mixtures such as petroleum, as well as an earlier DOE synthetic fuels program. DOE, EPA and industry have contributed to these programs. Accordingly, research under DOE's former synfuels program was implemented selectively in the area of environmental persistence and re-entrainment (in organisms). Chapter 3 of this report should be consulted for a more comprehensive evaluation of environmental persistence.

Because many difficulties exist in attempting to work directly with contaminated sediments, emphasis was placed first on understanding the principal differences in chemical composition of long-term versus short-term residues. Two laboratory approaches were taken. First, since PNL recognized that higher molecular weight (MW) compounds tended to be retained longer, we developed enrichment approaches to enable characterization of the higher MW compound in the organic compound distribution (Section 1.3.1.). Second, with compound distribution under control, we determined whether differences in toxicity were measurable between aquatic organisms residing in the sediment and those residing in the water column (Sections 1.3.2 and 1.3.3, respectively).

Beyond the laboratory approaches needed for initial experimentation, PNL recognized that other variables might be operative under field conditions. We subsequently undertook a stepwise approach to design appropriate field models for studying sediment to organism re-entrainment.

1.3.1 Chemical Characteristics and Acute Toxicity of WSF Enriched in Higher Molecular Weight Constituents

The initial composition of toxic materials in water following a spill of liquid fossil fuel, for example, consists largely of the most water soluble components. However, the less soluble components will distribute to sediments where they may have greater impact on chronic toxicity because of their environmental persistence. For coal liquids, aquatic organisms will be exposed to initially high and changing concentrations of low molecular weight phenols and aromatic hydrocarbons. These constituents will cause localized acute mortalities, while the more persistent compounds may cause more extensive chronic damage (Gray et al., 1983).

It is difficult, however, to study the more persistent compounds. In previous research, persistent compounds had not been categorized or identified, and reproducible separation had not been achieved. Accordingly, PNL devised a way of selectively enriching the WSF for heavier molecular weight compounds. Enrichment was based on controlled sequential aqueous extraction from a parent oil. Such sequential extraction provided a standardized way to examine simulated weathering while fixing the effects of several otherwise dynamic variables. "Weathering" in this sense might refer to a combination of partitioning processes that would be expected under either natural or laboratory conditions, e.g., volatilization to atmosphere or sorption on suspended oil microparticles.

For example, the initial WSF was prepared by slowly stirring 300 ml of a coal liquid with 30 liters of water for 4 hr. This WSF was siphoned from near the bottom of the carboy following phase-separation of the mixture for 1 hr. A second WSF was prepared by adding fresh water to the remaining oily material and bringing the volume back to 30 liters. This process of diluting, mixing and extracting the original material was repeated to prepare a series of seven sequential aqueous extracts. For the seven extractions thus prepared, concentrations of total organic carbon, total oil, phenols and aromatic hydrocarbons in the water were determined by analytical procedures described elsewhere (Bean et al., 1980, 1981a; Thomas, 1984). Concentrations of each group of compounds generally decreased with successive extractions (Figure 9). The rate of phenol removal was related to molecular weight; higher molecular weight phenols were not removed as rapidly as lower molecular weight phenols. Consequently, marked changes in the relative contributions of different classes of phenols to total phenols were observed in successive extracts (Figure 10). The relative contribution of lower molecular weight phenols

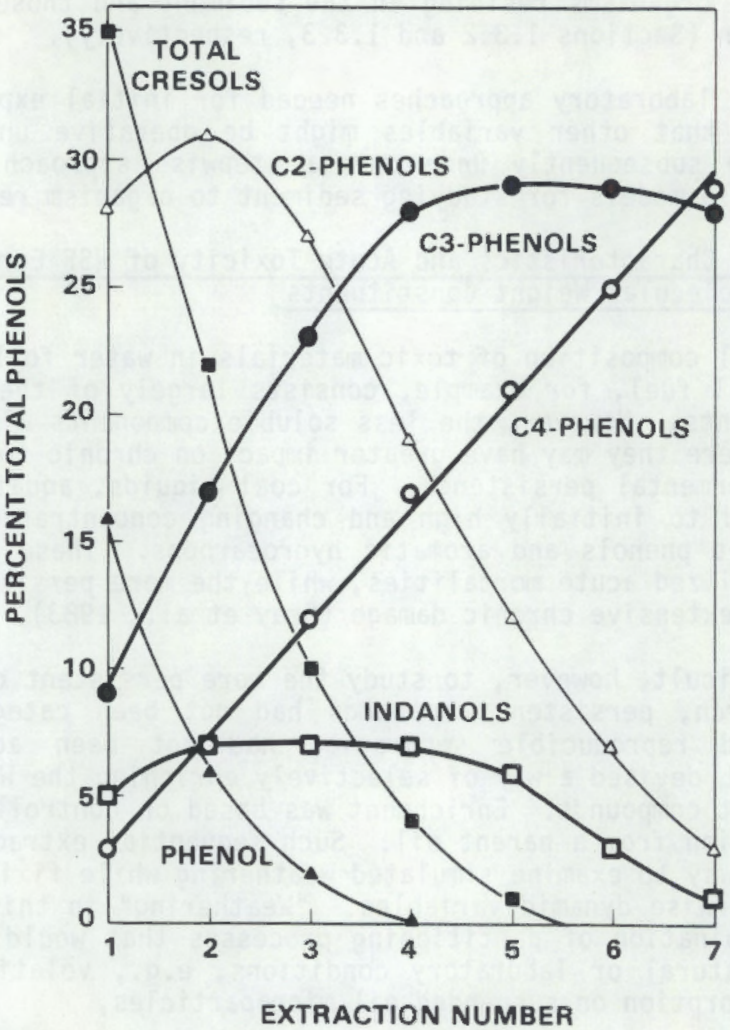


FIGURE 9. Percent of total phenols represented by various classes of phenols in sequential aqueous extracts of an SRC-II liquid. Each point represents the mean of two samples.

decreased rapidly with successive extractions, while the contribution of higher molecular weight phenols remained constant or increased. Aromatic hydrocarbons behaved differently; their concentration in the last extract was only 25% less than in the first extract (data not shown).

WSFs were less concentrated with each successive extraction, and consequently more of the WSF was needed to cause an acute toxic effect. The comparisons were made using a standardized bioassay procedure developed for screening purposes and based on mortality to the freshwater

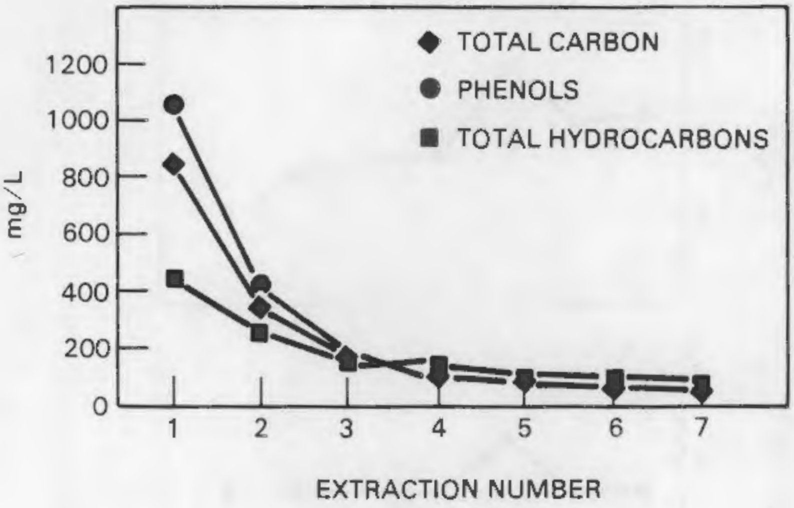


FIGURE 10. Levels of total organic carbon, phenols, and hydrocarbons decreased in water soluble fractions serially extracted from SRC-II.

invertebrate, Daphnia magna (see Section 2.1). However, based on TOC concentrations in exposure solutions, acute toxicities of the WSFs to D. magna were virtually constant for all extractions (Figure 11). Since phenol content was declining in subsequent extraction, the higher molecular weight, more persistent compounds (which included hydrocarbons and aromatic amines), thus appeared to be just as toxic as phenol itself.

1.3.1 Biological Effects of Persistent Organic Constituents in Laboratory-Scale Sediment/Water Systems

Having shown that successive aqueous extractions of an oil/water mix would become enriched in compounds of higher molecular weight, PNL attempted to demonstrate analagous findings in sediment/water mixes. We also expected to find differences in apparent toxicity for organisms living in the interstitial water of sediments, as compared to organisms living in the overlying water column. This latter expectation was based on PNL experience with marine petroleum contaminants (see Chapter 3).

The persistence of higher molecular weight classes in the contaminated sediments was confirmed (Dauble et al. 1983a). In these studies, 20 g of wet sediments typically were spiked with either 25 or 50 µl of coal liquid. Filtered water (500 ml) was added to the spiked sediment, mixed, and decanted after settling. The procedure was repeated six times using clean beakers. Sequential aqueous extraction of a "spiked" sediment generally showed similar chemical characteristics as the sequential extraction of an

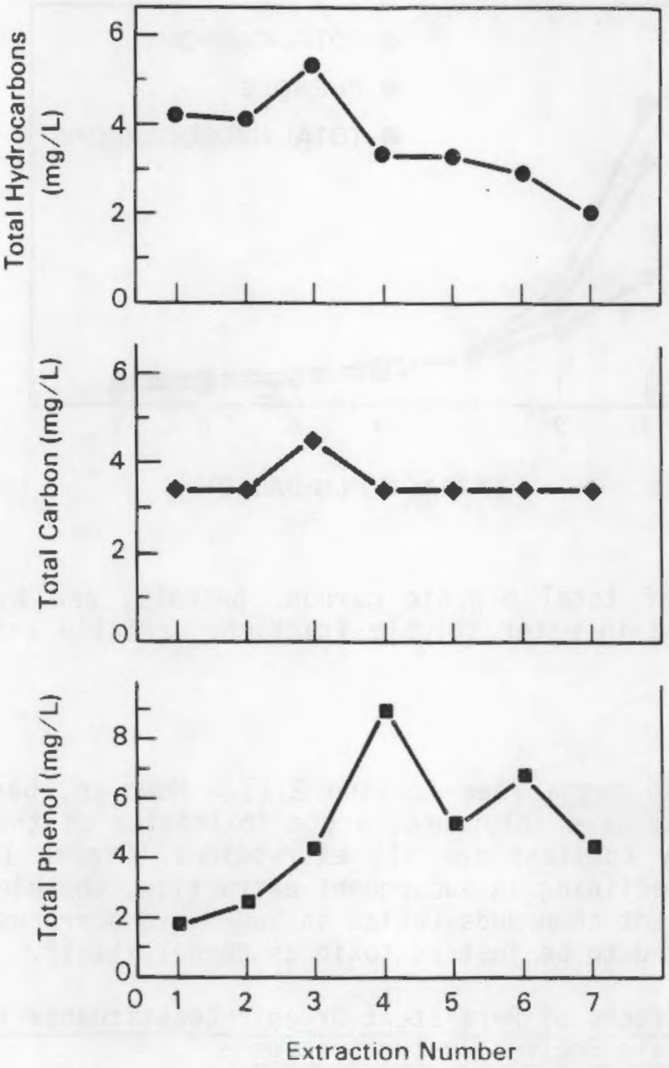


FIGURE 11. Toxicity of serial extractions of a coal liquid by standardized bioassay response. Graphs show the concentration needed to produce LRCT as a function of extraction number (Based on Gray et al., 1983).

oil mix. For example in the 4th, 5th or 6th sequential extraction, substituted indanols contributed a larger fraction of total phenolic compounds than did cresol or phenol itself. Some phenol was found, however, in even the later extractions. This was somewhat unexpected, and it may be explained either by enhanced recovery of phenol in the analytical scheme or by microbial degradation of substituted phenols (higher molecular weight) to phenol, itself (Dauble et al., 1983a).

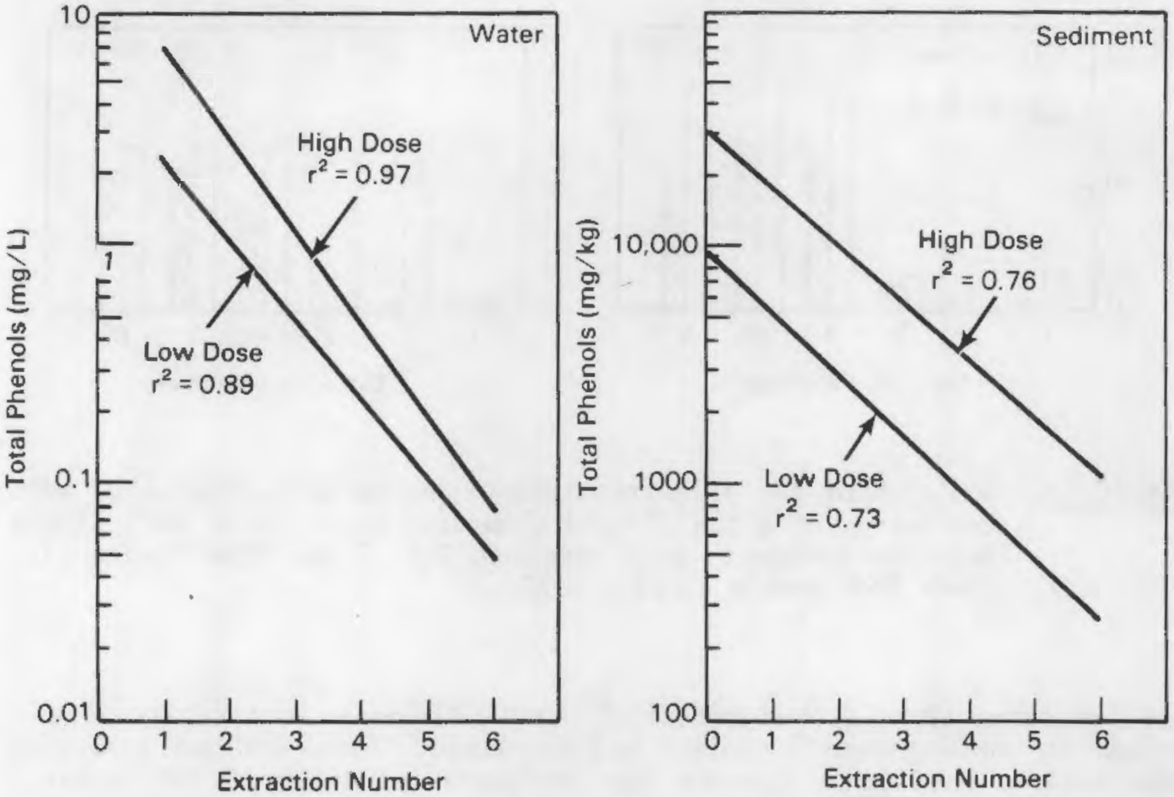


FIGURE 12. Levels of total phenols in sediment and in overlying water for serial extractions of sediments contaminated with either 1 µl (low) or 2.5 µl (high) coal liquid per gm of sediment.

The toxicity determinations with the sediment systems were particularly illuminating. In each of the six sequential extractions (WSF), the total of phenolic constituents decreased, in constant proportion, from one extraction to the next (Figure 12). A logarithmic decrease in total phenolics was also seen in the sediment (Figure 12). When the sequential extractions were bioassayed, they all retained measurable toxicity as shown by survival fractions of less than 100% in the higher dose group (50 µl spike). The 1st, 2nd and 3rd extractions where strongly toxic to Daphnia (Figure 13a), which live in the water column. No significant difference in toxicity was discerned among the three extractions. The 4th, 5th and 6th extractions were all less toxic, but they showed little if any detectable difference among the three.

When we examined chironomid larvae, which lived in the sediment fraction, sediments from the later extractions still showed appreciable toxicity, as evidenced, e.g., by only 60% survival and by a consistent difference in high and low dose groups (Figure 13b). Sediments in the 5th

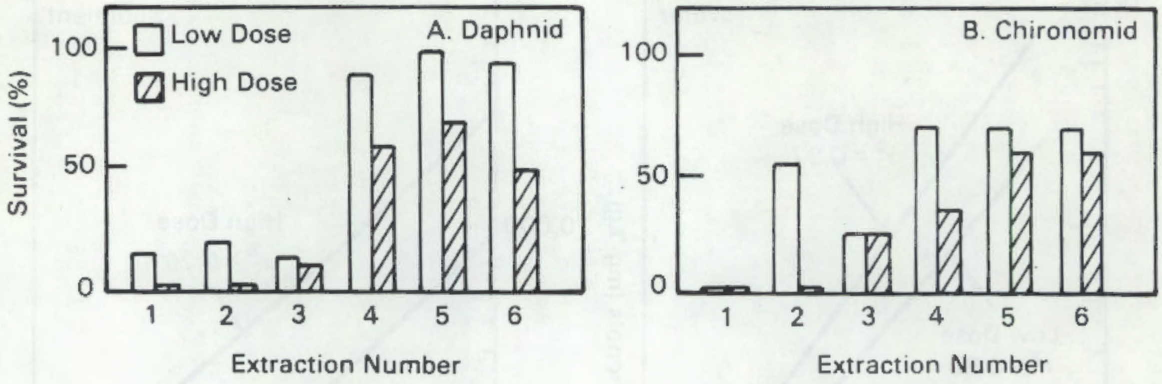


FIGURE 13. Toxicity of serial extractions of sediments contaminated with two levels of a coal liquid (low dose was 1 µl of coal liquid per gram sediment, high dose was 2.5 µl per gram sediment). (Data from Dauble et al., 1982c.)

and 6th extractions showed practically no difference in toxicity, even though the total phenolic content had decreased. These and the preceding observations on Daphnia suggest that sediment retention of the higher molecular weight compounds maintains the toxicity of sediment/water systems despite a sharply reduced total of phenolic constituents in the sediment. It was not clear from these experimental studies whether compounds of the higher molecular weight classes or their possible microbial (partial) degradation products accounted for toxicity in the later extractions.

Variability in the test results is probably explained by behavior and habitat preference of the two organisms studied. For example, at sublethal concentrations, swimming activity of daphnids may have facilitated the precipitation of minute, suspended particles as indicated by changes in the clarity of water in the test system. Thus, the rate of removal of micro-particulate oil from the water column by sedimentation might be affected by biotic activity in addition to physical and chemical phenomena discussed earlier. In the sediment- dwelling organisms, behavior as well as long-term survival might be affected by low concentrations of phenolic constituents.

2.0 Responses of Individual Organisms

2.0 RESPONSES OF INDIVIDUAL ORGANISMS

The use of any single organism or procedure to assess the potential hazard of a chemical substance or mixture can no longer be defended scientifically. Over the past 10 years, appropriate methods have been rapidly evolving for both single chemicals and complex materials (Cairns et al., 1978; Eaton et al., 1980; Southworth, 1981b; Bergman, 1984; Bartell, 1984). Assessment of impacts from toxic substances is now viewed as a multi-stage process that also includes physicochemical and other characterizations, illustrated in earlier sections of this report and in the literature (See also Branson, 1978; Brungs and Mount, 1978; Stern and Walker, 1978). In aquatic systems, the determination of toxic response is also viewed as a multi-stage, or "tiered" procedure, in which the series of tiers increase in complexity and, hence, in the degree of required effort. Appropriate procedural tiers are generally viewed as: 1) acute toxicity (i.e., bioassay) tests on individual organisms; 2) chronic tests on individual organisms, either limited or extended in duration; 3) microcosm studies on communities of organisms (Brungs and Mount, 1978); and 4) ecotoxicity studies conducted at location in natural ecosystems (Levin, 1982). We are here concerned with items 1 and 2. Items 3 and 4 will be considered in Chapter 3 of this report.

In using the tiered procedure, a priority system must be developed to generate the required data. Because efforts and costs increase with complexity, the initial characterization of a toxicant is usually conducted at the individual organism level by acute or chronic testing. Individual effects on aquatic organisms can be manifested in numerous ways: mortality, inhibition of reproduction, lowered survival of young, reduced growth rate, increased pathologic or physiologic dysfunction, alteration of behavior, bioaccumulation in tissue, increased susceptibility to predation, and inability to cope with environmental stresses. Of these several measurement "end-points," PNL selected a tier based on determination of acute toxicity, growth and reproduction effects, and behavioral changes.

2.1 ACUTE TOXICITY DETERMINATIONS

Acute toxicity tests, representing the initial and simplest tier, established a foundation for other experimental procedures developed in the course of this program. Other investigators have also found such tests useful as a guide in the design of future studies (Kimerle et al., 1978), and several such studies have been based on complex mixtures (Becker and Crass, 1982; Becker et al., 1983; Giddings et al., 1980; Gray et al., 1983; Millemann et al., 1980; Parkhurst et al., 1979). One organism, the cladoceran, Daphnia magna, was selected for acute toxicity determinations because of characteristics conducive to good experimental design and rapid results.

We next compared the relative toxicity of WSFs from various complex mixtures, in this case coal liquid products, with those from petroleum and other fuel products. Some important chemical differences in these fuels were described in Sections 1.1 and 1.2 of this report and in earlier publications (Giddings, 1982b; Strand and Vaughan, 1981). Besides mixing regimes (discussed in Chapter 1), other physical and chemical factors affecting the determination of toxicity were also controlled, i.e., temperature (Ullrich and Millemann, 1983) and water quality (Becker et al., 1983). With good control over physicochemical factors that might otherwise bias the determination of comparative toxicities, we determined that differing toxicological characteristics were due to chemical differences in the complex mixtures. Results of these studies at PNL (States et al., 1981; Dauble et al., 1983c) and other data from the literature (Parkhurst et al., 1979; Giddings and Washington, 1981) indicated that WSFs of various coal liquids posed a greater risk of acute toxicity to D. magna than identically prepared WSFs of shale oil, crude oil, or No. 6 and No. 2 fuel oils. Some of the data are shown in Figure 14. Identical preparation and exposure methods were the key consideration because of their overwhelming importance in controlling toxicological responses of organisms (see Section 1.2). To provide reliable comparison, the procedures for data in Figure 14 involved a slow, short-duration mixing regime. The slow regime generated toxic WSFs with high concentrations of water-soluble components (primarily phenolic compounds) characteristic of coal liquids. Thus, under rigorously controlled conditions, it was possible to establish that WSFs from coal liquid were more toxic than WSFs from other reference fuels.

All WSFs generated in our determinations were themselves complex mixtures of several organic compound classes, and toxicity could not be attributed to any one chemical class (see Sections 1.3.1 and 1.3.2). Instead, biological responses resulted from exposure to interacting toxic components. For example, the WSFs generated from these materials were more toxic to daphnids than were phenols or various cresols tested as separate compounds (DeGraeve et al., 1980). Low molecular weight phenolics did, however, comprise a relatively high percentage of the total organic carbon (TOC) in solution for all WSFs in our tests. The low solubility of constituents of the reference fuels is reflected in low total carbon concentrations. While the same mixing regimen may not be entirely adequate for dispersing more lipophilic components of other fuel oils, lipophilic components seem generally to be less toxic (Neff, 1979). A more rigorous mixing regime would have reduced toxicity differences in WSFs and would have led to underestimation of actual toxicity (Section 1.2).

2.2 EFFECTS ON GROWTH AND REPRODUCTION

Chronic toxicity studies represented the second tier in determining potential hazard. A variety of organisms were considered by PNL, and in several cases new experimental procedures had to be developed (Dauble et al., 1982a, 1982b, 1983b, 1984b). Because of incremental rise in time and effort for the chronic studies, the effort required careful tailoring to specific aquatic organisms to obtain maximum information. The studies were also designed to approximate specific field situations. The determinations included: growth and reproduction of the fathead minnow (Pimephales promelas) and rainbow trout (Salmo gairdneri); growth and productivity of a

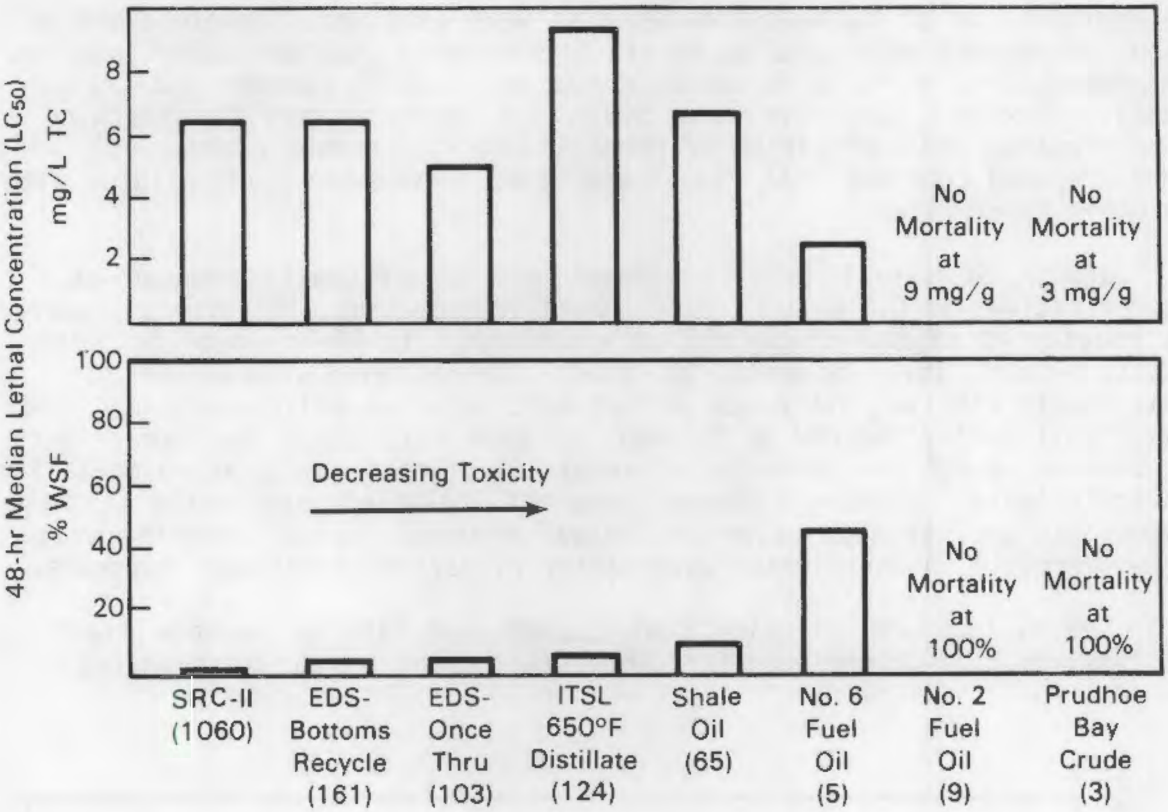


FIGURE 14. Comparative toxicity based on LC_{50} (D. magna) of coal liquids, shale oil and reference fuel oils expressed as % water-soluble fraction (WSF) or mg/L total organic carbon (TOC) that elicited the toxic response. Numbers in parenthesis indicate relative concentrations (mg/L TOC) of the respective WSFs (from Dauble et al., 1983c).

freshwater algae (Selenastrum capricornutum); growth and reproduction of two aquatic midges (Chironomus tentans and Tanytarsus dissimilis); and effect of environmental variables on growth of a freshwater bivalve (Corbicula manilensis).

2.2.1 Sublethal Effects on Fish

Fish are the most visible resident of aquatic environments and are widely used to determine the toxicity of soluble materials. Fish are of interest for studying effects on growth and reproduction because, as vertebrates, they also represent surrogates for early development in people. We examined growth and survival of fathead minnows and rainbow trout chronically exposed to a dilute WSF (Dauble et al., 1983b). Effects on reproduction of fathead minnows also were examined. Continuous-flow exposure regimes were used to maintain relatively constant water quality parameters consistent with adequate toxicant levels (Becker and Crass, 1982). Exposure solutions were chemically characterized to determine concentration and composition of phenolic and hydrocarbon components. WSFs were prepared from the coal liquid described in Chapter 1 and diluted with Columbia River water.

Growth of larval fathead minnows was significantly reduced at concentrations of 0.3 mg/L of total phenolic compounds; the effect occurred at roughly 4% of the concentration established in 96-hr acute toxicity tests (Figure 15). Spawning of adult fathead minnows exposed to coal-liquid WSFs was inhibited at 1.3 mg/L total phenolic compounds, and was significantly reduced at 0.6 mg/L (Figure 16). Thus, adverse effects occurred at a WSF concentration of about 10% of that which caused mortality in acute tests. Spawning, however, was not inhibited permanently at these concentrations, because pairs of fathead minnows resumed spawning when transferred to uncontaminated water after 21 days of continuous exposure.

The minimum WSF dilution that caused mortality of rainbow trout embryos was time-dependent. For concentrations of 1 mg/L total phenolic

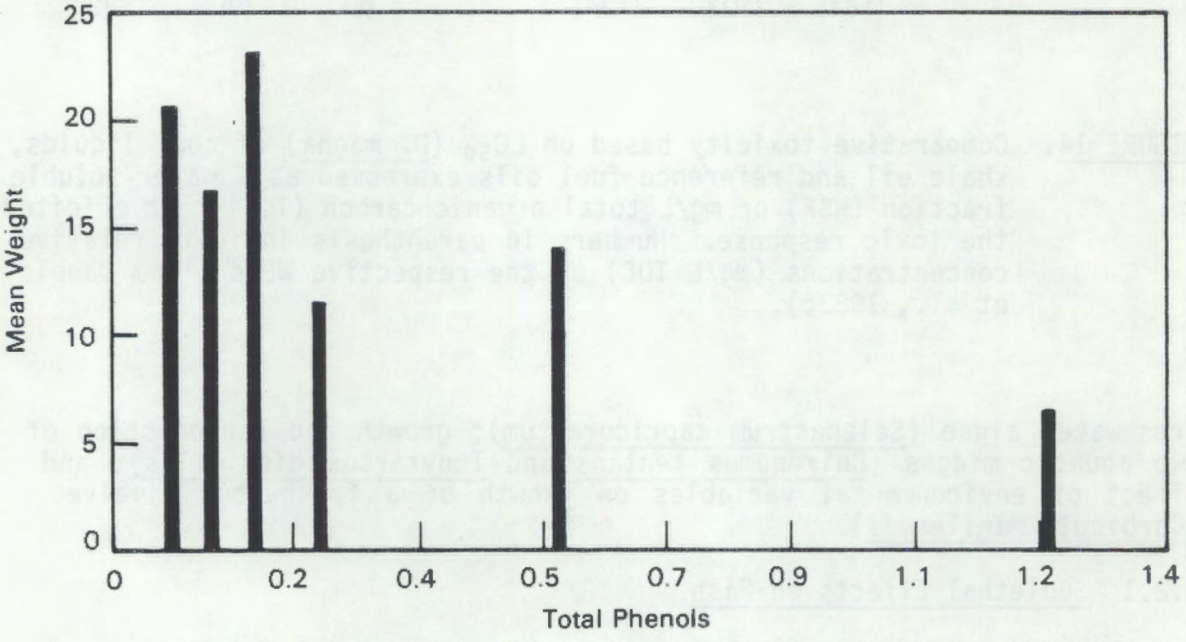


FIGURE 15. Weight of fathead minnow larvae at 30 days post-hatch following 32 days exposure to WSF from a coal liquid. Growth at 0.25 mg/L and higher total phenols was significantly lower than controls (based on Dauble et al., 1983b).

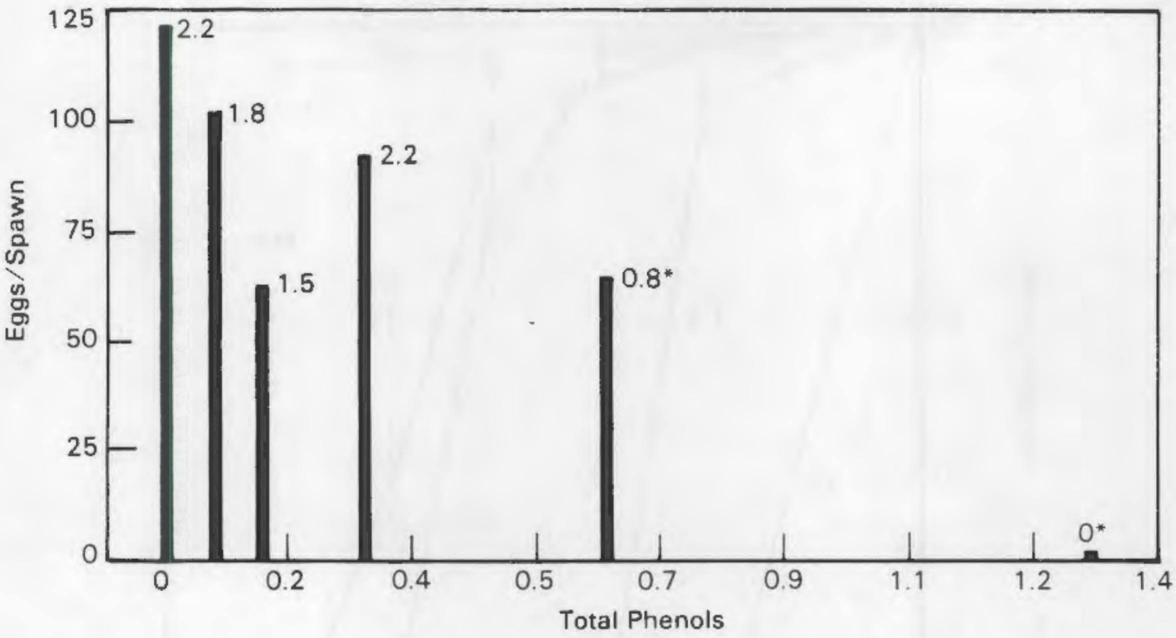


FIGURE 16. Spawning and egg production by fathead minnow exposed to water soluble fractions of a coal liquid for 21 days. Numbers indicate mean number of spawnings per female (based on Dauble et al., 1983b). Astrisk indicates significant change from controls.

compound, no embryos survived 14 days of exposure because of egg mortality or premature hatching. For 0.1 mg/L total phenolics, rainbow trout fry at the swim-up stage suffered rapid mortality after 28 days of exposure (Figure 17). Death of fry was partially due to fungal growth that clogged their gills. Because of differences in exposure concentrations and duration, and because the tests were conducted at different times, one cannot directly compare sensitivities of young fathead minnows to those of rainbow trout. However, the data suggest that fathead minnows were more tolerant of the WSF than rainbow trout, because they made a successful transition past the swim-up stage to active feeding stage, at exposures as high as 1.2 mg/L total phenolics.

As in the acute effects determinations (Section 2.1), these chronic effects determinations cannot be attributed to any single compound or compound class in the WSF. Phenolic compounds constituted about 95% of the organic carbon in solution, and the effects of aromatic and saturated hydrocarbons may be small per se. However, the WSF of the coal liquid appeared to be more toxic than major phenolic components either tested by us individually, or based on available literature (DeGraeve et al., 1980).

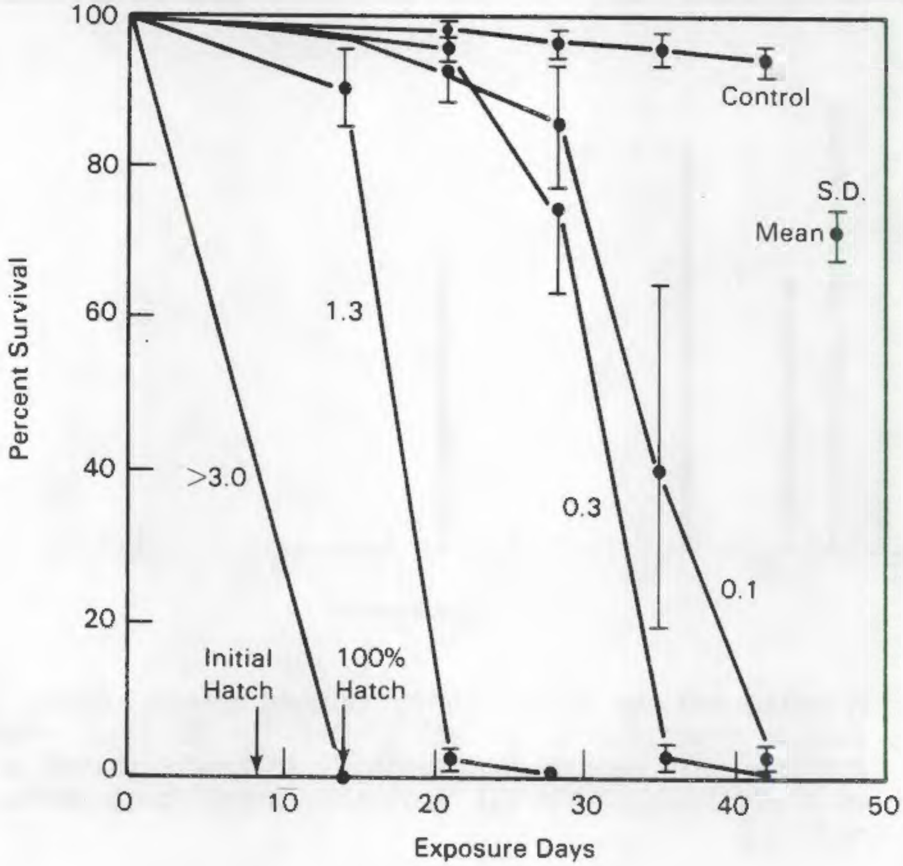


FIGURE 17. Mean survival of rainbow trout embryos during 42-day exposures to a water soluble fraction derived from a coal liquid. Exposure concentrations (adjacent to curves) are mg/liter estimated total phenols ± S.D. (based on Dauble et al., 1983b).

Exposure concentrations estimated by the dye photometric method showed that total phenolic compounds remained relatively stable in exposure solutions throughout the long-term exposures. However, repeated analyses of water chemistry by gas chromatography and mass spectrometry indicated that overall chemical composition varied. For these longer-term studies, a single general measure (such as total oil or total carbon) or a nominal dilution of a stock WSF would not provide an adequate understanding without chemical characterizations, also. Initially, the concentrations of readily soluble, low-molecular-weight phenolics, like cresol and phenol, were relatively high in exposure solutions. Later, higher-substituted phenolics and low concentrations of aromatic hydrocarbons dominated the chemical makeup (see Sections 1.3, 3.1). Biological degradation, and possibly some volatilization, selectively removed lower-molecular weight compounds, including those present in trace amounts. Although these compounds may be highly toxic at relatively low concentrations, their concentrations will never be maintained in nature because of physical and biological degradative processes. The present studies showed that effects associated with microbial growth and presence of adaptive bacteria must be considered when impacts of phenolic materials released to aquatic systems are being assessed (Dauble et al., 1983b).

2.2.2 Effects on Algal Productivity

Primary production in aquatic environments is largely dependent on algae, which derive energy from light and CO_2 to convert inorganic material to organic material while producing O_2. Any toxic material that limits primary productivity can be expected to affect secondary productivity of invertebrate and vertebrate consumers.

The unicellular freshwater green alga, Selenastrum, was selected for study (Gray et al., 1982). Relative toxicities of WSFs from a coal liquid, a Prudhoe Bay crude, and No. 6 fuel oil were also evaluated. Static toxicity determinations were based on modification of the standard EPA Bottle Test (algistatic) and used to quantify algal productivity. Population response was measured during and after exposure (5-day exposure, 9-day recovery) to WSFs prepared by the sequential extraction procedure described in Section 1.3.1. WSFs from the first and fifth extractions were used to represent, respectively, a simulated exposure of algal to recently-spilled or to weathered material. The WSFs inhibited algae growth at less than 1% dilution. "Weathered" WSF, i.e., WSF from the fifth extraction (Section 1.3.1), was less toxic than fresh WSF on the basis of similar dilution, reflecting the sequential removal of readily soluble compounds--primarily phenols. However, weathered WSFs were more toxic than fresh WSFs when total organic carbon (TOC) concentrations were taken into account. For example, the algistatic concentration was 16 mg TOC/L for weathered compared to 50 mg TOC/L for fresh WSF (Figure 18). Removal of readily soluble compounds left behind more complex hydrocarbons that were also inhibitory to algal recovery (Dauble et al., 1982a).

WSFs prepared similarly from Prudhoe Bay crude oil and No. 6 fuel oil were found to be less toxic than WSFs from the coal liquid (States et al., 1981). This can be accounted for, in part, by the greater solubility of chemical constituents in the coal liquid WSF. The relative TOC concentration in the coal liquid WSF was much higher than the TOC concentration in the two reference oils. For example, fresh WSFs from coal liquid contained 800 to 1050 mg/L TOC, of which about 90% were phenolic compounds. Weathered WSF contained 110 to 180 mg/L TOC, of which about 60% were phenols (Dauble et al., 1982a). These values compare to 3 mg/L TOC and <0.1 mg/L for phenolic compounds in the fresh WSF prepared from Prudhoe Bay crude, or to 4 to 5 mg/L TOC and 0.3 mg/L phenolics in the fresh WSF prepared from No. 6 fuel oil. Despite lower TOC concentrations in WSFs of the reference oils, algal growth was significantly reduced, but the algal populations returned to normal production levels after exposure ceased

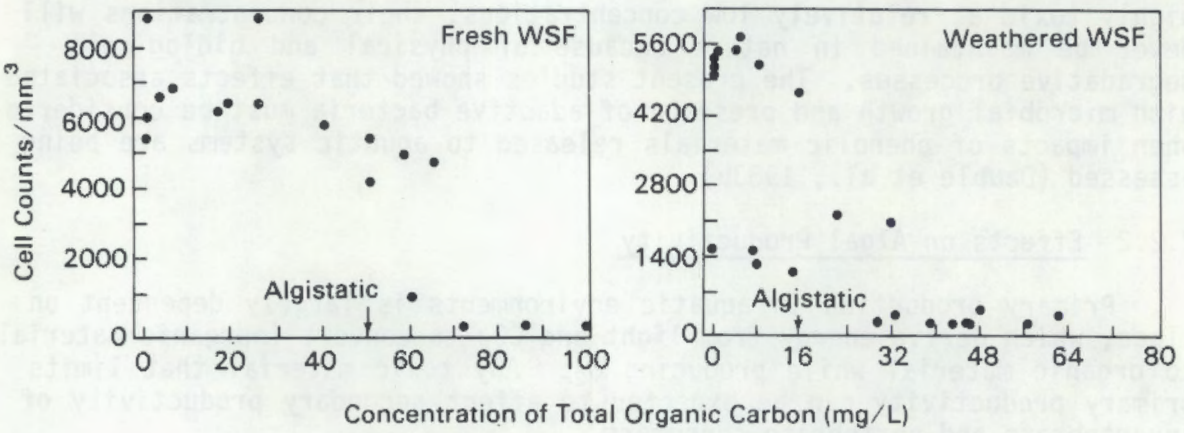


FIGURE 18. Cell counts (no./mm^3) vs. total organic carbon (TOC) concentrations (mg/L) after 9-day recovery for S. capricornutum exposed for 5 days to fresh and to weathered WSF of a coal liquid. Low levels of recovery were observed for control and exposed populations in one test (based on Gray et al., 1982).

(Gray et al., 1982). Lack of recovery was more noticeable for algae exposed to weathered WSFs. In all cases, growth suppression was apparently due to the combined presence of higher molecular weight phenolic compounds and more complex hydrocarbons (see Section 1.3.1). The effects of another coal liquid on Selenastrum also has been reported (Giddings, 1979; Giddings and Washington, 1981). In the studies cited, WSFs of the coal liquid inhibited algal photosynthesis at 1% full strength, but direct comparison of the data with the present PNL data is inappropriate because the source of the coal liquid and the method of WSF preparation differed.

We found evidence that low concentrations of the coal liquid in WSFs enhanced the growth of Selenastrum. Similar evidence has led others to the suggestion that bacterial decomposition of phenol and subsequent use of by-products as nutrients could increase algal growth (Kostyaev, 1973). Biodegradation in nature depends on several environmental factors (Buikema et al., 1979), including the presence of bacteria adapted to specific phenols because of prior pollution (Borighem and Vereecken, 1978). These factors will be considered in more detail in Chapter 3.

2.2.3 Sublethal Effects on Invertebrates

Secondary production in aquatic environments is based largely on invertebrate consumers that derive energy from algae, the primary producers. A toxic material may have greater impact on the secondary producer such as aquatic insect larvae or crustaceans than it might on the primary producer, where both primary and secondary production are important to ecosystem function.

PNL selected as representative secondary consumers the insects Chironomus tentans and Tanytarsus dissimilis, and the copepod, Daphnia magna. Again, WSFs were prepared by sequential extraction where the first and fifth extractions represented, respectively, a fresh material and a weathered material. The WSFs from each extraction were characterized by detailed chemical analyses (Dauble et al., 1982b). Exposures to diluted WSFs varied in duration among species: 14 days for C. Tentans (growth and survival), 14 to 21 days for T. dissimilis (survival and emergence), and 28 days for D. magna (survival and productivity).

Total carbon concentrations were 1120 ± 74 mg/L for fresh WSF compared to 126 ± 15 mg/L for weathered WSF. Total phenolic compound concentrations were about 1100 mg/L for fresh WSF compared to about 80 mg/L phenols for weathered WSF. Composition of phenolics in the two extractions also differed. Relatively low molecular weight compounds such as phenol, cresol, and C_2 phenols constituted 76% of the phenols in fresh WSF, but they constituted only 16% in the weathered WSF. The difference in total aromatic hydrocarbon concentrations between the two WSFs was not as great, e.g., about 2% of the TOC in fresh and about 5% in weathered WSF. The comparative contributions of various naphthalenes and higher substituted benzenes to the total were similar in both fresh and weathered WSFs (Table 1).

During the exposures of T. dissimilis, chemical changes were monitored in the exposure solutions over several consecutive days. Over a 7-day interval of exposure to fresh WSF, phenolics decreased from 9.7 mg/L to 1.0 mg/L. In contrast, during the same interval, weathered WSF declined from 7.1 mg/L to 4.2 mg/L. Chemical makeup also changed. In fresh WSF, phenols and cresols contributed >52% of the total phenolics at day 1, and declined to minor levels after 7 days. In weathered WSF, C_3 and C_4 phenolic compounds and indanols contributed the major fraction at day 0 and remained relatively constant over 14 days. When both types of WSF were diluted to 10 mg/L TOC, solutions of the weathered WSF contained over three times more aromatic hydrocarbons than did the fresh WSF. The major difference in the two solutions reflected the relative amounts of naphthalene and substituted naphthalenes (Dauble et al., 1982b). Similar chemical changes prevailed during exposures with similar insect larva, C. tentans, and the copepod, D. magna, in which chronic effects also were observed. Static exposure conditions were used for these particular determinations.

In survivors of all three species (Table 2), effects on growth, reproduction, or longer-term survival were measurable at WSF dilutions appreciably below those needed to produce acute toxicity in daphnids. Data in Table 2 should be compared against the 48-hr LC_{50} of 6.3 mg/L (total organic carbon) established as a benchmark for acute toxicity of the coal liquid to daphnids (see Section 2.1). Of greater interest, the weathered WSF was found to be from three to five times more toxic than the fresh WSF

TABLE 1. Phenols and aromatic hydrocarbon concentrations in fresh and weathered water soluble fraction (WSF).[(a)] All values are in mg/L.

Phenols	Fresh WSF	Weathered WSF	Aromatic Hydrocarbons	Fresh WSF	Weathered WSF
Phenol	160.3	<0.1	C_2 Benzenes	0.6	<0.1
Cresols	381.4	1.0	C_3 Benzenes	0.6	<0.2
C_2 Phenols	301.5	10.8	C_3 Benzenes	1.1	0.3
C_3 Phenols	107.9	21.1	Indan	0.6	0.2
C_4 Phenols	38.8	18.2	Tetralin	1.1	0.5
Indanols	59.6	5.6	Naphthalene	2.5	1.6
C_1 Indanols	40.0	12.9	C_5-C_6 Benzenes and C_1 Tetralins	3.9	1.2
C_5 Phenols	2.7	0.6			
C_2 Indanols	12.6	5.8	C_1 Naphthalenes	2.2	1.2
			C_2 Naphthalenes	1.1	0.2
Total phenols	1104.8	76.0	C_3 Naphthalenes and C_1-C_2 Fluorines	2.8	0.1
			Phenanthrene	0.5	0.1
			C_1 Phenanthrene	0.3	<0.1
			Fluoranthene	0.1	<0.1
			Pyrene	<0.3	<0.3
			Total aromatic hydrocarbons	17.5	5.2

(a) Based on Dauble et al., 1982b.

when exposures were referred to the concentration of TOC. Emergence time of T. dissimilis, however, did not seem to be different after exposures to fresh and weathered WSFs adjusted for equivalent TOC (see Dauble et al., 1982b).

Comparison of the data in Table 2 with data on the alga, S. capricornutum (Section 2.2.2), show that three aquatic invertebrates were considerably more sensitive than the alga to either fresh or weathered WSF, based on TOC. It is important to keep in mind that fresh and weathered WSFs do not have the same chemical composition. The weathered WSF was more toxic because it contained higher molecular weight phenols and other equally toxic but different compounds (Figure 19 and Table 1). These

TABLE 2. Comparison of chronic toxicity responses of three freshwater invertebrates exposed to weathered and fresh water soluble fractions (WSFs) from a coal liquid.(a)

Response	Total Carbon, mg/L	
	Fresh WSF	Weathered WSF
Growth (C. tentans)	5.1	0.6
Survival (T. dissimilis)	3.4	1.1
Reproduction (D. magna)	0.3	0.9

(a) Data presented as lowest concentration producing a significant effect when compared to control (from Dauble et al., 1982b).

observations have reference to practical situations: the data for fresh WSF would probably simulate exposure conditions for continuous leaks or discharge of a synthetic fuel oil, whereas the data for weathered WSF can be expected to be representative of a long-term spill situation where materials may, for example, persist in bottom sediments in the environment. Both conditions must be considered in experimental designs involving complex mixtures such as synfuel oils.

2.3 EFFECTS ON BEHAVIOR

The effect of a toxicant on the behavior of aquatic organisms is a sublethal response normally associated with third-tier studies (extended chronic studies). Behavioral studies are more difficult to design than toxicity tests, and they require special procedures for detecting and grading responses of experimental animals objectively (Brungs and Mount, 1978). Behavior represents an integrated response of an organism, impairment of which can lead to serious consequences for the organism's continued survival. For example, if reproductive behavior is impaired, survival of the population may be jeopardized. Two important considerations in behavioral toxicology involve the selection of suitable organisms and the use of appropriate monitoring and evaluation methods. For behavioral studies, PNL focused on two fish species (consumers) and three aquatic invertebrates (secondary producers).

2.3.1 Fish Avoidance

Fish typically select sites that provide favorable conditions for survival. Studies have shown that fish can detect and avoid adverse levels of a toxicant, given suitable gradients, although in some cases a toxicant may attract fish and lead to their death. A number of different behavioral

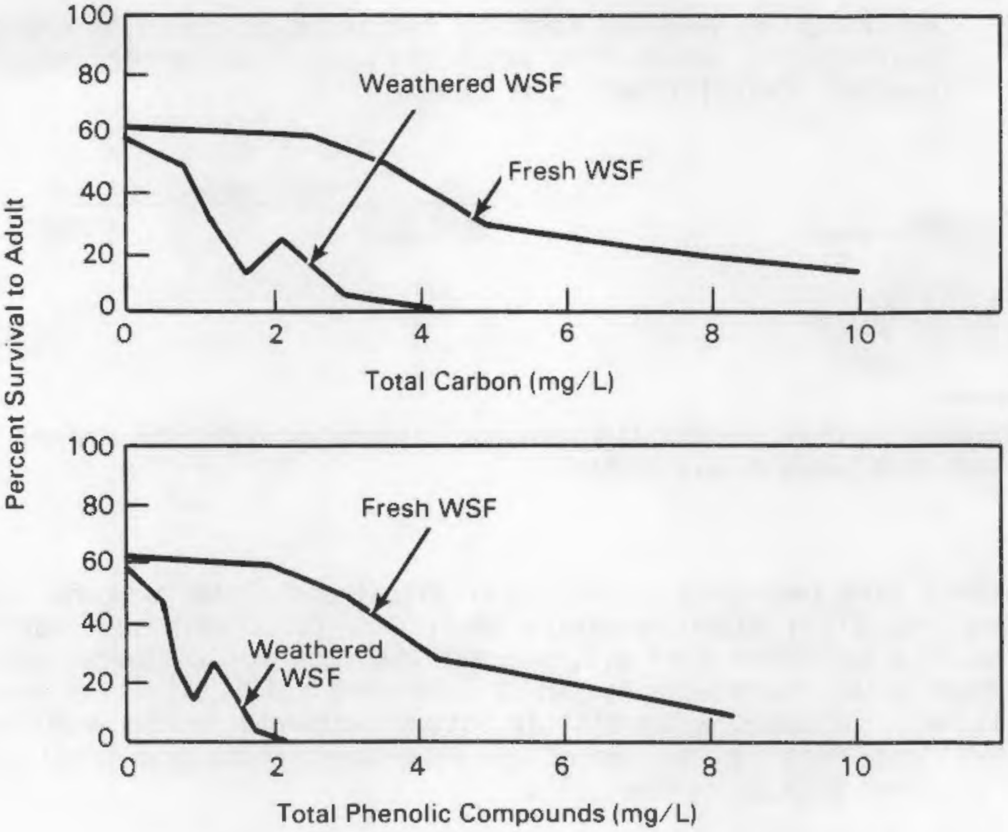


FIGURE 19. Comparative toxicities of fresh and weathered WSF of a coal liquid to Tanytarsus dissimilis. Trends shown as means of duplicate tests. The mortality response is graphed according to either total carbon or total phenolic compounds, both of which were determined in each sample (based on Dauble et al., 1982a).

effects are shown by fish exposed to toxicants, ranging from abrupt flight to latent narcosis (Olla et al., 1980).

Our objective was to determine the behavioral response of fathead minnow and rainbow trout exposed to concentrations of a coal liquid WSF ranging from sublethal to lethal levels. The displayed behavior pattern would then indicate the likelihood of fish survival or death following acute or chronic exposures in the environment (Dauble et al., 1982c; Gray and Dauble, 1983; Gray et al., 1984). It was anticipated that dissimilar species of fish might behave differently in response to a given toxicant.

For the fish behavioral studies, PNL developed a special nine-chambered rosette, about 150 cm in diameter with a central drain

(Dauble et al., 1982c). Baffles were placed to direct toxicant flow among chambers and to allow free movement of exposed fish. WSFs of a coal liquid were delivered to various chambers by a dilutor system. The location of fish among the different chambers, an avoidance or attraction response, was monitored by overhead video camera every 30 minutes (Figure 20).

Minnows normally aggregate in schools, and the schools responded to toxicant consistently in replicate tests. When the WSFs were introduced, fish moved almost immediately to control chambers (Figure 20). Because the fish preferred the center chamber, the degree of avoidance to the coal liquid WSF could be defined as relative reduction in center chamber use between exposure and non-exposure periods. The mean fraction of the fish utilizing the center during the exposure period was plotted against the center concentrations to provide a concentration-response curve, for which a fit was obtained with the logistic model. Model parameters were significant at $\alpha \leq 0.005$. Using the model, the concentration at which 50% of the fish avoided the center (avoidance concentration, or AC_{50}) and moved to the control zones was determined. For the fathead minnow, AC_{50} was 1.5 mg/L total phenols, with a 95% confidence interval of 1.4 to 1.7 (Dauble et al., 1984b). Fathead minnow showed no response at concentrations of 0.7 mg/L in the center chamber. The observed avoidance threshold was sufficient to protect fathead minnow from acutely lethal effects reported

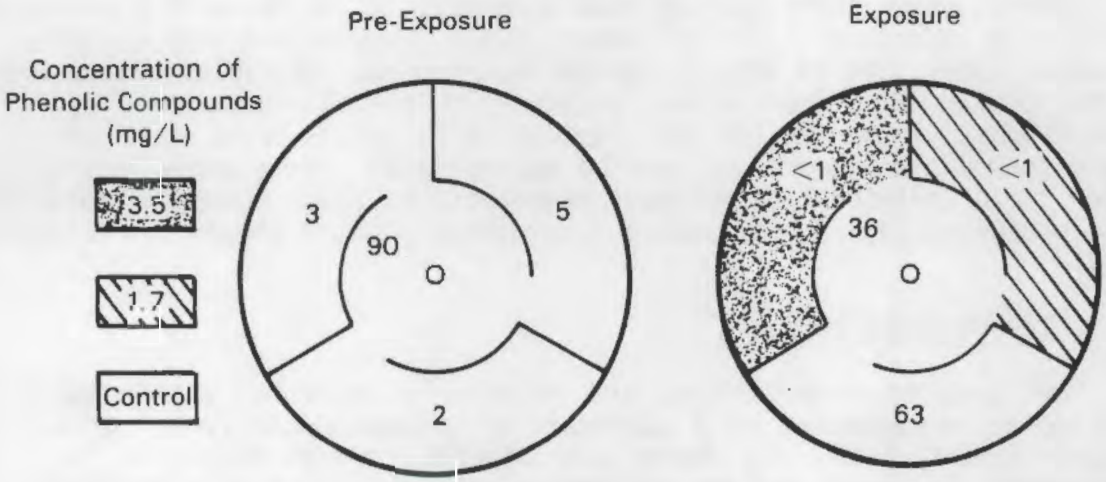


FIGURE 20. Schematic showing an avoidance response of minnows exposed to WSFs containing either 1.7 or 3.5 mg phenolic compounds per L. Contaminant enters and is mixed in the peripheral compartments, moves to the center compartment where it becomes further diluted, and exits via the central standpipe. Fish normally prefer the central compartment but move to the peripheral control chamber (no WSF) during exposure (distribution of fish shown). Actual device has nine peripheral chambers to provide randomization, and exposures are replicated at different series of concentrations. Representation is here simplified for clarity.

earlier for WSFs of this coal liquid (Section 2.2), but the fish did not avoid lower concentrations known to affect growth and reproduction (Figure 21).

The avoidance tests, when combined with other data generated in the preceding sections, revealed a graded series of responses for the fathead minnow (Figure 22). The most sensitive response measured was reduction in growth of juvenile fish. This was followed by reduced reproduction, and then by failure of normal avoidance.

Rainbow trout also preferred the center chamber during the pre-exposure period. However, they did not enter the control chamber during exposure concentrations of 3.1 and 6.3 mg/L phenols, and they remained in the center (~3.1 mg/L) despite over 50% mortalities (data not shown, see Dauble et al., 1984b). At lower concentrations, 2.4 and 4.6 mg/L phenols, fish still preferred the center chamber. Statistically significant ($\alpha \leq 0.05$) movement occurred only at much lower concentrations of toxicant, and movement was opposite to that expected at 2.2 mg/L phenols; in other words, the trout appeared to be attracted to concentrations of 2 mg/L total phenols, but the response was partially masked by aggressive activity among individuals. Thus, ecological factors can override avoidance tendencies determined in a laboratory apparatus.

Several other investigators have attempted to determine the avoidance of phenolic solutions by fish (Jones, 1951; Sprague and Drury, 1969; DeGraeve, 1982), and in most cases the response was variable. Since WSFs of the coal liquid are complex mixtures of phenolic compounds and hydrocarbons, comparison of PNL results with these tests involving single phenolic compounds may not be appropriate. For example, presmolt salmon avoid individual hydrocarbon components at lower concentrations than those presented here for a complex hydrocarbon mixture (Maynard and Weber, 1981).

2.3.2 Invertebrate Response

Most aquatic invertebrates are relatively immobile, either being attached to or dependent on a substrate or dispersed widely by currents. Because invertebrate life forms are diverse, their responses to contaminants in water are apt to vary widely. Clams feed by filtering algae and other organic material from water. They are also food, and like other bivalves, tend to bioaccumulate toxic materials. The National Research Council (NRC) has recommended their use for long-term monitoring of environmental contamination by fossil fuels (NRC, 1980). Because of its widespread distribution and abundance, the Asiatic clam, Corbicula, has gained the attention of toxicologists also in relation to bioaccumulation of persistent pollutants bound to bottom sediments (Cherry et al., 1980; Rodgers et al., 1980a). We also examined the behavioral responses of an aquatic insect (Diptera: Chironomidae) in this series of experiments. For aquatic insects, detection and avoidance of a toxic material during site selection and oviposition would help sustain numbers of important fish food

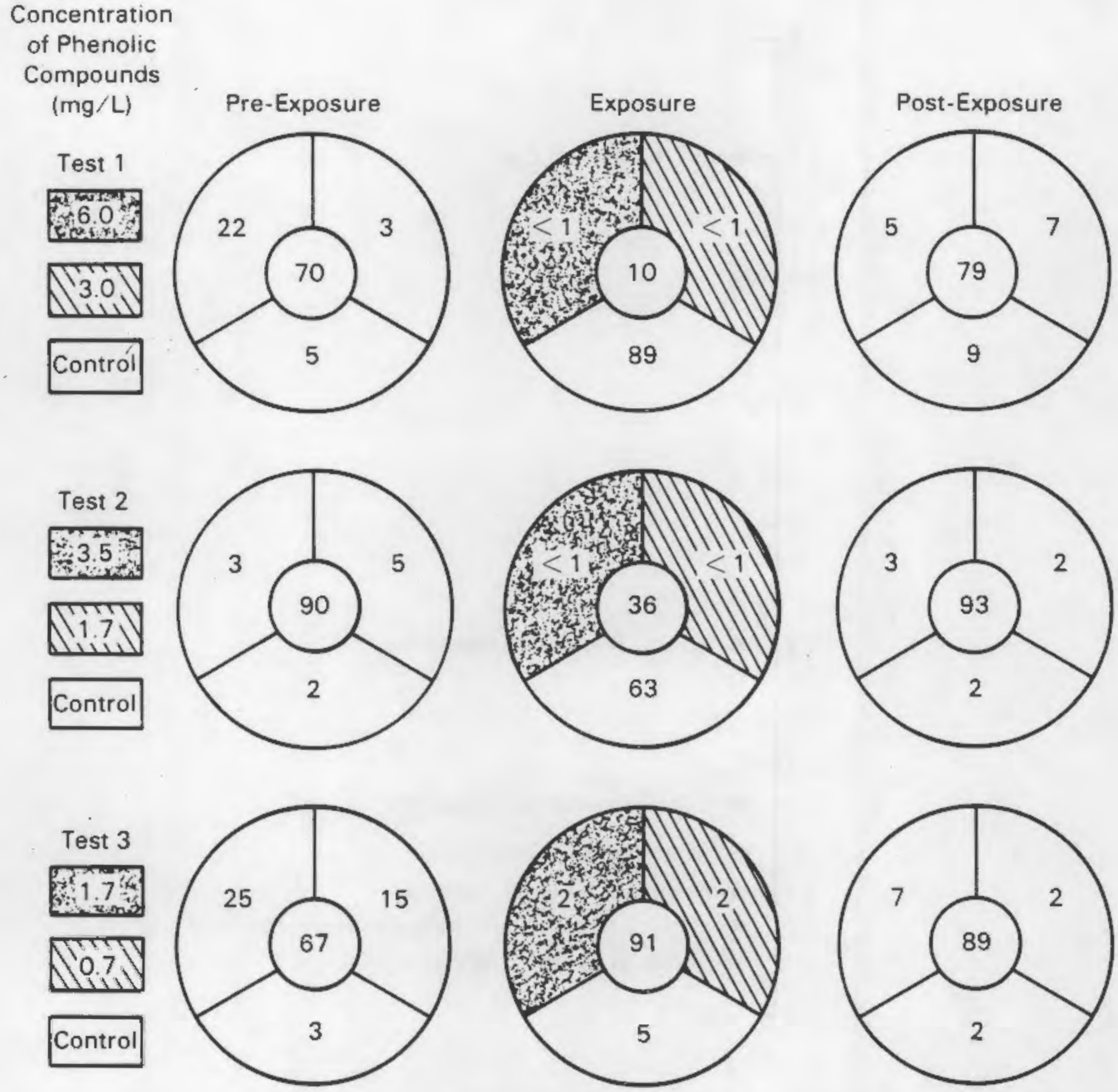


FIGURE 21. Avoidance response of fathead minnows exposed to WSF of a coal liquid at five different concentrations. Concentrations expressed as mg of total phenolic compounds per L. The 95% confidence interval for minimum avoidance concentration is 1.4 > AC_{50} > 1.6 (data taken from Dauble et al., 1984b).

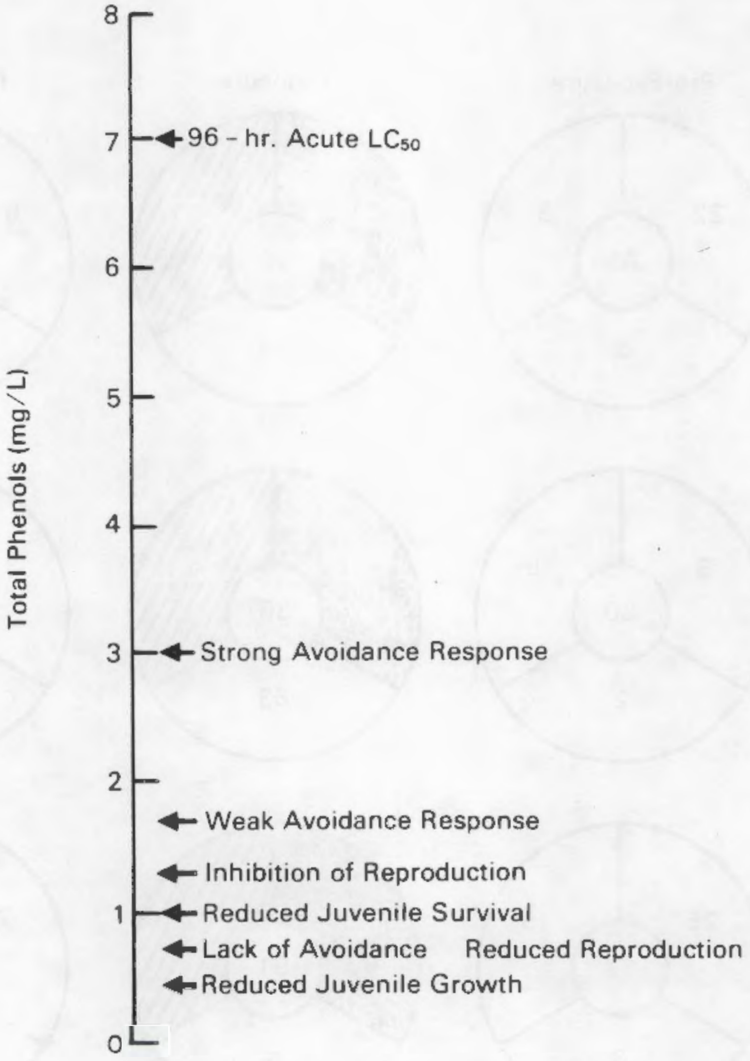


FIGURE 22. Comparison of the sensitivities of different biological responses in the fathead minnow to coal liquid WSF. Acute LC_{50} from Becker and Crass (1982) and chronic effects data from Dauble et al. (1983c).

organisms. Because many phenolic compounds degrade rapidly in water, insects able to detect coal liquids may avoid transient effects of spills by depositing eggs elsewhere.

Pacific Northwest Laboratory's experiments with the Asiatic clam had two objectives: first, to generate information for use in design of chronic effect studies, and second, to examine the effects of coal-liquid

WSFs on clam growth and behavior. In the first case, natural factors that influence the clam's activity have not been sufficiently studied to establish a foundation for examining clam response to a toxicant. In the second case, these and other clams are sessile and cannot avoid fuel spills, they can bioaccumulate toxic materials because they live several years, they will provide sufficient tissues for analysis of accumulated materials, and they serve as links in the food web to aquatic vertebrates (fish and mammals).

Three size groups of clams (12 mm, 20 mm, and 28 mm) were held in laboratory troughs at 10°, 20° and 30°C in both unfiltered river water with a natural phytoplankton food and in well water with a supplemental food. Growth and survival of clams was determined in relation to size, temperature, and food supply in two experiments each lasting 84 days (Dauble et al., 1984a).

Growth was minimal at low phytoplankton densities (~300 cells/ml), and clams in all size groups lost weight at 20° and 30°C. High temperatures of 30°C caused 100% mortality of small clams after 71 days, presumably because the clams received insufficient food to meet their increased metabolic requirements. Overall differences in growth appeared at high phytoplankton densities (>1000 cells/ml), and growth was maximum at 30°C (Figure 23). Small clams (12 mm) exhibited the greatest absolute increase in mean shell lengths at all test temperatures, and weight gains were similar to those of medium and large clams. Clams held in well water and fed trout chow at 117 mg/ml dry weight had an estimated conversion efficiency of 2.0%.

Having established optimal conditions for maintaining Corbicula, we exposed the clams to WSFs of the coal liquid for up to 14 consecutive days and retained them in river water to examine latent effects. Mortality occurred after 14 days exposure to 50 ppm total phenols, or levels three times greater than those acutely toxic to fathead minnows during 4-day exposures (Becker et al., 1983). Thus, clams were more tolerant than fish to synfuel WSFs. Part of this tolerance was due to valve closures, a behavioral response. Clams exposed to acutely lethal doses for 7 to 14 days often closed their valves, thus limiting the exposure and reducing the toxicant effects. No sublethal effects on growth of surviving clams was detected at exposure to 0.5, 5.0 or 50.0 ppm total phenol concentrations.

For the aquatic insect, Tanytarsus dissimilis, PNL attempted to establish a suitable experimental paradigm for determining avoidance. The determination depended on whether or not the insect would avoid placing its two egg masses (one 4 to 5 hr after emergence, and one 24 to 28 hr later) at contaminated sites, as compared to clean sites. Oviposition site preference (OSP) trials were made with groups and with single organisms (Dauble and Skalski, 1983). Results indicated that T. dissimilis displayed no preference for either the uncontaminated water in which they were reared, or water contaminated by an acutely lethal WSF (10 mg/L TC). Although avoidance was not documented, egg strands were shorter in the toxicant-spiked solutions, suggesting that the contaminant may have been

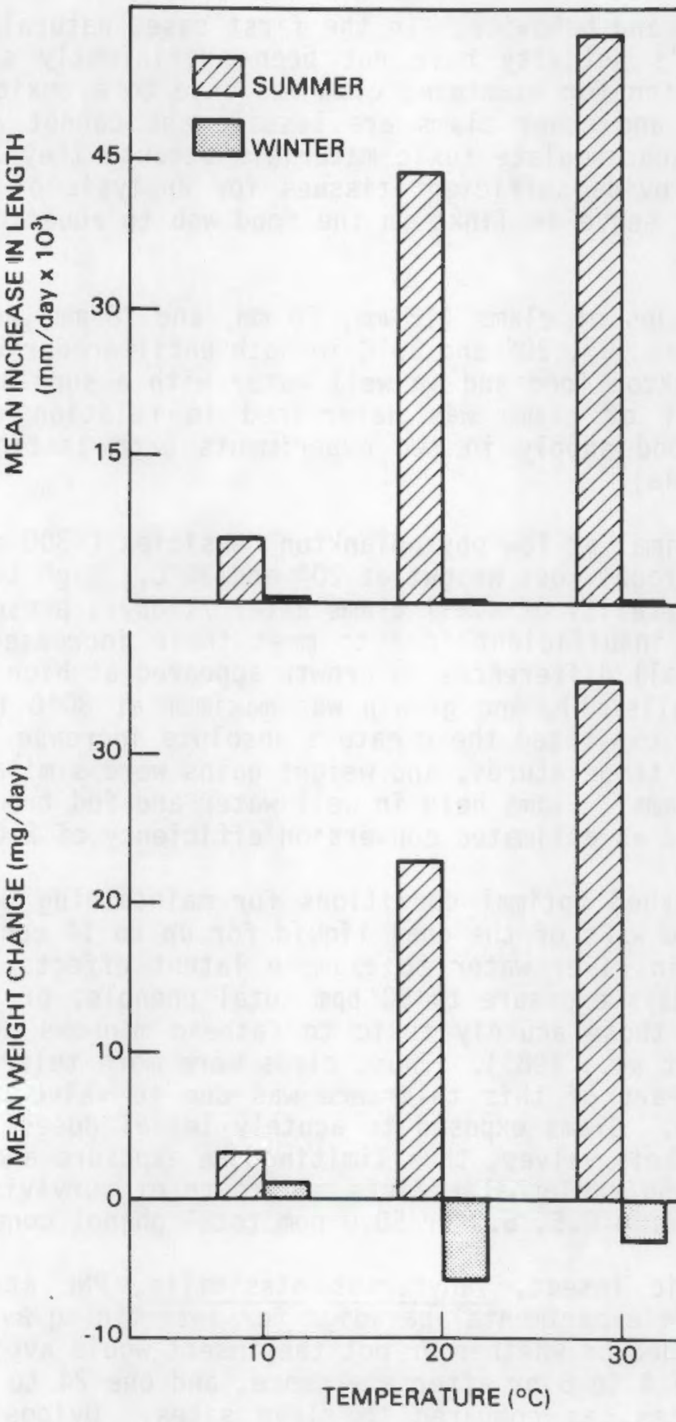


FIGURE 23. Relationship between season and temperature on growth rate of medium-sized clams. Values are summarized over 84 days (based on Dauble et al., 1984a).

detected after the oviposition act began (Figure 24). Reduced egg strand length may be attributable to inhibition of oviposition.

These laboratory studies indicate that *T. dissimilis* may be limited in their ability to detect and avoid areas contaminated with toxic, phenolic-based effluents. For this aquatic insect, avoidance behavior during ovipositioning may not play an important role in reducing acutely lethal effects of a coal liquid spill. However, toxicity thresholds observed for eggs or larvae may provide an adequate hazard assessment for bituminous materials released to aquatic environments. Detection mechanisms observed for other dipterans may differ, particularly if site selection of ovipositioning *T. dissimilis* is not based on chemical detection by the tarsus.

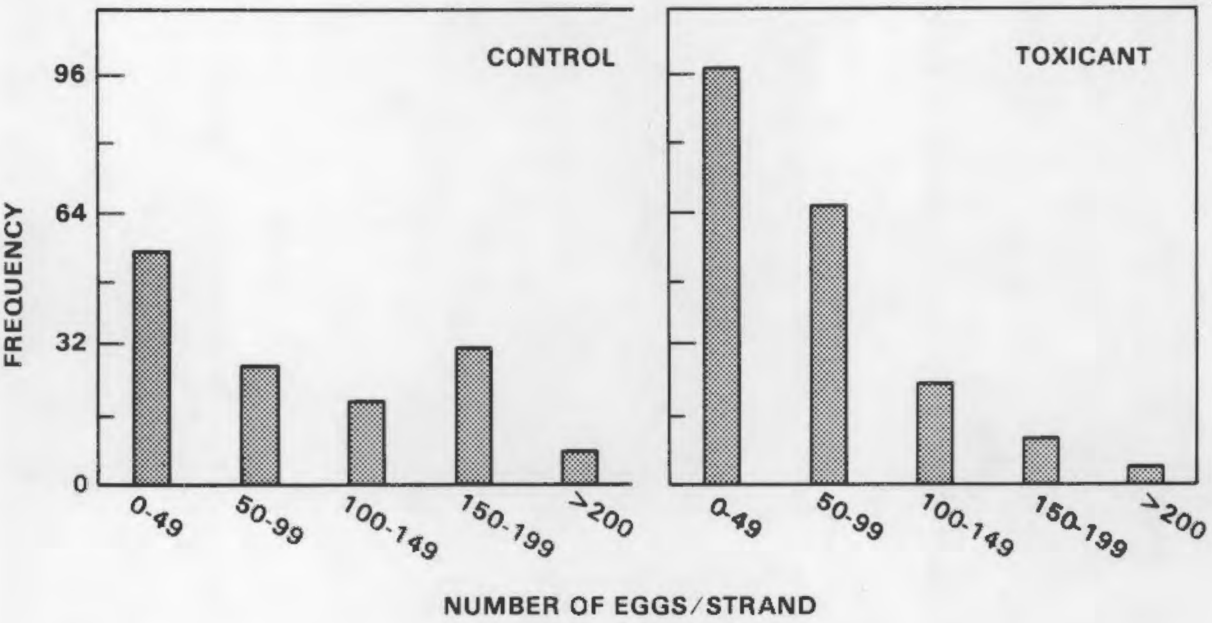
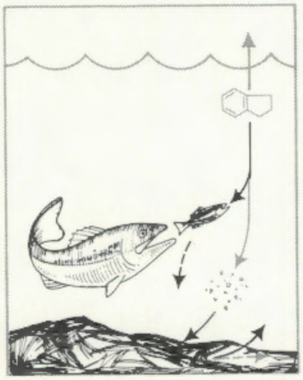


FIGURE 24. Frequency distribution of egg strand size in group oviposition site-preference trials (all tests combined) (data from Dauble and Skalski, 1983).



3.0 Systems-Level Determinations

3.0 SYSTEMS-LEVEL DETERMINATIONS

Given at present only a limited understanding of how processes affecting the persistence of organic compounds interact in an ecosystem, PNL concluded that there was need to develop a practicable approach to measuring systems-level responses. Since ecosystem and community studies of this kind have not generally progressed beyond the descriptive level, effort was placed on the quantitative development of a semi-natural aquatic ecosystem under outdoor conditions (discussed below). Laboratory studies like those discussed in preceding sections of this report necessarily imposed artificial conditions on the system under study. The studies are usually limited to single species and short-term experiments, because of the need to control numerous variables that might confound the determination of primary effect of the toxic materials used. In comparison, natural systems are much more complicated, making it difficult either to measure changes simply or to interpret results of experimental manipulations. For example, when an assemblage of species populations is present, long-range interactive effects may exist among the individual populations. These constraints present a dilemma to the research scientist attempting to understand how a toxic agent may affect a natural system.

In a flowing stream (lotic), for example, as compared to a laboratory beaker, a steady input of nutrient materials into the water maintains active algal and microbial metabolic activity. Early in the present studies, PNL had indications that such microbial activity might markedly affect the persistence and relative distribution of organic compounds in the sediment. This is recognized as an important environmental process, but in actual situations, its magnitude and direction are difficult to predict (Atlas, 1981; Cerniglia, 1981; Landrum and Scavia, 1983). Even in the presence of allochthonous sources of carbon, for example, microbial degradation is only partial (Horvath, 1972; Rogers et al., 1981), and concurrent biosynthesis of more complex compounds can also occur (Atlas, 1981). These same considerations may apply to organisms at higher trophic levels as well (Vaughan, 1984). Also, other environmental factors, such as photo-oxidation and volatilization, sometimes cause the disappearance of soil or sediment-bound materials (Lane and Katz, 1975; Jordan and Payne, 1980). In fish, photo-exposure of some xenobiotics (e.g., anthracene) may actually increase toxicity (Bowling et al., 1983).

In attempts to gain a more realistic understanding of how toxic organic mixtures affect ecosystems, PNL considered several approaches. Manipulation of a natural system is sometimes possible, but it was not in this case practicable with mutagenic materials. The effects of the manipulation might be environmentally unacceptable, and sampling would be prohibitively expensive. Controlled microcosms have been used in lieu of large-scale manipulations (Leffler, 1978; Taub and Crow, 1980; Taub et al., 1980; Bretthauer, 1980). The semi-natural stream system is a useful alternative to the controlled microcosm (Strand, 1982; Hoffman and Horne, 1980; Bowling et al. 1980; McIntire, 1975). It is one step closer to a natural system, but it is somewhat more difficult to sample reliably than the controlled microcosm. Uncontrolled microcosms have been used also (Lu et al., 1977, 1978), but they present many difficulties in interpretation of results (Vaughan, 1984). For present purposes, PNL undertook a limited effort to examine the fate and effects of a complex mixture in a semi-natural system involving artificial streams under partially controlled conditions (Strand, 1982).

The scale and volume of the system we designed for these initial tests (Figure 25) was kept small in order to permit replication and to limit the quantity of toxic material needed and the quantity of wastewater produced. The small scale necessarily involved some trade offs; i.e., the flow was low, and the surface area to stream volume ratio was unrealistically high, leading to possible boundary effects (National Research Council, 1980). On the other hand, the multiphasic system included flowing water, natural sediments, and biota representing several pre-determined species; it was exposed outdoors to natural light and temperature regimes, and source terms were controlled and quantified.

Two types of experiments were conducted in the semi-natural streams. In the first, natural sediments from the Columbia River were mixed with a coal liquid prior to being placed in stream channels. River water flowed

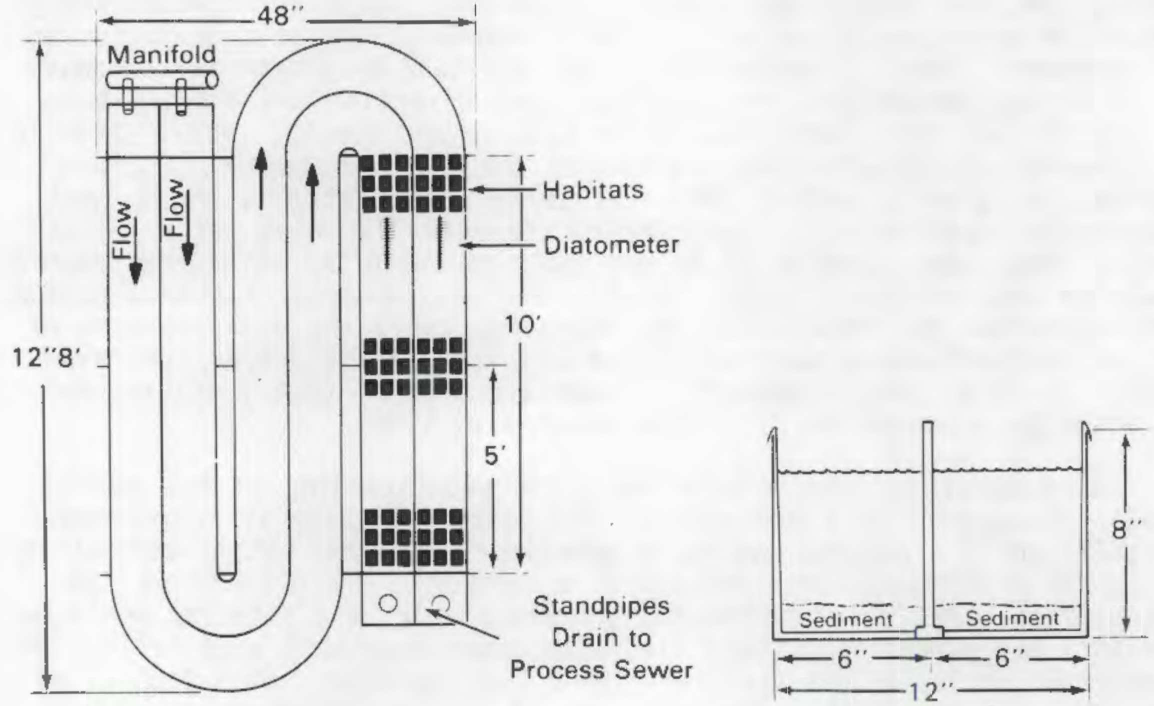


FIGURE 25. Schematic showing artificial stream channels.

through the streams for 42 days, during which time biological measurements and chemical analyses were conducted. We measured colonization, and we defined the chemical behavior of the coal liquid in the sediments. In the second experiment, streams were loaded with uncontaminated sediment and permitted to colonize with flowing river water prior to dosing the water with the coal liquid for a period of 120 hr. Lower concentrations of contaminant were used in this experiment, and chemical analysis was limited to simple dosimetry. Biological monitoring took place during dosing and following a recovery period. In both types of study, the aim was to examine the utility of both chemical and biological approaches for predicting system-level impacts and, in the case of the biological approaches, to examine the comparative effectiveness of several commonly used measures of biological response.

3.1 CHEMICAL CHANGES OVER TIME IN STREAM SEDIMENTS

Effects of combined natural processes (such as leaching, microbial activity, and algal growth) on concentrations of phenols and hydrocarbons were studied using the artificial stream system (Strand, 1982). Three streams were constructed, each containing a 1-inch layer of sediment impregnated with 2200, 440, or 88 mg/kg of coal liquid. A similar control stream was also constructed, and all four streams were allowed to flow for a period of 31 days. At the end of the experiment, sediments were sampled both in bulk (grab samples) and by collecting the top one-half and bottom one-half centimeter of sediment. The samples were analyzed for phenols and aromatic hydrocarbons; the results were then compared with samples taken initially.

Phenol, which is comparatively soluble, was depleted significantly in all three test streams at the end of the experiment (Figure 26). This was also true for phenolic compounds having up to six carbons (C_6) attached to the phenol ring. Phenol was depleted to less than one tenth of its initial concentration. Of interest was the increase over initial concentrations in phenolic compounds having side groups containing more than six carbon atoms, which was detected only in the low-dose stream. The control stream, which had no contaminant initially, also showed a measurable increase in its $>C_6$ fraction of compounds and an increase in phenol itself. No phenolic compounds detectable in the C_1 to C_6 range for the control stream. Evidently, biological activity had resulted in net production of higher molecular weight phenolic compounds, which responded to the phenol analysis procedure and some of the phenol, itself. The observations here are entirely consistent with literature indicating endogenous microbial production of such compounds (Neff, 1979, pp. 8-45).

The two streams initially contaminated at higher concentrations of coal liquid showed a decrease from beginning to end of the experiment, in all types of phenolic compounds (Figure 26). The $>C_6$ fraction did, however, show only a slight decrease, reflecting an offset due to endogenous production. Endogenous production of $>C_6$ compounds was further corroborated by comparing top and bottom sediment fractions (Figure 27).

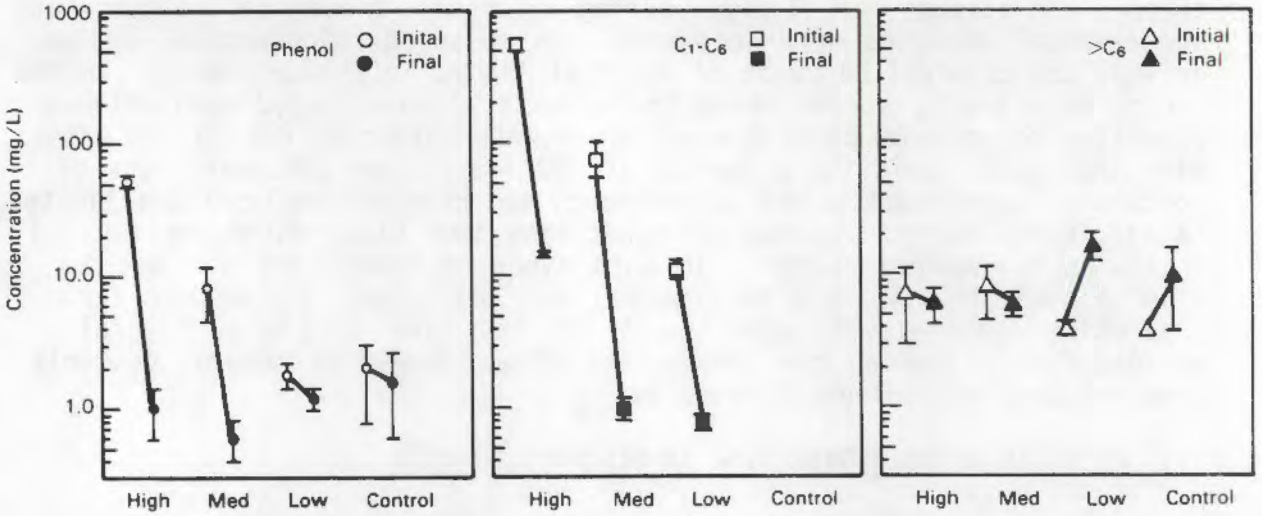


FIGURE 26. Initial and final concentrations of phenolic compounds in artificial stream sediments contaminated at three different levels initially. Compound classes were determined by GC/MS.

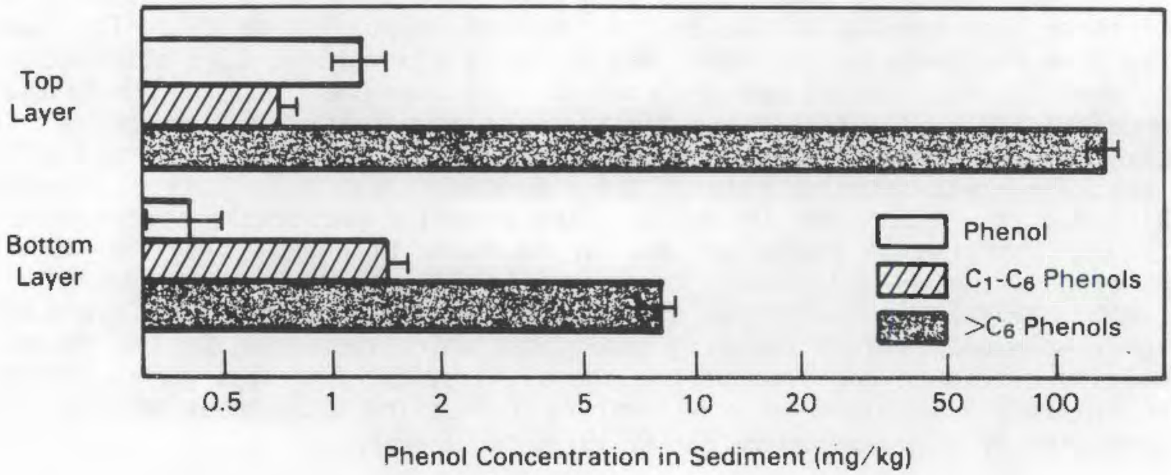


FIGURE 27. Distribution of phenolic compounds in top and bottom layers of sediment after 31 days. Sediments were contaminated initially at the low concentration of coal liquid (78 g/kg). Error bars represented ± 1 S.D. about the mean.

For example, concentrations of $>C_6$ phenols were always higher in the top layer of sediment, compared to the bottom layer, and it is in the top layer where one would expect to find the highest microbial metabolic activity.

Aromatic hydrocarbons remained essentially unchanged over the 31-day period (Figure 28). The lack of extensive leaching or microbial alteration indicates that aromatic hydrocarbons and other lipophilic constituents in sediments present possibly greater environmental concerns over long intervals than do the more water soluble phenolic compounds. Oil spill experience in marine environments supports such a concern (Atlas, 1981).

We have shown that the rate of removal of coal liquid residues depends upon the physical properties of the individual constituent types. Further, the data indicate that naturally occurring organic molecules can significantly affect the composition of weathered sediments.

3.1.1 Community Response Variables - Sediment Contamination (Expt. I)

Four widely considered biological response variables--community structure, biomass production, respiration (O_2 consumption), and primary production (chlorophyll)--were examined for their potential utility in signalling community response to coal liquid stress. In this experimental series, the coal liquid was uniformly mixed with Columbia River sediment at 88, 440, and 2200 mg/kg. The contaminated sediments and an uncontaminated

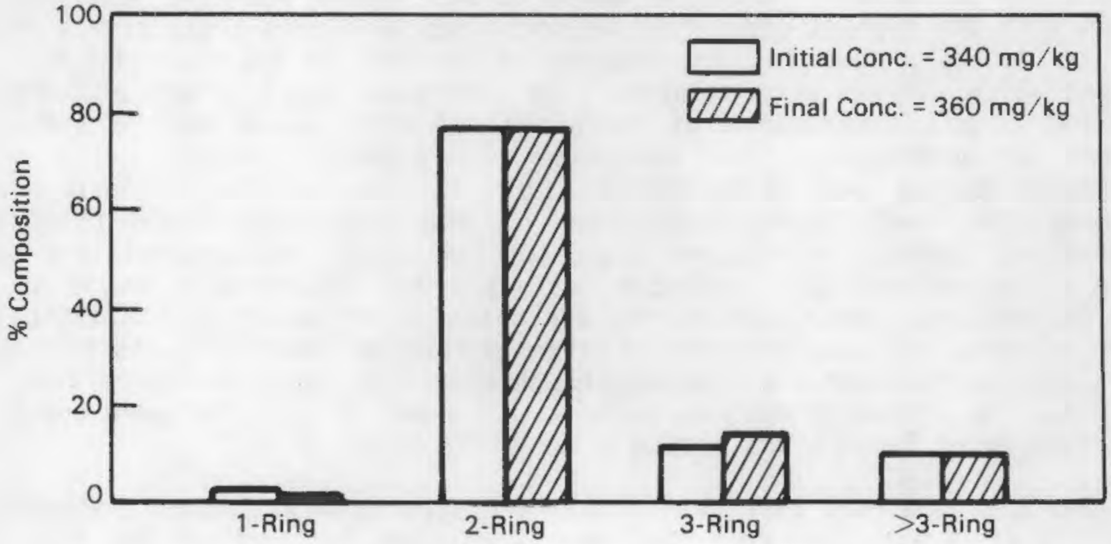


FIGURE 28. Distribution of aromatic hydrocarbons in sediments: initial versus 31-day exposure.

sediment control were placed in each of four stream channels. Columbia River water flowed through the streams, which were allowed to colonize, and samples were taken at 12, 24, and 42 days.

Benthic invertebrates would be most likely affected by the contaminated sediments; therefore, their colonization was monitored on glass habitats and their population was censused in sediment samples removed at the end of the experiment. Algal productivity is critical to the functioning of aquatic communities because it is a primary source of energy for the systems; therefore, biomass was measured on diatometer slides, periphyton was identified and counted, and both chlorophyll a and phaeophyton were measured. Zooplankton, another important structural component of aquatic ecosystems, were counted from water samples collected at termination of the experiment.

The control stream demonstrated a progressive colonization, as expected. Benthic macrofauna counts, on the glass habitats, increased with time; for example, midge fly larvae, mayfly larvae, and roundworms were detectable by day 12, and their numbers increased subsequently. Biomass as measured on diatometers increased from 1.2 to 2.5 gm/m^2 between day 12 and day 24, but it fell to less than 0.5 g/m^2 by day 42. The dominant alga was Asterionella formosa, which comprised 55% of the algal species present. Pennate diatoms and Synedra spp. each comprised 9% of the total.

In the contaminated streams, the coal liquid residues in sediment caused several effects on the community of organisms. Visual observations of the streams indicated gross differences, which could not be reliably measured in all cases. After 42 days, it was visibly evident that the stream with the highest concentration contained much more algal growth than did the control, and that the numbers of animals in the two higher concentration streams were reduced. The increased algal growth probably resulted from a combination of increased nutrient levels and reduced numbers of herbivores. For measurement purposes, a statistically detectable change was demonstrable after 12 days at the highest concentration level, based on chlorophyll a and phaeophyton determinations. Biomass and community structure (e.g., species counts) measurements did not prove to be statistically reliable, at least for practicable levels of sampling effort. These conclusions are based on estimates of statistical power of tests and sample sizes. Factors affecting power of tests include the alpha level, degree of detectable change, and sample variance (see Table 3). We strove to achieve tests with a power of 80% that were capable of detecting at least a 50% change at an alpha level of 0.05.

Tests of the null hypothesis (that the streams with treated sediments were not different from controls) were conducted for each of the four responses. Generally, the tests had very low power due to high variances (Table 3). Measurements of biomass, for example, did not show a significant difference at low and medium exposure levels, although a biomass change is suggested at the highest exposure level after 42 days. We concluded that there was no significant change in biomass after

TABLE 3. Mean and coefficients of variation (CV) for response variables from Experiment I.

Response Variable	Treatment	Overall(a) Mean	Overall(b) CV
Biomass (g)	Control	0.0053	42
	Low	0.0058	18
	Medium	0.0049	46
	High	0.0086	21
Chlorophyll a	Control	0.2598	29
	Low	0.3140	51
	Medium	0.3030	48
	High	0.4664	56
Phaeophyton	Control	0.0064	79
	Low	0.0271	61
	Medium	0.0321	130
	High	0.0794	171
Periphyton (Habitat modulea)	Control	61.5	51
	Low	58.5	37
	Medium	49.7	74
	High	57.7	98
Benthic Invertebrates (Mud Samples)	Control	281	70
	Low	11	55
	Medium	219	42
	High	2	100
Zooplankton	Control	806	18
	Low	817	7
	Medium	884	28
	High	1532	10

(a) Each entry is a mean of three sampling periods.

(b) Mean coefficient of variation from the three sampling periods.

exposure to oil, but this is questionable because of possible type II error. As an attribute of community structure, periphyton counts showed no reliable differences except possibly at 24 days. At 24 days, low and medium concentration streams had higher counts than either the control or high concentration streams but, again, variances were high. Chlorophyll a and phaeophyton in the high concentration streams showed no reliable changes at times other than 12 days, as mentioned above. Among the other attributes of community structure, macrobenthos counts, on the glass habitats, showed no detectable differences among treatments. Other differences also remained statistically questionable, except that zooplankton counts were detectably higher in the high concentration stream than in the other two streams.

In general, the statistical power of many of the determinations was very low (less than 20%; $\alpha = 0.05$), suggesting that the lack of detectable differences between treatments may have been indicative of type II errors (i.e., failure to reject the null hypothesis when it is false). Because of high variance in most of the measured responses, many more replicates would be required to give greater statistical power to the determinations. For example, in a highly controlled microcosm, nine true replicates would be required to detect a 50% change in biomass with 80% statistical power (Taub et al., 1980). Among the various responses measured, chlorophyll a and phaeophyton appeared to be useful indicators of system-level impact, at least for practicable effort levels.

3.1.2 Community Response Variables - Water Column Contamination (Expt. II)

For this series, the water column, and not the sediment, was contaminated by metering coal liquid into a baffled mixing unit during 5 days of exposure. The baffle approach to control partitioning has been described elsewhere (Vanderhorst et al., 1977; Oauble et al., 1981). Prior to the exposure, the artificial streams containing uncontaminated sediments and water from the Columbia River had been allowed several weeks to colonize naturally. The artificial streams were contaminated at two different levels, 6 and 16 mg/L total organic carbon; the control stream received no oil. Biological measurements again included biomass, chlorophyll a and phaeophyton (from diatometer slides) and, in addition, community respiration and net primary production. Species identification and counts were not performed for periphyton because Experiment I had demonstrated that their statistical power was low and the sample counting cost was high for such labor-intensive procedures.

Photosynthesis and respiration proved to be sensitive and statistically reliable measures of response in this series of experiments. They were assessed by measuring oxygen concentrations in light and dark chambers, which were inserted into the streams and sealed from the sediment. Circulation was provided, and small water samples were withdrawn for gas chromatographic analysis of dissolved oxygen. At both high and low concentrations of the oil, respiration exceeded gross O_2 production, resulting in a negative net production ($\alpha < 0.05$); and relative to controls, both gross and net O_2 production were depressed (Table 4).

Following a 9-day recovery period, the streams still appeared to show reduced respiration and photosynthesis, but some recovery had taken place and statistical tests failed to reject the null hypothesis of no effect. Net primary production (photosynthetic) could not be shown to differ from zero for either level of oil contamination. The control streams appeared to show higher net photosynthesis in July, when dosing took place, than in August. This may reflect seasonal differences in light or an effect of aging on stream communities.

TABLE 4. Means and coefficients of variation (CV) for response variables from Experiment II (N = 4).

Response Variable	Treatment	Mean	CV
Organic Dry Matter (ODM) (Treatment)	Control	0.0261	69
	Low	0.0241	79
	High	0.0418	45
Organic Dry Matter (Post-treatment)	Control	0.0213	105
	Low	0.0627	38
	High	0.0754	31
Chlorophyll a	Control	1.45	167
	Low	0.81	98
	High	1.29	176
Phaeophyton	Control	0.16	271
	Low	0.20	72
	High	0.44	92
Respiration (Treatment)	Control	-1.83	16
	Low	-1.29	35
	High	-1.24	26
Respiration (Post-treatment)	Control	-1.192	36
	Low	-1.095	11
	High	-1.168	23
Net Photosynthesis (Treatment)	Control	2.139	51
	Low	-1.535	18
	High	-1.262	28
Net Photosynthesis (Post-treatment)	Control	0.814	44
	Low	-0.088	895
	High	-0.478	145
Gross Photosynthesis (Treatment)	Control	3.966	31
	Low	-0.248	118
	High	-0.022	1774
Gross Photosynthesis (Post-treatment)	Control	2.006	16
	Low	1.008	88
	High	0.690	76

Biomass determinations were again relatively insensitive measures of community response. During the dosing interval, no statistically significant differences were measured. After the 9-day recovery period, biomass appeared higher in the streams receiving oil, as compared to the control, but the difference was not statistically significant. However, the power of this test was less than 50%. Analysis of covariance, adjusted for pre-dose differences between streams, also failed to detect significant differences between the treatments although the power of the test exceeded 95%.

At the end of Experiment II, macrobenthic organisms in the sediment were counted, but this, too, was not a sensitive measurement. Segmented worms and round worms were present in all streams, including the control. The control stream had, in addition, midge larvae and pupae, ostracods, clams, and mayfly nymphs. Streams treated with the oil had only a few midges, no mayflies, and only dead clams and ostracods.

Clearly in both Experiment I and Experiment II, the stress of the oil resulted in changes in the stream communities, including changes in macrobenthos, algal biomass, and community respiration and photosynthesis. These structural and functional changes were difficult to measure and validate statistically for several of the response variables, because of high inherent natural variability. For example, with four replicate streams at each test level, the statistical power of some of the tests we applied was too low to provide meaningful judgements about the measured changed (<50%). Measures of the community function (e.g., respiration, primary production) did provide useful indicies of effect. Measures of the community structure were ineffective because of the high variability (hence low power) and the relative high cost of identification and counting organisms. Biomass also showed low power and although individual samples are relatively inexpensive, the number of them required for reasonable resolving power is high (9 replicates are needed to detect a 50% change with 80% power).

Coefficients of variation (CV) for the response variables determined in the artificial streams were somewhat higher than those reported from studies of smaller microcosms, e.g., about 20% for such measurements as primary production and respiration in microcosms (Abbot, 1966; Giddings, 1980; Giddings and Eddlemon, 1979). Higher CV values, e.g., 25% to 70%, for example, have been reported for similar measurements in flowing water systems (Hoffman and Horne, 1980; McIntire, 1975; Rodgers et al., 1980). CV values above 50% have also been reported for the water chemistry of nutrients and pesticides in these systems (Giddings, 1980; Isensee, 1976).

Many of the microcosms reported as having CVs lower than 20% were more controlled and smaller than the artificial streams used in the present study. For example, synthetic sediments and distilled water were often used rather than natural sediments and water. In the present studies, use of natural sediments and water promoted a more realistic system but offered somewhat less control over the system. This increased the variability in parameter estimates. Although the CVs determined here are higher than those reported for other, more controlled microcosms, they are lower than those reported for field studies, which ranged from 40% to 200% (Eberhardt, 1978).

Given the differences among treatments considered here and the attendant CVs, estimates of the number of replications needed for studies of this type can be obtained from power calculations (Figure 29). With a CV of 50%, for example, 17 replicates per treatment group ($\alpha = 0.10$, 3 treatment groups) would be required to detect a 50% difference with a power of 80%. Methods based on response variables with lower variances and increased subsampling within each replicate may allow a reduction in the number of replicates needed to attain a test with a specified power.

In these experiments (Type II), respiration and primary production proved to be practicable and statistically reliable indicators of biological inhibition at the community level for TOC exposures ranging from 6 to 16 mg/L. In other studies, higher concentrations, e.g., 50 mg/L, were evidently needed (Giddings, 1982a) to inhibit biological response. Since respiration and production are only partial attributes of ecosystem-level responses, it is clear that additional experimental work will be required to perfect the state-of-the-art of measuring community response. It is possible that other measures of energy flow in the system may prove to be sensitive to stress response.

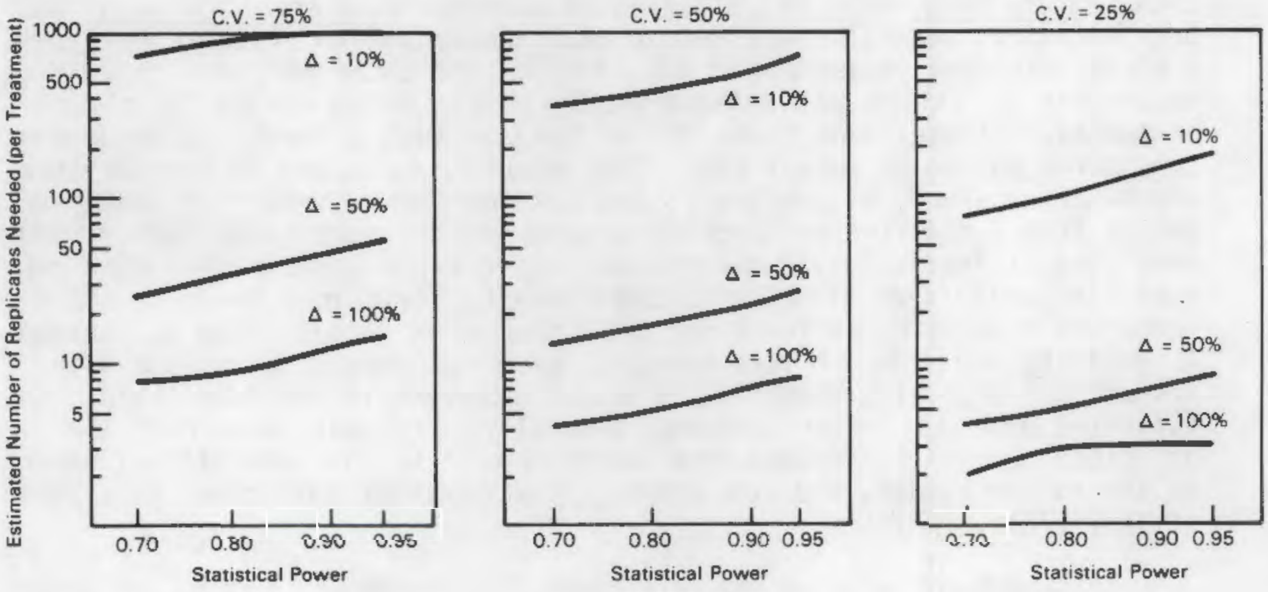


FIGURE 29. Statistical power curves for three different coefficients of variation (CV) (3 treatments, $\alpha = 0.10$). Because CVs for the community response variables measured are typically high, a large number of replicates would be required to detect reliable changes after exposure to a toxic mixture.

3.2 RE-ENTRAINMENT FROM SEDIMENT TO BIOTA UNDER NATURAL CONDITIONS

Data in this area are very limited and were derived mainly from PNL's petroleum research program. Sediment-to-biota transfer is a potentially important link in food chains to people. Re-entrainment takes place via microbial conversion, via uptake from the interstitial water of sediments, and via ingestion of bottom particulate matter. In aerobic sediments, as in soils, the complete conversion of complex hydrocarbons to CO_2 and water is known to require a suite of organisms and time for populations not initially present in great number to adapt and grow. Studies by PNL demonstrated, however, that microbial conversion was likely to be incomplete, even with an appropriate time interval, which may vary appreciably with temperature and other field conditions (Rogers et al., 1983). Rogers also demonstrated that during exposure, a wide variety of intermediate metabolites was produced (Rogers et al., 1983a). Further research will be needed to delineate metabolites and conditions that might be important for food chain uptake, however.

Only recently has information been available on the distribution of sedimentary compounds that find their way into organisms directly living in the sediments. For example, PNL was able to show that a detritus feeding clam, Macoma inquinata, and a burrowing polychaete, Abarenicola pacifica, both entrained several times the sediment concentration of three PAHs over a 60-day exposure (Augenfeld et al., 1982). The three PAHs were freed from impurities by silica gel chromatography before being sorbed to clean sediments. Tissue-bound forms of the PAH that were solvent insoluble were considered not to be parent PAH. They amounted to 1% and 9% for chrysene and benzo(a)pyrene, respectively, and 22% for phenanthrene. It would be useful from a modeling perspective to know whether such bound forms of PAH consisted of insoluble metabolites or rather represented parent compound that had partitioned into lipid constituents. Evidently the clam was a temporary reservoir, at least for PAH transfer to people. The polychaete A. pacifica would be of less concern, since an aquatic carnivore with hydrocarbon degrading capabilities would intervene in the food chain. In estuarine and salt water systems, generally, it would seem that the principal pathway for uptake from sediment will be via interstitial water to the filter feeder, and not directly from sediment particles (Roesijadi et al., 1978a, 1978b).

In subsequent work on New York Bight, fluoranthene, pyrene, and other PAHs of high MW found in the digestive gland of lobster sharply contrasted with the predominantly lower MW compounds like naphthalenes and biphenyls found in the liver of flatfish (Anderson, 1982). These differences were thought to reflect primarily habitat and feeding habits of the lobster. Differences in the metabolism of these compounds in lobster compared to fish were not investigated.

Volatilization from spill-contaminated sediments has been thought to be a factor reducing the likelihood of re-entrainment of hydrocarbons from sediment to biota (Dilling, 1977; Mackay and Wolkoff, 1973; Mackay and

Leinonen, 1975). Later studies, in which Henry's Law coefficients were determined for 2-, 3- and 4-ring PAH, suggest that volatilization cannot significantly reduce the sedimentary concentrations of the more carcinogenic 3- and 4-ring compounds (Southworth, 1979). Naphthalenes but not PCBs may be substantially reduced in aquatic sediments by volatilization (Konasewich et al., 1982; Southworth, 1979).

3.3 USE OF A MATHEMATICAL MODEL TO PREDICT BEHAVIOR OF A COAL LIQUID IN STREAM SEDIMENTS

The Aquatic Pathways Model (APM) is a mathematical representation of the distribution of organic materials among components of an aquatic ecosystem (Aaberg et al., 1983b). The model was developed as part of PNL's synfuels program. It is designed to describe the time-dependent distribution of several compounds simultaneously, and thus may be applied to screening and estimating the fate of compounds contained in complex mixtures. It has broad application to many compounds and to many aquatic ecosystems, including both flowing (lotic) and non-flowing (lentic) systems.

Many models described in the literature have been designed to assess environmental consequences resulting from introduction of a foreign substance (Bartell et al., 1981; Burns et al., 1982; Ditoro et al., 1981; Helton and Kaestner, 1981; Hicks, 1981; Mackay and Paterson, 1981; Neely, 1979; Niemczyk, 1980; and Onishi and Trent, 1981). From these models, individual submodels were selected for use in the Aquatic Pathways Model (APM). The APM has two main advantages over most other models: it requires simple input information, which is more apt to be available than complex information; and the use of such simple information allows the program to be run on a microcomputer, which makes the code portable and inexpensive to run. Since the APM code was first developed, the capabilities of microcomputers have increased greatly. Larger programs fit more easily on inexpensive microcomputers, making these programs more readily adaptable. The APM and its matrix method of calculation provide a straight forward and relatively fast solution to first order differential equations that form its base.

Phenolic compounds were chosen as the first class of chemicals to be investigated by APM because: they are more readily water soluble than other aromatics and hydrocarbons, they make up a large part of the water soluble fraction (WSF) of coal liquids, and they are highly toxic. The solubility of phenol in water is 93 g/L, versus, for example, 0.7 g/L for benzene; thus, phenolics will be dispersed rapidly if a spill occurs and are liable to affect an area larger than the slick itself. Even if undissolved oil is recovered, aquatic organisms will be exposed to dissolved phenols.

A literature search for applicable data on phenolic compounds was conducted concurrently with the model development (Aaberg et al., 1983b). Twelve phenolic compounds were considered in this model evaluation, and they have all been identified in aqueous suspensions of coal liquid used for the current studies (Strand and Vaughan, 1981). Toxicity of specific chemical species was not addressed in the model. In future modifications, toxicity data could be included and compared with predicted concentrations.

The sediment portion of the APM was applied to the sediment/water partitioning data from the stream studies described in Section 3.1 (Aaberg et al., 1983a). Since the experiment did not address uptake, estimates of uptake rates were required to provide the needed ratio. Published sediment/water turnover rates (Neely, 1979) were used. In the experiment, fine sediments were contaminated with coal liquid and loaded into artificial stream channels. They were allowed to settle overnight before flowing river water was introduced. Concentrations of phenolics and aromatic hydrocarbons were measured in sediment at the start and end (31 days) of the experiment. Concentrations in water were measured several times during the experiment. APM was run using projected day-1 sediment concentrations. A turnover rate was chosen and equilibrium partition coefficients were used to predict depuration rates. Simulations were compared with measured concentrations of phenolic compounds in water and with final concentrations in sediment. The simulations of water concentrations generally followed the trend from the measured data (Figure 30).

The least hydrophobic (lowest molecular weight) aromatic hydrocarbons were only partially removed from sediments. The concentration of substituted benzenes was reduced by about one-half, and the concentrations of naphthalene and C_1 naphthalene were reduced by about one-third. None of the other aromatic hydrocarbons showed significant reduction after the 31-day depuration period. Concentrations of aromatic hydrocarbons in the flowing water decreased with time, but were generally within a factor of 10 of initial concentrations. The experimental results do not strictly support a donor-controlled model for sediment release for aromatic hydrocarbons.

Although predictive models are generally limited because they are simplified assumptions and approximations of real situations, they can be used to determine order-of-magnitude variations in estimated quantities. As such, models can be used to guide environmental research by highlighting potential exposure pathways and mechanisms that are either very significant or of no consequence. The data indicated that donor control of leaching from sediment is applicable only for relatively mobile (soluble) compounds. Thus, use of bulk sediment properties is inappropriate for aromatic hydrocarbons. The latter compounds are not as mobile as phenols, and diffusion through a thin layer of sediment seems to be controlling release rates on all except the lightest compounds (benzenes). Two sediment compartments would be required to model the sediment interactions.

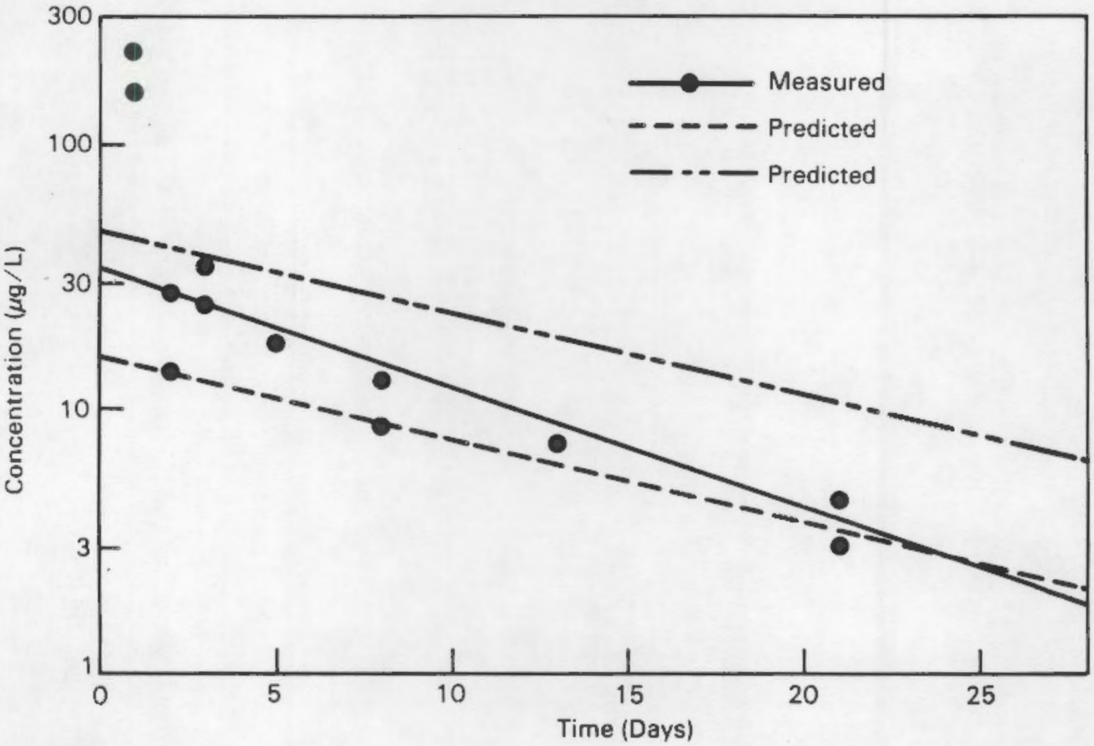
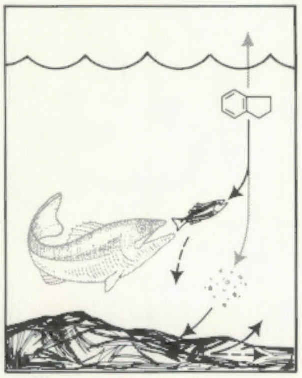


FIGURE 30. Concentration of C_4 phenol in experimental streams versus prediction estimated by the Aquatic Pathways Model. The upper and lower one-third segments of the stream are modeled as separate subcomponents.



4.0 Metabolism and Fate of Compounds in Complex Mixtures

4.0 METABOLISM AND FATE OF COMPOUNDS IN COMPLEX MIXTURES

Uptake and metabolism of constituent compounds are key considerations in understanding the environmental behavior of complex mixtures. However, systematic research on these important biological processes has been largely ignored. In an environmental pathway, for example, metabolism may not simply represent the removal of a constituent, as is generally assumed in current modeling studies (Aaberg et al., 1983b; Travis et al., 1983). Rather, it may sometimes lead to the production of by-products that are more retentive, more toxic, or both, compared to the parent compound (Gmur and Varanasi, 1982; Lu et al., 1978; Ludke et al., 1972; Varanasi and Gmur, 1980; Yoshimura and Yamamoto, 1973). These are important considerations in evaluating pathways for potential transfer of contaminants to people. They are also important from the standpoint of assessing the long-term ecological implications of contaminants spilled to the environment. Extant models are not competent to deal with the issues these considerations raise.

For microbial (Atlas, 1981; Cerniglia et al., 1981) and larger aquatic organisms (Vaughan, 1984), the literature on metabolism of some polycyclic organic compound classes has been reviewed, and the general features of metabolism for a few compound classes can now be outlined. In designing research on metabolism in food chain pathways, we commenced a selective program based on a current review (Vaughan, 1984). Research is currently in its early stages. Organisms are being selected to represent different feeding habits in major trophic levels (i.e., algae, zooplankton, clams, crayfish, and trout). The organisms are exposed in highly defined water and water-sediment systems to radiolabeled organic compounds, both singly and in complex mixtures. State-of-the-art analytical chemistry is applied to identify metabolites and parent compounds.

4.1 UPTAKE AND METABOLISM OF QUINOLINE IN FISH

PNL's initial work has focused on juvenile rainbow trout, which like other aquatic organisms have been thought to metabolize polynuclear aromatic hydrocarbons (PAHs) to hazardous intermediates with longer retention characteristics (Malins and Hodgins, 1981; Malins and Roubal, 1982). Metabolites of quinoline, a nitrogen containing heterocycle, here studied in fish, are also being studied in soil/plant systems under another PNL program. Quinoline was examined because current research has shown that the nitrogen-containing polyaromatic compounds are far more mutagenic than neutral PAH (Guerin et al., 1980, 1981; Later et al., 1982, 1983a, 1983b; Pelroy and Wilson, 1981). For these studies, a rapid, low-cost method was developed to analyze whole small fish and specific tissues or organs from the larger fish for both quinoline and hydroxylated quinoline. Caustic tissue digestates were treated with acetic anhydride, and the resulting acetylated hydroxy-quinolines, along with quinoline, were extracted into heptane and analyzed by capillary gas chromatography (GC) using a nitrogen/phosphorous specific detector (NPD) or by gas chromatography/mass spectrometry (GC/MS).

Uptake and depuration of quinoline showed patterns in juvenile rainbow trout (0.3 g) typical of other PAHs, with about one-third of the quinoline products remaining in the fish after the depuration interval (Figure 31). Tissue distribution of quinoline products was more interesting, and a substantial portion of effort was expended on chemical analysis, both to quantify tissue levels of parent compound and to identify metabolic products resulting from hydroxylation and conjugation. The radiolabel was present in all tissues examined (Figure 32), indicating the presence of either quinoline or its metabolic products.

Gills contained only a small portion of the body burden, indicating that the material was carried away by the blood. Levels of ^{14}C activity in the liver were low, but the gall bladder and bile contained over 60% of the burden following a 24-hr period of depuration. The low levels in kidney tissue indicate that kidney is not an important route for elimination. The portion of quinoline metabolites in muscle was surprisingly low, especially following 24 hr of depuration. The radiolabel in the gall bladder was nearly all associated with hydroxyquinoline compounds. This suggests rapid kinetics of transfer from liver to bile. Seven different hydroxylated quinoline metabolic products were isolated from the gall bladders. Thus quinoline, when taken up directly from water, was readily metabolized by

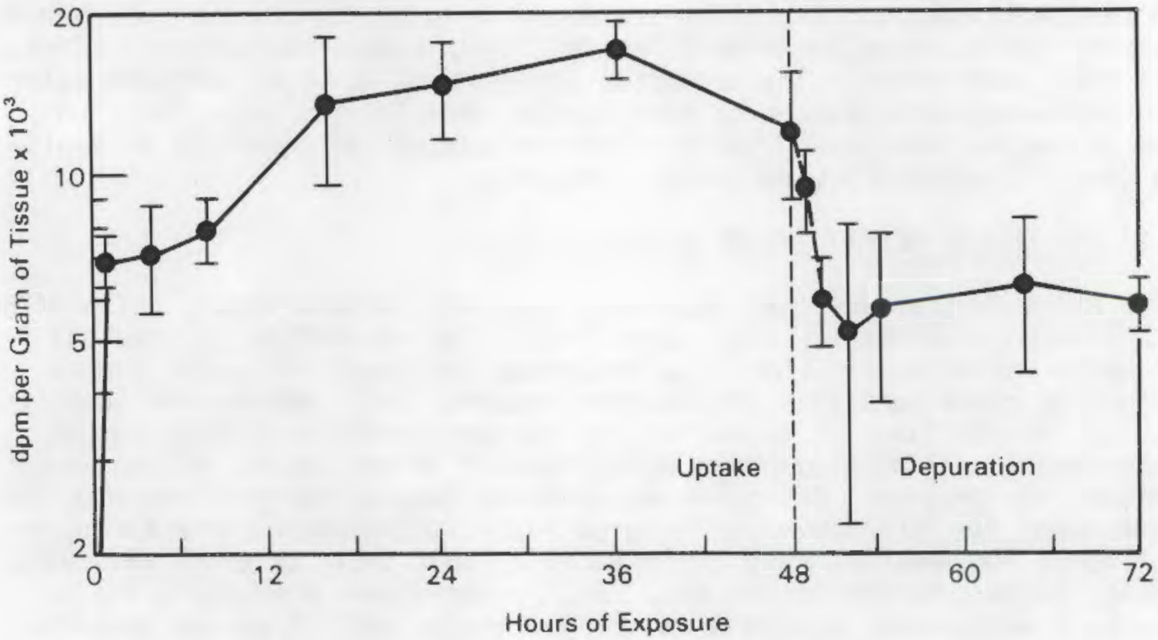


FIGURE 31. Uptake of quinoline and depuration of quinoline metabolites by juvenile rainbow trout (~0.3 g). Parent quinoline is no longer measurable in tissues at the end of the depuration interval.

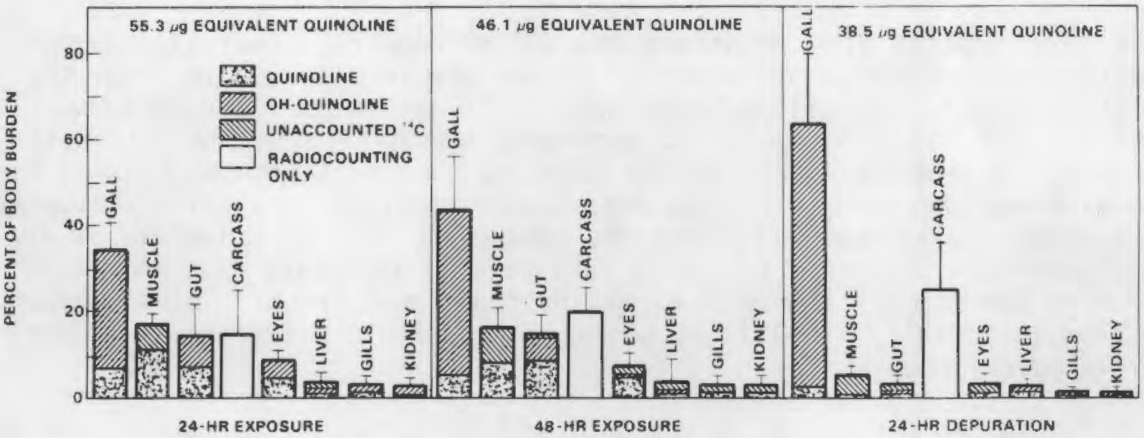


FIGURE 32. Tissue distribution of quinoline and its metabolites in juvenile rainbow trout (10 g) (based on Thomas et al., 1983).

liver enzyme systems. Uptake, and metabolism after ingestion may, however, differ from uptake via water because of alternate storage and elimination mechanisms.

Additional studies were conducted to determine if quinoline was taken up from the diet to the same extent as from the water column. Juvenile rainbow trout (10 g) were trained to accept food pellets that were dosed with 1 µCi of ^{14}C-quinoline or concentrations nearly equivalent to those taken up by a food organism (daphnids) after 48 hr exposure. The relative body burden of ^{14}C-quinoline achieved a plateau after 7 days feeding, and greatest concentrations were observed in the gall bladder and the gut (data not shown). Radioactivity in the gall bladder and the gut declined to 1% and 20%, respectively, of maximum concentrations after 4 days of feeding on noncontaminated food. Although parent compound plus metabolite concentrations in the muscle were relatively low in comparison to digestive organs, elimination was much slower.

In these PNL studies, thin layer chromatography has shown that quinoline metabolites exist as conjugates rather than as unbound hydroxylated quinolines. PNL has further found that the metabolites are <u>not</u> conjugated (as was expected) with glucuronic acid or with sulfate, since hydroxyquinolines are not released when the conjugate was treated with glucuronidase or with sulfatase enzymes. The unidentified conjugated material is currently undergoing insolation procedures so that its structure can be studied using direct-probe mass spectrometry and Fourier transform infra-red spectroscopy.

Diet may also be a significant route of uptake for quinoline, as results currently indicate (data not shown). Thus food chain transfer to fish from exposed prey organisms may be of concern. Overall, tissue distributions differed for dietary uptake compared to uptake from the water; however, the gall bladder was still the major storage site. Analyses are being conducted to determine whether quinoline metabolic pathways for dietary intake in the fish are similar to those observed for the water-uptake route. Studies are also in progress to examine the uptake of naphthol by rainbow trout and the uptake and fate of quinoline in the freshwater clam, Corbicula. It is particularly important that the results for fish not be generalized to organisms from lower trophic levels consumed as food (especially shellfish), because of possible differences in their physiological chemistry.

Procedures for isolating naphthol and its metabolic products from the tissues and organs of exposed fish also have been implemented based on use of the mass selective detector gas chromatograph, and upon specific derivitization procedures for isolation of altered naphthol residues.

4.2 BIOUPTAKE OF SPECIFIC COMPOUNDS IN THE PRESENCE OF COMPLEX WSFs

PNL conducted tests with juvenile rainbow trout (S. gairdneri) and the water flea, D. magna, to see if uptake and mobilization of single compounds when presented alone differed from estimates obtained with those same compounds presented as a complex mixture (water soluble fraction). Phenol, aniline, and quinoline were used as representative compounds because they are highly soluble, moderately toxic, and ubiquitous to many coal liquid products and corresponding wastes.

When juvenile rainbow trout were exposed to the single compound of interest as well as to the same compound in the presence of a coal liquid WSF, about 35% less radioactivity was accumulated when the coal liquid water solubles were present. The data (not shown) indicated that other lipophilic components compete with phenol for adsorption into tissue. Also, a larger portion of absorbed ^{14}C remained bound in trout tissue following single-compound exposure than when coal liquid components were present as well.

In contrast to the observations on phenol, the presence of other water solubles did not significantly influence either the uptake or the absorption of ^{14}C-aniline. This suggests that sites for absorption of aniline to trout tissue are limited and different from those for phenol.

Complementary studies with daphnids show that this organism accumulated less radiolabeled phenol in single compound exposures than in the presence of a complex coal liquid WSF (Figure 33). However, in biouptake experiments with quinoline and aniline, bioaccumulation was similar in single compound and complex mixture exposures. These studies suggest that any measure of the potential for bioaccumulation must consider all environmental variables, including the concentration of the toxic material and the fitness of the biological organisms exposed to the toxicant. The data show that BCFs determined from measurements with

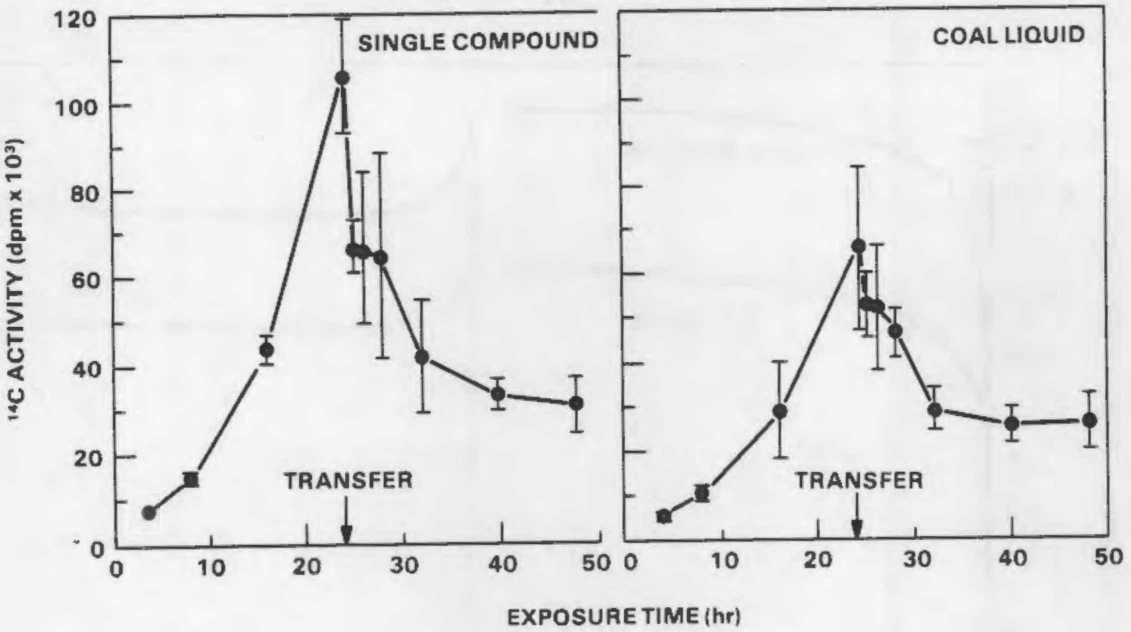


FIGURE 33. Uptake and depuration of phenol by daphnids exposed to phenol alone or to phenol in the presence of complex mixture.

pure compounds in the laboratory may seriously differ from BCFs in actual environmental situations.

4.3 ACCUMULATION OF PHENOL AND ANILINE BY ALGAE

The freshwater phytoplankter, Scenedesmus quadracauda was studied because phytoplankton can concentrate a variety of organic compounds from the surrounding water. Therefore, they can serve as a point of introduction of coal liquid residuals into aquatic food chains with ultimate transfer to people.

Uptake of both aniline and phenol by S. quadracauda was rapid in the first few hours and then slowed (Figures 34 and 35). Both compounds at the two concentrations examined showed virtually complete uptake after 4 hr. It is likely that the rapid uptake occurred primarily because of surface adsorption, as evidenced by the initially rapid elimination after transfer. However, both bound and adsorbed material would be available for food web transfer. When the phytoplankton were transferred to fresh media, radioactivity was lost rapidly. Elimination followed a biphasic course, with an initial rapid loss in the first 1 to 2 hr, followed by a slower elimination rate during the next 22 hr (Figures 34 and 35). Despite this rapid loss, a significant portion of radioactive material remained in the algae after the 24-hr depuration period. Uptake of phenol and aniline by

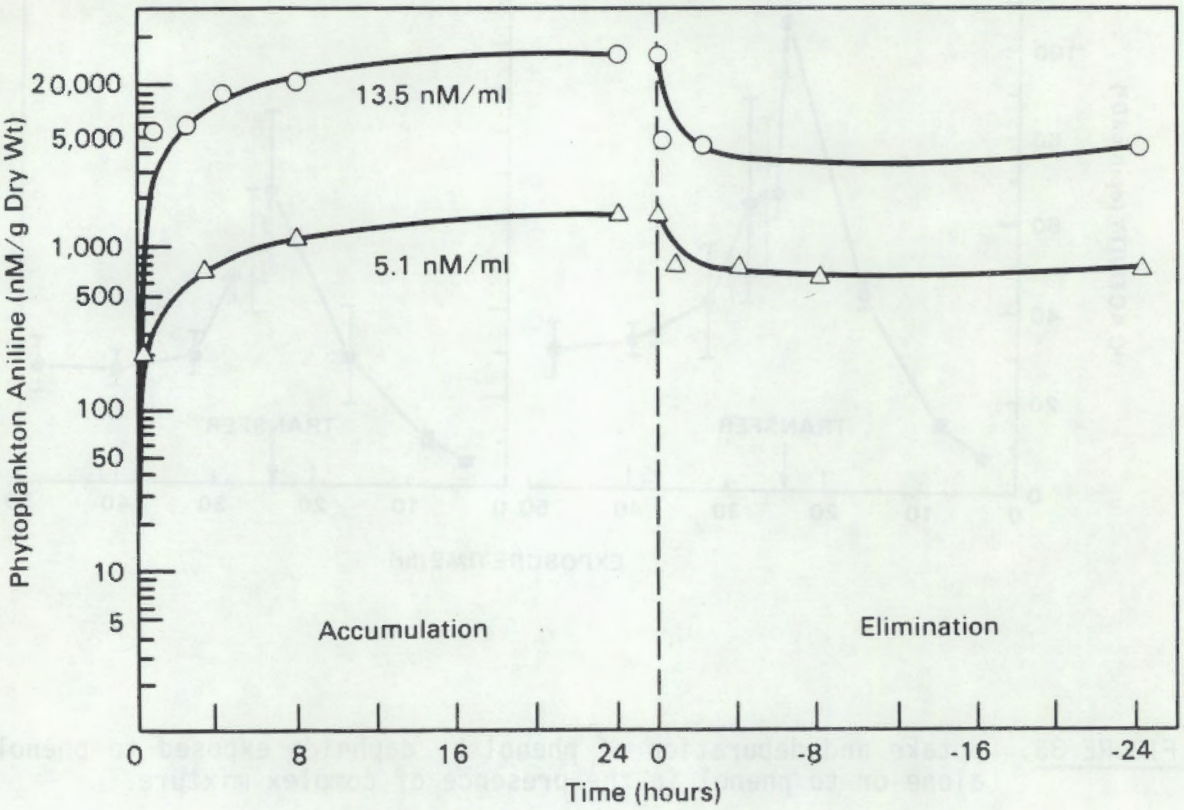


FIGURE 34. Accumulation and elimination of phenol by Scenedesmus quadracada. The rapid dynamics suggest that phenol was adsorbed to cell surfaces rather than being absorbed and incorporated into tissue.

S. quadracauda appears to be concentration dependent. When normalized to equivalent concentrations, bioaccumulation was 10 times greater for aniline than for phenol. Both phenol and aniline were metabolized with about 22% to 52%, respectively, of the accumulated compounds retained as parent material after 24 hr. Adjustment of the BCF values to account for metabolism yields values of 3.5 for phenol and 91 for aniline (Hardy et al., 1984).

These BCFs are within the range of other coal liquid components examined by Southworth et al. (1978), who studied the bioaccumulation potential of synfuel-generated azaarene compounds in the freshwater invertebrate Daphnia pulex. Since phenol and aniline have low octanol/water partition coefficients (Log p = 1.47 and 0.94 respectively), they would not be expected to bioconcentrate greatly in comparison to other coal liquid components. Equations used to estimate BCF from log p (Veith et al., 1980; Neely et al., 1974; Southworth et al., 1978) for a variety of

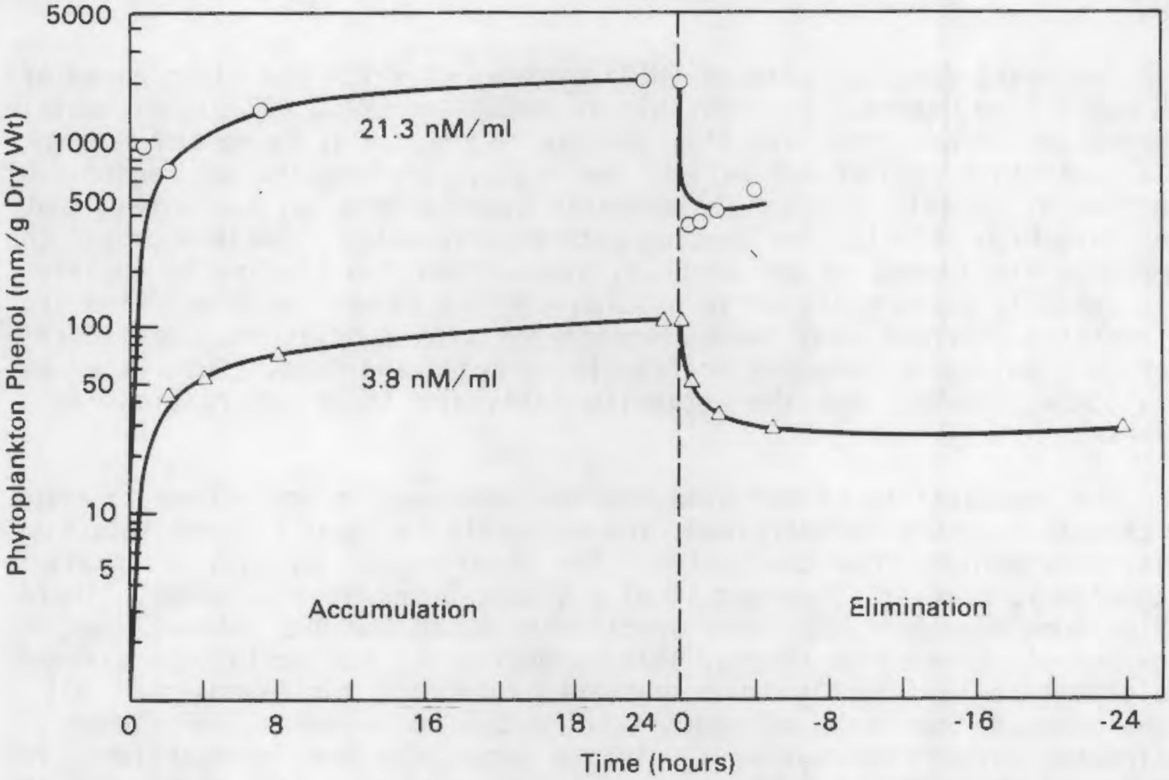


FIGURE 35. Accumulation and elimination of aniline by Scenedesmus quadracauda.

organisms, predict BCFs of 1.1 to 8.3 for phenol, i.e., generally in the lower end of our estimates.

4.4 SYSTEMS-LEVEL IMPLICATIONS

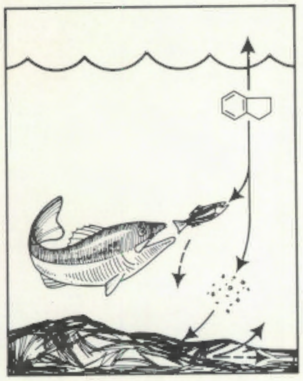
Of the PAHs taken up by fish and most other higher organisms, a variable fraction partitions into their lipid constituents, and a probably smaller fraction enters their metabolic pool (Lu et al., 1978). Over the long term, material may be recalled from lipid storage and metabolized. How important lipid retention is in relation to metabolism is unknown, particularly with respect to mutagenic compounds. The PAH compounds, the biphenyls, and the heterocyclic analogs of PAH are to some extent metabolized by all forms of life. The basic enzyme systems necessary to metabolize these compounds have been found in microbial populations, plants, insects, fish and mammals (Hatzios and Penner, 1982; Lu et al., 1977). Metabolic efficiency for these conversions varies greatly across phyla, however, and for food chain considerations there appear to be significant differences, for example, among fish and crustaceans (Vaughan, 1984).

The mixed function oxidase (MFO) system, on which the first phase of PAH metabolism depends, is inducible in mammalian (NAS, 1972b), microbial (Jordan and Payne, 1980) and fish species (Payne, 1976; Payne and Penrose, 1975). At this step of metabolism, the highly carcinogenic bay region diol epoxides of certain polynuclear aromatic hydrocarbons may be formed, and they have high affinity for binding with nucleic acids. Whether or not the compounds are formed or are blocked, and whether the binding to nucleic acid actually takes place or is blocked are not known. Both blocking and potentiated response have been demonstrated with complex mixtures added back to a mutagenic compound or fraction (Haugen and Peak, 1983; Later et al., 1983a, 1983b), but the metabolic basis for these observations is unknown.

The inducibility of MFO also enables some aquatic ecosystems to adapt to chronic exposure to petroleum, and evidently to adapt without selective loss of organisms from the system. The observations on this point are fragmentary, however (Stuermer et al., 1982b; Spies et al., 1982). There may be some degree of substrate specificity as to the MFO induced, judging from petroleum research (Payne, 1977). Whether or not ecological systems would respond differently to a synthetic fuel oil, for example, is not known because the range of specificities does not seem to have been delineated for any MFO system studied to date. Conjugation reactions, in the second phase of metabolism, are also common to most life forms, but the subsequent metabolic behavior of conjugates is not clear. For example, phenolic metabolites are usually excreted or further metabolized, but the ketones, which also form, tend to be retained. Anthrone, for example, is more persistent in tissues than its parent, anthracene (Lu et al., 1978). The longer-term fate of metabolites like anthrone is not clear, especially its implications for food chains.

With regard to long-term fate of PAH and related compounds in sediments or soils, complete degradation probably should not be assumed. Among microbial organisms studied in vitro, some species only partially degrade specific compounds or degrade only certain compounds. Mixed microbial populations under artificial conditions can completely metabolize a pure PAH to CO_2, but this evidently does not happen under ordinary conditions of plentiful carbon sources (Rogers et al., 1981; Horvath, 1972; Jordan and Payne, 1980). In field studies of petroleum spills, sulfur heterocyclic analogs of PAH and their alkylated metabolites persisted disproportionately to other oil compound classes, and these differences were not explainable on the basis of bioconcentration (Grahl-Nielsen et al., 1978; Dillon et al., 1978; Warner, 1975).

Subsequent to investigations described here and in Sections 3.1 and 3.2 on chemical changes in contaminated sediments, a new program dealing with long-term fate in soils and sediments was commenced. The work is in progress and results to date are reported in a separate publication (Rogers et al., 1983b).



5.0 References

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