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John A. Strand, III and
Burton E. Vaughan, Editors

October 1981

Prepared for the U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory
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ECOLOGICAL FATE AND EFFECTS OF SOLVENT REFINED COAL (SRC) MATERIALS: A STATUS REPORT

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Pacific Northwest Laboratory
Richland, Washington 99352

FOREWORD

As the second in a series of status reports, this document describes the most recent research findings (October 1977 to March 1981) as they apply to the ecological effects of coal conversion by the solvent refined coal (SRC) process. An earlier report, which summarized research conducted between May 1976 and September 1977, describes data on organic and inorganic constituents and acute toxicity.[(a)] Solvent refined coal materials used in these studies were collected at the U.S. Department of Energy (DOE) pilot plant in Fort Lewis, Washington, operated by the Pittsburg and Midway Coal Mining Company (P & M Co.). It should be noted that these materials may not necessarily represent future commercial SRC products. This work was sponsored by the Ecological Research Division, Office of Energy Research, U.S. Department of Energy.

(a) Becker, C. D., W. G. Woodfield and J. A. Strand. 1978. Solvent Refined Coal Studies: Effects and Characterization of Treated Solvent Refined Coal Effluent, FY-1977. PNL-2608, Pacific Northwest Laboratory, Richland, WA.

EXECUTIVE SUMMARY

EXECUTIVE SUMMARY

This summary gives results of research conducted at Pacific Northwest Laboratory (PNL) from October 1977 to March 1981 on the ecological fate and effects of solvent refined coal (SRC).

STATUS OF PHASE I AND PHASE II STUDIES

Research issues centering on direct liquefaction of coal are numerous and complicated; thus our research program was divided into four phases planned over several years. This document concerns Phases I and II (PNL 1980).

- Phase I--Variables Affecting Toxicity (chemical identifications, bioassay development, exposure methods)
- Phase II--Environmental Behavior (long-term fate in soils and sediments, long-term effects, organism uptake and retention, food chains)
- Phase III--Process Modifications (reference compounds, new materials)
- Phase IV--Field Monitoring (aquatic and terrestrial systems)

Research tasks in Phase I are largely completed, and several tasks in Phase II have commenced. There is some overlap between phases; e.g., screening tests may be recommenced as new coal liquid materials become available.

Samples of distillate blend (2.9 parts middle distillate to 1.0 part heavy distillate, unless otherwise specified) were collected by the Pittsburg and Midway Coal Mining Company from the Fort Lewis pilot plant during its operation in the SRC-II mode (DOE 1981, Sections 1.2.4.3, 2.2.1.1, and 4.1.3.7). The samples were obtained from raw product tankage accumulated over a series of runs conducted under operating conditions approximating the currently projected design for the SRC-II demonstration plant. The solid samples were obtained from an earlier pilot plant run under essentially the same process conditions as the liquid materials.

At the outset of these investigations, few adequate methods were available for exposing organisms to complex organic mixtures. Among the

factors found to affect organism response (e.g., mortality) were: 1) microbial degradation of organics during the course of long-term organism exposure; 2) the partitioning of specific toxic compounds at different rates depending on mixing intensity (fast or slow mix), and 3) the manner in which test organisms are exposed (static or flow-through with partial or continuous renewal). Factors 1 and 3 are commonly associated with long-term subacute studies; however, all three factors are markedly exacerbated by the complex chemical composition of coal liquids. The problems were serious enough to require special methods development for both chemical and biological assay.

Results of the short-term screening tests showed that there were marked differences in toxicity of the coal liquids tested in comparison to petroleum or other fuel oils. Water soluble fractions (WSF) of coal liquids were several orders of magnitude more toxic than were identically prepared fractions of Nos. 2 and 6 fuels oils (Sections 5.3.1 and 5.3.2), and the differences were corroborated by chemical characterizations [(Section 4.1 and Table 40) (see also PNL 1979; Mahlum 1981)]. WSFs were not stable chemically, and chemical characterizations required new methods to be developed, as described herein. For example, WSFs after repetitive extraction (water-leached WSFs) were usually found to be more toxic than WSFs as first prepared (initial WSF) where both had equivalent total carbon (TC). The greater toxicity was due to stable toxic compounds present in the water-leached WSF at relatively higher concentrations than in initial WSF (Section 5.2.3).

In these screening tests, toxicity was found to be directly related to the content of phenolic compounds present (Section 5.2.2); however, in other biological determinations (i.e., chronic toxicity, growth and reproductive effects), the results were complicated by both biological degradation of the phenols and complex partitioning of these and other compounds. For example, over 80 phenolic compound types were identified in the WSFs (see Section 4.1). Differential partitioning accounted for the differences in water-leached and initial WSFs. Total carbon determinations were an indispensable tool for range-finding in the chronic effects and other long-term biological determinations. They are also widely used as a basis for comparing toxicities of complex organic mixtures. However, except under highly defined conditions, as in

screening tests using only the initial WSF, TC determinations were wholly misleading for estimating toxicity. Where time differences of a few hours, or differences in such other exposure variables as aeration, settling, filtration, or mixing intensity entered into consideration, fractions of equivalent TC varied in toxicity by several orders of magnitude. Where phenols represented at least 3/4 or more of the TC, mortality in fish (fathead minnow) and daphnids was determined to be in the range of 1 to 10 mg/L TC (Sections 5.1.1 and 5.1.2). In these determinations, an important exposure variable was the intensity of mixing when adding oil to water. With petroleum, energetic mixing ("fast" mix) allowed more oil to enter the water column. The WSFs from fast-mix and slow-mix preparations killed daphnids at 10 mg/L. After filtration or aeration, the apparent toxicity of fast-mix WSF can amount to as little as 10%, approximately, of that determined before filtration or aeration. Filtration and aeration had negligible effects on the toxicity of slow-mix WSFs (Section 5.1.1). This observation may be of some relevance to industrial accident situations, in that the toxic constituents of coal liquids are likely to enter the water column in a manner more closely approximating the slow-mix conditions. Phenols comprised a larger fraction of the TC in slow-mix preparations.

Other acute effects that were examined included determination of lowest detectable mortality in midge larvae, Chironomus tentans (Section 5.1.3), and determinations of hatchability and abnormal embryogenesis in fathead minnow. Larval mortality of midge occurred at similar TC and phenol concentration as for fish and daphnids (i.e., 10 to 20 mg/L). Fish eggs presented difficulty in that a comparatively long time is required for either hatch (7 days) or for embryo development (21 days). Hence eggs were exposed to WSFs for a period of time comparable to that employed in the fish exposures (48 hours). Egg mortality was similar, at 18 to 26 mg/L TC. In otherwise similar experiments, abnormal embryogenesis was detected at TC concentrations of 180 mg/L. Salmon, rather than fathead minnow, were used for the latter determinations because their longer developmental period afforded better sensitivity in determining embryogenic abnormalities.

Phenols, although markedly toxic as a class, were sometimes present in such low concentrations that derivatization methods had to be developed for

their measurement. Derivatization methods were developed for several other compound classes as well (Sections 4.1 and 4.2). Benzenes and naphthalenes were found to be the dominant constituents in weathered and in settled WSF preparations (Section 5.2.2). In addition to phenols and lighter aromatic compounds, WSFs contained lower concentrations of several hundred aromatic compounds (many of unknown toxicology) and still lower concentrations of heteroatomic compounds such as anilines and pyridines (Section 5.2). Generally, phenols and lighter aromatics were significantly reduced by successive extractions that simulated the natural weathering process. In the later extractions, however, the heavier aromatics and heteroatomic species contributed greater proportions of the total carbon. These findings have profound significance in the design of chronic exposure studies because organisms exposed over long periods will experience a constantly changing spectrum of organic compounds. Such problems were most severe in fish exposures of 48 hours or greater. In these cases, toxic concentrations were evaluated in relation to the concentration of soluble constituents, which were determined periodically over the duration of exposure. For these reasons, it was found necessary to modify and re-evaluate conventional bioassay techniques and other approaches widely used to study less complex toxicants (Section 5.2).

Subacute (chronic) effects in fish were determined over 14- to 21-day intervals. Significant mortality in fathead minnow occurred at 1/10 the concentration of TC required for acute mortality, and significant decreases in weight were observed at 0.4 mg/L, or 4/100 the concentration required for acute mortality (Section 5.2.4). For these long-term exposures a mixing separation system was devised, operating on a flow-through basis. Phenols as dye complexible phenol were monitored daily, and as in acute exposures, the phenolic content exceeded 3/4 of TC (Section 5.2.2).

Subacute effects in chironomid larvae, which burrow in sediments, were detected at 0.1 to 0.3 mg/L of dye complexible phenol (Section 5.2.5). For these determinations, sediments were artificially contaminated, then subjected to a series of repeated mixing and water replacements before introducing the larvae. Retention of phenols and hydrocarbons were measured after each replacement.

In related studies, PNL and Oak Ridge National Laboratory determined the comparability of results from acute toxicity tests of daphnids to phenol and acridine. Results established small differences in the median lethal concentration (LC50) and slope term of the dose response curve. The differences, however, were minor and without biological importance. Also, differences in water quality at each laboratory did not appear to affect the toxicological responses measured.

Studies were initiated under Phase II of this program to determine long-term fate of coal liquids, specifically the compositional changes in WSFs of soil and sediment leachates. Some of these changes were substantial. For example, aniline sorption studies suggested that migration of organic nitrogen bases as might occur under spill conditons will be effectively retarded through certain (alfisol) soils. For these determinations, alkyl anilines were distinguished from alkyl pyridines by derivatization and GC/MS. Aniline, C-1 anilines, and C-2 anilines, as well as lesser quantities of C-2 pyridines and C-3 pyridines, were positively identified and contributed significantly to total organic carbon in WSFs. The SRC-II blended distillate contained approximately 8500 mg/L anilines, and equilibrated distilled water contained approximately 70 mg/L (Section 4.2). Sorption studies were carried out on the A1 and B1 horizons of Westmoreland silt loam and Fort Martin mine soils collected near Morgantown, West Virginia. Results indicated that hydrophobic interactions with organic matter controlled the extensive aniline sorption in these soils. Specific interactions with the mineral matrix predominated the B22t horizon (Section 4.3).

Certain other changes such as acidification also were found. Aqueous leaching studies were performed with non-gasified SRC-I mineral residue produced at the Fort Lewis, Washington, pilot plant from a bituminous western Kentucky coal. These studies were undertaken to evaluate methods used to characterize leachates from solid wastes produced by coal conversion, and to identify specific chemical and mineralogic properties of the mineral residue that may influence the physicochemical composition of the gasified mineral slag. The non-gasified SRC-I mineral residue was found to be predominantly pyrrhotite, a crystallographically complex iron sulfide (Section 4.4). It was also determined that significant changes in leachate composition may occur

with weathering, e.g., by oxidation of reduced iron and sulfur, suggesting that if residues are not gasified, long-term studies of the disposal of pyrrhotitic minerals should be undertaken.

Site-characterization studies wre also initiated on the proposed SRC-II demonstration site near Morgantown, West Virginia, to determine background chemical characteristics of local soils, riverine sediments, and surface waters (Section 4.5). Surface waters have been severely affected by acid mine drainage containing high levels of soluble heavy metals (Fe, Zn, and Cr), macro ions (Ca, Mg) and minor anions (F). The Westmoreland and Dormont soils, which are characteristic of upland areas of the site, were acid, high-base status soils with thick, organic-rich A horizons and B horizons with clay accumulation. The dominant soluble constituent in all soil materials was $CaSO_4$. Relative to other bodies considered contaminated with soluble Fe and Mn and exhaustible saturate and aromatic hydrocarbons, the Monongahela River sediments were dark, fine grained, reduced, and organic rich, containing comparatively high levels of aromatic hydrocarbons.

Additionally, feasibility studies were undertaken on ecosystems-level responses. The literature does not provide good guidelines; therefore, several approaches for the colonization of artificial stream channels were evaluated (Section 5.5).

Finally, studies were initiated to examine the effects of SRC-II liquid and SRC-I solid on germination and growth of barley plants (Section 5.6). These studies were useful for toxicity range finding, and may have implications for industry in connection with spill clean-up. For example, SRC-II liquids added to the soil layer 10 dm below the surface produced lesions, chlorosis, and necrotic tissue at both 1.48 L/m^2 and 14.8 L/m^2 treatment levels. These treatments also resulted in reduced grain yields and in grain with higher nitrogen content. However, yields increased during the second growing season following soil amendment, which suggests a toxicity decrease due to soil microbiotic and other processes. In the case of SRC-I solid product, results indicated that covering the solid with a soil overburden acted as a barrier to root penetration, whereas mixing the product 1:1 with soil produced no detrimental effect.

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ACKNOWLEDGMENTS

This status report summarizes investigations conducted by Pacific Northwest Laboratory for the U.S. Department of Energy, Washington, D.C., since October 1, 1977, under Research Contract No. DE-AC06-76RLO 1830. The research reported in section 5.1 was performed for the Environmental Protection Agency under a related services agreement with the Department of Energy (Contract No. DE-AC06-76RLO 1830). The project was under the administrative direction of Dr. John A. Strand and Dr. James B. States, Ecological Sciences Department. The principal research contributors to the studies reported herein were:

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SECTION 1.0
INTRODUCTION

1.0 INTRODUCTION

1.1 ADMINISTRATIVE OVERVIEW

To assure the ultimate environmental acceptability of processes developed to commercialize economically feasible coal conversion technologies, the Department of Energy (DOE) has established a program of investigation to assess SRC coal liquids for their potential biomedical and ecological impacts. DOE's envionmental program has been outlined generically for liquefaction processes in the Environmental Development Plan (DOE 1978) and has been addressed for SRC-I and SRC-II in a Project Environmental Plan (DOE 1979a, b). The environmental program proposed in the PEP was implemented jointly by the Offices of the Assistant Secretary for Environment (ASEV) and Fossil Energy (ASFE).

A number of organizations have been selected to evaluate the biomedical, environmental and safety aspects of SRC. The Pittsburg and Midway Coal Mining Co. (P & M Co.) has been assigned responsibility for industrial toxicology. Enviornmental Research and Technology (ERT) has been given responsibility for providing various environmental engineering support and evaluation services relating to coal liquefaction. Oak Ridge Operations has been assigned responsibility for writing the required Environmental Impact Statements and the filing for Environmental Protection Agency (EPA) Construction Permits.

In this context, Pacific Northwest Laboratory (PNL) has been charged with responsibility for:

- generating a chemical, biomedial, and ecological data base for materials from the SRC-II process, and
- developing and applying appropriate protocols for the interpretation and use of these data for assessing the health and environmental impacts of the SRC-II process.

Pacific Northwest Laboratory initiated chemical studies with SRC materials in FY-1976. Ecological studies were initiated in FY-1977 as part of an expanded synfuels research effort. Biomedical studies began in FY-1978. As an outgrowth of this research, PNL was requested to develop detailed Program Plans for the evaluation of potential biomedical and ecological impacts of the

SRC-I and SRC-II processes. The SRC-I Preliminary Program Plan was submitted in January 1981 for review by ASEV and ASFE. The Detailed Environmental Program Plan for SRC-II was published in October 1980 (PNL 1980).

This status report provides a summary of the most recent findings as they apply to the ecological fate and effects of SRC materials in the environment. This is the second in a series of status reports by PNL on the potential ecological effects of coal conversion. The initial status report (Becker, Woodfield and Strand 1978) summarizes data on the inorganic and organic constituents and the acute toxicity of SRC effluents obtained between May 1976 and September 1977 at the pilot plant operated for DOE by P & M Co. at Fort Lewis, Washington.

1.2 RESEARCH ASSUMPTIONS

The research program in ecological sciences, addressed here and in the Detailed Environmental Plan (PNL 1979), was undertaken as a generic study. However, consonant with available funding for this research, specific research tasks were prioritized for the SRC-II demonstration plant. Several reasons necessitated this approach. The principal reasons were the limited availability of suitable coal liquids and the differing design criteria applied to the SRC-II demonstration plant. Early in our program, a rather detailed base of information became available as to how this new technology would operate for the direct conversion of coal to synthetic fuel oils (ERDA 1977). It should be noted that there are few direct liquefaction plants in existence, and that none exist operating to the design standards or scale planned for the SRC-II demonstration plant. The SRC-II plant was planned by its designers to operate as a much cleaner facility than coking or gasification facilities currently in operation. The products and process streams also would differ substantially from gasification or coking processes.

Early in the development of this research program, and reflecting the differing perceptions of technology designers and environmental scientists, DOE set up a coordination task force to help develop a commonality of understanding and to help delineate actual from perceived environmental issues.

This task force was comprised equally of environmental and technology representatives. Deliberations of the task force had an important bearing on the priorities established for specific research tasks, and they led to publication of the Detailed Environmental Plan mentioned above.

Although the ecological research program is generic and allows data derived from the program to be applied to coal liquefaction issues, we initially placed greater emphasis on aquatic ecological concerns rather than on terrestrial ecological concerns. This decision, established in deliberations of the task force mentioned above, was based on the following considerations:

- Principle materials likely to enter the environment will be: coal liquids from accidental spills during loading and transportation of the commercial product and spent residues (solids) from gasifier units, which are part of the liquefaction facility.
- In the East, waterways will be principle corridors for movement of bulk coal liquids.
- Leachates from stored spent residues will potentially contaminate groundwater; their organic content will be low, but mineral fraction enrichment may be of chief concern.
- Sulfur will be internally recycled and removed as a solid by-product in the facility. Sulfur gases likely to be released will be in compliance with current federal standards.
- "Fugitive" emissions (i.e., valve leaks of process water and coal liquids) will be collected and incinerated. Incinerator residues will be treated the same as gasifier residues.
- Because of major operational differences and major differences in control technology, existing pilot plant facilities will not provide a realistic basis for the design of trend monitoring programs appropriate to the SRC-II plant operation.

1.3 OUTLINE OF THE PRESENT STATUS REPORT

Section 2.0 is a discussion of the major environmental issues and implications of SRC technology development. Section 3.0 is a description of the pilot plant operating conditions under which the SRC source materials were obtained for ecological experimentation. Sample relevance to pilot plant operations and to the eventual demonstration and commercial situation is also discussed. Section 4.0 presents information on the inorganic and organic properties of SRC materials and their fate in aqueous, sediment, and soil systems, with particular reference to the West Virginia coal region. Section 5.0 provides results of investigations to determine potential toxicity of SRC liquids in aquatic systems. Preliminary studies of the effects of SRC-I solid materials on vegetation are also presented.

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SECTION 2.0
ECOLOGICAL ISSUES AND IMPLICATIONS

2.0 ECOLOGICAL ISSUES AND IMPLICATIONS

Two objectives of the Ecological Research Program for Coal Liquefaction are: 1) to establish effects on the natural environment (ecosystems) and 2) to establish relationships among organisms in food chains to man, whereby contaminants reach man indirectly (indirect health effects). The issues below represent major ecological questions asked by environmental scientists who have reviewed this program over the past several years. The commentaries represent our judgement about each issue in light of three years study of the direct liquefaction process. In discussing the data implications below, we have drawn on appropriate chemical, biomedical and ecological information developed in the integrated PNL program. Most of the information is now available in documentary form.

1. From an ecological viewpoint, what contaminants are the most important?

Screening tests have shown that liquid products and process water are the most toxic materials on a weight basis. They are several orders of magnitude more toxic than certain solids, petroleum, or shale oil (see also PNL 1979, p. 24, Table 1; Mahlum 1981, pp. 3, 40, Table 17). An extremely large number of hydrocarbons are present, but for many of these specific toxicity information is unavailable. The hydrocarbon mixtures also present major analytical difficulties because of oil/water immiscibility, complex partitioning phenomena, and the fact that the spectrum of partially soluble (toxic) compounds is continuously changing with time. Investigations arising out of this program have shown several compound classes of either food-chain or ecosystem concern that are present in coal liquids (Later, Lee and Wilson 1981; Pelroy and Wilson 1981). These are: 1) the potentially carcinogenic group of polyaromatic hydrocarbons (PAH), "other nitrogen-containing polyaromatic compounds with more than four rings" (NPAC), and polyaromatic amines (PAA); and 2) the ecologically noxious group of phenolic and heteroatomic (anilines and pyridines) compounds. Over 80 phenolic compounds have been identified.

2. What effect will sulfur gases, particulate and hydrocarbon emissions have on terrestrial ecosystems?

Sulfur gases would be the most phytotoxic material, but sulfur gases released from the facility are estimated to be negligible in relation to ambient concentrations of sulfur in air (see Item 1, above). While the hydrocarbon classes identified above are not expected to enter atmospheric effluents, phenolic compounds might be of concern since animals are highly sensitive to these compounds. Some animals detect phenols at concentrations in the parts per trillion range (Buikema, McGinnis and Cairns 1979), and it is conceivable that off-flavors, for example, imparted to herbage in this way, might affect grazing behavior. Particulates would also be of concern. Small particulates (<1 μm) are a highly efficient adsorbing medium. Experience has shown that they also bind tightly to green leaf plants (Vaughan, Wildung and Fuquay 1976; Vaughan et al. 1981). However, in the clean-air, forested region of Fort Lewis, Washington, where the SRC pilot plant is located, atmospheric contaminants cannot be detected above regional ambient levels (Alsid, Snowden and Assoc. 1979). Hence, potential problems of atmospheric effluent origin will depend on magnitude of scale-up to commercial operation or on relaxation of control technology measures. Appropriate studies are scheduled for a later phase of the research program.

3. In what way are potential airborne contaminants likely to enter human food chains?

Experience with metals and radioelements has shown that air-to-green leaf-to-animal is the more important pathway for airborne contamination entering the human food chain. For the reasons stated above, airborne contaminants are unlikely to enter human food chains at meaningful concentrations. However, for purposes of accident analysis, laboratory data should be obtained for uptake of reference PAHs, NPACs, and PAAs from air to leaf, and from leaf to herbivore. This data should also be re-evaluated in the light of possible technology changes.

3a. Will they persist in the human body?

If measurable amounts of the compound classes indicated above are present in foodstuff, molecular weight and solubility data suggest that they would be retained. However, body retention data are unavailable and will have to be estimated from animal data.

3b. What kinds of food chains require investigation?

In West Virginia, for example, and other locations, recreational sources of food such as crayfish, catfish, tree squirrels, and game birds sometimes constitute a significant part of the diet. These food-chain possibilities may need to be evaluated in later research phases. However, unless the scale-up from pilot plant to commercial facility suggests a significant increase in airborne contamination, these studies will be postponed.

4. What contaminants are likely to affect aquatic ecosystems?

Certain compounds of low molecular weight (MW) in the phenol and aniline classes and the "light ends" (benzenes, xylenes) will be acutely toxic to aquatic organisms. These compounds have reasonably well-established toxicities in the parts per million range, and they are probably the most important constituents for acute effects. Chronic effects (embryo development) are about 10 times more sensitive. The low-MW compounds will disperse hydrologically and are likely to be of transient concern.

The PAH, NPAC and PAA classes and the high-MW phenols are comparatively insoluble and will sink to the bottom where they may accumulate. Toxicity of complex mixtures and the heavier molecular forms cannot be readily inferred from the simple parent compound. Their toxicity to aquatic organisms is unknown, as are their release rates from sediments. There is evidence of significant biodegradability for the phenol class in sediments. The other compound classes of heavy MW probably also biodegrade, but data are incomplete. These sorbed contaminants will be brought into intimate contact with the biological tissues and digestive enzymes of sediment dwelling organisms (detritivores). This is potentially important in recycling from sediments.

4a. Will any of these compounds be accumulated or recycled?

PAH, NPAC, PAA and high-MW phenols are representative of compounds that have the potential for entering fish via sediment recycling. These compound classes are all rather insoluble, but compounds in these classes are metabolized. The PAAs and PAHs show strong correlations with carcinogenicity induced experimentally. These heavy MW compounds are probably biodegraded in sediments, although data are incomplete.

4b. Will any of these compounds be important in human food chains?

It is not clear whether long-term (indefinite) accumulation is a realistic expectation because many of the compound classes appear to be degradable. Whether or not contamination of man or his resource organisms occur will depend on the relative rates of sediment input biodegradation. Degradation can conceivably take place without lasting effects on the aquatic ecosystem, provided spilled amounts do not exceed the conversion capacity of particular sediment systems. Conversion capacities (biodegradation) are not yet known.

5a. Will there be adverse long-term effects on ecosystem structure (diversity)?

Current research shows that the prevalent toxic compounds are the lower MW phenols. These compounds biodegrade rapidly, and they disperse hydrologically in large river systems. However, odor detection of phenols is remarkably sensitive, i.e., in the tens of parts per billion range. This feature and the comparatively slow release of higher MW phenols from contaminated sediments, suggest the real possibility of ecosystem-level effects. Such effects might occur through imparting off-flavors to the food base of higher level aquatic organisms or through avoidance/attraction phenomena. For these reasons, behavioral studies on fish are now under development in this research effort. Fish are particularly sensitive to odorous substances, and current data suggest attractant responses to soluble coal liquid materials in the fractional parts per million range.

The carcinogenic compound classes do not appear likely to cause ecosystem effects because the period of induction, or period of critical accumulation needed to produce a cancer, can be shown to be far longer than the lifetime of potentially affected animals in the environment. Carcinogenic investigations on fish are needed for safety assessment purposes, as fish are sometimes found with cancers in polluted waterways. These compounds may be of greater concern for indirect human health effects (see below).

5b. Is there a long-term human health concern?

The PAH, NPAC, and PAA compound classes are likely to enter human food chains, but if so, their relative abundance and solubility characteristics suggest that tissue concentrations are likely to be low. Uptake retention data on these compound types are needed either in food organisms or in other organisms referable to food sources. These investigations are scheduled for Phase III of the research program.

6. Are long-range changes in buried solid waste from liquefaction likely to alter ecological risk considerations?

At this point, the question cannot be answered specifically because local geographic and ecological factors must be considered. Nevertheless, some general features are emerging based on soil amendment studies in progress with SRC residue, and a larger body of related experience with retort residue from shale and processing. These features are discussed below. Due to terrain (hydrology), soil minerology, and environmental variables (precipitation and temperature), some geographic locations will be more satisfactory than others as burial sites. The major ecological concerns are similar to those described above, but will result either from the contamination of ground water via leachates or from inhibiting the terrestrial productivity of rehabilitated land (restoration). Pollutant transfers will need to be evaluated for non-agricultural situations such as recreational food chains (see 3b, above). This may be an especially important consideration where revegetation is practiced.

6a. Will soil microbial and geochemical processes enhance the pollution potential of leachates?

In soils, like those of West Virginia, sorption studies show that the organic nitrogen bases (aniline, PAAs) are retarded in their migration. This feature and microbial degradation would tend to lessen the likelihood of these organic compounds leaching to groundwater where they might have further ecological impact.

Certain other changes can be shown, especially acidification of leachates, which are highly dependent on minerological, soil microbial, and other physical factors. The mineral content of coal feedstock becomes very important in this connection. For example, pyrrotite and its weathering products can conceivably lead to conditions analogous to acid mine drainage, with consequent, severe impacts on terrestrial productivity and water quality. For SRC residues, the extent of these reactions in soils needs to be quantified before the potential ecological risk can be evaluated.

6b. Can soil microbial and geochemical processes be manipulated to mitigate the pollution potential of mineral or hydrocarbon residues?

This question cannot be answered definitively but there are promising leads. For example, several years' experience in the closely related problem of spent shale (after restoration) indicates that edaphic factors and botanical selections can be manipulated in arid locations to significantly modify leachate composition and facilitate revegetation.

7. How should we monitor for long-term ecological trends in the terrestrial environment?

There may be long-term adverse changes in the performance of ecosystems located near coal liquefaction facilities. The problem is that such changes are just as likely to result from other industrial activities (e.g., coal mining and combustion). Changes attributable to a liquefaction facilitity are more likely to be related to its scale of operation. No suitable facility exists at present, and the SRC pilot plant at Fort Lewis, Washington, operates

well within current federal standards. Efficient monitoring approaches cannot be designed in advance without site specific knowledge such as terrain, soil minerology, plant types, and important animal or fish life. In our Detailed Environmental Plan for SRC-II (PNL 1980), a monitoring approach was laid out for the former demonstration facility planned at Fort Martin, West Virginia, but this effort now is not likely to proceed. Nevertheless, some general principles can be specified.

A specific spectrum of ring compounds (PAAs, PAHs, NPACs, phenols, and anilines) peculiarly distinctive for the coal liquefaction process can now be identified and differentiated from other hydrocarbon contaminants in sediment where the compounds accumulate. Hence, in addition to air sampling and water sampling for these constituents, appropriate sediments should also be collected and analyzed. Second, terrestrial baseline studies should be initiated from 3 to 5 years in advance of start up of the liquefaction facility. These studies should focus on trends in primary productivity, changes in key habitats, and changes in key animal abundance. They should be carried out in a systematic and statistically defensible manner independent of ecosystem modeling desires. The 3- to 5-year period for preoperational data collection is extremely important (and less than optimal) for tracking normal variability in observational measurements. Third, we believe it is important to validate and document local food chains, both recreational and agricultural, for the reasons outlined above (3b).

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SECTION 3.0
SRC SOURCE MATERIALS FOR ECOLOGICAL EXPERIMENTATION

3.0 SRC SOURCE MATERIALS FOR ECOLOGICAL EXPERIMENTATION

3.1 PILOT PLANT CONDITIONS

The SRC-II liquid source materials used in present studies were collected in March, 1979, by the Pittsburg and Midway Coal Mining Co. from the Fort Lewis, Washington, pilot plant. The materials were obtained from tankage accumulated over a series of pilot plant runs extending from October 1978. All of the runs had the same set of target operating conditions with the goal of accumulating large-volume samples for in-field power generation and combustion testing. Unless otherwise specified, a middle-distillate to heavy-distillate blend ratio of 2.9 to 1.0 was determined from the average yield ratios of the runs during the accumulation period. For a description of the SRC-II process and pilot plant operations refer to Moschitto (1978) and Weimer et al. (1980).

The SRC-I (solid) samples were obtained in March 1977 from product storage at Ft. Lewis. These materials were produced during a pilot-plant run started in December 1976 under the SRC-I process conditions.

3.2 REPRESENTATIVENESS OF PILOT PLANT SOURCE MATERIALS

Uncertainties associated with obtaining representative samples from a single pilot plant run were much reduced because the source materials were accumulated over a long series of similar pilot plant runs. However, other difficulties associated with long-term accumulation and representative source material included: 1) material from all modes of operation (ranging from steady-state to upset conditions) were accumulated, and 2) some unavoidable product variability was introduced since operating conditions were not precisely duplicated from run to run.

3.3 RELEVANCE OF SOURCE MATERIALS TO DEMONSTRATION AND COMMERCIAL-SCALE MATERIALS

Pilot plant processing conditions were relatively similar to demonstration design conditions during material balance run periods. These periods accounted

for approximately one-fifth of the pilot plant operations time during accumulation. The remaining operations time involved generally similar operating conditions, although a portion of the accumulated material was produced during plant upset conditions and, therefore, was considered nonrepresentative.

While detailed demonstration-plant design criteria for product composition have yet to be specified, it is suggested that products of pilot and demonstration plants should be similar for given boiling range cuts, although perhaps different in relative proportions. However, the lower nitrogen and oxygen content of the pilot plant coal (Powhatan No. 5) when compared to demonstration design coal could affect the distillate concentration of heteroaromatic compounds. Also, the fact that pilot plant source material contains less heavy distillate than does demo design product is of particular importance because the heavier ends are more difficult to upgrade (hydrotreat) and are usually associated with greater genotoxic activity (Weimer et al. 1980).

3.4 REFERENCES

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SECTION 4.0
ENVIRONMENTAL CHEMISTRY AND FATE

SECTION 4.0

ENVIRONMENTAL CHEMISTRY AND FATE

Non-occupational health effects associated with SRC operation will be determined by environmental factors governing the form, transport, and persistence of SRC materials and wastes--factors which also mediate exposure to man. Accordingly, the research described below is an attempt to determine the fate of disposed solid wastes and spilled SRC materials, and it necessarily focuses on water soluble, persistent materials with greatest potential for mobility and incorporation into water and food supplies.

Initially, aqueous equilibrations of SRC-II liquid material and SRC-I non-gasified mineral residue were subjected to chemical characterization. Subsequently, laboratory studies were performed on the interaction of aqueous equilibrates of SRC-II liquid and SRC-I non-gasified mineral residue with soil materials, isolated suspended sediments, and bottom sediments. These studies were designed to identify effects of specific sorption reactions, ion or induced-ion exchange reactions, and toxicity of water soluble, biologically active materials derived from liquid and solid wastes. Results of these experiments have applicability to the environmental fate and effects of biologically active compounds released under different scenarios from product spills and solid waste disposal.

This section includes a description of field studies that were designed to evaluate the background chemical regime in surface waters, soils, and sediments of the Fort Martin, West Virginia, demonstration site. The results of these studies provide understanding of the important factors governing the potential impact on soils and sediments and the transport to surface and ground waters of soluble SRC-II components from material spill and waste disposal methods.

4.1 DETERMINATION OF PHENOLS AND CARBOXLYATED PHENOLS IN AQUEOUS SUSPENSIONS OF SOLVENT REFINED COAL

R. M. Bean, K. Shiosaki and R. G. Riley

BACKGROUND

Solvent refined coal (SRC) liquids contain relatively high concentrations of phenols (Bean et al. 1981; Giddings et al. 1980). Phenols have been demonstrated to be acutely toxic to aquatic organisms at the mg/L level (DeGraeve et al. 1980). Since several processes for the liquifaction of coals are already in the development stage, it is increasingly important to have methods for the rapid and sensitive analysis of phenolic materials in waters contaminated with SRC materials. Such methods would facilitate environmental studies of the fate and effects of SRC materials.

Although phenols have been directly analyzed in coal-derived liquids using both gas (Buryan, Macak and Nabivach 1978) and liquid (Schabron, Hurtubise and Silver 1979) chromatography, most of the recent methods for analysis of phenols in trace concentrations have involved derivatization procedures. Matsumoto and Hanya (1980) used silylation in the analysis of sediments for phenols. Trace phenols in water have been determined using the heptafluorobutyryl derivative (Lamparski and Nestrick 1978) and the acetyl derivative (Coutts, Hargesheimer and Pasutto 1979, 1980; Krijgsman and Van de Kamp 1977). The procedures used in our studies of aqueous suspensions of SRC-II were adapted from the latter methods, in which the phenols were directly derivatized with acetic anhydride under strongly alkaline conditions. Modifications to the procedure were required to avoid losses of phenols during removal of nonphenolic bases and neutral compounds present in aqueous suspensions of SRC. The method has been extended to include monocarboxylated phenols which were recovered as the acetyl methyl esters.

EXPERIMENTAL

Aqueous SRC Suspensions

The SRC liquid used to prepare the suspensions was a 1:1 mixture of middle and heavy distillates obtained from the Fort Lewis, Washington, pilot plant. The suspensions were prepared in two ways: 1) by gently stirring with a Teflon® rod 30 mL SRC with 29.7 L water for 24 hours (gentle mixing), and 2) by homogenizing in a blender 8 mL SRC with 1 L water for 1 min before adding to 29 L water, stirring for 10 min (rigorous mixing) and allowing to settle for several hours. The suspensions were sampled by glass syphon into sample jars prior to analysis. The suspensions were also used for toxicity testing of freshwater biota (Bean et al. 1981).

Analysis of Suspensions for Phenols

The suspensions (50 mL) were treated with 0.4 mL of 12.5N NaOH solution and extracted once with 20 mL n-hexane. Freshly distilled acetic anhydride (0.5 mL) was added and the mixture shaken for 1 min. Then the sample was extracted once with 5 mL n-hexane to recover the acetates. The solvent was changed to 1 mL n-heptane containing n-pentadecane as internal standard. Analysis was by capillary gas chromatography (GC) using a 30 m x 0.25 mm SP2250 column at 4°C/min from 70°-200°C. A Hewlett-Packard 5985 GC/Mass Spectrometer operating in the electron impact mode was used for characterization of phenols and acid-phenols.

Analysis of Suspensions for Hydroxybenzoic Acids

After the acetylated phenols were extracted with n-hexane, the remaining solution was acidified with 1 mL conc. H_2SO_4 and then extracted twice with 5 mL methylene chloride. The combined extracts were transferred to a diazomethanation apparatus described by Fales, Jaouni and Babashak (1973) and then evaporated to 3 mL. Using 0.5 mL 5N NaOH, 100 mg of N-methyl-N'-nitro-N-nitrosoguanidine was used to generate the diazomethane derivitizing agent in the apparatus. Reaction was complete after 1 hour at 0°C. The methylene chloride sample was then changed to n-heptane and analyzed by GC as above using n-pentadecane internal standard.

Preparation of Phenol Acetate Recovery Standards

Approximately 1 g of the phenol to be derivatized was placed into a 25 mL Corex® centrifuge tube with Teflon®-lined screw cap. Five mL silylation-grade pyridine and 9.2 mL freshly distilled acetic anhydride were added, and the mixture was capped and shaken to dissolve the phenol. The reaction was heated to 100°C for 30 min and, upon cooling, was diluted with 20 mL water in a separatory funnel and extracted 3 times with 10 mL benzene. The combined extracts were washed with 20 mL 5% (w/v) HCl solution, then with 20 mL 5% (w/v) NaOH. After drying over Na_2SO_4, the benzene was evaporated under a stream of dry N_2, leaving an oily residue. The residue was streaked onto a 20 x 20 cm preparatory layer plate and developed with 50 vol % hexane in diethyl ether. The acetate was observed under UV light, and the affected plate area was removed and eluted with diethyl ether. After the ether was removed, the sample was dried for 1 hour in a drying pistol over refluxing acetone. Most of the acetates were recovered as oils. Gas chromatography of each of the prepared acetates resulted in only one peak with a stable baseline.

Preparation of O-Acetyl-p-hydroxybenzoic Acid Methyl Ester

Five hundred mg p-hydroxybenzoic acid was heated in a sealed glass tube with 5 mL boron trifluoride in methanol (Supelco®) at 70°C for 15 min. The BF_3 was hydrolyzed with 10 mL water and the aqueous phase extracted with ether. After drying over Na_2SO_4, the ether was removed under a stream of N_2, and the resulting crude ester derivitized with pyridine and acetic anhydride as above. After separation on the preparatory thin layer chromatography (TLC) plate, the product was crystalized from benzene/hexane.

RESULTS AND DISCUSSION

Figure 1 shows a total ion chromatogram of the derivatized phenol acetate extract from a saturated aqueous extract of SRC. Suggested structures for the phenol acetates were assigned on the basis of the mass of the molecular ion and on the fragmentation pattern. The presence of a prominent M-42 peak (loss of ketene moiety) in the spectrum was deemed to be sufficient grounds for identification of a component as a phenol. Over 80 individual phenol components were

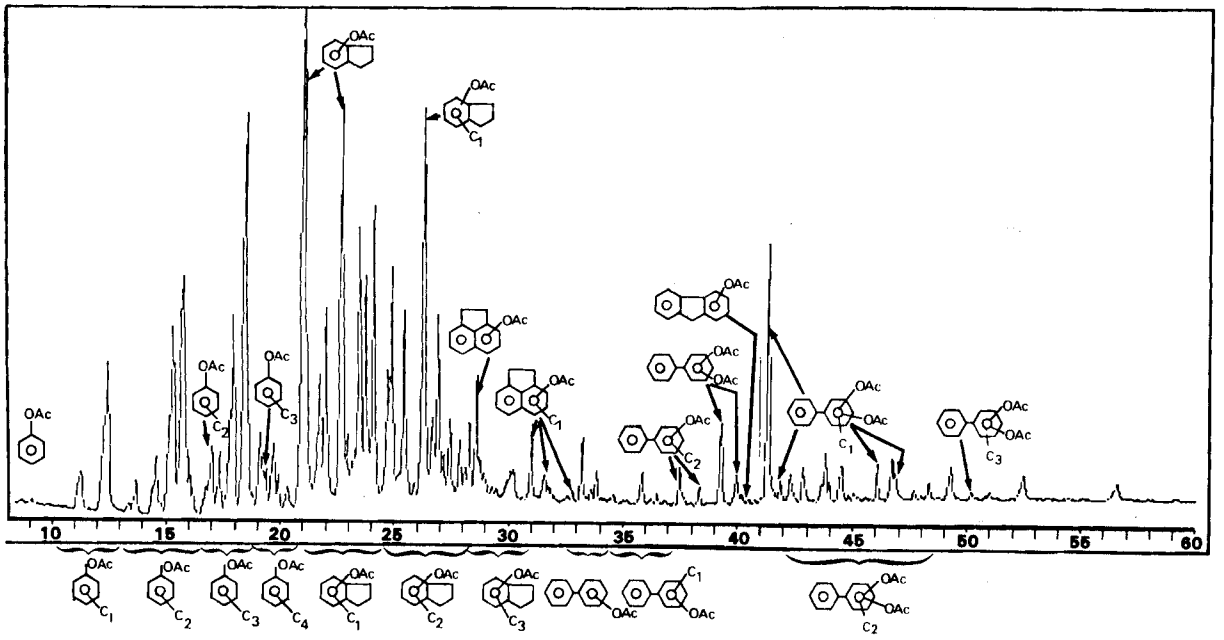

FIGURE 1. Total Ion Chromatogram of Derivatized Aqueous Extract of Solvent Refined Coal

resolved by the chromatographic column. We have not attempted to identify specifically each component but only to classify the phenols structurally according to ring type and degree of alkyl substitution. This is readily accomplished from considerations of molecular weight and fragmentation. Figure 2 shows the spectrum and fragmentation assignments made in the identification of a dimethyl phenol acetate. This figure also shows fragments arising from the loss of ketene and a prominent fragment from the loss of both ketene and a methyl group. The structural classifications of all phenols quantifiable by flame ionization detection are listed in Table 1.

Four phenolic components containing carboxylic acid groups were found in the esterified material derived from the aqueous esterification by diazomethanation. The spectrum shown in Figure 3 of the acetylated methyl ester of a hydroxybenzoic acid illustrates the fragment types that permitted the identification of these component types. In addition to the hydroxybenzoic acid, we found mono- and dimethyl-substituted components as well as a trace of material with a molecular ion consistent with an indane hydrocarbon skeleton.

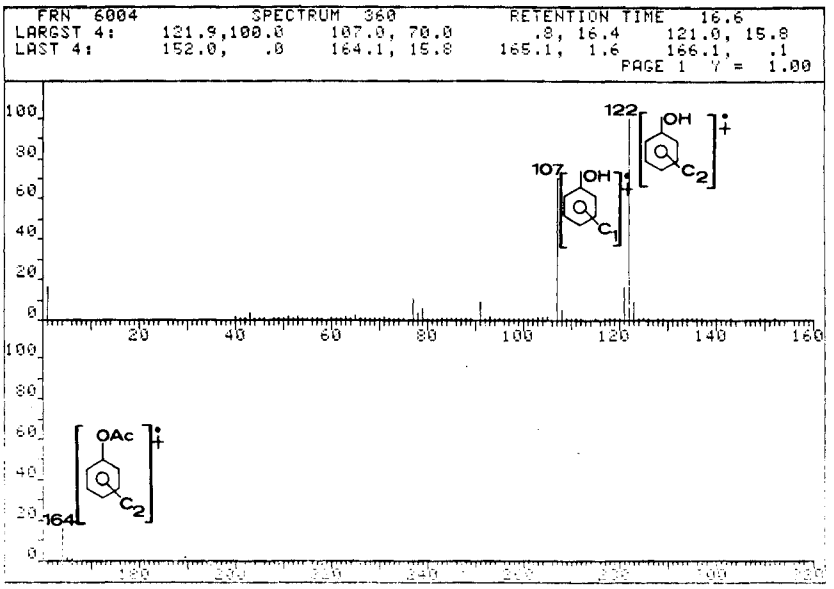

FIGURE 2. Mass Spectrum of Dimethylphenol Acetate, Showing Principal Fragments

TABLE 1. Recovery of Phenol Acetates from Aqueous Solution Using NaOH (Phenol concentrations were approximately 2 µg/mL)

Phenol	Recovery,[(a)] %
Phenol	71
p-Cresol	84
3,4-Dimethylphenol	89
2,3,5-Trimethylphenol	77
Resorcinol	20
5-Indanol	91
Naphthol	74

(a) Recoveries calculated by comparing the detector reponse of the analete acetate to the expected response determined from the synthesized acetate compound.

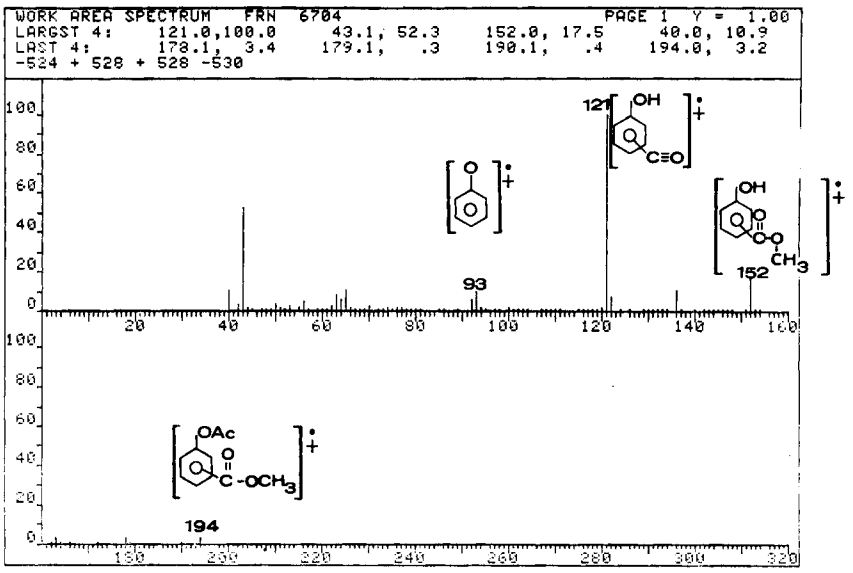

FIGURE 3. Mass Spectrum of O-Acetyl-p-hydroxybenzoic Acid Methyl Ester, Showing Principal Fragments

Recovery of Phenolic Components

Recent methods involving the methylations of phenols in aqueous media (Coutts, Hargesheimer and Pasutto 1979, 1980; Krijgsman and Van de Kamp 1977) have employed sodium or potassium carbonate as the alkaline agent. While carbonate was satisfactory for accomplishing quantitative acetylation, severe phenol losses were observed when the alkaline solution was extracted with either toluene or hexane prior to derivatizing for removal of interferring basic and neutral components of SRC material. Where carbonate was used, the losses were particularly large for the higher methyl-substituted phenols. Only 50% recovery of 3,4-dimethylphenol and 10% recovery of 2,3,5-trimethylphenol were obtained. However, Table 1 shows that recoveries of phenols were acceptable when sodium hydroxide solutions of the phenols were extracted with hexane prior to the addition of acetic anhydride.

Recovery of hydroxy phenols was too low to consider direct aqueous acetylation, an acceptable analytical method for these compounds. Catechol and hydroquinone could not be recovered using this procedure. Thus, the phenol analysis of SRC suspensions using this technique was largely limited to the

monohydroxy compounds. The effect of SRC components on the recovery of phenolic compounds was investigated by adding 2 µg/mL m-cresol to an SRC suspension. After subtracting the m-cresol present in the original SRC suspension (28 µg/mL), recovery of added cresol was 108%. Recovery of hydroxybenzoic acids as the acetylated esters was investigated by applying the 2-step derivatization procedure to 0.5 µg/mL p-hydroxybenzoic acid in NaOH solutions and by comparing the gas chromatographic response to that determined from a synthetic sample of the derivative. Recovery was 79%.

Determination of Phenols in SRC Suspensions

Quantification of phenols in the SRC suspensions was accomplished by comparing the sum of the chromatographic peak areas from each component type to the peak areas obtained from reference phenols added to sodium hydroxide solution and subjected to the analytical derivatization procedure. Phenol and the three cresols were quantified by reference to individual compounds, but the dimethylphenols were determined from the averaged response of 3,4- and 2,3-dimethylphenol. All other alkyl phenols were referenced to the response of 2,3,5-trimethyl phenol. 5-Indanol was the reference for the indanols and substituted idanols. Naphthol was used to determine the phenylphenols/acenaphthols.

Phenol concentrations obtained for the SRC suspensions are given in Table 2. The results, when compared to the total organic carbon content of the aqueous suspensions, show that phenols constitute a large fraction of the organic matter in water suspensions. This may account for the higher aquatic toxicity of SRC suspensions when compared to similar extracts of fuel oils (Giddings et al. 1980).

The carboxylated phenols were quantified using p-hydroxybenzoic acid as a reference. Concentration of these compounds was lower than for phenols. The concentration of the most abundant compound, a methylhydroxybenzoic acid, was 0.94 mg/L in the suspension prepared by gentle mixing. The total concentration of all caboxylated phenols found was 1.22 mg/L. Thus, compound types only contribute about 1% of the organic carbon in the SRC suspension.

TABLE 2. Determination of Phenols in Aqueous Suspensions of Solvent Refined Coal

Component	Concentrations, mg/L	
	Gentle Mixing	Vigorous Mixing
Phenols	7.80 ± 0.09	2.10 ± 0.01
Methyl phenols	13.74 ± 0.03	5.15 ± 0.03
C_2-Phenols	18.96 ± 0.14	5.07 ± 0.01
C_3-Phenols	12.91 ± 0.32	2.91 ± 0.06
C_4-Phenols	3.27 ± 0.08	0.71 ± 0.01
Indanols	14.07 ± 0.50	2.88 ± 0.13
Resorcinols	1.79 ± 0.11	0.50 ± 0.05
Acenaphthols/ Phenylphenols	0.83 ± 0.05	0.09 ± 0.01
Total Phenols	73.4	19.4
Total Organic Carbon as Phenols	57.9	15.3
Total Organic Carbon in Suspension	75.8	31.0
Percent Organic Carbon as Phenols	76.4	49.4

Application of the Method to Analysis of SRC for Phenols

Extraction of a solution of 0.1 g SRC liquid (2.9:1 middle to heavy distillate) with 0.01N NaOH produced a phenolic solution which could be derivatized using the acetylation procedure. The analysis of the resulting phenol acetates by gas chromatography is reported in Table 3 in terms of weight percent of SRC. Although the contribution of phenols to this SRC blend was substantial (22.3%), phenol contribution to the total organic material was not as high as in the corresponding aqueous extracts (Table 2), where phenols constituted half or more of the organic carbon (76.4%). Thus, it is apparent that contacting

TABLE 3. Phenol Composition of SRC Liquid 2.9:1 Blend

	% of SRC	Std. Deviation (n=3)
Phenol	2.86	0.07
Cresols	5.81	0.08
C_2-Phenols	5.99	0.03
C_3-Phenols	2.83	0.07
C_4-Phenols	1.84	0.05
Indanols	2.85	0.13
Acenaphthols/ Phenylphenols	0.08	0.01
TOTAL	22.26	0.44

bituminous materials with water can produce profound changes in the composition of the resulting aqueous suspension. This should be kept in mind when conducting environmental or toxicological research.

4.2 ANALYSIS OF ALKYL ANILINES IN SRC-II AND ITS CORRESPONDING AQUEOUS EXTRACT

L. J. Felice

BACKGROUND

The water soluble constituents of SRC are potentially the most environmentally mobile and, as such, deserve emphasis in the study of the fate of SRC materials. In studies conducted at Pacific Northwest Laboratory (PNL), alkyl phenols were shown both to be a major component of an SRC-II blended distillate and also to contribute to the majority of organic carbon in an aqueous extract of the distillate (Bean et al. 1981). In an effort to further characterize the water soluble fraction of SRC liquids, the composition of the water soluble nitrogen compounds was investigated. Alkyl anilines and pyridines were identified in the SRC-II distillate and shown to contribute significantly to the water soluble fraction. Both alkyl pyridines and anilines have been previously identified in coal-derived liquids by using gas chromatography/mass spectrometry (GC/MS) (White, Schweighardt and Shuty 1978; Paudler and Cheplen 1979). However, the similarity of the electron impact mass spectra of the alkyl anilines and pyridines and the lack of a complete set of standard compounds prompted the development of an alternative analytical method that would unambiguously differentiate anilines from pyridines. An approach using chemical derivatization with acetic anhydride combined with GC or GC/MS proved to be successful and resulted in the quantification of alkyl anilines in the SRC-II distillate and aqueous extract.

EXPERIMENTAL

Acetylation Procedure

The SRC-II aqueous extract (50 mL) was adjusted to pH 12 with 5N NaOH and extracted twice with CH_2Cl_2 (1 x 50 mL, 1 x 25 mL). The CH_2Cl_2 extracts were combined, back extracted with 1M HCl (40 mL) and the HCl extract, then adjusted to pH 12 with 5N NaOH. Acetic anhydride (0.5 mL) was added, the

mixture shaken, and left standing at room temperature for 5 to 10 min. The solution was extracted twice with CH_2Cl_2 (1 x 20 mL, 1 x 10 mL), and the combined CH_2Cl_2 extracts were dried over Na_2SO_4 and concentrated under a stream of nitrogen. Individual compounds in the concentrated extract were analyzed by GC and GC/MS following the addition of benzanilide as an internal standard. Extraction of organic nitrogen compounds from the SRC-II blended distillate was accomplished by dissolving the distillate in CH_2Cl_2 and following the above procedure.

SRC-II Distillate

An aqueous extract of the 2.9:1 middle to heavy distillate blend was prepared by mixing 1 part SRC-II blend with 100 parts distilled water (v/v) and stirring for 24 hours.

GC and GC/MS Analyses

GC analyses were performed on a Hewlett-Packard 5880A equipped with flame ionization and nitrogen/phosphorus detectors. A 60 m glass capillary SP2100 column was used for all GC analyses. A Hewlett-Packard 5985 system with 7900/7920 multidisc drive and operating in the electron-impact (EI) mode was used for GC/MS analyses. A 50-m methyl silicone fused-silica column was used with the column outlet running directly into the mass spectrometer source.

RESULTS AND DISCUSSION

The extraction procedure used in this study yielded nitrogen-enriched fractions when applied to both the SRC-II blended distillate and to an aqueous extract of that distillate. GC/MS analysis of the organic nitrogen fraction showed a number of major components with electron impact mass spectra consistent with an alkyl aniline or alkyl pyridine structure. However, distinction between pyridine and aniline could not be made on the basis of GC or GC/MS because of the similarities in the EI mass spectra. In Figure 4, the GC profile of the organic nitrogen fraction from an SRC-II aqueous extract is presented along with the profile of an acetylated extract. The acetylated anilines were shifted to much longer retention times and gave characteristic EI mass spectra showing a strong M-42 fragment for loss of ketene.

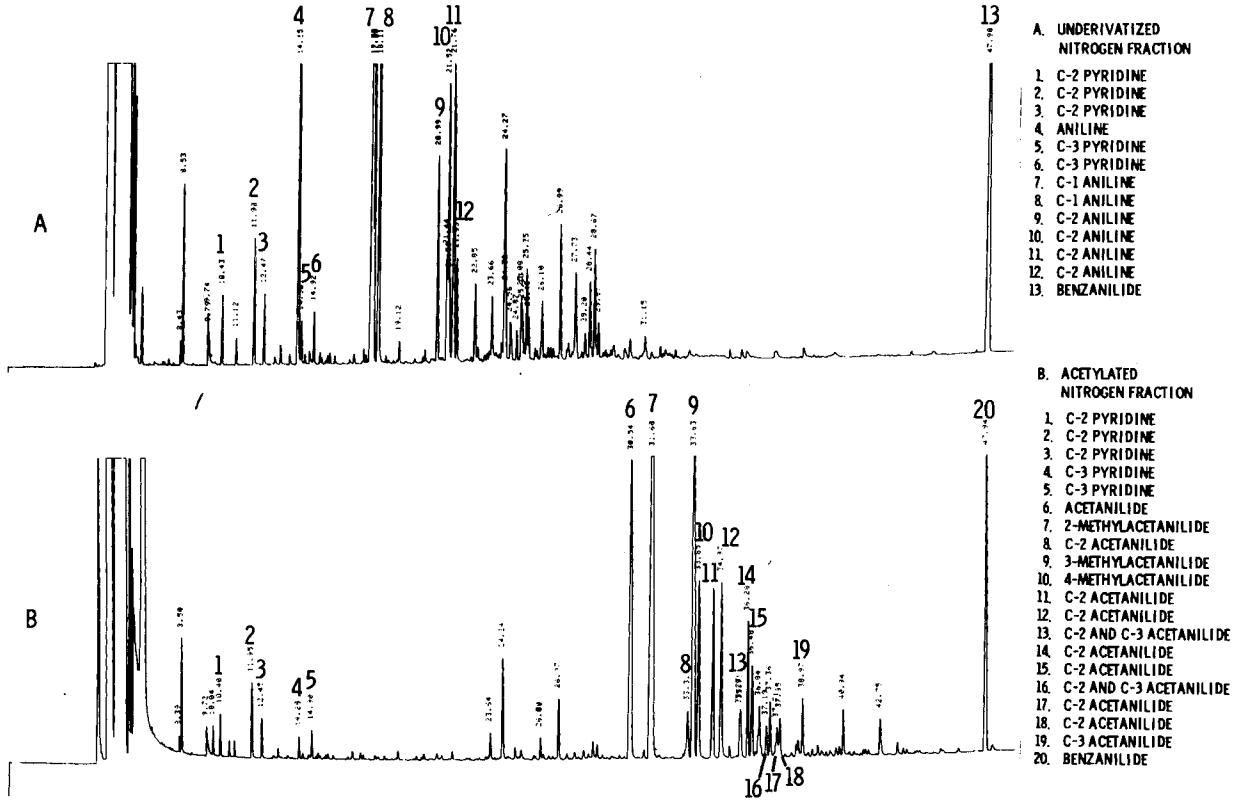

FIGURE 4. Gas Chromatograms of Organic Nitrogen Compounds from SRC-II Aqueous Extract

The acetylation approach was also found suitable for the quantitative analysis of anilines and was used for the quantification of alkyl anilines in the SRC-II blend and in the SRC-II aqueous extract (Table 4). Standards were available for the quantification of acetanilide, 2-methylacetanilide, and 4-methylacetanilide; however, the determination of 3-methylacetanilide was based on the 2-methyl and 4-methyl derivatives. Quantification of C-2 acetanilides was based on data obtained for 2,6-dimethylacetanilide and 2,4-dimethylacetanilide. A number of relatively small GC peaks were identified as C-3 acetanilides but were not resolved from other components and could not be quantified. Several compounds in the SRC-II were tentatively identified as C-2 and C-3 pyridines based on GC/MS data and on the fact that they were not influenced by treatment with acetic anhydride. The pyridines have not yet been quantified, but they appear to be present at much lower concentrations than the anilines.

TABLE 4. Analysis[a] of Anilines in SRC-II Blended Distillate and Corresponding Aqueous Extract

Compound	SRC-II Distillate		SRC-II Aqueous Extract	
	Analysis I	Analysis II	Analysis I	Analysis II
Aniline	1,170	1,025	11.4	14.3
2-Methylaniline	2,340	2,545	21.0	25.1
3-Methylaniline	1,735	1.660	14.5	16.2
4-Methylaniline	400	445	4.5	4.4
C_2-Aniline	2,555	3,350	14.5	18.9

(a) Concentration in µg/mL.

4.3. SORPTION OF COAL-DERIVED ORGANIC BASES ON SOILS OF THE WEST VIRGINIA COAL REGION

J. M. Zachara and L. J. Felice

BACKGROUND

To determine the fate of SRC materials in aquatic and terrestrial environments, it is necessary to characterize the physicochemical interactions of SRC effluents with soils and sediments. In an effort to define these interactions, a series of soil sorption studies was initiated with aniline and phenol and selected alkyl-substituted isomers. These compound types were shown to account for the majority of water-soluble organics in the SRC-II blended distillate (2.9:1). Sorption studies of aniline with soil collected near Morgantown, West Virginia, site of the proposed SRC-II demonstrations plant, were completed. Sorption studies with several alkyl-substituted anilines are nearing completion. This report will briefly describe the results of the aniline ($C_6H_5NH_2$) sorption studies and will evaluate the applicability of the Langmuir and Freundlich equations to the adsorption isotherms obtained.

The Freundlich equation has been used extensively to describe the sorption of neutral and, to a lesser extent, polar organic compounds (Hasset et al. 1980; Zierath, Hassett and Banwart 1980; Karickhoff, Brown and Scott 1979) by soils and sediments. This equation can be used to calculate the partition coefficient, K_d, which is specific to the soil or sediment used. When applied to the sorption of hydrophobic organic compounds, K_d can be converted to another constant, K_{oc}, by normalization to the amount of organic carbon present in the solute ($K_{oc} = K_d$/percent organic carbon.) For many neutral organic compounds, K_{oc} has been shown to be independent of the sorbate used and can be directly related to compound solubility and the octanol/water partition coefficient (K_{ow}) (Karikoff, Brown and Scott 1979). The Langmuir equation is also frequently used to describe adsorption phenomena in soils and sediments. This equation is useful for calculating adsorption maxima and binding energy terms and is most often applied to the sorption of polar organic compounds and charged inorganic species.

EXPERIMENTAL

Field Sampling

The Westmoreland silt loam (Ultic Hapludalf) and a mine soil that covered a recently reclaimed strip mine were sampled on the Ft. Martin site near Morgantown, West Virginia. The Westmoreland and other closely associated alfisols occupy most of the undisturbed upland areas on the Ft. Martin site. These soils have formed from weathered Paleozoic shale and sandstones of the Conemaugh Group (Casselman and Glenshaw Formations). The mine soil was a heterogeneous mixture of soil and subsoil materials that had been removed from the mine site, stockpiled during mining operations, and spread to a depth of approximately 40 to 50 cm as soil cover over mine refuse during subsequent strip mine reclamation. The A1-Ap (0 to 25 cm), the B1 (25 to 38 cm), the B21t and B22t (38 to 68 cm) horizons of the Westmoreland, and the upper 38 cm of the mine soil, were sampled.

Soil Analysis

Samples from each of the individual horizons and the mine soil were air-dried and sieved to less than 2 mm. They were then analyzed for pH (saturated paste); amorphous and crystalline iron and aluminum oxides (dithionite/citrate/bicarbonate method, atomic adsorption spectrophotometry); particle size distribution (centrifugation); readily soluble materials by batch equilibration (plasma emission spectroscopy); and organic carbon (wet combustion). Additional analyses in progress include cation exchange capacity (Ba-saturation, atomic absorption); amorphous iron and aluminum oxides (acid ammonium-oxalate, EDTA, atomic absorption); total inorganic carbon (wet combustion); bulk and clay mineralogy (x-ray diffraction); total elemental content (spark source mass spectrometry, wet digestion, atomic absorption); and specific surface area (glycerol sorption).

Sorption Studies

Sorption studies were accomplished by shaking 1 g of soil with 5 mL of an aqueous aniline solution (10 to 400 µg/mL) on a reciprocal shaker at 25°C for 4 hours. The samples were then centrifuged for 5 min at 7700 g, decanted, and

the supernatant centrifuged again at 7700 g for 20 min. The supernatant was analyzed for aniline by direct liquid chromatographic analysis with UV detection.

RESULTS

Select soil characteristics of Westmoreland silt loam and Ft. Martin mine soil are presented in Table 5. The Westmoreland has an acid upper or A horizon with an accumulation of organic carbon. Organic C is lowest in the B22t horizon (0.15%) where higher levels of total extractable iron (1.30%) exist. No distinct accumulation of clay-sized materials was found in the B1 or B22 horizons. Silt-sized materials predominated in all horizons, reflecting the shale parentage of the Westmoreland. The mine soil was of higher pH (6.10) and, in comparison to the Westmoreland, contained high levels of total extractable iron. Highest concentrations of fine-grained materials were found in the mine soil, which contained 22.15% clay.

Aniline sorption isotherms for the Westmoreland and Ft. Martin mine soils are presented in Figure 5. The isotherms depict quantity of aniline adsorbed per g of dry soil (x/m in µg/g) versus the concentration of aniline remaining in solution after equilibration (C_{eq} in µg/mL). Sorption of aniline in the A1 and B1 horizons and in the mine soil was nearly linear over the concentration range studied. These isotherms are similar to the L (normal or Langmuir)

TABLE 5. Select Soil Characteristics of Westmoreland Silt Loam and Fort Martin Mine Soil

Soil	pH (Sat. Paste)	Particle Size Distribution, % Sand	Silt	Clay	Organic C, %	Fe_d, %	Al_d, %
Westmoreland							
A1-Ap	4.88	23.96	60.68	14.35	2.03	0.960	0.227
B1	5.23	22.33	64.03	13.64	0.976	0.964	0.282
B22t	5.11	41.08	44.61	14.31	0.150	1.30	0.265
Mine Soil	6.07	23.02	54.84	22.15	0.538	2.41	0.169

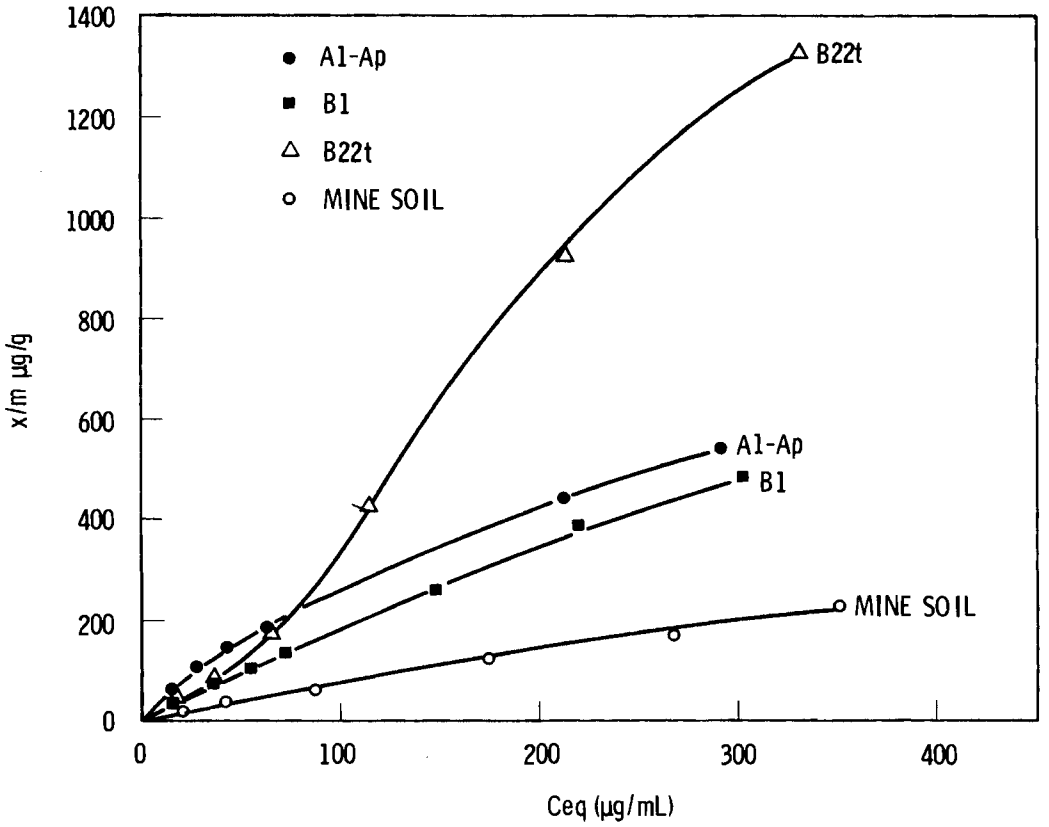

FIGURE 5. Aniline Sorption Isotherms for Westmoreland Silt Loam and Fort Martin Mine Soil

curves of Giles et al. (1960) and were the most commonly obtained isotherms in sorption studies from dilute solution where available sites for adsorption become a limiting factor. Of these soils (A1-Ap, B1, and mine soil) and over the concentration range studied, sorption was greatest in the A1 horizon where maximum quantities of organic carbon existed (2.03%). In contrast, sorption of aniline was anomalously high in the B22t horizon, where an S-curved isotherm was obtained (Giles et al. 1960). The S-curved sorption isotherms may occur when: 1) the solute molecule is monofunctional, 2) the solute is arranged vertically in a regular array in the adsorbed layer, and 3) strong competition for substrate sites occurs between the solute and solvent or with another adsorbed species.

Aniline sorption was well described by the Freundlich equation

$$C_s = K_d \cdot C_{eq}^{1/n}$$

where

C_s = concentration of aniline adsorbed by the soil in μmoles/mL

and K_d and 1/n are constants

Freundlich isotherms for the Westmoreland and mine soils are presented in Figure 6. Regression coefficients ranged from 0.982 to 0.999 (A1-Ap indicating a close fit of isotherm to the experimental data. Values of K_d and 1/n were calculated from the intercept and slope of the Freundlich isotherms, and K_{oc} was calculated by normalization to percent organic carbon. These are presented in Table 6. If aniline sorption was controlled by the organic fraction alone, K_{oc} values would be comparable in all horizons. This is not the case. The high K_{oc} for the B22t horizon clearly indicated sorption is controlled by the mineral fraction.

Sorption data presented in Figure 5 were plotted according to the Langmuir isotherm (Figure 7). The Langmuir equation can be written as

$$C_s = \frac{K_1 K_2}{1 + K_1 C_{eq}}$$

where

C_s = the concentration of aniline sorbed (μg/g)

C_{eq} = the equilibrium solution concentration (μg/mL)

K_1 = a constant related to the binding energy

K_2 = the adsorption maximum (μg/g).

The Langmuir plots for each of the horizons could be divided into two linear segments, which suggested the presence of at least two types of sorptive sites. Curve inflections for the A1-Ap, B1, and mine soil occurred at a

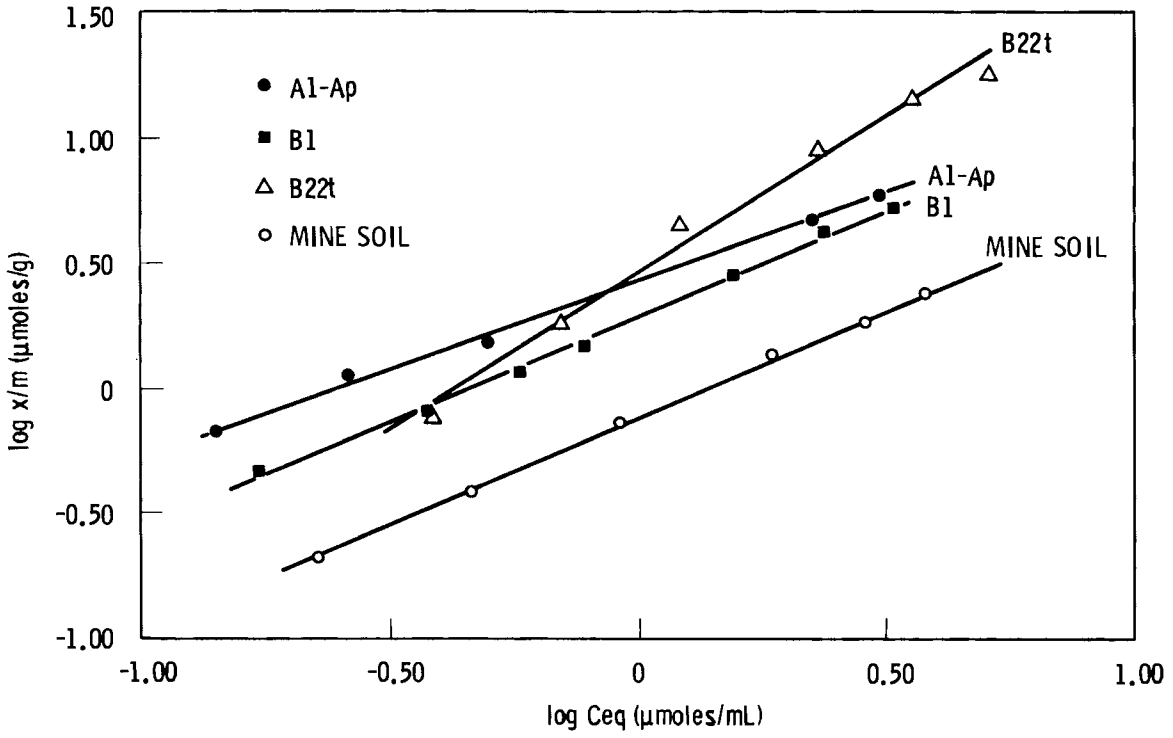

FIGURE 6. Freundlich Sorption Isotherms for Westmoreland and Silt Loam and Fort Martin Mine Soil

TABLE 6. Aniline Sorption Parameters for Westmoreland Silt Loam and Fort Martin Mine Soil

Soil	Organic C, %	K_d (Freundlich)	K_{oc}	(x/m) max[a] No. 1, µg/g	No. 2, µg/g
Westmoreland					
Al-Ap	2.03	2.606	128.4	1204	1161
Bl	0.976	1.905	195.2	1465	2712
B22t	0.150	2.890	192.6	4192	4376
Mine Soil	0.538	0.750	139.4	825.4	812.7

(a) Aniline adsorbed per g of dry soil.

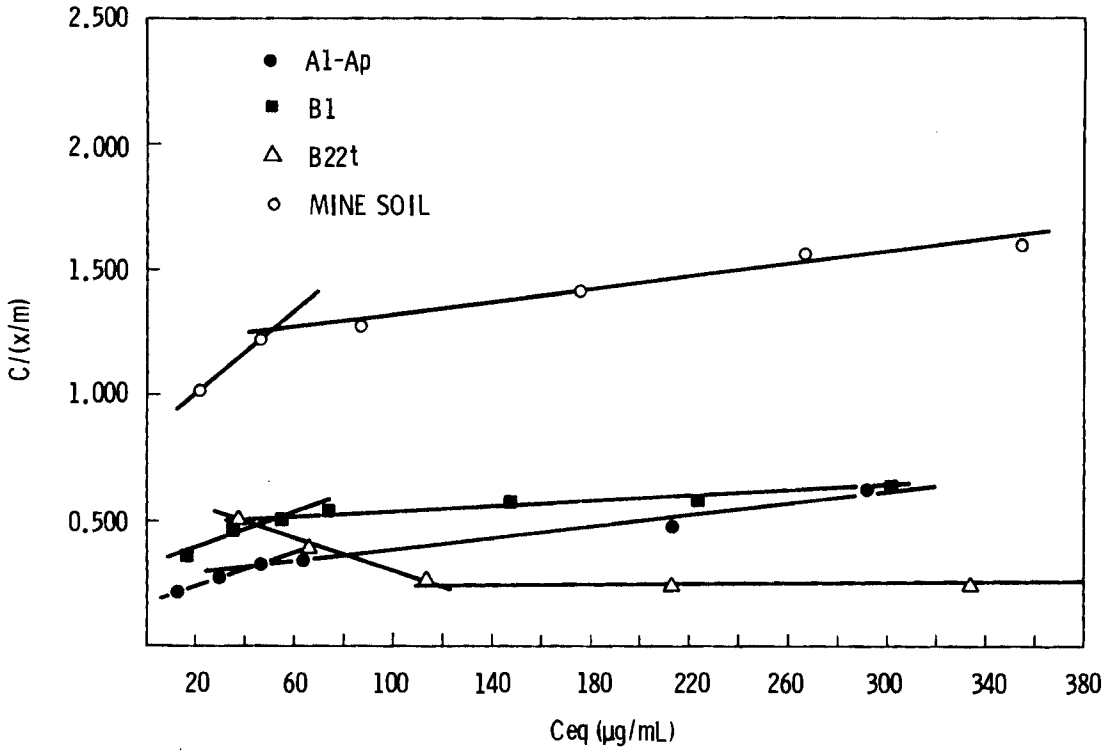

FIGURE 7. Langmuir Sorption Isotherms for Westmoreland Silt Loam and Fort Martin Mine Soil

similar solution concentration (∿50 µg/mL), but differed significantly for the B22t horizon (∿112 µg/mL). Several methods, including those of Syers et al. (1973) and Griffin and Burau (1974), were used to evaluate these two-part isotherms, each with limited success. Average sorption maxima for each of the horizons as calculated by these techniques are presented in Table 6. Of significance was the increasing aniline adsorptive capactiy of the Westmoreland soil with depth. This trend was inverse to the distribution of organic carbon. The mine soil adsorbed the least amount of aniline.

DISCUSSION

Aniline sorption isotherms for the soils and soil horizons studied could be represented by the Freundlich equation. The resulting K_{OC} values (Table 6), however, suggested that while hydrophobic interaction with organic material may control sorption in the Westmoreland Al-Ap and the mine soil,

interaction with the mineral matrix may be responsible for binding much greater quantities of aniline in the B22t. Both sorption processes occurred in the B1 where intermediate values of K_{oc} and sorption maxima (x/m, max) were calculated. Higher aniline sorption in the B22t did not correlate directly with any of the soil chemical or physical parameters evaluated. We anticipate that analyses in progress, specifically the determination of amorphous iron hydrous oxide, will facilitate this interpretaion. In contrast, Moreale and Van Bladel (1979) ascribed aniline sorption in soils solely to the organic fraction, thus suggesting that the extent of sorption was governed by the ratio of hydrophobic to hydrophilic organic surfaces.

Two-part Langmuir isotherms were applied to aniline sorption in all horizons studied. Moreale and Van Bladel (1979) studied European soils and obtained results similar to those shown in Figure 7 for the first segment (part) of the low concentration (C_{eq} = 0 to 50) isotherms. Although two-part isotherms are commonly attributed to the presence of two populations of sorbing sites or two distinct retention mechanisms (Holford, Wedderborn and Mattingly 1974; Holford and Mattingly 1975), other investigators (Posner and Bowden 1980; Harter and Baker 1977) argue that desorbed species and variable-potential charged surfaces will alter the sorption of solute molecules and cause this apparent effect. The negative slope of the low concentration Langmuir isotherm for the B22t horizon, Figure 7, (C_{eq} = 38 to 120 µg/mL) results from the increase in percent adsorption

$$\frac{C_o - C_{eq} \times 100}{C_o}$$

over the concentration range. This may result from strong competition between aniline and H_2O for the adsorption sites. Deviation from the Langmuir isotherm could also result from specific sorption by reaction with amorphic iron oxides or preferential fixation of the protonated organic base by phyllosilicates (Bache and Williams 1971). At any rate, sorption at low concentrations (C_{eq} = 38 to 120 µg/mL) in the B22t may violate the underlying assumptions of the Langmuir equation that 1) adsorption energy is constant and independent of surface coverage, and 2) interactions do not occur between adsorbate moelcules.

Further studies are necessary to clarify adsorption mechanisms in these soils and the microbial persistence of adsorbed aniline in discrete soil horizons. However, these sorption studies suggest that the B horizons of certain alfisols of the eastern coal region may effectively retard migration of organic nitrogen bases potentially released from SRC liquids under spill conditions.

4.4 AQUEOUS LEACHING STUDIES WITH NON-GASIFIED SRC MINERAL RESIDUE

J. M. Zachara and T. R. Garland

BACKGROUND

The proposed ground disposal of solid wastes (mineral residues and gasified mineral slag) from the solvent refining of coal may create the potential for contamination of surface and ground waters through the leaching of soluble organic and inorganic residual compounds. Evaluation of the potential ecological and human health effects that may occur requires a complete understanding of: 1) the chemical and microbiological processes effecting solubilization within solid waste landfills with time and weathering, and 2) the identity and chemical form of soluble components released and their transport and persistence in soils, sediments, and substrata.

The Pacific Northwest Laboratory approach to evaluate these potential ecological and human health effects will be carried out in two phases. First, the chemical composition of leachates will be determined. In these initial studies, batch equilibrations will be used to assess and identify the total quantity of readily soluble materials present in the residues and their rate of release, and single-phase columns will be used to estimate the composition of leachates potentially produced at solid waste disposal sites. In the second phase, the environmental transport and fate of identified soluble components will be evaluated using batch sorption studies to generate adsorption isotherms for select components. Also, incubation studies will be undertaken to identify the microbiological persistence of organics. Subsequently, mixed-phase column studies will be initiated to identify the mobility of soluble components through soil, subsoil, and substrata.

Studies presented in this report include mineralogic characterization and batch and column leaching of the non-gasified SRC-I mineral residue produced at the Fort Lewis, Washington, pilot plant. Present plans call for SRC-I mineral residue to be gasified to produce hydrogen for process uses and to convert the inorganic material present to a glassy, relatively inert fly ash.

It is expected that this will essentially destroy the organic hydrocarbon compounds present in the mineral residue. The converted residues (gasifier fly ash and slag) are proposed to be landfilled for disposal. In the absence of gasified mineral residues representative of those produced at demonstration or commercial facilities, studies of ungasified filter cake were undertaken to evaluate methodologies suitable for identifying the quantity and chemical composition of leachates released from SRC solid wastes. It is understood that the results of these investigations will not represent the types and amounts of leachates which might be leached from gasified mineral residue.

EXPERIMENTAL

The material used was non-gasified SRC-I mineral residue (filter cake) produced at the Fort Lewis, Washington, pilot plant and sampled in December 1976. The coal feed was a bituminous western Kentucky coal produced at the Pittsburg and Midway Colony Mine.

For x-ray diffraction analysis, approximately 10 g of less than 150 μm (100 mesh) mineral residue was washed four times with benzene and twice with acetone. Final drying of residue was from acetone. X-ray diffractograms were obtained by scanning 2θ angles of 2° to 65° with both dry powder mounts on aluminum trays and dried slurries on glass slides.

Batch equilibrations were performed with a 10:1 solution to solids ratio (100 mL of deionided H_2O:10 g of residue) and were sampled over a 200-hour period. Equilibrations received constant agitation using a desk top reciprocal shaker while temperature was maintained at 25°C ± 1°C. Sample aliquots were removed after 1, 2, 4, 8, 24, 48, 60, 72, 144, and 192 hours of equilibration. Column leaching studies were performed with 10 cm^3 columns packed with the SRC-I mineral residue and leached at a flow rate of 0.1 mL/hour through successive column volumes. Chemical analyses of batch extracts and column leachates included pH, redox potential (ion selective electrodes), conductivity (E.C. bridge), organic and inorganic carbon (CO_2 pyrolysis unit), anions (ion chromatography), and cations and trace elements (plasma emission spectroscopy).

RESULTS

The non-gasified SRC-I mineral residue was found to be predominantly pyrrhotite, a crystallographically complex iron sulfide with an approximate formula of FeS. Pyrrhotite is formed by reaction of pyrite (FeS_2) with hydrogen present in the organic matter of the coal or the liquid and gaseous hydrocarbons produced during the conversion process. Hydrogen sulfide (H_2S) is a by-product of this reaction. An x-ray diffractogram of the residue is illustrated in Figure 8. Major peaks at 2.07, 2.65 and 2.98 Å are those of pyrrhotite; the single maxima at 2.07 Å identifies the structure as hexagonal, which is typical of higher temperature formation (>300°C). Additional minerals present included quartz (3.34 and 4.25 Å), kaolinite (7.13 and 3.57 Å), an expanding 2:1 phyllosilicate (17.7 Å), and possibly gypsum (2.89 Å). Absent are carbonates and pyrite, mineral phases commonly found in coal.

Table 7 presents a comparison of selected chemical parameters of the batch equilibration after 24 hours and in the first column volume of leachate. Additional species measured, but not presented, include Ba, Cr, Cu, Li, Ni, Pb, Zn, NO_3^{2-}, $S_2O_3^{2-}$, and SCN^{2-}. Column leachates (v/v_o = 1) and batch extracts (24 hours) of the mineral residue are slightly basic (pH 8.0 to 8.4) and contain notable quantities of soluble salts (Na^+, Ca^{+2}, Cl^-, and SO_4^{2-}). Boron, strontium, fluoride, and organic carbon are released in high levels. This is particularly apparent in the column studies where 70 mg/L boron, 22 mg/L strontium, 23 mg/L fluoride, and 196 mg/L organic carbon were present in the first column volume.

Figure 9 depicts characteristic release profiles of selected solubles with time in the batch system and with increased leaching in the column system. The major cations (Na and Ca) approach equilibrium in the batch system after approximately 48 hours (Figure 9a). With the exception of aluminum (data not shown), most other soluble inorganic cations and anions follow a similar trend. Organic carbon also reaches a maxima after 48 hours, but decreases rapidly thereafter (Figure 9b), which probably reflects microbial activity. In contrast, boron increases steadily with time. In the column studies, maximum quantities of most of the soluble species are released along with readily

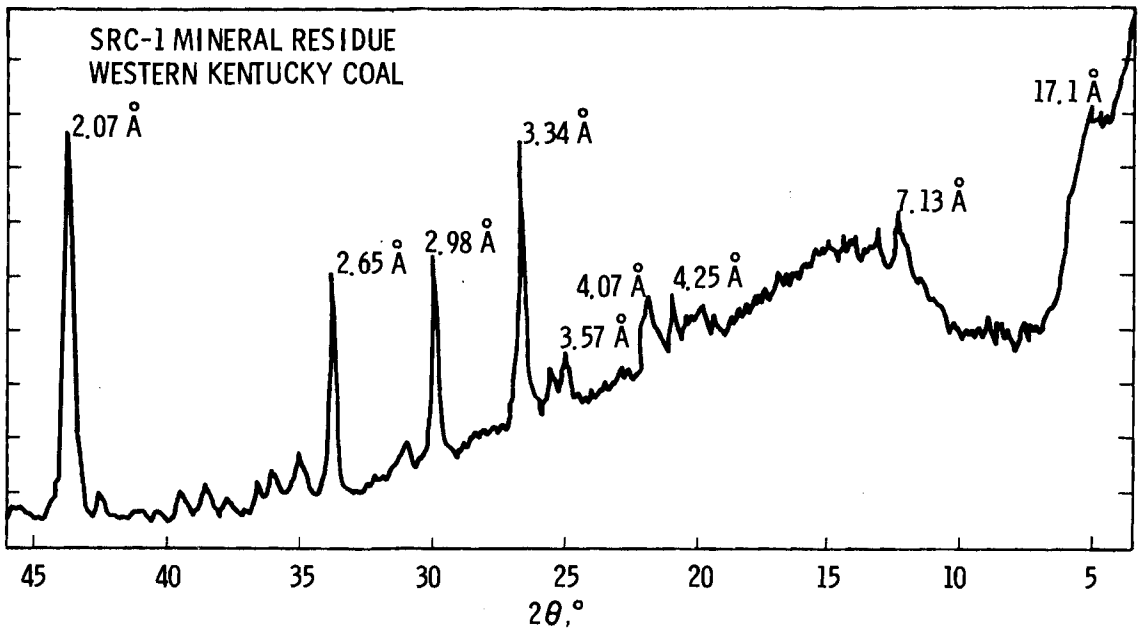

FIGURE 8. X-Ray Diffractogram of SRC-I Mineral Residue

soluble salts (Na^+ - Cl^-) in the first pore volume of leachate (Figures 9c and 9d). This trend holds for organic carbon, fluoride, and boron (data not shown). Sulfate follows an inverse trend, with an apparent approach to equilibration with a solid phase, probably gypsum ($CaSO_4 \cdot H_2O$).

The total quantity of soluble materials released in the column and batch system differed significantly. In the column studies, 1.79 mg/g of soluble inorganic and 135 mg/g of soluble organic materials were released in the first three pore volumes, as compared to 7.70 mg/g of inorganic and 0.360 mg/g of organic materials released after 48 hours of batch equilibration.

DISCUSSION

Aqueous leaching studies showed that the batch system released a greater quantity of readily soluble materials from the mineral residue, but the column leachates more appropriately simulated the probable composition of solutions which could enter the environment after leaching a mineral residue pile. Batch studies of the non-gasified SRC-I residue reached equilibration after approximately 48 hours, and at this time had solubilized approximately two times more

TABLE 7. Chemical Composition of Batch Extracts and Column Leachates from Non-Gasified SRC-I Mineral Residue

Chemical Composition[a]	Batch at 24 hr	Column in $v/v_o = 1$
pH	8.40	8.07
Conductivity (m mhoes)	0.75	3.27
Inorganic C (mg/L C)	4.9	13.6
Organic C (mg/L C)	21.0	196
Al	1.46	0.17
B	5.07	70
Ca	360	1400
Fe	0.018	0.030
K	4.50	34.8
Na	18.3	372
Mg	1.64	27.9
Mn	0.066	0.540
Si	5.82	6.42
St	2.35	21.5
Mo	0.084	1.28
Cl	65.0	1360.0
F	<0.60	23.3
SO_4	742	1658

(a) All elements expressed in mg/L.

organic and five times more inorganic materials than were released from the column in the first three volumes. After 48 hours, apparent microbial activity in the batch system increased, thereby reducing levels of soluble organic carbon.

Column studies indicate that the initial leachates ($v/v_o = 1$) contained elevated levels of soluble salts, organic carbon, boron, and fluoride, which decrease rapidly with leaching. Elevated concentrations of soluble organic

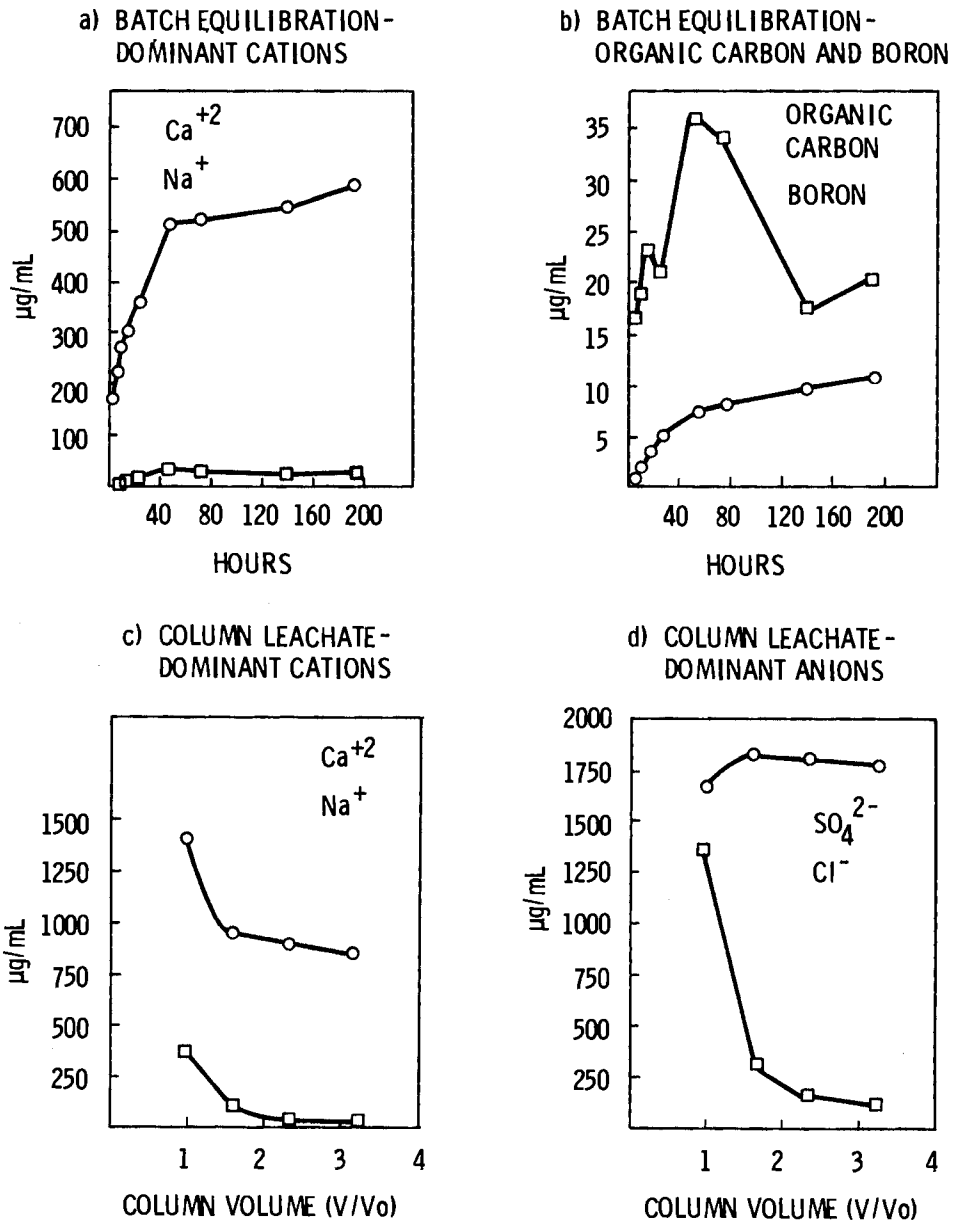

FIGURE 9. Release Profiles of Selected Solubles from SRC Mineral Residue in Batch and Column Systems

carbon may reflect, to a certain degree, residual process solvent still contained in the residue. Although the soluble organic-carbon fraction has not been characterized, the sustained environmental release of leachates containing these levels of organic carbon (≃196 mg/L as C) could be of potential ecological or human health concern. However, current developmental plans entail

gasification of organic residuals present in the mineral residue to low Btu gases, thus increasing the energy efficiency of the process.

Both boron and fluoride are elements commonly enriched in coals (Nicholls 1968). Boron has been shown to accumulate in coals in association with organic material (Gluskoter 1975). However, the boron content of illite (a mixed 2:1 phyllosilicate) in Illinois coals has been used as a paleosalinity indicator (Bohor and Gluskoter 1973). Cavallano et al. (1978) evaluated trace element contents in coals from Illinois and western Kentucky. These coals averaged 54 μg/g fluorine, and most of this was found in the heavier specific gravity fractions, suggesting an association with the inorganic or mineral fraction. No mineralogic residence was suggested, but fluorite (CaF_2) is a possibility.

Detailed interpretation of the fluxes of inorganic species from the residue is difficult unless one evaluates the mineral species found in the coal feedstock. Coals from the Illinois Basin, which includes the western Kentucky fields, commonly contain from 9.4 to 22.3 wt % of mineral matter. Of this percentage, 52% are phyllosilicates, 25% are sulfides and sulfates, 9% are carbonates, and 15% is quartz (Gorman and Walker 1972; Rao and Gluskoter 1973). The clay minerals, sulfides, and carbonates all undergo thermal decomposition reactions and, under aerobic conditions, normally convert to more oxidized and soluble phases. It is unknown whether similar reactions will occur in the SRC process, where moderate temperatures and high pressures occur and reducing conditions prevail.

Although the composition of the non-gasified SRC-I mineral residue used in these studies could be very different from that produced at commercial SRC-II facilities (where differing coal feeds would be used and gasification of the filter residue would occur), the long-term effects of the disposal of this pyrrhotitic mineral residue should be considered. Although natural alteration products of pyrrhotite include pyrite, marcasite, iron sulfates, carbonate, and oxides, it is unclear whether oxidation can be microbially mediated as in the case of pyrite. In any event, pyrrhotite is thermodynamically unstable under aerobic conditions, and oxidation of ferrous iron and sulfide sulfur contained will lead to acid production upon weathering and,

thus, to chemical conditions analogous to acid mine drainage. The other minerals present in the residue (kaolinite, quartz, 2:1 phyllosilicates, and gypsum) have a relatively poor pH buffering capacity and will likely contribute little to ameliorate these effects. It must be recognized, however, that material to be landfilled at a demonstration or commercial facility is expected to have a completely different character than the mineral residue.

The laboratory leaching methodologies utilized in the study provide a preliminary evaluation of potential water quality problems associated with leachate from SRC solid waste materials. Batch and column leaching, if performed under both aerobic and anaerobic conditions as a function of time and flow rate, can qualitatively bracket conditions anticipated in a waste disposal site. However, results of laboratory leaching studies must be viewed as only indicative of the general chemical character of leachates which may be produced under field conditions. These data are a necessary first step in assessing the need for additional research. The complex interactions between climate, biologic activity and natural weathering processes occuring over time in a landfill site necessitate that field studies (e.g., lysimeter or cassion) be applied to quantitatively evaluate potential impacts on water quality that may result from SRC solid waste disposal.

4.5 SITE CHARACTERIZATION STUDIES AT THE FORT MARTIN SITE, MORGANTOWN, WEST VIRGINIA

J. M. Zachara, T. R. Garland, R. M. Bean and R. E. Wildung

BACKGROUND

The Fort Martin site, 3 miles north of Morgantown, West Virginia, was proposed for the construction of a demonstration-scale SRC-II process plant. Proximity of this site to the Monongahela River and to Morgantown itself raised concern over the potential environmental degradation that could arise from this development. A major issue was possible effects of product spills and the disposal of gasified solid wastes to soils, surface waters, and ground waters local to the Fort Martin site.

Factors governing impact on soils and transport to surface and ground waters of soluble SRC-II components include: 1) climate; 2) physicochemical properties of the product or solid waste; 3) physicochemical and mineralogic characteristics of soil, subsoil, and underlying substrata mediating sorption and water movement; 4) chemical and microbiologic persistence of soluble organic components; and 5) hydrology of the vadose zone governing water infiltration and runoff. However, concise identification of impact requires understanding of the background chemical regime of local soils, sediments, and surface waters. This understanding is essential in the Morgantown area where surface waters have been severely affected by past coal mining and other industrial activities, and where significant areas of soil cover have been disturbed by recent strip mining. These effects must be clearly differentiated from possible impacts resulting from SRC liquid and solid effluents. The results of initial studies designed to evaluate the background chemical regime in surface waters, soils, and sediments on and in immediate proximity to the Fort Martin site are presented in this report.

EXPERIMENTAL

Water and Sediment Samples

Water samples and bottom sediments were collected from surface waters draining or in proximity to the proposed SRC-II demonstration plant site (Figure 10). Sampling locations on Robinson's Run, Crafts Run, Crooked Run and the Monongahela River are shown in Figure 10. Only sediment samples were collected on the Monongahela River.

Analyses performed concomitant with sampling included temperature and stream flow rate (with the exception of the Monongahela). Dissolved oxygen, pH, and redox potential were determined within 2 to 4 hours of sample collection using the appropriate ion-selective electrodes. Samples were maintained at 4°C for further analysis.

Interstitial waters from Monongahela River sediments (M-1, Figure 10) were expressed (pressure system) both in the field on the day of collection and in the laboratory 7 days later. In the field, this was performed using an ultrafiltration cell with a PM 10 membrane and compressed air for pressure. Laboratory extractions were performed with sediment that had been stored at 4°C and used N_2 for pressure. Aliquots expressed in the laboratory were immediately acidified using concentrated Ultrex® HCl. Dissolved oxygen, pH, and redox potential were determined on interstitial waters immediately after collection.

Additional chemical analyses performed on filtered (0.22 μm) water samples and interstitial waters included dissolved organic carbon, total organic carbon (CO_2-pyrolysis), conductivity (standard resistance bridge), major cations and trace elements (inductively coupled plasma emission spectroscopy) and anions (ion chromatography). Organic material was removed from subsamples of 35- to 50-g subsamples by Soxhlet extraction with methanol, then with a mixture of benzene and methanol. The organic extracts were then separated over silica gel into saturate and aromatic hydrocarbons, and these fractions were then separated into individual components using capillary gas chromatography (Bean, Blaylock and Riley 1980).

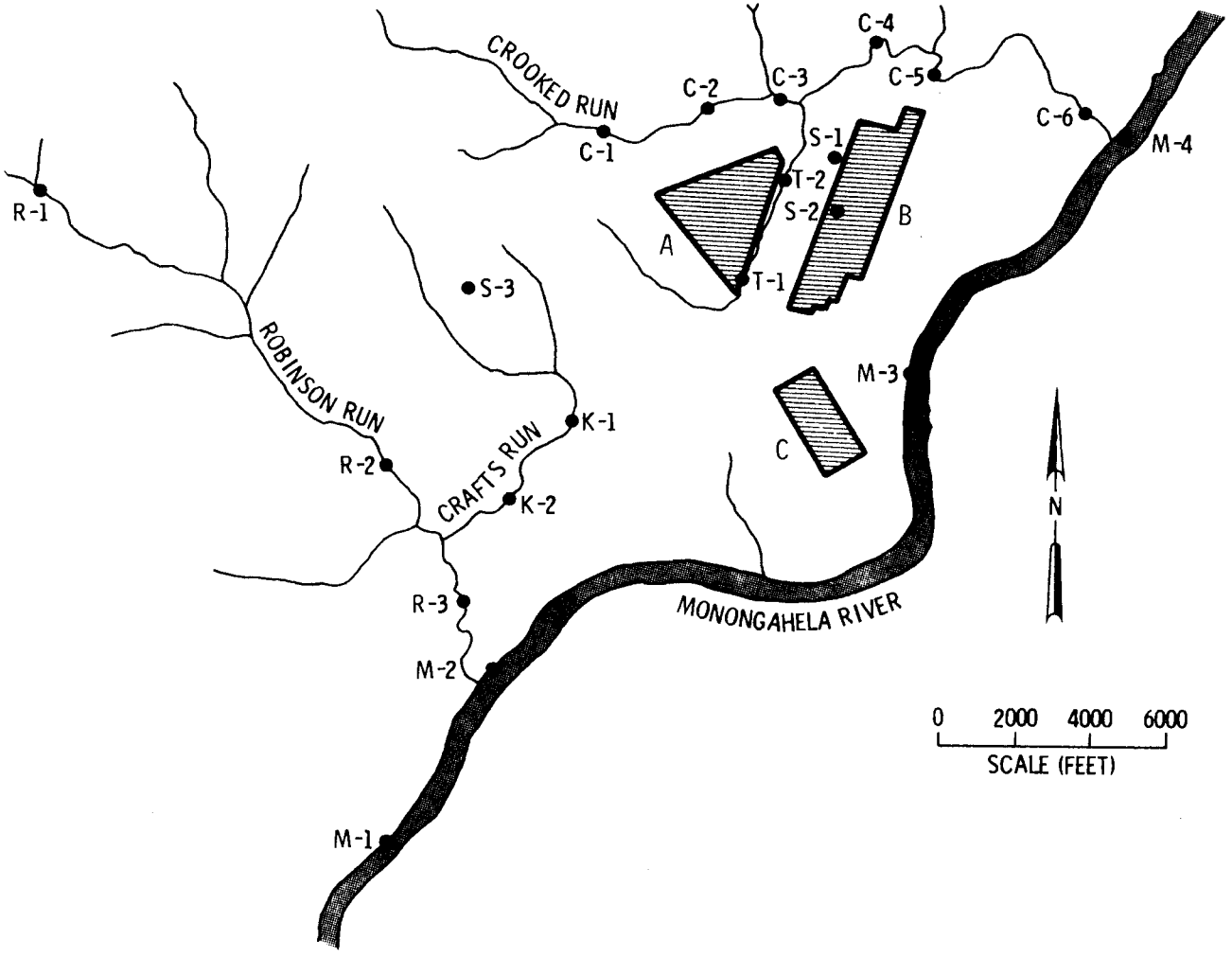

FIGURE 10. Sampling Locations on Proposed SRC-II Demonstration Plant Site: (A) Proposed Slag Disposal Area, (B) Demonstration Process Area and Commercial Expansion, and (C) Coal Stockpile

Soils

Soil samples were collected (Figure 10) from the three main upland soil series located on the proposed development site: Westmoreland (S-1) and Dormont (S-2) silt loams, and reclaimed mine land (S-3). The A1-Ap, B1 and B22t horizons were sampled in the Westmoreland and Dormont soils, while a composite of the upper 30 cm was taken from the mine soils.

Batch equilibrations using a 5:1 solution to solids ratio (100 g H_2O and 30 g soil) were performed on the less than 2 mm soil materials, which had been maintained at their field moisture status. Equilibrations were performed at 25° ± 1°C on a reciprocal desk top shaker. Aliquots were extracted after 1, 2, 6, 24, 48, and 72 hours. Aliquots removed were immediately centrifuged, and pH, redox potential, and conductivity were measured. Filtered samples (0.22 µm) were maintained at 4°C until analysis of dissolved organic and total carbon, major cations and trace elements, and anions was complete.

Physicochemical Characterization of Soils and Sediments

At this time, we are still characterizing the total elemental content; total organic material, total quantity of the various forms of extractable Al, Fe, Mn; particle size distribution; and mineralogy of the soils and sediments used in these experiments. These results will be published at a later date.

RESULTS

A summary of the chemical composition of surface waters, sediment interstitial waters, and soil extracts from the Fort Martin site is presented in Table 8. Figure 11 depicts the relative redox status of these waters and extracts based on platinum electrode readings. Surface waters on the site are affected by acid mine drainage. Robinson's Run from just above R-2 and Crafts Run beginning at K-2 (Figure 10) are acid waters down to their confluence with the Monongahela River. This acidity results primarily from discharge from abandoned underground coal mines. Crooked Run is not significantly affected and maintains a slightly alkaline pH regime characteristic of most natural watersheds of the region. Water quality data from lower Robinson's and Crafts

TABLE 8. Concentration Range of Dissolved Constituents in Surface Waters, Sediments, Interstitial Waters, and Aqueous Soil Extracts from the Fort Martin Site

Analysis	Acid Streams	Alkaline Streams	Monongahela Sediment Interstitial Waters	Aqueous Soil Extracts	
				A+B Horizons	Mine Soil
pH	2.9-4.24	7.3-8.0	7.0-7.9	4.8-5.2	5.8-6.0
Cond (μ mho/cm)	1000-1730	200-450	200-340	27-42	311-316
Org C (mg/L)	3.9-6.4	0.2-8.1	7.6-30	15.5-19.5	12.5-14.5
Inorganic C (mg/L)	<.1	14.1-41.4	56.9-60.5	<0.5	.54-.87
Cl^-	3.15-10.7	5.38-10.1	9.7-13.3	.06-.47	.57-.72
F^-	.66-1.65	.11-.26	.33-.51	.10-.32	.34-.36
SO_4^{2-}	1400-2350	88-207	3.62-4.83	7.47-17.4	134-135
Al	33-81	<.003-.025	<.003-.23	.016-2.13	0.6-.15
B	.08-.24	.03-.47	.06-3.1	.021-.032	.015-.021
Ba	.018-.072	.035-.094	.21-.39	.010-.145	.035
Ca	314-414	42-78	36-54	2.79-4.84	43.2
Cr	.042-0.78	<.001-.027	<.001-.056	DL-.015	.016
Cu	.015-.11	<.001-.003	.06-.27	DL	ND
Fe	23-146	<.001-.029	.016-12.7	.001-1.84	.004
K	1.0-16	0.6-13	2.2-4.7	.308-.943	2.16-2.21
Li	.15-.18	.003-.032	.009-.019	.004-.012	.004-.01
Na	9.1-71	4.2-19	13.-19	.985-1.23	1.67-1.82
Mg	73-110	5.5-27	8-13	.221-.293	9.4-9.5
Mn	2.3-5.2	.05-8.9	1.2-3.3	.016-.248	.216-.238
Mo	.026-.064	.002-.082	.008-.019	DL	<DL
Ni	.25-.45	.002-.014	.001-.034	DL-.013	.010-.015
Pb	.10-.23	<.005-.008	.005-.46	.019-.097	.006-.012
Si	11-33	3.5-4.0	3.3-6.3	2.06-4.00	1.95-2.09
Sr	1.8-2.8	.11-.94	.22-.42	.015-.027	.112
Zn	.45-.96	.002-.064	.006-.29	DL-.047	.009-.017
NO_3^-	0.71-.1.16	.46-2.33	.10-.21	.76-7.19	4.66-4.75
PO_4^{2-}				.06-.12	.06

(a) All elements expressed in mg/L.

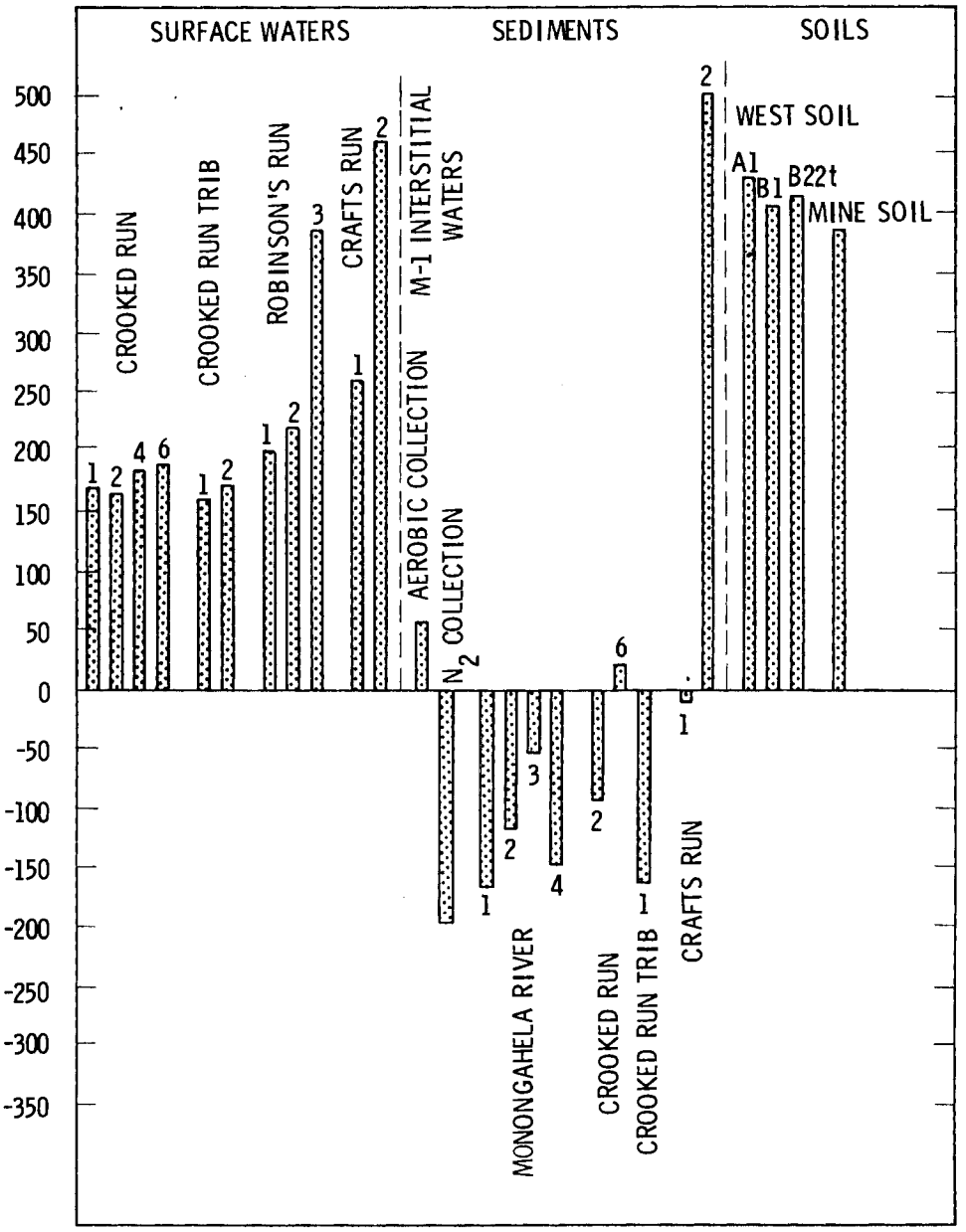

FIGURE 11. Relative Redox Status of Surface Water, Stream and River Sediment and Surface Soil - Platinum Electrode Readings

Runs and from mine discharge at R-2 and K-2 is summarized under acid streams in Table 8. Data from the headwaters of Robinson's and Crafts Runs and all of Crooked Run are provided under alkaline streams.

In addition to having lower pH, acid streams are higher in conductivity (5x), F^- (5x), SO_4^{2-} (10x), Al (10^4x), Ca (8-10x), Fe (10^4x), Mg (7 to 10x), Si (5 to 10x), and most trace metals (Cr, Cu, Mo, Ni, Pb, and Zn) than are unimpacted alkaline waters. The unusually high quantities of soluble iron and other trace metals results primarily from the microbially catalyzed oxidation of pyrite (Moon and Lucostic 1979; Biesecker 1966). The resulting acid conditions retard the hydrolysis and precipitation of these metals and additionally encourage dissolution of Ca and Mg from carbonates and Al and Si from aluminosilicates. In contrast, alkaline streams of the area contain higher levels of inorganic carbon (HCO_3^-), which contribute significantly to the anion-cation balance of these waters. Barium, boron, potassium, and chloride are found in nearly equal concentrations in acid and alkaline waters. Acid waters [Robinson's Run (R-3) and Crafts Run (R-2)] are generally more oxidized (Figure 11) than normal surface waters (Crooked Run), which probably reflects the low values of the Fe^{+2}/Fe^{+3} redox ratio.

Monongahela sediments were dark, fine-grained, organic-rich sediments with a mild odor of sulfide. Platinum electrode readings (Figure 11) were negative, indicating a relatively anaerobic redox status. In contrast, sediments from the acid reaches of Crafts Run (K-2) are highly oxidized due to rapid, turbulent stream flow and precipitation of hydrous oxides ("yellow boy") of iron over the stream bottom. The chemical composition of interstitial waters extracted from M-1 was sensitive to the pressure gas used (compressed air, N_2) and reflected the reducing character of these sediments. The ion balance in the interstitial water was dominated by Ca^{+2}, Mg^{+2}, Na^+, and HCO_3^- and was comparable in conductivity to alkaline surface waters. Concentrations of barium, boron, lead, and copper were slightly elevated while Mn, Fe, and organic carbon were present in high concentration and were sensitive to the extraction procedure. The highest values of soluble Fe (12.7 mg/L) and Mn (3.3 mg/L) were obtained with the N_2 gas where the redox status of the extracted interstitial water was negative (Figure 11).

Samples from M-1 and M-2 were analyzed for aromatic hydrocarbons. A chromatogram of the aromatic components from M-1 are shown in Figure 12. The complex pattern shown is significantly different from those obtained from

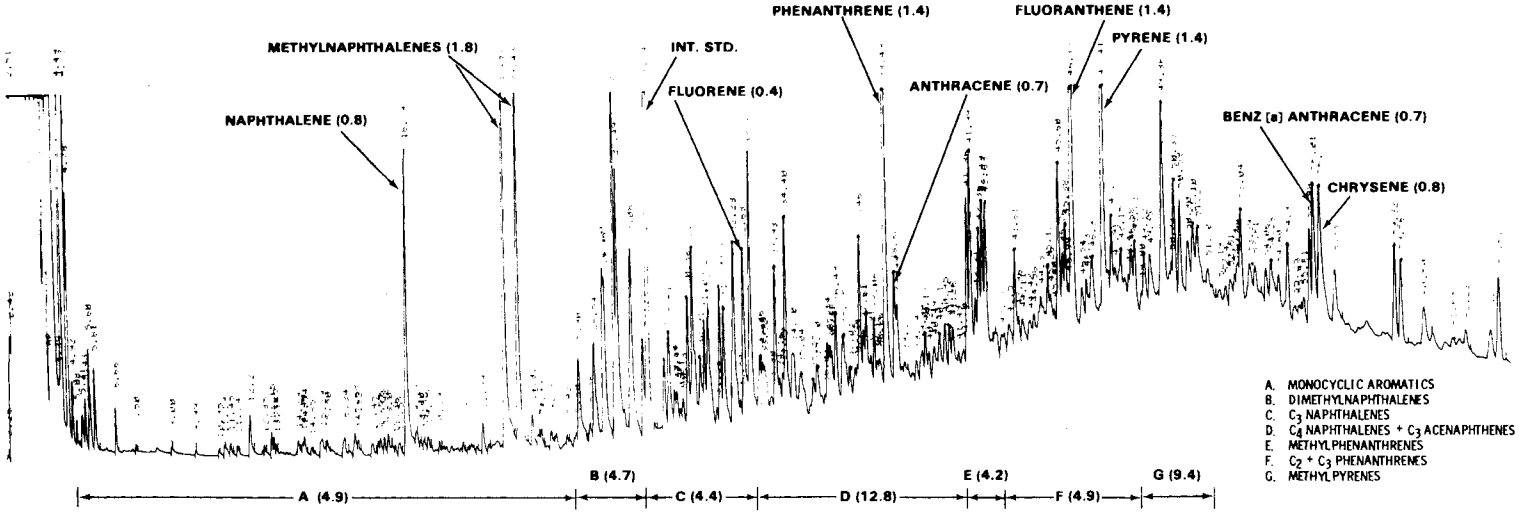

FIGURE 12. Capillary Gas Chromatogram of Aromatics Fraction From Monongahela River Sediment (Station M-1). (Component concentrations are shown in parentheses as mg/kg dry sediment weight)

aqueous extracts of SRC-II liquids, but the distributions and relative abundances of the naphthalene and substituted naphthalenes are very similar to those from a crude oil. In three subsamples, levels of aromatic hydrocarbons at M-1 were 52.0 ± 32.0 mg/kg dry sediment; the corresponding saturate-hydrocarbon content was found to be 7.6 ± 1.9 mg/kg. Duplicate subsamples from M-2 contained 32.4 and 18.8 mg/kg aromatics, and 5.6 and 3.9 mg/kg saturates. Weathering of the hydrocarbon fraction is evident from the prominence of pristane in the saturate chromatogram and the relatively high contribution of three- and four-ring aromatic components to total aromatic concentration in the sediments.

The Westmoreland and Dormont soils are acidic alfisols (Ultic Hapludalfs) and are characteristic of the upland areas of the Fort Martin site (U.S. Department of Agriculture 1978). These are high base status soils >35% with a thick, organic-rich A horizon (A1-Ap 0 to 25 cm) and B horizons with clay accumulation (B1, B21t, and B22t 25 to 68 cm). Soils have formed from mixed sandstone and shale residuum from the Conemaugh Group (Pennsylvanian). Batch extracts (5:1) of the natural soil materials are generally more acidic and more highly oxidized than the mine soil (Table 8, Figure 11). The dominant soluble constituents in all soil materials sampled were Ca^{+2} and SO_4^{2-}, with lesser quantities of Na, Mg, K, Mn, Si, and NO_3. The mine soils released approximately five times more solubles than either the Dormont or Westmoreland silt loams. With the exception of the mine soil, 5:1 batch extracts contained lower concentrations of these constituents than did either surface waters or sediment interstitial waters.

DISCUSSION

The degree of transport of soluble organic or inorganic components from a waste pile or point of spillage will depend upon interaction with the soil and aquatic systems of the area. Soil cover in proximity to the proposed process and to coal stockpile and ash disposal areas on the Fort Martin site will likely be a mosaic of the Westmoreland, Dormont, and mine soils. The natural soils (alfisols) with their accumulation of organic carbon and fine-grained clay materials will be important in mediating sorption reactions. The acidic

pH regime (4.8 to 5.2) will affect the mobility of water-soluble organic components present in SRC liquids, including organic bases and phenols. The reduced pH (4.8 to 5.2) and prominence of an ionic balance in the soil batch extracts dominated by neutral salts ($Ca\text{-}SO_4$) suggest that these soils will not be strongly buffered against pH reduction that may be induced by acid leachates from coal stockpiles or sulfide-containing mineral wastes.

Surface waters on the site are both acid and alkaline, and sediments are oxidized and reduced. Accordingly, the mobility and chemical form of both organic and inorganic residuals released by SRC operations will differ significantly. For example, hydrolyzable inorganic elements (i.e., Fe^{+2}, Ni, Zn) if released to acid waters will likely remain soluble. In contrast, concentrations of these same species would be significantly reduced in alkaline waters by hydroxide and possibly by carbonate solubility controls. Comparable processes will occur with soluble organics. Nitrogen-containing organic bases (anilines) will be protonated below a pH of approximately 5. Protonation will render these compounds more soluble but it also will increase their potential for exchange and possible retention by negatively charged colloids (i.e., phyllosilicates). Great differences will exist in the sorption of inorganic and organic components, the solubility of inorganic components, and the persistence of organic components in oxidized and reduced sediments. Most influential will be differences in the microbial populations (aerobic vs. anaerobic), chemical characteristics of the sediments (hydrous oxide vs. fine-grained, organic-rich carbonaceous materials), and chemical form of prominent redox couples (SO_4^{2-}/S^{2-}, Fe^{+3}/Fe^{+2}, and Mn^{+4}/Mn^{+2}).

The concentrations of hydrocarbons found in the Monongahela sediments are quite high, even when compared to other water bodies that are considered contaminated. Levels of aromatic hydrocarbons about one order of magnitude lower than Monongahela River sediments have been reported for intertidal sediments of the Duwamish River, Washington. Concentrations of individual aromatic compounds such as pyrene and benzanthracene were similar.

Analysis of subsamples of sediments for phenols by caustic extraction and formation of the phenol acetates by direct aqueous derivatization did not

reveal phenols to be present in concentrations >10 mg/kg wet sediment. Similarly, the Soxhlet extracts of the sediment did not give any response when chromatographed on a capillary column connected to a nitrogen-phosphorous detector. These results, taken together with the petroleum-like hydrocarbon patterns observed for the Monongahela sediments, suggest that a number of criteria can be used to investigate Monongahela sediments for the presence of SRC-II-derived contaminants. A change of aromatic hydrocarbon distributions, coupled with the appearance of significant concentrations of nitrogen bases and/or phenols, could provide evidence for the accumulation of coal-derived liquid residuals in the environment.

These limited background studies have shown that the Fort Martin site is not pristine and that complex chemical differences do exist between natural and disturbed soils and between individual surface water systems. Clearly, the chemical controls on surface waters and sediments must be accurately defined to assess impact of SRC-II development. Results discussed in this report are based on limited sampling of stream waters and sediments, and additional research emphasis must be directed toward understanding the chemical regime of these systems on a seasonal basis. However, these initial field studies can be used to plan additional, detailed field sampling programs and, more importantly, to design laboratory experiments aimed at predicting the behavior and fate of both organic and inorganic residuals potentially released to the environment at Fort Martin.

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SECTION 5.0
ECOLOGICAL EFFECTS

SECTION 5.0

ECOLOGICAL EFFECTS

Studies described below were designed to assay complex SRC-II liquids, selected chemical fractions, and/or specific compounds and reference commercial oils. Test organisms included the freshwater green algae, Selenastrum capricornutum; the zooplankter, Daphnia magna; two sediment-dwelling detritivores, Chironomus tentans and Tanytarsus dissimilis; the fathead minnow, Pimephales promelas; rainbow trout, Salmo gairdneri, and chinook salmon, Onchorynchus tshawytscha. These species are accepted reference test organisms and most have considerable experimental history. The two benthic detritivores were selected as species representative of the water/sediment interface, where coal liquids released to the environment are expected to accumulate. The three fish represent eastern and western species.

Toxicity tests were designed to: 1) provide a means of biologically screening raw SRC-II liquids and chemical fractions, 2) provide a reference data base likely to be required for the generic environmental impact statement and various federal and state permits, and 3) provide a data base useful to federal and state regulatory agencies in the setting of environmental standards and in implementing the National Environmental Policy Act (NEPA) 1969.

Chemistry was integrated with toxicity tests because partitioning of SRC-II liquids into water and sediment in test systems was found to vary with time and compound type. For example, animals chronically exposed (e.g., 30 days) were found to be exposed to a different distribution of specific toxic compounds than were animals acutely exposed (e.g., 48 to 96 hours). Toxicity results were also found to be altered depending on the rigorousness of the mixing used to introduce pollutants to water. For these reasons, a special approach based on dilution index ratios was developed for dealing with toxicity comparisons among these complex toxicants. Additionally, small weighing/leaching lysimeters were designed to determine if SRC-II liquids and SRC-I mineral residues

(solid wastes) were deleterious to plant growth when introduced to soil in various amounts. This information is needed for soil decontamination procedures and cleanup following accidental spills in the field.

5.1 DEVELOPMENT OF EXPOSURE CRITERIA AND RANGE-FINDING FOR AQUATIC BIOTA (ACUTE TOXICITY)

D. Becker, W. E. Fallon, R. M. Bean, D. W. Crass, A. J. Scott, J. R. Skalski, J. B. States and C. S. Abernethy

BACKGROUND

Acute toxicity tests with liquid SRC-II blends require application of procedures that meet accepted standards for toxicity testing (e.g., APHA et al. 1976; ASTM 1979a; EPA 1975) with some modifications required by the uniqueness of the product and the test organism. Relatively insoluble petroleum products and synfuels present special problems in obtaining representative stock solutions for conducting acute tests. Hence, procedural and statistical design modifications were required for the acute toxicity tests.

Initial work involved examining experimental procedures for preparing stock solutions of liquid coal synfuel blends (e.g., the SRC-II process), which were subsequently diluted with water for exposure of test organisms (see "Biological Testing," below; also Bean et al. 1981a). As a result, stock solutions for acute toxicity tests were routinely prepared at 20°C by slowly mixing 300 mL of a selected synfuel blend and 29.7 L of filtered (5μ) river water for 4 hours. After the solution stood 1 hour, 18 L of the water soluble fraction (WSF) were siphoned from the center of the jar as stock. The complex hydrocarbon mix in stock and exposure solutions was then monitored on the basis of total carbon (TC) and total dye-complexible phenolic compounds (total phenols).

Acute Screening studies were first conducted with an SRC-II blend consisting of 1 part middle distillate to 1 part heavy distillate. Subsequent work, however, has employed a SRC-II blend consisting of 2.9 middle distillate to 1 part heavy distillate. The WSF derived from the 2.9:1 blend is predominated by phenolic compounds and contains lower concentrations of aromatic hydrocarbons, saturated hydrocarbons, and nitrogen compounds. WSFs of stock

solutions usually contain about 1,100 mg/L TC and 700 to 800 mg/L dye-complexible phenols. In contrast, WSFs identically prepared from crude oil in Reference Toxicant tests usually contain less than 50 mg/L TC and lesser amounts of phenols.

The acute toxicity data as developed here may be used to: 1) establish a data base to sustain a reasonable inference regarding the acute hazard to aquatic organisms at different trophic levels and life stages that might occur after a spill of coal-derived synthetic fuel into surface waters; 2) complement and support the first objective with concurrent and comprehensive chemical characterization of toxicant solutions used during acute exposure of aquatic organisms; 3) generate comparable toxicity and chemical data for other complex organic fuels, including synthetic, shale, and petroleum-based fuels; and 4) develop test protocols incorporating new investigative procedures and methods for data expression that are applicable to acute toxicity tests with aquatic organisms, chemical characterizations, and hazard assessments for any complex bituminous mixtures.

EXPERIMENTAL

Experimental methods are developed and applied in Environmental Chemistry and Fate Task (Sec. 4.0) and Ecological Effects Task (Sec. 5.0). Experimental details described below are applied to acute, chronic, and comparative effects studies (Sec. 5.1, 5.2, and 5.3).

Design

Values for 48- or 96-hour LC50 (based on mg/L TC in WSF derived from SRC blends) and 90% confidence intervals were usually obtained by computerized probit analysis. Other methods of data analysis were employed when required. Three special concepts the "lowest rejected concentration tested" (LRCT), the "dilution index" (DI), and the "relative hazard" (RH)--were developed for use with synfuel studies. These are discussed below.

Certain documented bioassay methods such as the probit analysis for estimating LC50 (Finney 1971) have proven to be restrictive for constant application to data generated by screening-type tests. The probit method, for

example, required mortality between 0% and 100% in at least three test concentrations. This requirement cannot be practically met in the present work for two reasons: 1) variations in the overall concentration-response relationships are extremely large, depending on partitioning effects (discussed in Section 4), and 2) the dose response curve is extremely steep for water soluble fractions of a given phenolic composition. For these reasons we chose to measure the toxicity of SRC blends in some tests by determining the LRCT, which is defined as the lowest test concentration of TC that caused a statistically significant ($\alpha = 0.05$) increase in mortality compared to control organisms. In the case of invertebrates, where 24 animals were exposed to a given concentration, four mortalities would establish the test concentration as the statistically valid LRCT (see discussion 5.1.1). However, an additional step was necessary to compare the relative toxicities. This step employed using the initial concentration of organic carbon (or an equivalent measurement) in stock solutions in conjunction with the LRCT, and was referred to as the dilution index (DI):

$$DI = \frac{TC}{LRCT}$$

where TC is the total carbon in stock solutions and LRCT is the lowest rejected concentration tested ($\alpha = 0.05$).

The DI can be interpreted as a minimum dilution of stock solution that still results in a statistically significant ($\alpha = 0.05$) increase in mortalities of test organisms.

It should be noted that the LRCT is based on an experimental design where toxicant concentrations are usually increased systematically by a factor of two. Such dilutions permit determination of toxicity over a test concentration range of more than one order of magnitude, but a 0.5 serial dilution reduces sensitivity of the computed LRCT. Thus, because of design, LRCT (and DI) must differ by at least a factor of four to be considered real and not experimental artifacts.

A further step was developed to compare relative potency of different SRC blends with different treatments or other products. Since different treatments and different products usually produced different chemistry in aqueous suspensions, methods of estimating potency that assumed fixed dose-response relationships (e.g., Finney 1978) were not entirely feasible. Instead, we compared two DIs by their ratio, expressed as "relative hazard" (RH), as

$$RH_{xy} = \frac{DI_x}{DI_y}$$

where x and y are stock solutions representing different treatments or materials. If the RH of x over y is 15, then x is 15 times more toxic than y, and the aquatic system would need a dilution capacity 15 times greater to present equal estimated hazard after a spill.

DI and RH will receive greater emphasis in continuing studies, particularly in the Reference Materials task (see Section 5.3).

Biological Testing

Water used in acute tests for stock preparation and exposure of organisms was filtered Columbia River water. Acute tests under standard conditions were conducted at 20°C, 7.3 to 8.2 pH, 65 to 80 mg/L ETDA hardness as $CaCO_3$, 60 to 70 mg/L alkalinity as $CaCO_3$, and dissolved oxygen near saturation (9 to 10 mg/L). Quality characteristics of Columbia River water were relatively stable throughout the year and usually remained within these limits.

Standard test organisms used in acute toxicity tests were the fathead minnow Pimephales promelas (a vertebrate consumer), the cladocerian Daphnia magna (an invertebrate consumer), and larvae of the midge fly Chironomus tentans (a benthic detritivore). Stocks were propagated and reared at our laboratory. Other aquatic species were sometimes tested under the category of Reference Organisms, with methods appropriate to the species and life stage.

Acute tests with fathead minnow were 96-hour duration and were initially under static or replacement conditions (see Section 5.1.2) but evolved to flow-through conditions because of dissolved oxygen and declines in TC. Flow

through tests were conducted in glass aquaria (67 x 30 x 7.5 cm) containing 25 L of exposure solution delivered by gravity feed from a proportional dilutor. Each test required six dilutions of stock solutions and a control (seven aquaria). Twenty minnows measuring <50 mm fork length were temperature acclimated and placed in each aquaria. Positions of aquaria and distribution of fish were randomized.

Acute tests with daphnids and midge larvae were 48-hour duration and conducted under static conditions in glass jars containing 200 mL of exposure medium. Normally 24 organisms were exposed to each of six exposure concentrations plus a control. Disposition of six organisms per jar, 4 jars for each concentration, provided a 7 x 4 randomized grid distribution and placement test design. A water bath was used for temperature control.

Water quality characteristics were determined at the start of each test. Mortality, temperature, and chemical variables (TC and phenol concentrations) were monitored to provide quality control and correlation of effects with chemistry of exposure solutions. A 24-hour post-exposure period was used with fathead minnows and midge larvae to determine recovery or delayed mortality.

Chemical Monitoring

The selection and application of specific analyses depend on the SRC-II concentrations employed during aquatic testing and the quantity of material available.

The following procedures were employed for analyses of SRC-II material in water mixtures used for acute screening, chronic screening, and comparative effects tasks:

Carbon Analyses

Concentrations of total carbon (TC) were determined by direct injection of aqueous samples on Beckman or Dohrmann carbon analyzers. Total carbon (TC) was determined by difference, or after removal of inorganic carbon. Concentrations of SRC-II materials in water were determined as TC after standard deletion of total carbon indigenous to the water.

For aquatic effects studies, dosimetry was based on the concentration of TC derived from SRC-II materials in water.

Organic Solvents

All solvents employed are Burdick & Jackson "distilled in glass®."

Total Oil by Infrared (IR) Spectroscopy

Aqueous samples were extracted with carbon tetrachloride (CCl_4) and the absorbance of the extract at 2927 cm^{-1} determined. This provided an estimate of total oil concentrations based on calibration curves of whole SRC-II liquid in CCl_4 (Simard et al. 1951). Determinations of total oil by IR provided an indication of the total mass of bituminous material in water and also monitored the reproducibility and stability of test solutions.

Total Oil by Ultraviolet (UV) Spectroscopy

Aqueous samples were extracted with hexane and the absorbance of the extract at 333 nm determined. This provided an estimate of concentrations of SRC-II components (e.g., higher aromatics) absorbing at this wavelength. This technique was investigated because the highly aromatic nature of coal and coal conversion products indicated the occurrence of UV-absorbing chromophores.

Photometric Phenols Assay (Dye Phenols)

Aqueous samples were buffered to pH 10 and treated with α-aminoantipyrene and potassium ferric cyanide, which react with certain phenols to form a colored complex. Absorbance at 510 nm was measured. This provided an estimate of total phenols based on the response of phenol on a calibration curve (APHA et al. 1976). This rapid and simple procedure was used extensively to monitor levels of phenols in stock and test solutions of SRC-II materials in water.

Phenols Analysis by Gas Chromatography

Analysis for phenols by gas chromatography permitted quantitative and qualitative determinations of specific phenols and phenol classes present in

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SRC water soluble fractions. When applied over time to SRC test solutions, the method was used to monitor the degradation or leaching of labile or hydrophilic phenol species and the persistance of the more stable and lipophilic species.

Analyses of Hydrocarbons

Water soluble extracts of SRC-II liquids contain relatively high concentrations of aromatic hydrocarbons and lower concentrations of saturate hydrocarbons. Hexane extracts of aqueous samples were reduced in volume under N_2 and fractionated into aromatic and saturate fractions on silica gel (Warner 1976). Individual hydrocarbons or classes were determined by capillary gas chromatography (GC) on a Hewlett Packard 5880 GC using 30 M x 0.25 mm ID WCOT column coated with SP2100 liquid phase.

Analysis of Volatile Hydrocarbons by Purge and Trap Technique

Analysis of volatile hydrocarbons was performed to provide an estimate of the hydrocarbon load in aqueous samples which can be removed (volatilized) by aeration and agitation. A modification of commercially available Enviro-Test Equipment Co. apparatus was utilized. Aliquots were added to freshly boiled and cooled deionized water to a final volume of 35 mL. Nitrogen was bubbled through the sample at 40 mL/min for 20 min and the purged volatiles were trapped on a short, stainless steel Tenax® column. The Tenax® column was then fitted with a syringe needle and the trapped volatiles were eluted into the injection port of a Hewlet Packard 5985 GC/MS system by heating in a flow of helium carrier gas. Gas chromatography was on a 1.8 m x 2 mm, 3% SP2100, 80/100 mesh Supelcoport column.

5.1.1 DESIGN AND RANGING EXPERIMENTS: CRITERIA FOR COMPARING DIFFERENT EXPOSURE REGIMES

Background

A series of experiments was conducted with the initial SRC-II blend to: 1) quantify changes in toxicity elicited from SRC/water mixtures by varying mixing energy; 2) examine physical and chemical variables influencing toxicity

by appropriate chemical analyses and through the physical separation processes of filtration and aeration; and 3) derive quantitative criteria to compare toxic responses (see Bean et al. 1981a).

The toxicity of petroleum suspensions in water will vary with the method of preparing test solutions. Higher concentrations of oil in water usually occur after violent mixing rather than after gentle mixing. Regardless of how solutions are mixed, dispersions of oil in water are relatively unstable compositions that consist of suspended water-insoluble droplets, solvated molecules, and "accommodated" molecular aggregates. Further, the composition of suspensions of oil and water and the resultant toxicity to aquatic life can be significantly altered by filtration through 0.45μ filters or by aeration.

Since these features were known to apply to petroleum suspensions (e.g., crude and refined oils in commerce), it was necessary at the beginning to examine their effects on suspensions of synfuel blends in water. The results would provide an initial basis for "standardizing" further research on acute toxicity, particularly when preparing exposure solutions for a series of acute toxicity tests involving different SRC-II blends and different aquatic test organisms.

Experimental

A 1:1 blend of middle and heavy distillate was the SRC-II material used. The experiments consisted of two treatments. The first was 2 x 2 x 5 factorial design to determine the effects of alternate mixing regimes (fast and slow mix), filtration (filtered and nonfiltered) and phase separation time (0.5, 2, 8, 24, 32 hours) on the chemical, physical, and toxic properties of SRC suspensions. Similarily, a 2 x 2 x 5 factorial design was used to determine the effects of mixing regime, aeration (aerated and nonaerated) and settling time (0.5, 2, 8, 24, 32 hours) on the SRC suspensions. The work was conducted in four steps: 1) fast mix--filtered versus nonfiltered; 2) slow mix--filtered versus nonfiltered; 3) fast mix--aerated versus nonaerated; and 4) slow mix--aerated versus nonaerated. Chemical, physical, and toxicological probes were used to characterize mixtures for each treatment. Acute tests with the water flea, Daphnia magna, constituted the toxicological probe.

Fast Mix--Filtered Versus Nonfiltered

Eight mL of SRC liquid was added to 1 L of river water in a high-speed blender and homogenized for 1 min. The slurry was added to 29 L of river water in a 50-L glass carboy, stirred for 10 min, and the phases were allowed to separate for 5 min. After phase separation was complete, duplicate samples were withdrawn at different time intervals by siphon from the center of the aqueous stock solution for chemical characterization and toxicity testing. One of the duplicates was filtered through a 0.45μ Millipore® filter under slight pressure at each time interval.

Fast Mix--Aerated Versus Nonaerated

Treatment was as above through phase separation. The aqueous phase was then siphoned into two 12-L glass bottles. One bottle was continuously aerated by airstone at approximately 150 mL/min. Corresponding aerated and nonaerated samples were periodically withdrawn for analysis and toxicity testing.

Slow Mix--Filtered Versus Nonfiltered

Thirty mL of SRC liquid was added to 29.7 L of water in a 50-L glass carboy and mixed for 24 hours with a bent, teflon®-coated rod at approximately 90 to 95 rpm. After 5 min for phase separation the aqueous solution was siphoned from below the oil-water interface into two 12-L bottles. Corresponding samples, one of which was filtered as before, were withdrawn for analysis and testing.

Slow Mix--Aerated Versus Nonaerated

Thirty mL of SRC liquid was treated as above through phase separation. The aqueous stock was siphoned into two 12-L jars, one of which was aerated as before. Aerated and nonaerated samples were periodically withdrawn for testing and analysis.

Exposures of young D. Magna (±12-hour-old) were for 48 hours in 200 mL of solution in glass jars. Serial dilutions of stocks were based on the total carbon (TC) content, calculated by multiplying the measured stock TC value by a dilution factor. Portions were drawn for acute tests 0.5, 2, 8, 24 and 32 hours after phase separation. Portions were drawn simultaneously from

aerated and nonaerated stocks or filtered and nonfiltered stocks. Acute tests were conducted with five dilutions from each portion plus two controls (representing 12 exposures). Portions were differentially diluted to produce nominal concentrations of 0.625, 1.25, 2.50, 5.0 and 10.0 mg/L TC. Twenty-four daphnids (six daphnids per jar) were exposed to each concentration and to the controls. Jars were positioned in a randomized grid and held at 10°C during organism exposure.

Results and Discussion

Fast Mix--Filtration

A brown-colored suspension resulted from fast mixing the SRC with water. Filtration produced a clear, colorless water solution. Analytical results for stock solutions and filtrate at 0.5 and 32 hours are in Table 9. The first three measurements listed are concentrations for hydrocarbon classes. Benzenes and naphthalenes were determined by the purge-and-trap (P&T) method, and the saturate hydrocarbons and phenanthrenes/pyrenes were determined by capillary chromatography. P&T data were obtained within a few days after stock preparation; the capillary data were obtained from samples allowed to stand longer. When concentrations of mono- and dicyclic aromatics determined by the two methods were compared, the values obtained by the capillary method were lower than those from P&T by as much as 50%. Data for saturates and phenathrenes/pyrenes were probably also low because of the time lapse between the preparation and analysis of the relatively unstable suspensions.

The last three measurements in Table 9 indicate the total SRC material in the stock solutions. Both IR and UV methods were calibrated with the original SRC material. Different total SRC values for the same samples were measured by IR and UV because different properties of the SRC blend are used for quantitative measurement. IR measures absorbance of aliphatic carbon-hydrogen bonds, while UV is sensitive to polycyclic hydrocarbons and other chromophores having significant absorbance at 330 nm. Relative differences among IR, UV, and TC values can, therefore, give qualitative information about physical and chemical changes in SRC water suspensions resulting from treatments. These differences also illustrate the difficulties in determining actual concentrations of complex materials in solution.

TABLE 9. Concentrations of SRC and Hydrocarbon Types in Fast-Mix Stock Solutions--Nonfiltered (NF) Versus Filtered (F)

Analysis	0.5 Hr After Preparation		32 Hr After Preparation	
	NF	F	NF	F
Saturates (GC)	0.25	nd[a]	.02	nd
Benzenes/naphthalenes (P&T)	4.12	1.05	2.30	1.53
Phenanthrenes/pyrenes (GC)	1.02	nd	0.32	nd
Total SRC (IR)	58[b]	13	36	26
Total SRC (UV)	66	0	16	0
Total carbon	53	35	42	39

(a) nd = not detected.
(b) From 2-hr data.

The biological data generated during the fast-mix filtration experiment is presented in Figure 13. Since the carbon concentrations in exposure solutions were determined by stock dilution, differences in carbon concentrations in the stocks after preparation and/or treatment were not a factor in daphnid mortality. Rather, differences in mortality at the same TC level must be interpreted on the basis of qualitative differences in carbon compounds to which the organisms were exposed. A significant difference occurred between the nonfiltered and filtered fast mix SRC suspensions at all settling times tested.

Filtration (Table 9) reduced saturate and polycyclic hydrocarbons to less than detectable levels (<0.01 mg/L per component), and reduced benzenes and naphthalenes substantially. Although TC, IR, and UV methods for total SRC in the nonfiltered fast-mix suspension provided similar data initially, there was little agreement after either filtering or standing for 32 hours. Filtration through 0.45μ filters reduced UV absorbance at 330 nm to zero, and caused substantial reduction in material absorbed in the IR at 2927 cm^{-1}. TC was lowered by either filtration or standing, but in either case, the reduction

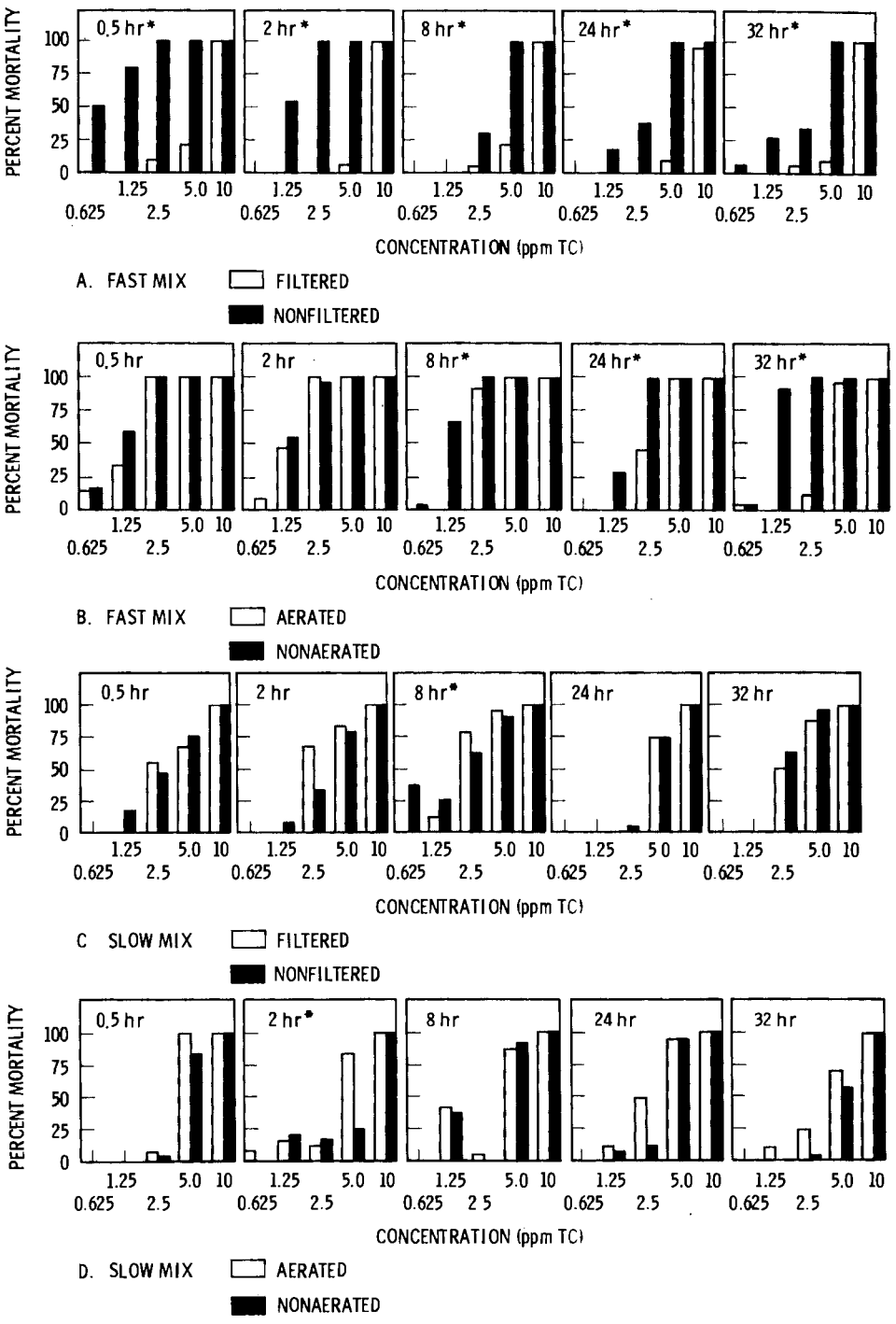

FIGURE 13. Acute Toxicity of Dilutions of Fast-Mix Stock Solution to Daphnia magna. Time in upper left corners of each square refers to the period after stock preparation that the acute test was initiated. Significant ($\alpha < 0.05$) differences between treatments are indicated with an asterisk.

was less than indicated by IR or UV analysis. Correspondence between the drop in IR value for total SRC and saturate concentration, and the drop in UV value for total SRC and polyaromatic concentration in Table 9, was constistent with the removal of these insoluble components by filtration, leaving behind materials more transparent to the spectral probes used. Comparing the analyses of nonfiltered suspensions at 0.5 and 32 hours (Table 9), it is evident that standing had a pronounced effect on composition. Capillary GC, IR and UV analyses indicated that the 32-hour settling period resulted in removal of insoluble, suspended components from the WSF.

Filtration also significantly affected the toxic properties of the SRC suspension (Figure 13). Although near total daphnid mortality occurred from 32-hour exposure to both nonfiltered and filtered material at 10 mg/L TC, acute toxicity below this level was sharply reduced by filtration at all sampling times. Mortality and chemical data for the filtered suspension indicated that the mixture of toxic chemicals in the water produced by filtration was independent of settling time prior to filtrations. Acute toxicity of the nonfiltered suspension also appeared to decline wth settling time. This suggests that suspended material in the stock solutions contributed to daphnid mortality, and their removal was the main reason toxicity declined after filtration.

Fast Mix--Aeration

Gentle aeration affected the chemical composition of stock solutions, but only after aeration continued for some time (Table 10). Differences in toxic response between aerated and nonaerated material were not significant ($\alpha < 0.05$) until the 8-hour aeration (Fig. 13B). Aerated and nonaerated material differed little in total SRC values or in hydrocarbon content after 0.5 hour aeration, but all values decreased greatly at 32 hours. Daphnid toxicity did not reflect the substantial loss in carbon produced by aeration since all exposures at subsequent intervals were based on the same TC levels.

Since SRC suspensions for the two fast-mix experiments were prepared by blending, mortality and chemistry of initial stocks should be similar. However, values for total SRC (by IR), saturate hydrocarbon, and phenanthrenes were substantially higher in the initial stock for the aeration treatment even

TABLE 10. Concentrations of SRC and Hydrocarbon Types in Fast-Mix Stock Solutions--Nonaerated (NA) Versus Aerated (A)

Analysis	0.5 Hr After Preparation		32 Hr After Preparation	
	NA	A	NA	A
Saturates (GC)	0.70	0.50	0.06	nd[a]
Benzenes/naphthalenes (P&T)	4.79	4.08	2.27	nd
Phenanthrenes/pyrenes (GC)	3.85	4.98	0.62	0.08
Total SRC (IR)	75	72	29	11
Total SRC (UV)	56	65	16	3
Total carbon	47	45	20	13

(a) nd = not detected.

though TC was somewhat lower (47 versus 53 mg/L). Also, total daphnid mortality from exposure to untreated stock occurred at 2.5 mg/L TC, compared to 5 mg/L TC in the filtered treatment. The evidence suggests substantially more SRC material in the initial stocks for the aeration treatment than the TC measurements indicated; thus, dilutions based on TC actually contained more toxicants. This disparity may relate to placing the initial stock for the fast-mix aeration experiment in two separated vessels (one aerated, one not), while that for the fast-mix filtration experiment remained in its initial container.

Slow Mix--Filtration

Stock suspensions generated by stirring slowly for 24 hours were almost clear, translucent solutions, which slowly darkened to brown over 32-hours. Analytical data in Table 11 show that filtration had little effect on IR, UV, or TC values. Although filtration reduced some hydrocarbon types, benzenes and naphthalenes were predominant before and after filtration. Further, no significant change in IR, UV, or TC was produced by filtration after 32 hours.

The data also suggest that benzenes and naphthalenes were reduced more when the stock stood for 32 hours than by filtration.

Daphnid mortality (Fig. 13C) was consistent with the analytical data. In general, no significant difference ($\alpha < 0.05$) in toxicity occurred between filtered and nonfiltered suspensions. The exception, 8 hours after preparation, is due to an unusually high mortality at the lowest TC concentration.

A large discrepancy occurred between the IR and UV results (Table 11); both were less than the measured TC. The low UV value indicated low concentrations of high molecular weight aromatic components. Further, neither the spectroscopic values nor the TC of the slow mixed stock changed with time or filtration. Hydrocarbon analyses accounted for little of the TC.

Slow Mix--Aeration

There was little difference in the analytical measurements of nonaerated and aerated slow-mix stock after 0.5 hour of aeration (Table 12), and little material could be found in the aerated stock after 32 hours with P&T or capillary chromatography. Also, ultraviolet and IR absorbance and TC values changed only slightly. Aeration had little effect on toxicity (Fig. 13D), with only one of five test (t = 2 hours) showing a significant difference ($\alpha < 0.05$) in toxic response.

Contribution of Phenols to TC

This study showed that most organic carbon present in the water was not accounted for by hydrocarbon analytical procedures. Data for the slow-mix experiments indicated that a large portion of the carbon was water soluble since it was not removed by filtration. It was also nonvolatile since it was not lost by aeration. This suggested that for the slow-mix experiments, most organic carbon in the suspension consisted of phenolic compounds. Capillary chromatographic analysis of some remaining samples of phenols isolated as their acetates revealed a complex mixture of phenolic constituents. Phenol analyses converted to equivalent organic carbon concentrations were compared with the TC obtained for the SRC suspension (Table 13). Phenols represented a substantial portion of the organic carbon in all samples examined representing

TABLE 11. Concentrations of SRC and Hydrocarbon Types in Slow-Mix Stock Solution--Nonfiltered (NF) Versus Filtered (F)

Analysis	0.5 Hr After Preparation		32 Hr After Preparation	
	NF	F	NF	F
Saturates (GC)	nd[a]	nd	0.02	nd
Benzenes/naphthalenes (P&T)	4.58	2.60	1.57	0.75
Phenanthrenes/pyrenes (GC)	0.09	nd	0.03	nd
Total oil (IR)	54	54	54	54[b]
Total oil (UV)	3	3	1	2
Total carbon	75	72	71	68

(a) nd = not detected.
(b) 24-hr sample.

TABLE 12. Concentrations of SRC and Hydrocarbon Types in Slow-Mix Stock Solutions--Nonaerated (NA) Versus Aerated (A)

Analysis	0.5 Hr After Preparation		32 Hr After Preparation	
	NA	A	NA	A
Saturates (GC)	nd[a]	nd	nd	nd
Benzenes/naphthalenes (P&T)	6.57	5.27	1.99	0.04
Phenanthrenes/pyrenes (GC)	0.06	0.05	0.03	0.01
Total oil (IR)	65	61	65	58
Total oil (UV)	4	5	4	3
Total carbon	83	80	83	84

(a) nd - not detected.

TABLE 13. Organic Carbon Contribution to SRC Suspensions as Phenols

Mix	Total organic C, mg/L	Organic C as phenol, mg/L	% as phenol
Fast Mix			
nonfiltered ($t^{(a)}$ = 2 h)	44	8	18
filtered (t = 2 h)	31	15	48
nonaerated (t = 32 h)	20	7	35
aerated (t = 32 h)	13	7	54
Slow Mix			
nonfiltered (t = 0)	76	60	79
filtered (t = 0)	75	58	77

(a) t = time after preparation of stock suspension that sample was taken.

almost 80% of the TC in the two slow-mix stocks. Phenols accounted for a much larger fraction of the organic carbon than the saturate and aromatic hydrocarbons found by the GC analyses in Table 9 through 12, and contributed substantially to the organic carbon in the fast-mix suspension.

Expression of Toxicity

In analyzing the toxicity of a pure, water soluble substance, the computed LRCT measures intrinsic toxicity of the substance. However, in the case of a complex, multicomponent, largely water-insoluble material like SRC, the LRCT measures only the toxicity of various components in aqueous suspensions. As our analytical data show, component concentrations can be highly variable depending upon the method of stock preparation, and can have an appreciable effect on the resulting LRCT. Tables 14 and 15 report LRCT values for the various acute tests performed.

Although LRCT may be used as a measure of the toxicity of the SRC components in aqueous suspensions, the relative toxicity resulting from one treatment to another must still be compared. Different treatments can result in

TABLE 14. Lowest Rejected Concentration Tested at $\alpha = 0.05$ (LRCT), the Dilution Index (DI) and the Relative Hazard (RH) of the Nonfiltered to Filtered Stock Solutions Prepared by Fast and Slow Mixing. Values of LRCT are expressed as mg/L TC.

Setting Time, hr	Fast Mix					Slow Mix				
	Filtered		Nonfiltered		RH	Filtered		Nonfiltered		RH
	LRCT	DI	LRCT	DI	NF:F	LRCT	DI	LRCT	DI	NF:F
0.5	4.93	7.0	0.54	98.1	14.0	2.52	28.5	1.22	61.5	2.2
2	7.94	3.9	1.14	38.6	9.9	2.51	26.2	2.44	30.7	1.2
8	2.23	14.8	4.61	10.4	0.7	2.52	28.9	0.62	118.5	4.1
24	6.70	5.0	2.22	20.0	4.0	4.97	14.4	5.00	14.4	1.0
32	7.78	4.9	1.01	41.6	8.5	2.48	27.2	2.50	28.4	1.0

TABLE 15. Lowest Rejected Concentration Tested at $\alpha = 0.05$ (LRCT), the Dilution Index (DI) and the Relative Hazard (RH) of Nonaerated to Aerated Stock Solutions Prepared by Fast and Slow Mixing. Values of LRCT are expressed as mg/L TC.

Setting Time, hr	Fast Mix					Slow Mix				
	Aerated		Nonaerated		RH	Aerated		Nonaerated		RH
	LRCT	DI	LRCT	DI	NF:A	LRCT	DI	LRCT	DI	NF:A
0.5	1.13	39.4	1.19	39.1	1.0	5.10	15.2	4.74	17.4	1.1
2	1.32	29.5	1.17	38.5	1.3	1.20	71.9	1.28	72.3	1.0
8	2.50	8.4	1.27	21.7	2.6	1.25	64.8	1.25	67.2	1.0
24	2.41	5.6	1.22	18.4	3.3	2.50	32.8	5.02	17.5	0.5
32	4.84	2.6	1.28	15.2	5.9	2.48	33.7	4.97	17.1	0.5

different types of toxic chemicals present and in different total amounts of material in suspensions. Therefore, an index of relative toxicity such as the DI is useful because it employs both the initial concentration of organic carbon in the stock solution and the LRCT. Calculated DI values for the acute tests are included in Tables 14 and 15.

The utility of LRCT and DI for comparing toxicity of complex mixtures can be seen from data generated by fast-mix stocks. In Table 14, the LRCT of nonfiltered fast-mix solutions was almost always lower than the LRCT of the corresponding filtered solutions. The average LRCT for the filtered fast-mix solution (5.9) was three times higher than the nonfiltered (1.9) solution. This shows that filtration changed the mixture of toxic components to one that was less toxic. Analytical data generated for the fast-mix filtration experiment (Table 9) showed marked reductions of saturate and aromatic hydrocarbons as well as UV absorbing material, even though reduction in TC was not as great.

It is also useful to compare the DI of filtered and nonfiltered stocks as a ratio, expressed as a relative hazard (RH). Thus, the RH of the initial nonfiltered to filtered material at 0.5 hour is 14.0 (Table 14), and the average RH for the five samples is 7.4. In other words, an aquatic system requires 14 times dilution capacity to reduce toxicity of nonfiltered stock to that of filtered.

In contrast, filtration of the slow-mix stock solution (Table 14) produced little change in toxicity, hence, in the mixture of toxic components (average LRCT for nonfiltered, 2.4; average for filtered, 3.0). The DI also indicated little change in overall toxicity of the SRC suspension. The average RH was 1.9, considerably lower than the corresponding fast-mix case, even when data for 8 hours were included, where a low LRCT was recorded. These conclusions are largely substantiated by data in Tables 11 and 12, which indicate little change in chemistry or carbon concentration as a result of filtration since the major organic constitutents were water soluble phenols.

The toxicity of chemicals in solution from the aeration experiments, as well as overall toxicity of stock solutions, also can be analyzed using LRCT, DI, and RH. As aeration of the fast-mix stock proceeded, LRCT values rose to

more than four times the initial sample value, whereas the LRCT for nonaerated stock remained essentially constant. The 8-, 24-, and 32-hour samples showed a significant difference between aerated and nonaerated treatments. The DI for the aerated fast-mix experiment became appreciably lower than the initial value after 8 hours, while RH values steadily increased. However, differences in DI between aerated and nonaerated slow-mixed stocks were small at each sampling time, yielding consistently low RH values.

In studies relating to the toxicity of largely water-insoluble bituminous materials (including petroleum, shale oil, and liquified coal), the concepts of LRCT, DI, and RH are valuable for comparing different procedures for preparing exposure solutions. In many cases, the method of introducing bituminous materials to water may have more influence on the measured toxicity than on the chemical composition of those materials. Thus, a determination of the range of toxic response through use of LRCT and DI measurements would be prerequisite to a comparative study of toxicity in aquatic systems using different liquid fuels.

5.1.2 EXAMINATION OF PROCEDURES FOR ACUTE TOXICITY WITH FATHEAD MINNOWS AND SRC LIQUIDS

Background

The purpose of this study was to identify the best method of exposing fish to water soluble fractions of coal synfuel blends in order to obtain reproducible and comparable results in a continuing test series (see Becker and Crass 1981).

In an initial series of static acute tests, dissolved oxygen (DO) levels in 25 L aquaria dropped from saturation values (9 mg/L and up) to less than 1 mg/L during the standard 96-hour exposure, thus subjecting fathead minnows to low oxygen stress. After partial (3/4) renewal of test solutions every 24 hours, DO values still dropped to unacceptable levels. Bacteriological samples taken with DO samples indicated that a rapid build-up of bacteria coincided with oxygen deficits and reduction in phenol concentrations. The bacteria apparently utilized the phenol as a nutrient source while consuming

dissolved oxygen. Oxygen deficits did not occur in companion static tests with daphnia and midge fly larvae in jars containing 200 mL solution.

When exposure solutions were renewed in each aquarium every 24 hours, DO levels remained satisfactory through the first 2 days, but often dropped to 2 to 3 mg/L before each subsequent replacement. In addition, temporary removal of test fish while solutions were changed added an additional stress influencing 96-hour median lethal concentration (LC50) values.

Based on these experiences, we conducted a series of abbreviated tests to examine the influence of experimental methods on results of acute tests, expressed as mortality of fathead minnow at 24-hour intervals during a 96-hour exposure period. Maintaining DO and total phenol concentrations in test solutions while minimizing fish disturbance were main concerns.

Experimental

The following treatments were examined in two consecutive test series. Each treatment represents one aquarium containing 20 fathead minnows (except where noted). The first test series involved seven different treatments: 1) 12-hour solution renewal; 2) 24-hour solution renewal; 3) 3.5 volume additions per day (flow-through); 4) 9.0 volume additions per day (flow-through); 5) 24-hour solution renewal, with light aeration; 6) 24-hour solution renewal, fish pretreated with a bactericide; and 7) 24-hour solution renewal, reduced biomass (10 fish). The second test series, contained five treatments: 1) no toxicant, fish only (static); 2) 12-hour solution renewal; 3) 24-hour solution renewal; 4) 9.0 volume additions per day (flow-through); and 5) exposure solution without fish (static). The first test provided initial data for evaluation. The second test replicated critical treatments from the first series and provided data on two additional treatments.

Results and Discussion

First Series

Dissolved Oxygen. DO levels were high only during the initial 24 hours for all treatments (Figure 14). Treatments involving total renewal of exposure solutions every 24 hours (aeration, low fish biomass, and pretreatment of fish with a bactericide) had the lowest DO levels at 72 and 96 hours, respectively.

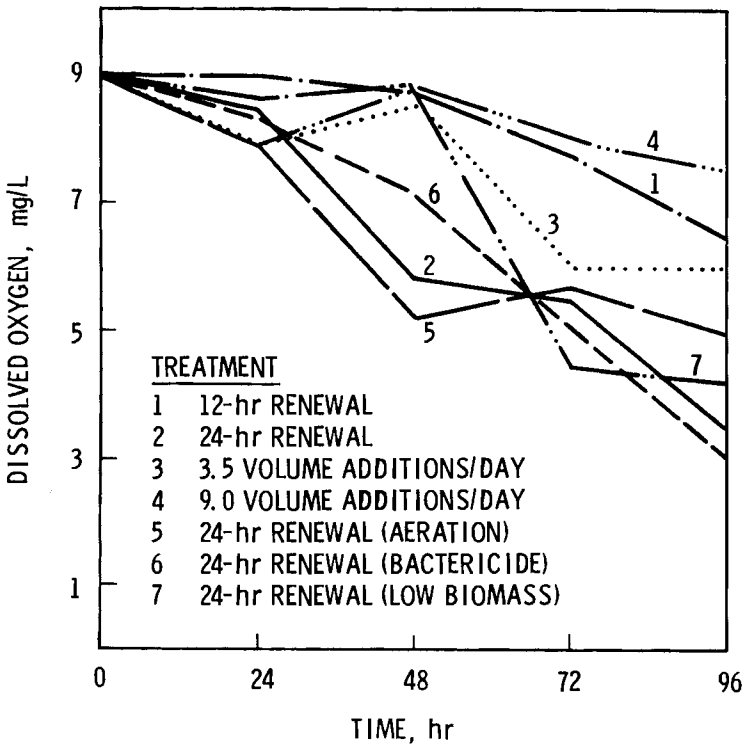

FIGURE 14. Dissolved Oxygen Levels During Each Treatment First Test Series

Although replacement of test solutions returned DO to initial levels (9.0 mg/L), they declined to progressively lower levels each 24-hour period, with the lowest levels present at termination. Acceptable DO levels were maintained with either 12-hour renewal or with 9.0 and 3.5 volume additions per day treatments.

Interrelations. All treatments except flow-through exhibited similar relationships among DO, total phenol, and mortality of fathead minnows (Figure 15). As oxygen and total phenol levels dropped, mortality of minnows increased so that 50% kill was reached at either 72 or 96 hours. Fish mortality did not exceed 10% in flow-through treatments. However, DO and total phenol concentrations declined progressively more at 3.5 than at 9.0 volume additions per day.

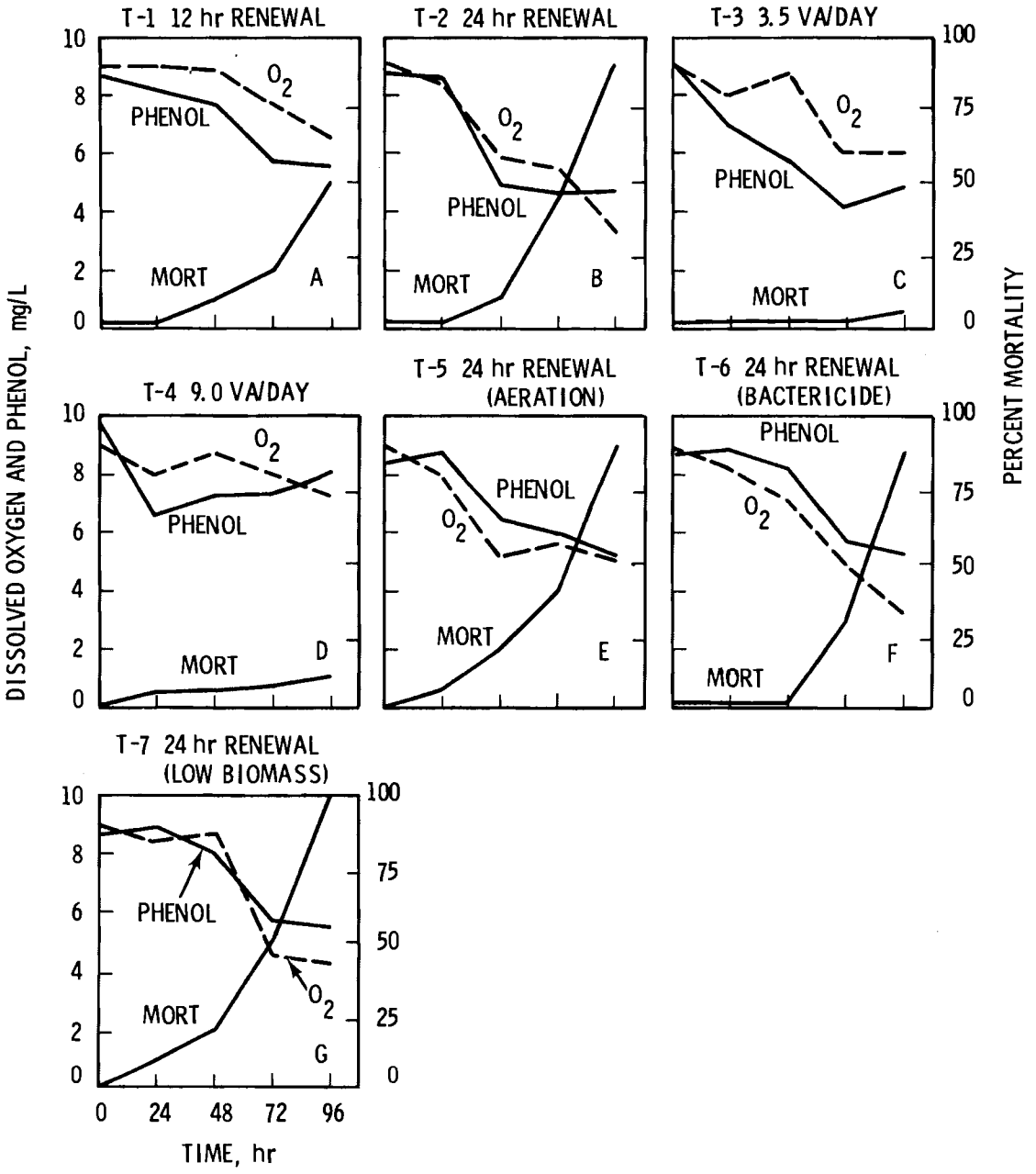

FIGURE 15. Interrelationship of Dissolved Oxygen, Phenol, and Fish Mortality During Each of Seven Treatments, First Test Series

Declines in total phenol during static tests corresponded to declines in DO. Although replacement of test solutions returned total phenol to original levels, the trend was similar to that of oxygen in that progressively lower phenol levels occurred over successive replacements until test termination at 96 hours.

Remarks, Treatment 1. Complete renewal of stock solution each 12 hours maintained suitable DO levels, but, as in other static treatments, total phenol declined. Although oxygen levels remained acceptable, fish mortality increased sharply between 48 and 72 hours, which was also the trend in other static treatments. Increased handling of fish when solutions were changed daily may have imparted stress and contributed to mortality.

Remarks, Treatment 2. Complete renewal of stock solutions each 24 hours was standard procedure for initial acute tests. DO and total phenol declined despite periodic replacement of stock, and fish mortality was high. Presumably mortality was influenced by the toxicant and low DO acting in concert. Bacterial populations that appeared after 24 hours (monitored in earlier test) apparently utilized the total phenol component and, in combination with fish respiration, reduced oxygen levels.

Remarks, Treatments 3 and 4. Flow-through treatments differed from static treatments by automatically and gradually providing a number of volumetric additions daily with minimal disturbance of fish. The 3.5 volume additions per day treatment was less capable of maintaining DO and total phenol levels than 9.0 additions per day. A smaller exposure chamber with a flow through rate of 9.0 volume addition per day might yield constant DO and total phenol levels, yet would require similar or lower volumes of stock solution than were used in the static tests.

Resistance of fathead minnow to the WSF was greater in flow-through than in static treatments, even though measured total phenol concentrations were initially similar. Thus, results of flow-through tests were not comparable with those of static tests.

Remarks, Treatments 5, 6 and 7. Modifications of the 24-hour renewal treatment did not produce results that differed significantly from the 12- to 24-hour renewal treatments. Mildly aerating a static aquarium with an airstone maintained DO levels to a degree, but posed a potential problem through oxidation of total phenols, changes in pH, or loss of volatile organic compounds during the 96-hour exposure. Pretreatment of fish with a bactericide to reduce bacteria associated with the mucus and/or metabolic waste delayed onset of mortality until 48 hours but did not affect DO or total phenol. Reducing number of test fish from 20 to 10 showed no effect.

Analysis. Statistical analysis indicated that fish mortality was high under lower DO regimes over the 96-hour exposure period. A significant ($\alpha = 0.05$) negative correlation occurred between DO concentrations and mortality ($r = 0.827$).

Fish mortality did not appear to be directly associated with total phenol levels. A nonsignificant ($\alpha = 0.05$) negative correlation was found between total phenol concentration and observed mortality ($r = 0.490$).

Second Series

Dissolved Oxygen. Again DO levels remained high only during the initial 24 hours for all treatments (Figure 16). Moreover, DO remained high under static conditions with 20 fathead minnows and no toxicant, indicating that fish respiration alone did not reduce oxygen concentrations. Also, DO was maintained over the 96-hour exposure period with a flow-through rate representing 9.0 volume additions per day.

Replicate tests involving replacement of test solutions each 12 or 24 hours again failed to maintain DO levels. The greatest oxygen decline occurred in the static solution containing only toxicant. This represents either high oxygen demand of the WSF, development of bacteria utilizing the WSF as a nutrient source and consuming oxygen, or both.

Interrelations. Replicate treatments (T-2, T-3 and T-4; see Figure 17) showed relationships among DO, total phenol level, and fish mortality comparable to those in the first test. Mortality of fish in the flow-through treatment reached 25% in 96 hours.

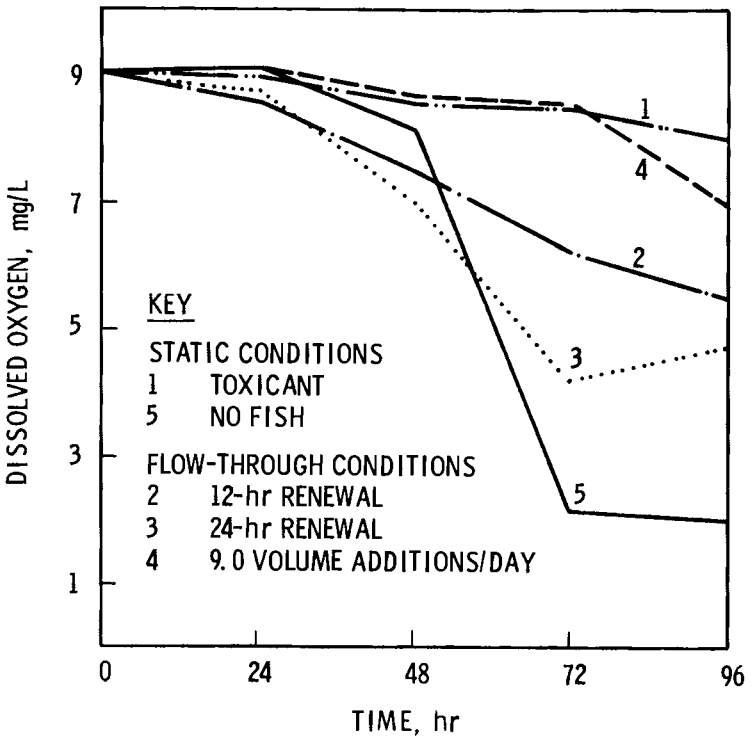

FIGURE 16. Dissolved Oxygen Levels During Each Treatment Second Test Series

Remarks, Treatment 1. There was no toxicant in this control. DO levels remained high. Fish were dipped from the exposure chamber to a bucket each 12 hours and returned. Two fish died in 24 hours, suggesting that dipping is a mortality factor associated with static tests when solutions are changed on a 12- or 24-hour basis. Fish respiration had little effect on DO.

Remarks, Treatment 5. The decline in DO under static conditions was accompanied by a decline in total phenol level, and was presumably associated with bacterial degradation during 96 hours.

Analysis. Treatments were insufficient to correlate DO or total phenol concentration with fish mortality. No statistically significant difference was found in fish mortality between the 12- and 24-hour renewal treatments. However, fish mortality in renewals differed significantly from that in other treatments.

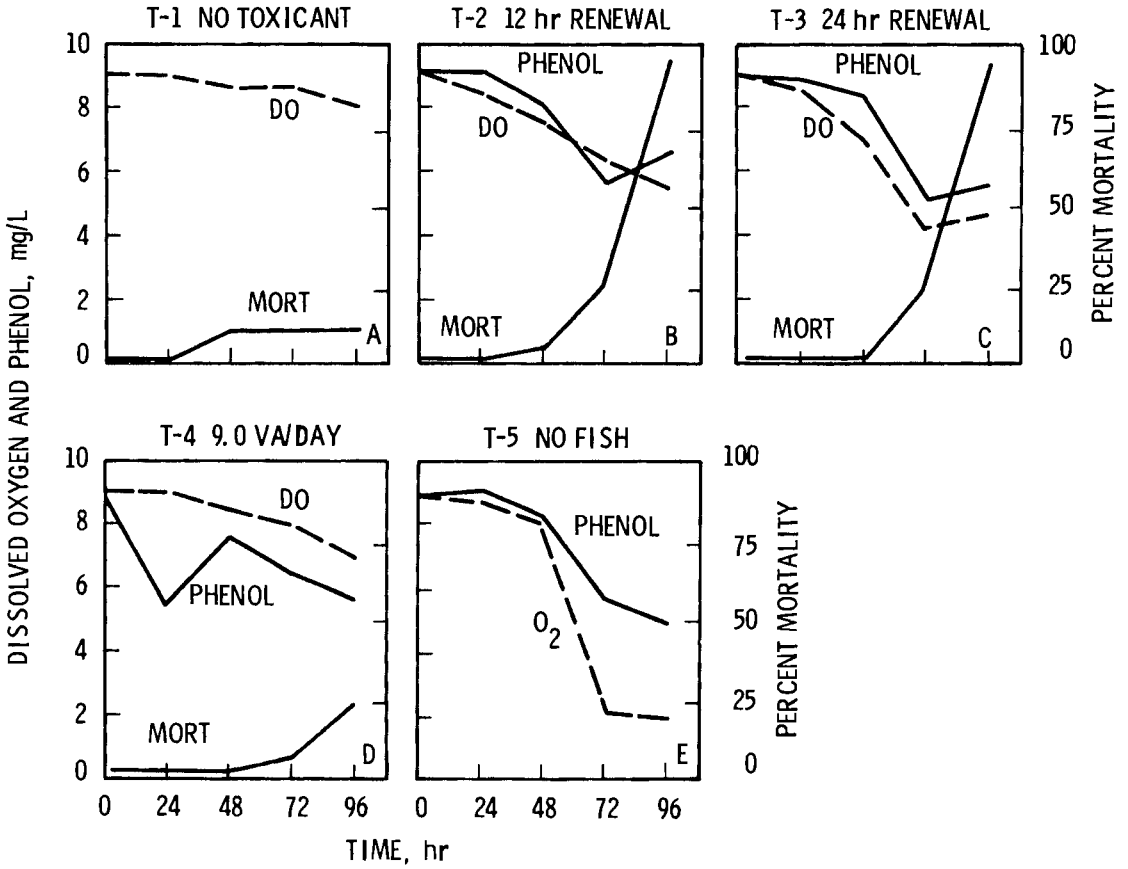

FIGURE 17. Interrelationships of Dissolved Oxygen, Phenol, and Fish Mortality During Each of Five Treatments, Second Test

For statistical assessment, data from each test, each with 1 degree freedom (df), were combined to provide cumulative results based on a Chi-square analyses of 2 df. Flow-through treatments differed significantly from both renewal methods. The 12-hour renewal treatment differed significantly from the 24-hour renewal.

Acute toxicity tests can be conducted by at least four procedures (ASTM 1979a): 1) static, in which test solutions and organisms are placed in chambers and kept there for the exposure period; 2) recirculation, in which each test solution is continuously circulated through an apparatus to filter, aerate and sterilize the water; 3) renewal, in which test organisms are periodically exposed to fresh solution, usually once every 24 hours, by transferring fish

or replacement of solution; and 4) flow-through, in which the test solution flows into and from the test chamber continuously at a monitored rate. Application of different acute test procedures with the same toxicant and test organisms are likely to yield different results more often than not, particularly if the method influences toxicity during exposure.

In general, static acute tests provide the most easily obtained measure of toxicity, but cannot last longer than 96 hours because DO, toxicant, and pH may change, and because degradation may occur and metabolic products may accumulate. Flow-through acute tests are preferable with toxicants that are highly volatile, unstable in aqueous solution, removed by test organisms during exposure in significant quantities, or have a high oxygen demand. Use of recirculation and renewal techniques may be justified in other cases (ASTM 1979a). Low DO stress should be avoided (Scheier and Burton 1973), particularly with fish as the test organism.

Toxicity tests for acute studies are conducted with the WSF derived from the first extraction, as described under Section 5.1.1, "Design and Ranging Experiments." Theoretically, the first extraction represents the most hazardous, short-term condition to aquatic life arising from an accidental spill.

These tests permit evaluation of certain properties associated with the WSF of synfuel blends that help establish procedures for conducting acute toxicity tests with fish.

Conclusions

Under static conditions:

- Unacceptable declines in DO and total phenol (e.g., total dye-complexable phenols) concentrations occur over 96 hours.
- DO declines are not a result of fish respiration.
- Decline in total phenol is not due to loss of volatile compounds.
- Fish mortality is inversely correlated with declines in DO and total phenol level, and is greater over 96 hour under static than flow-through conditions.

- Test solutions support the growth of microorganisms, presumably due to nutrients provided by phenol components of the WSF.
- Development of microorganisms is related to declines in DO and total phenol.

Under renewal conditions:

- Complete renewal of exposure solutions every 24 hours does not prevent DO and total phenol declines.
- Complete renewal of exposure solutions every 12 hours retards, but does not entirely prevent, DO and total phenol declines.
- Transfer of test organisms every 12 hours is stressful to fish and contributes to mortality.
- The pattern of fish mortality is similar to that for fish exposed under static conditions.
- Renewal requires maximum expenditure of time and manpower.

Under flow-through conditions:

- Replacement of 3.5 volumes daily will not maintain adequate DO and phenol levels.
- Replacement of 9.0 volumes daily will maintain acceptable DO and phenol levels.
- Fish mortality is lower over 96 hours than under static conditions.

The interrelated attributes of DO and total phenol in our studies are evident in other toxicological studies with phenol and related compounds. According to Buikema, McGinniss and Cairns (1979), who cite supporting references: 1) once phenols or phenolic compounds enter an aquatic system, microbial action (primarily from species of Pseudomonas) decomposes them rapidly; 2) microbial degradation can be a more significant problem in static and static-with-renewal toxicity tests than in continuous-flow tests; and 3) low oxygen levels increase the sensitivity of fish and various aquatic invertebrates to phenol.

The words "total phenol" in our analyses refer to total dye-complexable phenol in a complex mixture of different phenolic compounds. Preliminary data suggest that actual total phenol concentrations may exceed dye-complexable phenol by 70%. While the literature indicates that most phenolics are easily degraded by bacteria and some are metabolized by fish, others are refractory to biological degradation (Thurston et al. 1979).

The data indicate that future acute toxicity tests involving fish and WSFs of coal synfuel blends should be conducted under flow-through conditions. This method provides relatively stable DO and phenol levels over the standard 96-hour exposure and avoids needless stress to test organisms. Therefore, it generates reproducible data.

Although fish undergo some stress during solution change, the static method with 24-hour renewal might be used where flow-though units are not available. If so, 48-hour tests are more desirable than 96-hour tests because low DO and phenol levels are likely to occur at 72 and 96 hours as bacterial populations are established. Solution replacement retards rather than prevents microbial degradation.

5.1.3 EXAMINATION OF THE EFFECTS OF WATER QUALITY ON ACUTE TOXICITY OF SRC WATER SOLUBLE FRACTIONS

Background

Differences in water quality can influence the results of acute and chronic toxicity tests with aquatic organisms. This consideration is important in assessing the toxicity of chemically complex synfuel blends on the basis of their WSFs because water quality is a factor in both stock preparation (solubility) and exposure (toxic action) solutions. Water quality may also influence fish susceptibility to a toxicant (resistance mechanisms) prior to and during exposure. Such changes may not prove biologically or ecologically significant, but they must be assessed.

Surface water quality will vary from one site to another. Different laboratories conducting toxicity test programs with synfuels may also use water with different quality features. Accordingly, the effect of water quality values on acute toxicity of WSFs from SRC-II was examined.

Experimental

The variables were temperature, pH and hardness. Fathead minnows, daphnids, and midge larvae were tested at 10°, 20° (standard) and 25°C. Fathead minnow and midge larvae were tested under acidic conditions at pH 6.0 and daphnids at pH 6.5, compared to standard alkaline conditions at pH 7.2 to 7.8. All three organisms were tested in hard water at ≃180 to 220 mg/L $CaCO_3$ hardness, compared to the normal standard of ≃55 to 75 mg/L $CaCO_3$ hardness.

Test temperatures were obtained by mixing heated and chilled supply water automatically by thermal control units in flow-through systems and by placing exposure chambers in a water bath. The pH levels were lowered with sulfuric acid (H_2SO_4), and were maintained automatically with a pH control unit in flow-through systems. Hardness was increased by adding a salt mixture (EPA 1975). For flow-through systems, a concentrated batch was mixed in a plastic tank prior to delivery to exposure chambers along with stock solutions.

Test organisms were gradually acclimated to and held at least 2 weeks under each variable condition (temperature, low pH, high hardness) before testing. Water used in preparation of stock solutions and in exposure chambers for stock dilution was altered to correspond to the variable being examined.

Results

Designations for acute tests (Tables 16, 17, 18, and 19) were: A = standard, B = temperatures, C = pH, and E = hardness. Results unsuitable for probit analyses and changes in protocol are indicated in the tables where appropriate. For example, data were initially obtained for daphnids from 48-hour exposures with a 24-hour post-exposure period to identify delayed mortality. However, transfer of daphnids to untreated water appeared to cause stress or starvation; therefore, post-exposure retention for daphnids was discontinued. Also, tests with midge larvae changed from 96-hour to 48-hour exposures after initial trials.

LC50 values in Tables 16 through 19 show satisfactory goodness-of-fit to the probit model. Where probit is not possible, LRCT values alone are listed.

Standard Conditions

Data obtained under standard conditions are grouped in Table 19. These values provide a foundation for comparing acute toxicity of the 2.9:1 blend with that of other synthetic and petroleum-based fuels.

Based on average LC50s, daphnids were the most sensitive organism tested (3.3 mg/L TC), followed by fathead minnow (11.1 mg/L TC) and midge larvae (13.7 mg/L TC). Daphnids also provided the most consistent data from a 48-hour exposure period. Considerable variation occurred among tests with other reference organisms. In the case of fathead minnows, LC50 values were lower under static and partial renewal conditions in early tests (A Series) than in subsequent series where flow-through conditions were used. Tests with midge larvae were often unsuitable because of two recurrent phenomena: cannabalism among controls and at low test concentrations, and narcosis at high test concentrations. In the latter, organisms were apparently anesthetized during exposure but recovered during post-exposure maintenance. Narcosis was an

TABLE 16. Probit Analysis of Acute Toxicity Tests with WSFs of a 2.9:1 SRC-II Blend and the Cladoceran *Daphnia magna* (Tabulated Values are mg/L total carbon)

Test Description[a]	LC50[b]	95% Confidence Interval	LRCT (α = 0.05)
A-1 standard			
48 hr	2.44	2.11 - 2.69	2.2
48/24 hr	2.03	1.81 - 2.25	2.2
A-2 standard			
48 hr			2.5
48/24 hr			1.4
B-1 25°C 48 hr			4.5
48/24 hr	4.91	3.86 - 8.28	4.5
B-2 10°C 48 hr			--
48/24 hr			--
B-3 20°C 48 hr	4.93	4.35 - 5.94	2.7
48/24 hr	4.74	4.22 - 5.57	2.7
B-4 25°C 48 hr	4.64	4.15 - 5.21	3.4
48/24 hr	3.48	3.06 - 3.93	2.5
B-5 20°C 48 hr	2.96	2.50 = 3.40	1.9
48/24 hr	2.37	1.82 - 2.80	1.9
B-6 10°C 48 hr	12.33	9.50 - 5.21	9.0
48/24 hr	15.71	10.30 - 91.78	6.7
C-1 pH 7.8 48 hr			2.1
pH 6.5 48 hr	6.50	5.56 - 8.15	2.9
C-2 pH 7.8 48 hr	2.91	2.44 - 3.32	2.1
pH 6.5 48 hr	3.26	2.88 - 3.63	2.1
E-1 76 mg/L $CaCO_3$			
48 hr	2.98	2.65 - 3.30	2.8
230 mg/L $CaCO_3$			
48 hr			2.1
E-2 74 mg/L $CaCO_3$			
48 hr			2.1
230 mg/L $CaCO_3$			
48 hr	1.54	0.11 - 2.52	2.1

(a) The 24-hr post-exposure period ended with B series. Transfer to freshwater probably caused some mortalities through stress and starvation.
(b) Data are omitted where unsuitable for probit model.

TABLE 17. Probit Analysis of Acute Toxicity Tests with a WSF of 2.9:1 SRC-II Blend and the Fathead Minnow Pimephales promelas (Tabulated values are mg/L total carbon)

Test Description[a]	LC50[b]	95% Confidence Interval[b]	LRCT ($\alpha = 0.05$)
A-1 standard			
96 hr	8.75[c]	8.24 - 9.31[c]	
96/24 hr	8.23	7.48 - 9.07	6.0
A-2 standard			
96 hr	9.44	9.43 - 10.43	
96/24 hr	9.94	9.42 - 10.43	10.7
A-4 standard			
96 hr	9.85	9.20 = 10.56	--
96/24 hr	8.28	7.87 - 8.70	7.7
B-1 25°C 96/24 hr			9.4
B-2 10°C 96/24 hr	11.73	10.44 - 14.09	7.7
B-3 20°C 96/24 hr			11.4
B-4 25°C 96/24 hr	11.87	11.18 - 12.67	11.5
B-5 20°C 96/24 hr			11.5
B-6 10°C 96/24 hr			18.0
C-1 pH 7.8 72 hr			11.8
pH 6.0 96/24 hr	16.30	15.25- - 18.20	11.8
C-2 pH 7.7 96 hr	15.73	14.07 - 17.86	--
96/24 hr	14.25	12.76 - 15.95	10.2
pH 6.0 96 hr	14.79	11.52 - 19.76	--
96/24 hr	14.15	13.01 - 15.44	10.2
C-3 pH 7.2 96/24 hr			8.2
pH 6.0 96 hr	12.92	8.27 - 19.80	--
96/24 hr	12.35	8.12 - 18.23	10.2
E-1 50 mg/L $CaCO_3$			
96 hr	10.08	9.21 - 10.87	8.2
155 mg/L $CaCO_3$			
96 hr	14.72	13.39 - 16.27	12.8
E-2 55 mg/L $CaCO_3$			
96 hr	11.70	7.80 - 16.24	10.2
96/24 hr			
189 mg/L $CaCO_3$			
96 hr	12.67	11.66 - 13.75	10.2
96/24 hr	11.22	10.24 - 12.17	10.2
E-3 55 mg/L $CaCO_3$			
96 hr	14.34	13.36 - 15.44	12.8
96/24 hr	13.47	12.60 - 14.42	12.8
200 mg/L $CaCO_3$			
96 hr	11.46	7.92 - 16.58	12.8
96/24 hr	11.54	7.30 - 14.79	10.2
260 Standard			
96/24 hr	12.62	11.46 - 13.84	--

(a) A-1 was a static test, A-2 was a 24-hr partial renewal test, A-4 and the B-Series were 24-hr complete renewal tests; all subsequent tests were flow-through.
(b) Data are omitted where unsuitable for probit model.
(c) Calculated with moving average method.

TABLE 18. Probit Analysis of Acute Toxicity Tests with a WSF of a 2.9:1 SRC-II Blend and Midge Larvae, Chironomus tentans. (Tabulated values are mg/L total carbon)

Description [a,b]	LC50[c]	95% Confidence Interval	LRCT (α = 0.05)
A-2 standard/sand			
48 hr	16.86	11.49 - 24.75	17.5
96 hr	11.58	5.63 - 28.82	12.3
96/24 hr	10.04	4.75 - 20.47	8.6
standard/no sand			
48 hr	17.79	12.19 - 25.97	17.5
96 hr	11.08	6.82 - 18.01	17.5
96/24 hr	9.18	5.05 - 16.67	17.5
A-4 standard/sand			
48 hr	11.48	7.92 - 16.68	11.25
96 hr	8.37	7.41 - 9.38	6.3
96/24 hr	9.76	7.60 - 12.53	6.3
B-1 25°C 96/24 hr	6.20	4.65 - 7.41	8.4
B-2 10°C 96/24 hr	(a)		20.0
B-3 20°C 96/24 hr	(a)	(a)	6.3
B-4 25°C 48/24 hr	13.40	9.82 - 17.35	16.9
B-5 20°C 48/24 hr	14.19	12.48 - 16.08	16.9
B-6 10°C 48/24 hr	(a)	(a)	---
C-1 pH 7.8 48/24 hr	9.45	6.43 - 11.33	9.8
pH 6.0 48/24 hr	32.70	24.84 - 77.56	9.8
C-2 pH 7.8 48/24 hr	17.48	15.70 - 19.50	9.8
pH 6.0 48/24 hr	17.38	15.49 - 19.53	9.8
E-1 76 mg/L $CaCO_3$			
48/24 hr			12.3
230 mg/L $CaCO_3$			
48/24 hr	33.97	28.12 - 53.21	24.0
E-2 74 mg/L $CaCO_3$			
48/24 hr			9.8
220 mg/L $CaCO_3$			
48/24 hr			9.8

(a) Clean sand was added to exposure jars as substrate, Tests A-2 to B-4; plastic tubes used to isolate individual midge larvae, Tests B-5 to C-a; papaer discs were provided as substrate, tests C-2 to termination. Changes were made in effort to lower cannibalism.
(b) Tests reduced to 48-hr exposure with B-4.
(c) Data are omitted where unsuitable for probit analysis.

TABLE 19. Summary of LC50 Values from Acute Toxicity Tests Conducted With a WSF of a 2.9:1 SRC-II Blend under Standard Conditions (Tabulated values are mg/L total carbon)

Daphnia 48 hr			Fathead minow 96/24 hr[a]			Midge larvae 48/24 hr		
Test	LC50	DI[b]	Test	LC50	DI	Test	LC50	DI
A-1	2.44	368	A-1	8.23	109	B-5	14.19	78
B-3	4.93	233	A-2	9.24	91	C-1	9.45	138
B-5	2.96	375	A-4	8.28	93	C-1	17.48	61
C-2	2.91	368	C-2	14.27	70			
E-1	2.98	3.63	E-3	13.47	74			
			260	12.62	98			
Sample size		5		6			3	
LC50		3.25		11.13			13.71	
S^2		0.938		7.07			16.293	
95% CI		2.04-4.45		8.34-13.92			3.68-23.73[c]	

(a) For fathead minnow, test A-1 was static, test A-2 was 24-hr partial renewal, test A-4 was complete renewal, and tests C-2 and E-3 and #260 were flow-through.
(b) Dilution Index = TC of Stock Solution/LC50.
(c) The large confidence interval for midge larvae results, in part, from the few replicate tests (3) under standard conditions that fit probit model.

inconsistent feature that occurred more strongly in some high concentration exposures than in others. Cannibalism was largely eliminated by adding paper disks to test containers.

Temperature Variable

Analysis of temperature effects (B Series) was done in two ways. Chi-square was used where exposure levels remained constant from one test to another. This permitted detection of changes in either LC50 or the concentration-response curve. However, TC concentrations were increased in some tests because of lower mortality in a preceding test, usually in relation to a high

(25°C) or low (10°C) temperature compared to the standard 20°C. In these instances, we examined the LC50 and slope curve directly, where non-overlapping 95% confidence intervals indicated a significant difference.

Most conparisons indicated a difference in toxicity to daphnids due to temperature, although the slopes appeared to be similar for all tests. Some difference was apparently due to the range of response within similar tests, as indicated by B-3 and B-5, both conducted at 20°C (Table 16). While there appeared to be little real difference in toxicity to daphnids at 20° and 15°C, the coal liquid was clearly less toxic at 10°C.

Fathead minnow showed considerable variation in the temperature series, and several tests gave insufficient kills (Table 17). The LC50 for A-4 was smaller, and the slope for B-2 was less, when each was compared with the other two tests. When compared by Chi-square (first way), test B-5 (20°C) was statistically different (α = 0.05) from test A-4 (20°C), B-1 (25°C), B-2 (10°C) and B-4 (25°C).

Direct comparison of midge larvae data (Table 18) was limited. By Chi-square analysis, tests A-4 (20°C) and B-2 (10°C) were significantly different at $\alpha<0.05$. Tests B-4 (25°C) and B-5 (20°C) were the only 48/24-hour tests (48-hour exposure, 24-hour post-exposure) with acceptable results, yet they had 17% kill among controls due to cannibalism.

pH Variable

Tests involving low pH (C-Series) were conducted simultaneously in pairs, one representing standard conditions (Table 16). Thus, exposure concentrations (mg/L TC) were identical, and Chi-square analysis was used to compare organism response. Daphnids could be acclimated successfully only to pH 6.5. Significant differences in one pair of tests, but not the other, was due more to the slope of the concentration-response curves than to LC50. Differences in pH had little affect on toxicity.

Hardness Variable

Tests involving high water hardness (E-Series) were also conducted in pairs. Chi-square analysis showed significant (α = 0.05) differences except

for fathead minnow, Test E-2, which was significant at $\alpha = 0.10$. The coal liquid was less toxic to the three reference organisms when prepared and delivered in hard water than in soft, standard water.

Analytical Chemistry

Chemical analyses were conducted for major phenolic and aromatic hydrocarbon compounds in stock solutions at different temperature, pH and hardness regimes. Generally, differences in composition of WSFs under different water quality conditions were minimal. Surprisingly, temperature had relatively little effect. Concentrations of phenolics were similar at 10°to 25°C. However, aromatic hydrocarbons, especially the heavier compound classes, were higher in the stock mixed at 10°C. Although the differences appeared to be real, they were not large.

Dilution Indices

Mean DIs for the standard test series (Table 19) were 341 for daphnids, 89 for fathead minnows, and 92 for midge larvae. Values for fathead minnows and midge larvae were essentially similar, although midge larvae were generally the most resistant of the three reference species.

The DI is sensitive to changes in the denominator (in this case, the LC50). A DI equal to one indicates that a 50% kill over the exposure period is produced by an undiluted WSF. Considerable dilution of WSFs obtained from the SRC-II blend was required before TC levels below acute toxicity were reached. The relatively high DIs reflected the high solublity and/or toxicity of phenoloic compounds in the coal liquid.

Discussion

All stocks were identically prepared, including those involving water quality variables. Exposure values were calculated for TC equivalence, and stocks were diluted as required for acute tests; thus, differences in statistical values associated with temperature, pH, and hardness were probably due, in large part, to variations in physiological responses of test organisms. For example, daphnid activity was greatly reduced at 10°C, which possibly affected uptake of toxicants. Hardness may also influence uptake rates because all test organisms appeared to be less susceptible in hard water than in soft.

The similarity in chemical composition of stocks prepared with the SRC-II liquid under contrasting temperature, pH and hardness regimes suggested that water quality was an inconsequential factor affecting the overall chemical composition of WSFs prepared for our screening tests. This is an important consideration for evaluating acute impacts of liquified coal spills on aquatic life in different geographical areas.

However, physical as well as chemical properties affected mixing. The SRC-II liquid tended to sink or remain suspended in the water column at 10°C, but it formed a surface slick at 20° and 25°C. The slightly higher concentration of heavier hydrocarbons in the 10°C stock solution was probably due to diminutive oil droplets dispersed in the water column. Soluble phenolics would be less affected by suspension. The differences were not reflected in stock TC values, which were 1040 and 1100 mg/L for 10° to 25°C stocks, respectively, in one comparison.

5.1.4 ACUTE TOXICITY OF SRC-II BLENDS TO EGGS AND EMBRYOS OF FATHEAD MINNOW AND CHINOOK SALMON

Background

Fish eggs are just as apt to be acutely exposed to liquid synfuel spills as are adult fish and other aquatic organisms. Fish eggs may survive brief exposures to toxicants that are lethal to fry, juveniles, and adult fish because the egg chorion may prove to be relatively impermeable. However, subsequent development may be abnormal, teratogenic individuals may be produced, and sublethal effects may appear in yearling fish.

Effects cannot be assessed solely by counting dead eggs after acute exposure. Depending upon the rate of embryogenesis, which varies among fish species, exposed eggs must be monitored through hatching and at least until fry become free-swimming. If greater test sensitivity is desired (e.g., detection of possible alteration of immune response in yearling fish), an extended rearing period is required. Relatively large numbers of test organisms (fertilized eggs) must be used to monitor sublethal responses during subsequent development.

Experimental

Tests were initiated to evaluate the effects of acute exposure of WSFs of the SRC-II blend on eggs of fathead minnow and chinook salmon. The relative susceptibility of eggs and of juvenile fish to WSFs can then be compared.

Thin sheets of clear polyethylene were fitted to the inner, concave surface of PVC pipe in which adult minnows spawn. The sheets were removed as soon as adhesive eggs were deposited, usually in 12 hours, and cut into sections containing about an equal number of eggs ($\simeq$50). Eggs on each section were counted. Sections were then floated, egg-side down, on surfaces of exposure solutions in 200-mL glass jars for 48 hours. They were then removed, rinsed, placed in jars containing fresh, filtered water at 20°C, and monitored daily through hatching until fry issued. Under normal conditions, hatching occurred in 7 days at 20°C. Mortality, development rate, and malformation were compared with controls.

Chinook salmon eggs were held in vertical-flow incubators at 10°C. Eggs were removed and exposed in groups of 20 in glass jars containing toxicant dilutions on the 22nd day after fertilization (eyed stage) for 1 hour. The eggs were then rinsed, placed in compartments in the incubators, and monitored 32 days through hatching.

Special statistical analysis of multidependent stage tests was required to assess at least four stages: egg loss, egg mortality, fry mortality and fry abnormality. Each stage of embryogenesis was conditional on the success of the preceding stage. Thus, egg mortality depended on egg loss; fry survival depended on egg hatching; and fry abnormality depended on fry surviving.

Results and Discussion

Results from a range-finding test with fathead minnow eggs are summarized in Table 20, which shows the analytical steps required. Data are shown only to test termination. Note that "egg loss" appears to be a function of exposure concentration. LRCT (α = 0.05) values for all stages were either 18 or 26 mg/L TC. The LC50 for egg mortality was 37.5 mg/L TC (34.1 to 40.9 C.I.), while that for fry mortality was 22.9 mg/L TC (17.0 to 30.9 C.I.) when eggs were exposed to the WSF within 24 hours of fertilization.

The fathead minnow egg test represents the simplest method involving fish embryogenesis for comparing relative toxicity of WSFs of liquid synfuel materials. The same egg test can be used for salmonid eggs, which incubate at lower temperatures and require up to 2 months of development before hatching.

Results of chinook salmon egg range-finding exposures are presented in Table 21. There was no egg loss, as in tests with recently fertilized fathead minnow eggs. Thus, only three developmental stages were examined statistically: egg mortality, fry mortality, and fry abnormality. Again, each stage of embryogenesis was conditional on the success of the preceding stage. With the brief exposure period used (1 hour), and the relative impermeability of the salmonid egg chorion, higher TC concentrations were required to produce a direct effect. LRCTs were 445 ppm TC for egg hatch and 178 ppm TC for abnormal

TABLE 20. Results of Exposing Fathead Minnow Eggs to Dilutions of a WSF Containing 1000 mg/L TC Prepared from a 2.9:1 SRC-II Blend

Concentration (mg/L TC)	Sample Size (A = B+C)	Eggs Lost (B) (%)	Eggs Retained (C = D+E)	Eggs Mortality (D) (%)	Eggs Hatched (E = F+G)	Fry Mortality[a] (F) (%)	Fry Survival[a] (G = G+I) (%)	Number of Fry	
								Normal (H)	Abnormal (I) (%)
Control									
0	54	0(0.0)	54	7(13.0)	47	0(0.0)	47(100.0)	46	1(2.0)
18	58	5(9.0)	53	3(6.0)	50	11(22.0)	39(78.0)	36	3(8.0)
26	80	13(16.0)	67	20(30.0)	47	35(75.0)	12(26.0)	9	3(25.0)
37	65	9(16.0)	56	28(50.0)	28	23(82.0)	5(18:0)	0	5(100.0)
53	74	17(23.0)	57	51(90.0)	6	5(83.0)	1(17.0)	0	1(100.0)
75	73	8(11.0)	65	62(95.0)	3	3(100.0)	0(0.0)	--	--
		LRCT=18 ppm TC		LRCT=26 ppm TC		LRCT=18 ppm TC		LRCT=26 ppm TC	

(a) Based on hatch.

TABLE 21. Results of Exposing Chinook Salmon Eggs for 1 hr to Dilutions of a WSF Containing 890 mg/L TC Prepared from a 2.9:1 SRC-II Blend

Concentration (mg/L TC)	Sample Size (A = B+C)	Eggs Mortality (B) (%)	Eggs Hatched (C + D+E) (%)	Fry Mortality[a] (D)	Fry Survival[a] (E = F+G) (%)	Number of Fry	
						Normal	Abnormal (%)
(Control)							
0	40	3 (8.0)	37 (92.5)	0	37 (100.0)	37	0 (0)
178	40	3 (8.0)	37 (92.5)	0	37 (100.0)	22	15 (40.5)
267	40	3 (8.0)	37 (92.5)	0	37 (100.0)	11	26 (70.3)
356	40	4 (10.0)	36 (90.0)	0	36 (100.0)	0	36 (100.0)
445	40	13 (33.0)	27 (67.5)	0	27 (100.0)	0	27 (100.0)
534	40	31 (78.0)	9 (22.5)	0	9 (100.0)	0	9 (100.0)
623	40	39 (98.0)	1 (2.5)	0	1 (100.0)	0	1 (100.0)
712	40	40 (100.0)	0 (0.0)	--	-- --	--	--
801	40	40 (100.0)	0 (0.0)	--	-- --	--	--
		LRCT = 445 ppm TC[b]			NA	LRCT = 178 ppm	

(a) Based on hatch.

fry production. There was no mortality of chinook salmon fry that hatched. Abnormality was manifest primarily through smaller size and reduced eye pigmentation.

The longer developmental period of salmonid eggs permits examination of time-to-hatching as a sublethal effect (Table 22). By means of Chi-square contingency table analysis, an overall test for a difference in hatching-time patterns for the control (c) and the first five exposures (T_1 through T_5) was found significant at $\alpha = 0.05$ ($X_{15} = 24.29$). Subsequent analysis showed the hatching-time pattern between the control (c) and T_1 and T_4 was significantly different at $\alpha \leq 0.05$.

Based on egg mortality (yolk coagulation), fathead minnow eggs appear to be more resistant to the WSF of a 2.9:1 blend (48-hour exposure) than are adult fish (96-hour exposure) (see Section 5.1.3). However, exposure times differ, and the data are not directly comparable. Acute studies with fish eggs normally require 48-hour exposures or less. Eggs of the fathead minnow are well suited for toxicity testing because they are easily obtained when required and develop rapidly at 20°C for assessment of "delayed effects." The method requires refinement, but it holds promise as a reliable way to assess the relative acute toxicity of SRC-II blends. Additional tests must be conducted to establish a firm data base.

TABLE 22. Analysis of Hatching Time Patterns of Chinook Salmon Eggs Exposed One Hour to WSF Dilutions (see Table 21).

Treatment	Hatching Date				Total Eggs Hatched
	Nov. 8-11	Nov. 12	Nov. 13	Nov. 14-17	
C	0	7	22	8	37
T_1	6	5	25	1	37
T_2	4	7	23	3	37
T_3	3	4	19	10	36
T_4	4	3	11	9	27
T_5	0	1	5	3	9

Chinook salmon eggs show potential for studies of delayed teratogenic and carcinogenic effects following brief exposure to WSFs of synfuel blends. However, the range-finding test indicates that sample sizes must be substantially increased for more definitive studies. A TC level of 178 ppm results in 40% incidence of abnormal fry. Although few eggs are needed to detect such effects, sample sizes will have to increase significantly to detect carcinogenesis and to establish "safe levels" for SRC liquids and related materials.

5.1.5 USE OF REFERENCE SPECIES TO DETERMINE ACUTE TOXICITY OF SRC-II LIQUID BLENDS

Background

Three species were originally selected as standard test organisms for routine acute toxicity testing. The three species represented different trophic levels, were widely distributed in aquatic habitats, were readily cultured, and were routinely used in toxicity testing laboratories. Because responses of aquatic species may vary widely, occasional tests with other organisms as "reference species" provide a basis for intraspecies comparison. For example, LC50s and LRCTs of the fathead minnow, a warm-water cyprinid, may not be comparable to that of rainbow trout, a cold-water salmonid. However, if the response is similar, either species might be used in acute toxicity testing with equal effectiveness, and in event of a spill, the hazard posed to both species could be similar.

Other species may be more suitable for routine acute tests with SRC-II WSFs than the standard species initially selected. Although fathead minnow and daphnids provide fairly consistent data, midge larvae do not. The response of midge larvae is influenced by cannibalism at control and at low-toxicant levels and by the phenomenon of survival narcosis, a condition in which larvae "sleep" during exposure and revive upon placement in fresh solution. Thus, tests with midge larvae may yield results that are invalid. We are presently seeking to improve methods for acute tests with midge larvae and for a more suitable and representative detritivore.

EXPERIMENTAL

Methods for conducting acute toxicity tests with reference species are identical to those used for standard species, e.g., 96-hour flow-through for fish and 48-hour static for invertebrates (see Section 5.1).

RESULTS AND DISCUSSION

The only reference species used to date is juvenile chinook salmon (*Oncorhynchus tshawytscha*), a cold-water species reared and exposed at 10°C.

The LRCT was 3.8 mg/L TC. Thus, juvenile chinook at 10°C were more sensitive than fathead minnow tested at 20°C, which gave LRCT values under standard conditions of about 8.9 to 13.3 mg/L TC. The range of toxic response (95% C.I.) was also much narrower for juvenile chinook (∿2 to 4 mg/L TC) than for fathead minnow (∿7 to 14 mg/L TC).

Although juvenile chinook salmon are more sensitive than fathead minnow to WSFs of SRC-II blends, their distribution is limited to the northwestern United States and they are available only seasonally. They have limited use in extended toxicity test programs to define aquatic hazard of synfuel blends. The rainbow trout (Salmo gairdneri) is a more representative cold-water fish species for such tests. Additionally, aquatic organisms representing different ecological niches should be evaluated.

5.2 DETERMINATION OF CHRONIC TOXICITY REPSONSE IN AQUATIC BIOTA

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These studies were directed to evaluate potential sublethal and chronic effects of material derived from the Solvent Refined Coal (SRC-II) process on freshwater organisms. Species studied to date include a green algae (Selenastrum capricornutum), a zooplankter (Daphnia magna), two benthic macroinvertebrates (Chironomus tentans and Tanytarsus dissimilis), and the fathead minnow (Pimephales promelas). These species represent various trophic levels and are found in a wide variety of freshwater habitats.

An initial objective of the chronic effects studies was to develop reproducible yet realistic methods of presenting SRC-II-derived materials to test organisms. We evaluated two mixing regimes and characterized the derived test material both chemically and biologically. Screening tests were conducted with water soluble fractions (WSFs) designed to simulate chemical composition after initial contact of SRC-II liquid and water, and also with WSFs designed to represent chemical composition after SRC-II liquids had been subjected to prolonged contact with the aqueous environment. Organism exposure levels in test containers were monitored to evaluate changes in biological systems due to bacterial and chemical degradation. Flow-through test regimes were conducted to determine the potential effects of SRC-II liquids on various life stages of fish. We also initiated a series of experiments to examine interaction of sediments and SRC-II liquids and the resultant biological effects. All tests to date have been conducted with a 2.9:1 blend of middle to heavy distillate obtained from a pilot plant at Fort Lewis, Washington.

SRC-II toxicological data were of benefit to the DOE in the preparation of the Environmental Impact Statement for the SRC-II Demonstration Project at Fort Martin, West Virginia. SRC-II data are particularly useful for assessing potential impacts of accidental releases of SRC-II material to the Monongahela River and other waterways. These same data will also benefit the Environmental

Protection Agency (EPA); toxicological data (including consideration of fate and food-chain transfer) are or soon will be used by states to "set" water quality standards as they implement NEPA guidelines. For these complex organic mixtures, the development of test protocols, procedures, and methods applicable to toxicity testing and chemical characterization of complex organic mixtures has already been of use to EPA in their attempts to standardize toxicity testing.

5.2.1 EVALUATION OF BIOASSAY MIXING METHODOLOGIES

Background

Early experiments showed that the method of mixing test material SRC-II liquid with water prior to exposing organisms affected the physical and chemical characteristics and toxicity of the resulting extract (Bean et al. 1981a). Mixing methods used for acute toxicity tests may not provide a test material representative of that to which organisms are exposed after long-term interaction of complex organic material and water. Accordingly, we evaluated two mixing methods as potential simulators of weathering and dilution in nature. The first method consisted of a series of sequential batch mixes where water and SRC-II liquid were mixed and the aqueous fraction (water-soluble fraction and oil-in-water dispersion) was periodically removed. An equal volume of water was added to the remaining oil fraction to generate the next aqueous fraction. The second method involved a system where SRC-II liquid was continously recirculated in contact with flowing river water and the aqueous fraction was continuously drawn off.

Experimental

Chemical Characterization

Chemical analyses of aqueous fractions of SRC-II stock solutions included: total carbon (TC); SRC oil concentrations by infrared spectroscopy (IR); phenols by dye photometric and gas chromatography techniques; and saturate and aromatic hydrocarbons by silica gel and gas chromatography.

Concentrations of various chemical components of SRC material generally decreased with each extraction and water replacement (dilution) in the batch mix, and with time (volume of wash) in the recycle mix. TC concentrations tended to stabilize after five replacements in the batch mix and after about 120 hours at flows of 70 mL/min in the recycle mix (Figures 18 and 19). Phenol concentrations tended to stabilize sooner and with fewer replacements than did TC. The rate of loss of TC and dye complexible phenol concentrations was similar for both mix methods. However, total oils as measured by IR tended to remain at a relatively higher level in the recycle mix than at comparable TC

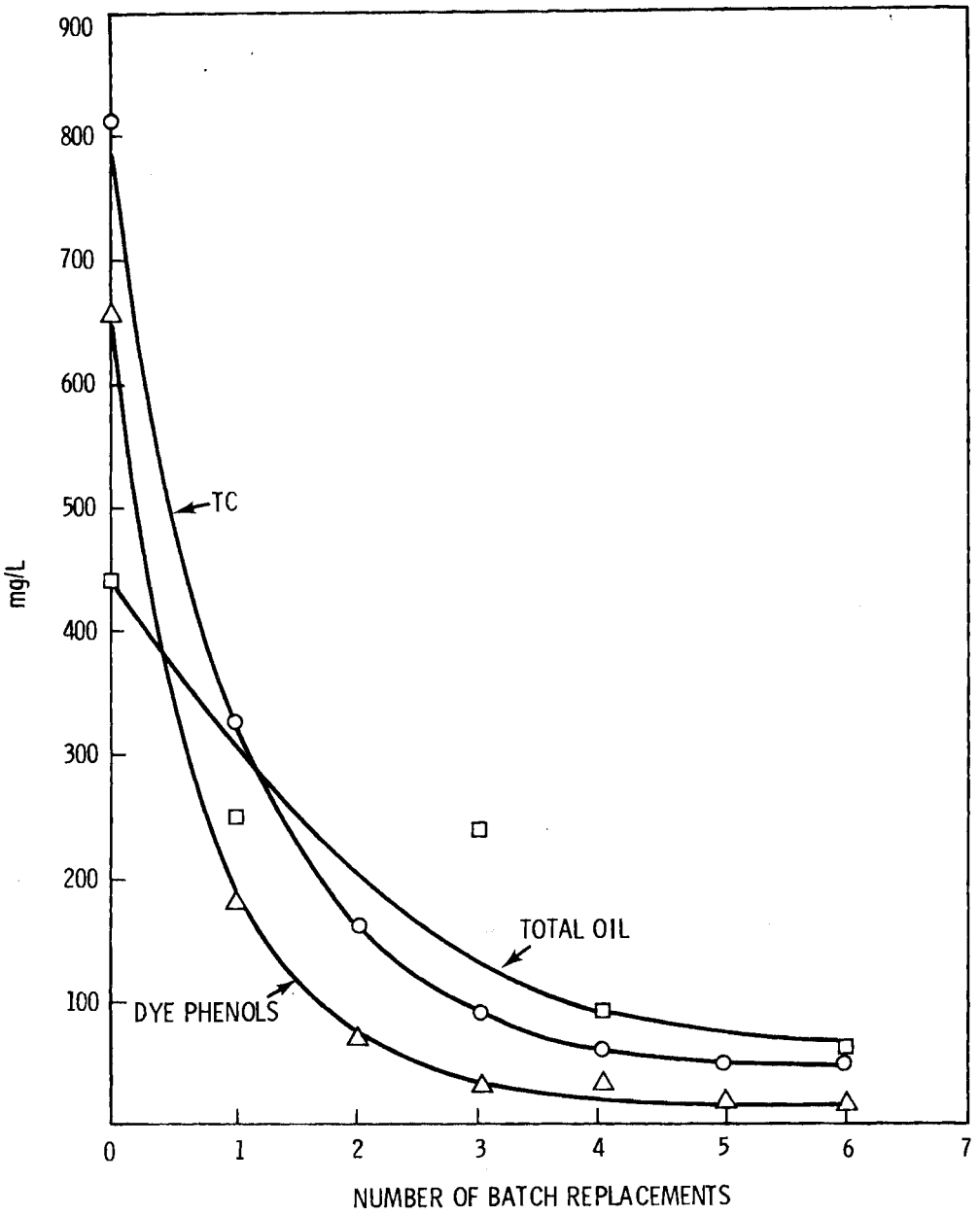

FIGURE 18. Chemistry of the Water Soluble Fraction in the Batch-Replacement Series

concentrations in the batch mix. Examination of the recycle WSF indicated that it contained more floating and dispersed particles than the WSF generated in the batch replacement series.

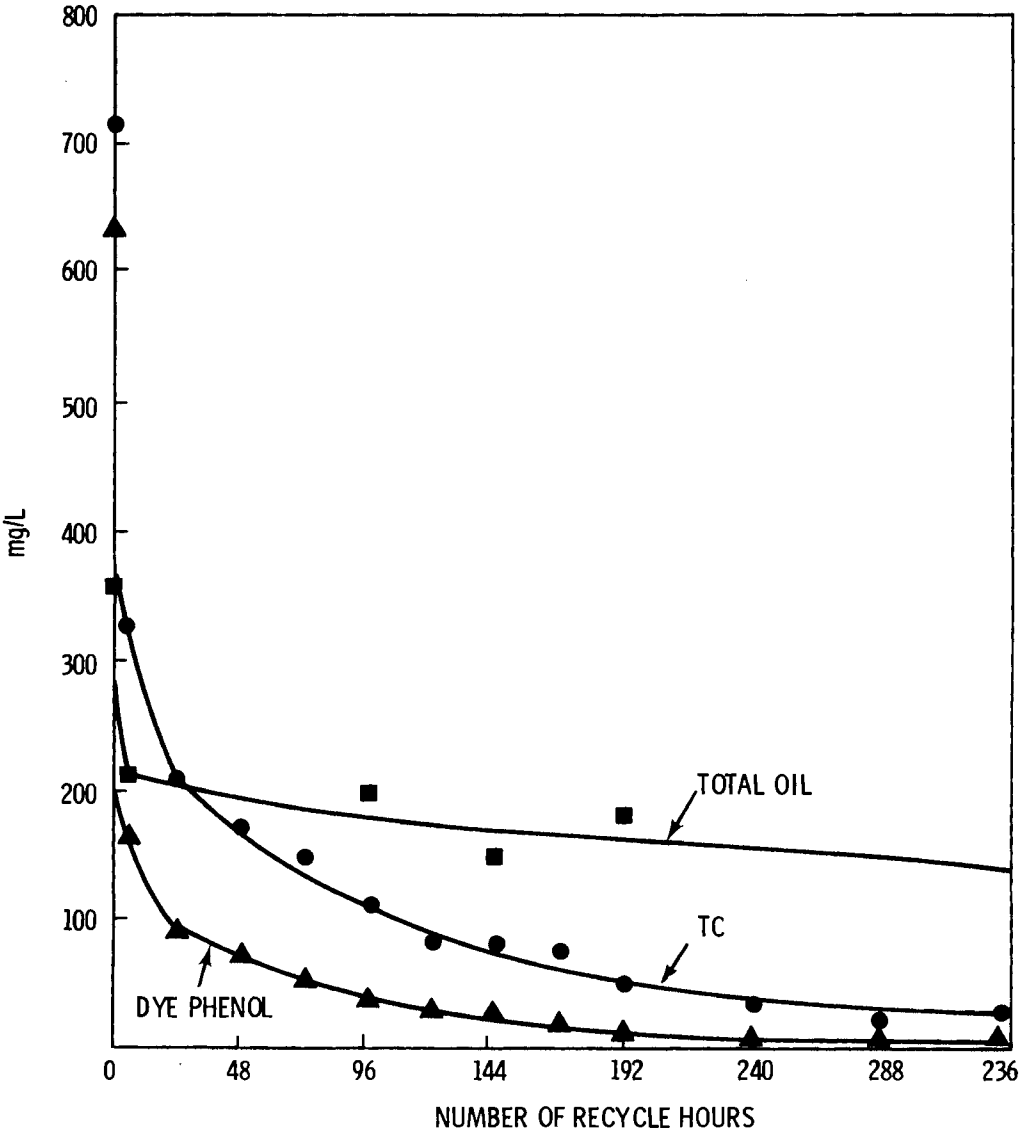

FIGURE 19. Chemistry of the Water Soluble Fraction in the Recycle-Mix Series

Biological Characterization

Exposure solutions for biological screening were obtained by diluting the stock WSF with filtered (5μ) river water. Because concentrations of each successive stock solution differed, test solution concentrations were based on TC so comparisons among successive WSFs could be obtained. Biological data are

presented as the lowest rejected concentration tested (LRCT) or lowest concentration tested in which a mortality significantly different from controls was observed. Concentrations of TC, phenol, and total oil (IR) in exposure solutions were calculated based on percent dilution of stock WSF. Although only acute toxicity (48-hour exposure) was determined, the intent of the test series was to indicate general trends in toxicity so that methodology could be developed for chronic exposure.

Results and Discussion

Batch Replacement Series

Relative toxicity of the WSFs to Daphnia magna remained similar during the replacement series based on TC concentrations (Table 23). However, since the stock solutions were less concentrated with each successive replacement, more of the WSF was needed to cause a toxic effect. Although phenol concentrations declined through the replacement series, total oil concentrations were generally higher with each replacement.

Recycle Method

Toxicity of WSFs generated by the recycle method increased over time based on TC and phenol concentrations (Table 24). This occurred despite the observation that total oil concentrations (by IR) in the recycled stock solutions remained relatively constant through 192 hours of recycling (see Figure 19). In exposure solutions generated after prolonged recycling of the WSF, total oil concentrations were up to ten times greater than in the exposure solutions generated after short-term recycling. This can happen because the dilution of stock to a given exposure concentration was based on the decreasing levels of TC in the recycled stock.

The primary difference in results between the two mixing methods was that based on TC, toxicity of stock solutions generally increased as the original SRC-II material was leached with water in the recycle series but remained relatively constant over time in the batch replacement series. The recycle-mix WSF produced an emulsion which was not as evident in WSFs obtained in the batch series. The surface oil film produced by the recycle mix entrapped some D. magna, which resulted in floatation and death.

TABLE 23. Toxicity Trends in the Batch-Replacement Series Shown as Lowest Concentration Causing Mortality Significantly Different from Control

Number of Replacement	% Dilution of Stock Solution	LRCT, mg/L[a]		
		TC	Phenols	Total oil
0	0.4	3.4	2.7	1.8
1	1.0	3.4	1.9	2.5
2	2.8	4.5	2.0	4.3
3	3.8	3.4	1.3	9.0
4	5.6	3.4	2.0	5.1
5	7.5	3.4	1.0	6.7
6	7.5	3.4	0.8	4.4

(a) Calculated values based on dilutions of stock solution.

TABLE 24. Toxicity Trends in the Recycle-Mix Series Shown as Lowest Concentration Causing Mortality Significantly Different from Control

Hr of Recycle	% Dilution of Stock Solution	LRCT, mg/L[a]		
		TC	Phenols	Total Oil
0	3.2	2.5	2.2	11.5
48	3.2	1.4[b]	0.6	5.9
96	5.1	1.4[b]	0.5	10.1
144	4.7	1.0	0.3	7.1
192	6.4	0.8[b]	0.2	11.6
240	8.6	0.6[b]	0.1	--

(a) Calculated values based on dilutions of stock solution.
(b) Lowest concentration tested.

Although the recycle mix may be a more realistic version of leaching, the batch replacement mix appeared more reproducible. The batch replacement WSF could be generated with a reasonably consistent chemical composition and was a more efficient method of producing test material. Detailed chemical analyses associated with sequential batch mixes are discussed in Section 5.2.2.

5.2.2 ANALYSES OF SUCCESSIVE AQUEOUS EXTRACTS OF SRC-II LIQUID

Background

Investigations of the chronic effects of SRC-II materials required a system or apparatus that would produce a simulated weathered oil and would provide aqueous extracts approximating those that might occur in nature over time. One approach taken was to generate successive aqueous extracts of SRC-II liquid for use in bioassays and chemical characterization studies. This section describes the generation and chemical characterization of these extracts.

Experimental

Generation of Successive Aqueous Extracts

In accordance with a standard protocol, 300 mL of the 2.9:1 middle to heavy distillate blend of SRC-II liquid was added to 29.7 L of filtered Columbia River water in a 13 gal carboy and stirred gently for 4 hours. After settling for 43 hours, an aliquot (Extract #1) was siphoned from the center of the aqueous phase for chemical and biological testing. Approximately 90% of the remaining aqueous phase was washed and replaced with an equal volume of fresh river water. The system was then mixed, settled, and sampled as before to yield Extract #2. This procedure was repeated until seven aqueous extracts were obtained.

Chemical Methods

Total carbon values were determined on a Beckman® 915B carbon analyzer.

Detailed analysis of the aqueous extracts for specific phenolic constituents was accomplished by derivatization to the phenol acetates and capillary gas chromatography of the derivatized sample.

Analysis of the aqueous extracts for hydrocarbons was accomplished by solvent extraction and capillary gas chromatography. Hexane extracts of the were separated into saturate and aromatic fractions using a modification of the procedure described by Warner (1976), which is routinely employed in these

laboratories for analyses of hydrocarbons in sediments and seawater. Analysis of the extracts for total oil was accomplished by extraction with carbon tetrachloride (CCl_4) and quantification by infrared (IR) spectroscopy.

A rapid estimate of total phenols (dye phenols) in the extracts was achieved with a modification of the direct dye photometric method (APHA, AWWA and WPCF 1976). Correlation of this rapid photometric technique with more detailed gas chromatographic analyses of phenols is discussed below.

Results and Discussion

Table 25 summarizes the results of chemical analyses conducted on the seven sequential extracts of SCR-II liquid. The extracts were complex mixtures of phenols and aromatic and saturate hydrocarbons. Concentrations of TC, total oil, phenols, and hydrocarbons generally decreased with successive extractions.

TABLE 25. Chemical Characterizations of Seven Successive Aqueous Extracts of an SRC-II Liquid (All values in mg/L)

	Aqueous Extraction Number						
Analysis	1	2	3	4	5	6	7
TC	809	324	161	89	57	46	45
Total Phenols	1069.6	406.7	188.5	85.8	58.5	39.0	26.2
Dye Phenols	660	184.9	69.6	32.9	20.1	14.2	9.7
Total Oil	442.5	247.5	152.7	235.7	90.8	88.9	--
Total Aromatic Hydrocarbons	12.28	11.9	8.5	12.0	9.8	8.9	9.6
Total Saturate Hydrocarbons ($\times 10^{-3}$)	103	47.5	46.0	29.3	15.5	11.2	25.7

The concentration of individual phenols or classes of phenols in the extracts were also determined. Although the concentrations of all phenols and phenol classes decreased in successive extracts, the correlation of concentration with extract number varied from logarithmic to nearly linear with increasing molecular weight (Figure 20). Consequently, marked changes in the relative contributions of the different classes of phenols to the total phenols were observed in successive extracts (Figure 21). In general, the relative contribution of lower molecular weight phenols decreased rapidly with successive extractions, while the contribution of heavier phenols remained constant or increased. These differences were attributed to increasing oil-to-water distribution coefficients as the average molecular weights of phenol classes increased.

Verification that concentrations of phenols in the aqueous and oil phases of the extracts had achieved equilibrium was determined by application of the multiple phase equilibration calculations decribed by McAullife (1971). Following a series of phase equilibrations (aqueous extractions), concentrations of solutes or classes in the extracts were determined. These data were plotted against extraction number. The plotted data generated a straight line (Figure 22) thus confirming that the solutes were in equilibrium when sampled. This procedure also allowed equilibrium coefficients to be estimated.

In addition to high levels of phenols, the extracts contained lower concentrations of several hundred aromatic hydrocarbons (Figure 23). Analyses indicated generally linear reductions in concentrations of aromatics with successive extractions (Figure 24). The degree of data-scatter observed in these analyses may be due to the physical disintegration of the oil phase, which increased during later extracts.

Very low levels of saturated hydrocarbons were also found in the extracts. The composition of the saturate fraction was a typical distillate envelope of nC_{10} through nC_{24} saturate hydrocarbons.

Calculations of total phenols and total aromatic hydrocarbons were converted to the corresponding total carbon as a check for organic carbon balance (Table 26). These data revealed a trend which has important experimental and environmental implications. In the first three extracts, phenols

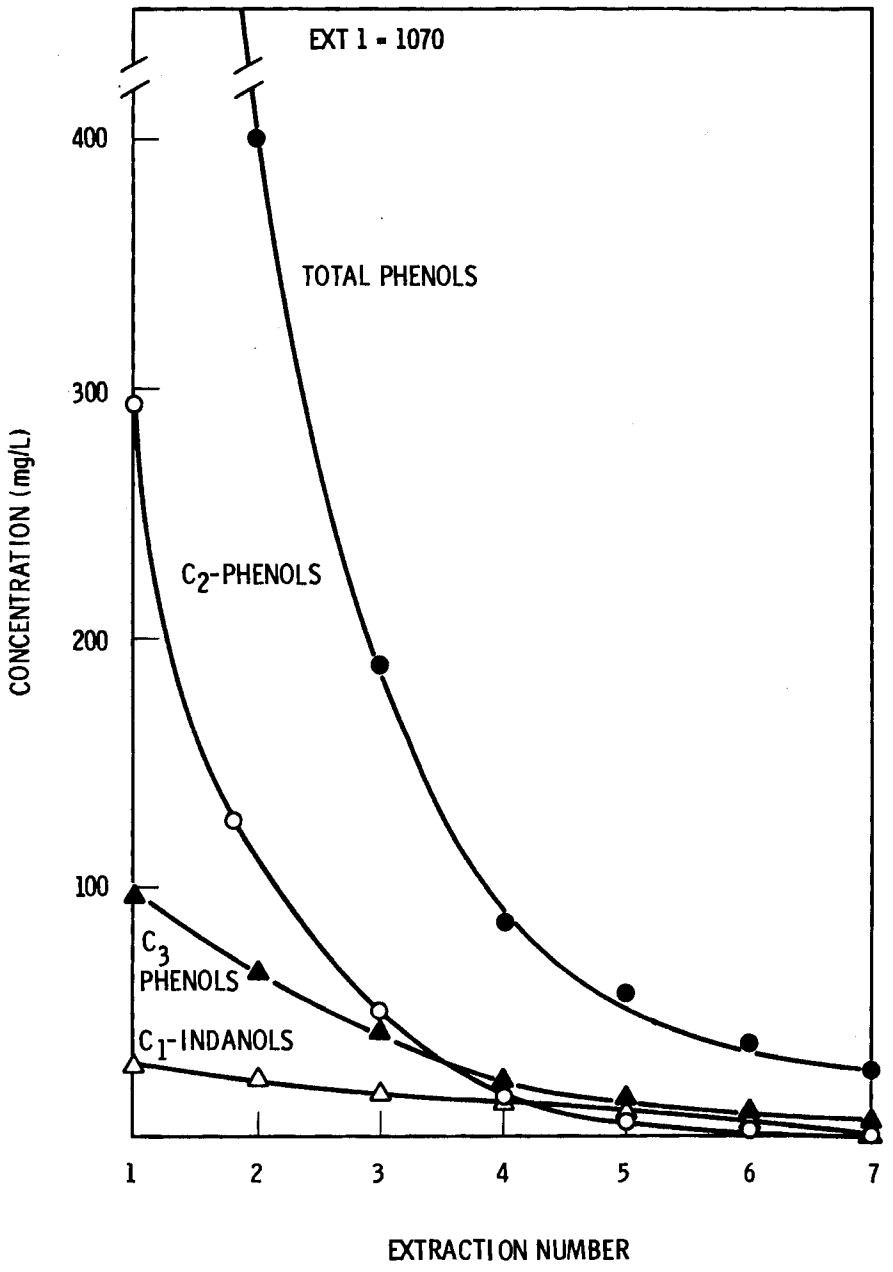

FIGURE 20. Concentrations of Total Phenols and Three Phenol Classes in Seven Successive Aqueous Extracts of an SRC-II Liquid

accounted for in excess of 90% of the total carbon while aromatic hydrocarbons contributed less than 5%. However, in subsequent extracts there was a clear trend toward equalization of the percent contribution to TC for these two classes. Moreover, phenols and aromatic hydrocarbons accounted for

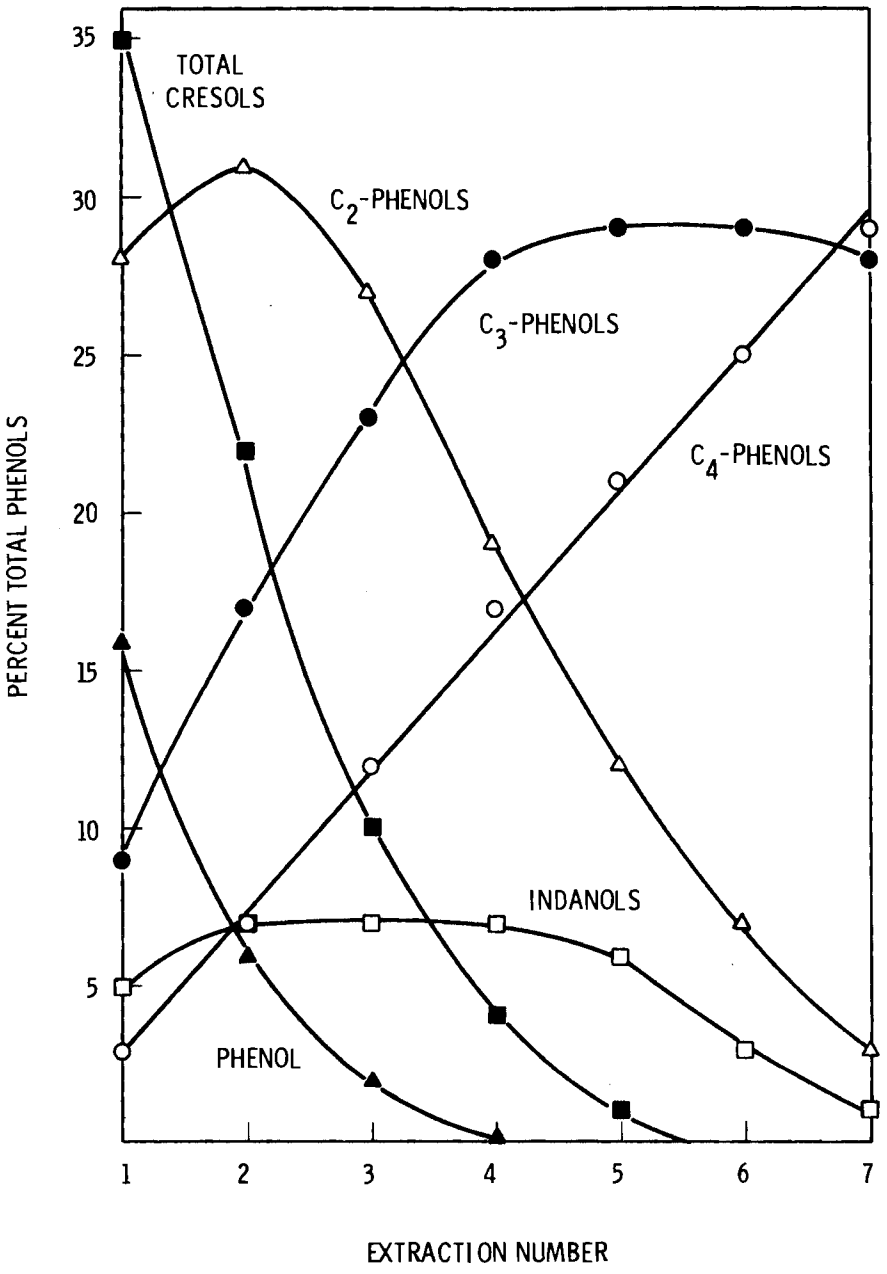

FIGURE 21. Percent of Total Phenols Represented by Various Classes of Phenols in Seven Successive Aqueous Extracts of an SRC-II Liquid

essentially all the TC in early extracts, but for considerably less of the TC in later extracts. This indicated that all the extracts contained low concentrations of other organic solutes, which contributed significantly to the TC load of the later extracts. Nitrogen compounds, especially anilines, sulfur

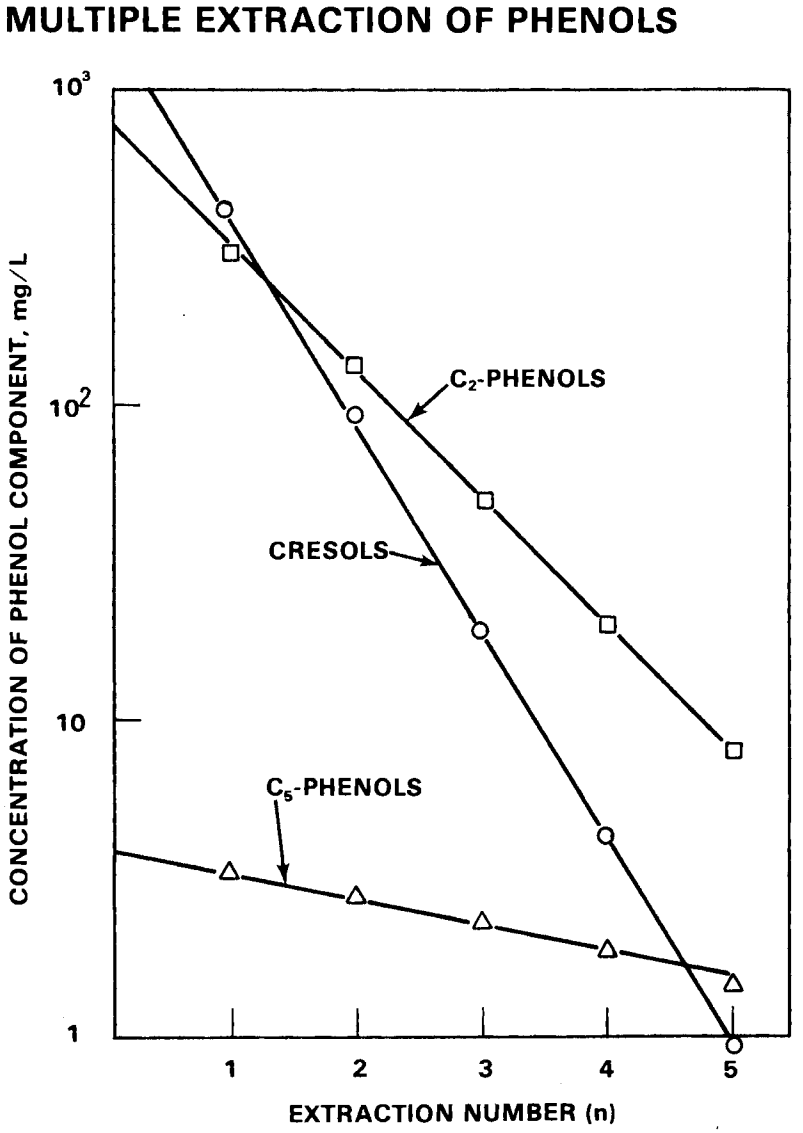

FIGURE 22. Partitioning of Three Phenol Classes Between Aqueous and Oil Phases for Five Successive Aqueous Extractions of an SRC-II Liquid

compounds, and heavier polynuclear aromatic hydrocarbons were likely candidates since they have been identified in coal and SRC-II liquids.

To observe the effects of settling time on the composition of the extracts, the first aqueous extract was also sampled after a 1-hour settling time. Tables 27 and 28 compare the phenolic and aromatic hydrocarbon composition of extract #1 after 1 and 43-hour settling times. Virtually no change was observed in the phenolic composition. In contrast, the longer settling

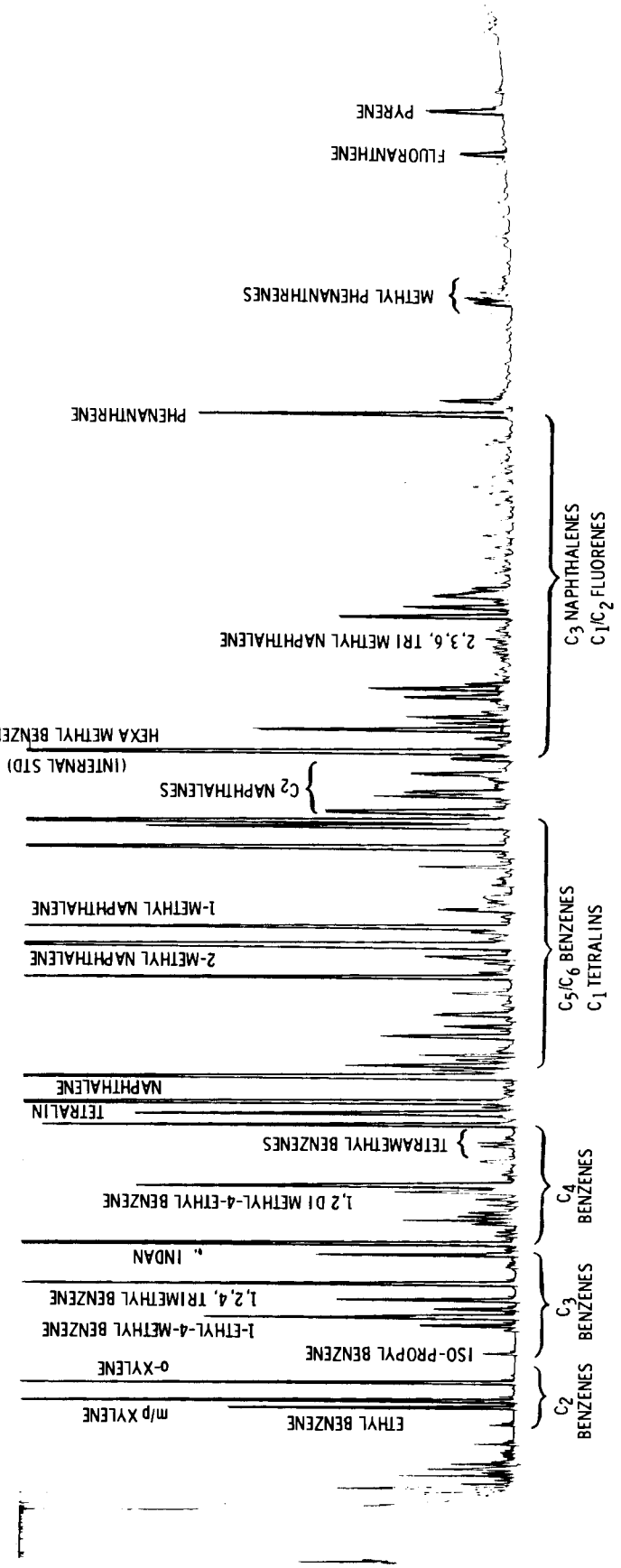

FIGURE 23. Capillary Gas Chromatogram of the Aromatic Hydrocarbon Fraction Isolated from an Aqueous Extract of an SRC-II Liquid

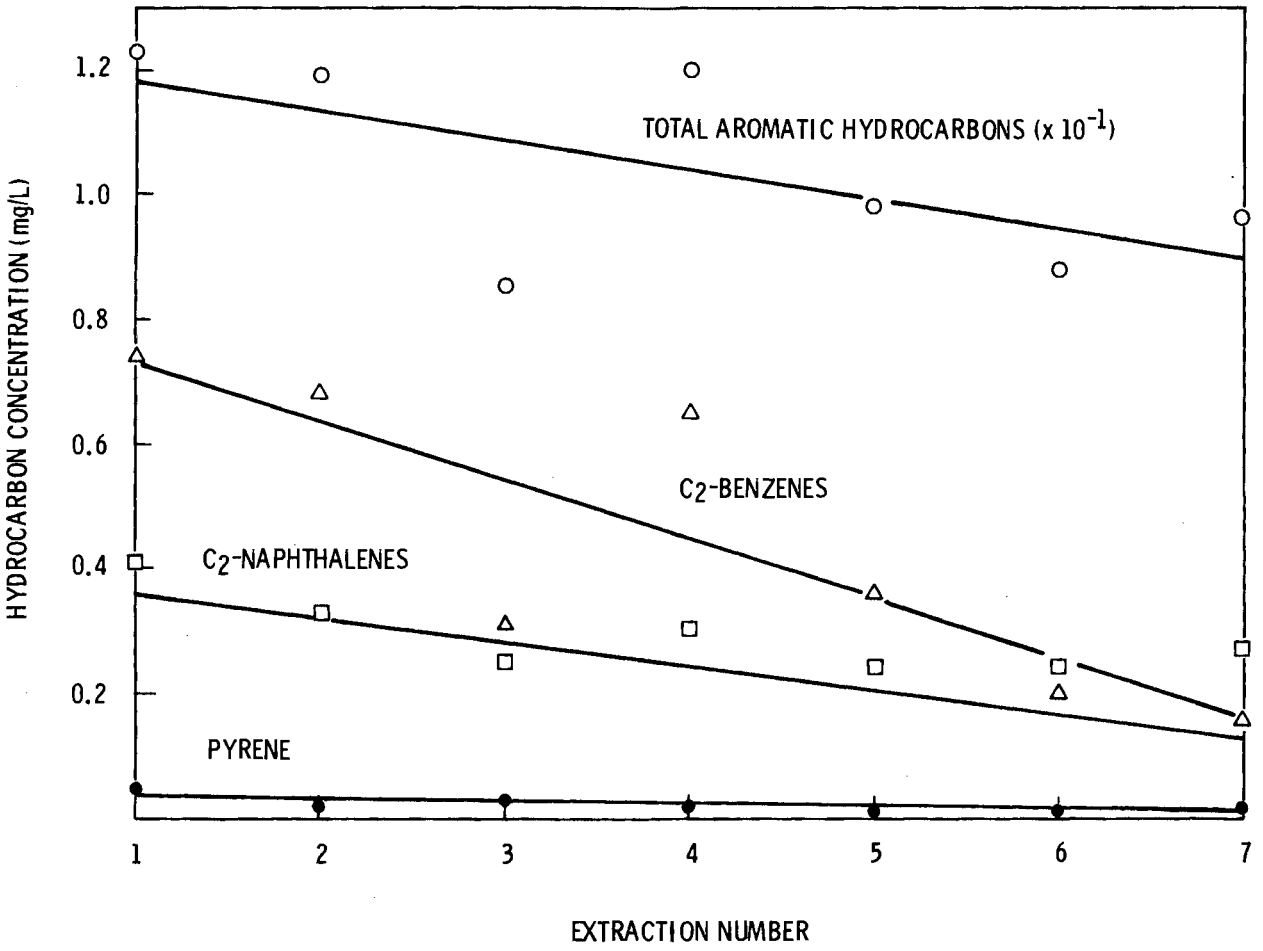

FIGURE 24. Concentrations of Total Aromatics, C_2-Benzenes, C_2-Naphthalenes, and Pyrene in Seven Successive Aqueous Extracts of an SRC-II Liquid

time was found to markedly reduce the concentrations of all aromatic hydrocarbons. Moreover the percent reduction was by no means constant for 11 classes of aromatics. Reduction in concentrations of lighter aromatics ranged from 12% to 75%, while all classes heavier than C-1 naphthalenes were reduced in excess of 80%. These data illustrate the need for invariant protocols for conducting bioassays of oil-in-water systems.

These data imply that a spill of an SRC-II liquid would initially insult aquatic organisms with a massive, transient dose of low molecular weight phenols and aromatic hydrocarbons. However, the greatest potential for

TABLE 26. Total Organic Carbon Balance in Seven Successive Aqueous Extracts of an SRC-II Liquid

Extraction Number	TC (mg/L)	% TC As Phenols	% TC as Aromatic Hydrocarbons
1	809	103.6	1.4
2	324	99.1	3.4
3	161	93.0	4.8
4	89	77.0	12.4
5	57	82.3	15.8
6	46	68.0	17.6
7	45	46.7	19.6

environmental damage probably would be from persistent, long term leaching of heavier aromatics and heteroatomic species. Chronic bioassays of SRC-II liquids should be designed with understanding of the fact that organisms will be exposed to a constantly changing spectrum of organics. Moreover, owing to the complexity of SRC-II, this spectrum will only asymptotically approach zero or constant composition. Moreover, it is reasonable to expect that the persistence of SRC-II organics in the aquatic environment would increase if source materials were to come into contact with sediments.

APHA, AWWA and WPCF (1976) provides a convenient and rapid photometric analysis for total phenols (as phenol) in aqueous samples based on the absorbance (510 nm) of a colored complex. Although the method suffers from certain limitations (Faust and Mikulewicz 1967a and b), it can be routinely used as a dosimetry tool for determining dye complexable phenols in standardized, initial aqueous extracts (Ext. #1) of SRC-II liquids. When correlated with detailed gas chromatographic analyses of phenols, the method provides a rapid estimate of total phenols and, therefore, total organic carbon in initial aqueous extracts. However, since the sensitivity of response varies inversely with molecular weight, as well as with the identity and position of substituents,

TABLE 27. Effect of Settling Time on the Aromatic Hydrocarbon Composition of an SRC-II Liquid Aqueous Extract

Component	Concentrations (mg/L) After 1 Hr	Concentrations (mg/L) After 43 Hr	% Change
C_2-Benzenes	0.84	0.74	12
C_3-Benzenes	0.79	0.67	15
Indan	0.96	0.74	23
C_4-Benzenes	1.91	0.95	50
Tetralin	1.99	0.98	51
Naphthalene	3.96	2.70	32
$C_{5,6}$-Benzenes / C_1-Tetralins	9.01	2.23	75
C_1-Naphthalenes	4.33	1.67	61
C_2-Naphthalenes	2.48	0.41	83
C_3-Naphthalenes / $C_{1,2}$-Fluorenes	6.78	0.85	87
Phenanthrene	1.35	0.18	87
C_1-Phenanthrenes	0.52	0.06	88
Fluoranthrene	0.28	0.03	89
Pyrene	0.60	0.05	92
TOTAL AROMATIC HYDROCARBONS	35.82	12.28	66

its applicability to a series of compositionally changing, sequential extracts was questionable. However, excellent linear correlation was found between concentrations of total phenols as determined by GC and by the dye method for the seven extracts. Excellent linear correlation was also found between absorbance at 510 nm and molar hydroxyl concentrations. Although linear correlations were excellent for data pairs for extracts #1 through #7, in all cases plots were found to pass much closer to the origin when data pairs from extract #1 were

omitted. This is reasonable in that extraction #1 is dominated by high concentrations of phenol, cresols and C_2-phenols (Table 28) and, with the exception of meta-substituted species, the dye method is especially sensitive to these lower molecular weight phenols.

TABLE 28. Effect of Settling Time on the Phenolic Composition of an SRC-II Liquid Aqueous Extract

Component	Concentrations (mg/L) After 1 Hr	Concentrations (mg/L) After 43 Hr	% Change
Phenol	169.17	168.07	1
o - Cesol	80.65	86.16	7
p - Cresol	169.42	168.04	1
m - Cresol	123.79	120.04	3
C_2-Phenols	292.08	294.42	1
C_3-Phenols	95.93	96.91	1
C_4-Phenols	33.12	34.86	5
C_5-Phenols	3.28	3.16	4
5-Indanol	52.78	52.67	1
C_1-Indanols	28.75	29.78	4
TOTAL PHENOLS	1059.41	1069.63	1

5.2.3 TOXICITY COMPARISONS OF WATER SOLUBLE FRACTIONS DERIVED FROM FRESH AND WATER LEACHED SRC-II LIQUIDS

Background

Mixing tests showed that physical and chemical composition of the WSF from SRC-II could be expected to vary with duration of exposure to the aquatic environment. Compounds present after initial contact of SRC-II liquid and water differed from compounds that persisted over time. These physical and chemical changes should be considered in designing chronic toxicity tests because toxic response to the altered material may also differ.

To observe a range of effects anticipated after introducing SRC-II liquid to water, we examined two water soluble fractions (WSFs) derived from the same SRC-II liquid. Experiments were designed to compare biological effects of chemicals initially present in the water after a spill with effects of chemicals expected after SRC-II liquid had been exposed to the aquatic environment. The multiple extraction procedure or batch replacement method described in Section 5.2.1 was used to simulate "initial" and "water-leached" WSFs of SRC-II liquids (Dauble et al. 1981a). Chemical analyses of WSFs were conducted to characterize the system. Exposure solutions were also analyzed to evaluate differences in biological response. Analytical chemistry is described in detail by Bean et al. (1981a).

Experimental

Biological response of several freshwater organisms to WSFs derived from the fresh and water-leached SRC-II liquid were monitored. Test organisms included two insects (*Chironomus tentans* and *Tanytarsus dissimilus*), a zooplankter (*Daphnia magna*), and a green algae (*Selenastrum capricornutum*). Invertebrate tests were conducted at 20°C with a 16:8-hour light to dark cycle. Algae tests were conducted at 24°C under continuous light. All tests except the daphnid life-cycle test were static (i.e., no replacement).

Chronic screening tests emphasized sublethal effects to the organism and long-term effects to the population. Growth and survival of *C. tentans* were determined over 14-day exposure periods that encompassed the developmental

period from second to fourth instar. Long-term survival, fecundity, and emergence timing were determined for T. dissimilis over a complete life cycle. Long-term survival and productivity of population units of D. magna were determined during 28-day exposure periods using a modification of ASTM (1979 b) procedures. Toxicity tests with S. capricornutum were conducted over a 5-day exposure period and 9-day recovery period according to protocols for Algae Assay Bottle Tests (Payne and Hall 1978; Miller, Greene and Shiroyama 1978).

Chemical Characterization

TC concentrations typically ranged from 1080 to 1220 mg/L in initial WSFs and from 112 to 140 mg/L in water-leached WSFs. Most organic carbon in the initial WSF was derived from phenolic compounds. A typical initial WSF contained about 1100 mg/L phenols compared to about 76 mg/L for a water-leached WSF. A comparison of phenol composition as determined by GC of the initial (first contact) and water-leached (fifth replacement) WSFs used for testing is shown in Table 29. Phenol composition differed in the two WSFs. Phenol, cresol, and C_2-phenols constituted 76% of the total phenols in the initial WSF but only 16% in the water-leached WSF. Differences in total aromatic hydrocarbon concentrations between the two WSFs were also detected.

Concentrations of phenolic and aromatic hydrocarbon compounds were monitored in biological test solutions to determine actual exposure concentrations during T. dissimilis and D. magna life-cycle tests. At the highest concentration tested (10 mg/L TC), compositional changes and a decrease in concentrations were noted in T. dissimilis exposure solutions. Although phenol and cresols contributed >52% of the total phenols in initial WSF solutions at day 0, they declined to negligible concentrations after 7 days (Table 30). Indanols and C_3-phenols comprised the major phenols present in water-leached WSF solutions. Their contribution to total phenols remained similar from 0 to 14 days. Water-leached WSF solutions contained over 3 times the level of aromatic hydrocarbons as initial WSF solutions when each was diluted to 10 mg/L TC. The differences in the two WSFs mainly involved amounts of naphthalenes present (Table 31).

TABLE 29. Phenol and Aromatic Hydrocarbon Concentrations in an Initial and Water-Leached Water-Soluble Fraction (WSF)

Components	Phenols, mg/L		Component	Aromatic Hydrocarbons, mg/L	
	Initial WSF	Water-Leached WSF		Initial WSF	Water-Leached 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_4-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-Phenols	2.7	0.6	$C_{5,6}$-Benzenes / C_1 Tetralins	3.9	1.2
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 / $C_{1,2}$-Fluorines	2.8	0.1
			Phenanthrene	0.5	0.1
			C_1-Phenanthrene	0.3	0.1
			Fluoranthrene	0.1	0.1
			Pyrene	0.25	0.3
			TOTAL AROMATIC HYDROCARBONS	17.4	5.4

TABLE 30. Concentration of Phenols in Initial (I) and Water-Leached (W) Static Exposure Solutions from T. dissimilis Life Cycle Experiments (all values in mg/L)

Components	Day 0		Day 7(a)		Day 14	
	I	W	I	W	I	W
Phenol	1.52	<0.01	<0.03	<0.01	<0.03	<0.01
Cresols	3.56	0.09	<0.07	<0.01	<0.07	<0.01
C_2-Phenols	2.69	0.90	0.25	0.38	0.22	0.17
C_3-Phenols	0.90	1.99	0.23	0.78	0.14	0.39
C_4-Phenols	0.27	1.63	0.14	1.09	0.09	0.68
Indanols	0.52	0.49	0.18	0.30	0.17	0.19
C_1-Indanols	0.26	1.38	0.24	1.11	0.15	0.82
C_5-Phenols	<0.01	0.06	<0.01	0.06	0.01	0.04
C_2-Indanol	<0.20	0.57	<0.20	0.44	0.20	0.32
TOTAL PHENOLS	9.72	7.11	1.04	4.16	0.77	2.61

(a) Initial day 7 values extrapolated from day 6 and day 8 concentrations.

Routine analysis of daphnid test solutions at organism introduction and 48 hours later when the toxicant was replaced also indicated rapid loss of phenols (Table 32). Only 20% of the original concentration of phenolics in initial WSF dilutions remained after 48 hours. Degradation in water-leached WSFs was not as rapid with 56% to 70% of the initial phenol concentration remaining after 48 hours.

Results and Discussion

Biological Screening

The lowest rejected concentrations tested (LRCT) when compared to the control are shown in Table 33. Since the initial WSF was more concentrated, with a TC load about 10 times greater than the water-leached WSF, more dilution was necessary to decrease toxicity below chronic levels. However, when exposure concentrations were adjusted for TC or total phenols, the water-leached WSF was usually more toxic than the initial WSF.

TABLE 31. Relative Concentrations of Aromatic Hydrocarbons in Static Exposure Solutions at the Outset of T. dissimilis Life-Cycle Experiment. (Concentrations declined to trace levels after 2 days)

Components	Initial WSF, mg/L	Water-Leached WSF, mg/L
C_4-Benzenes	0.03	0.05
Tetralin	0.02	0.08
Naphthalene	0.04	0.21
$C_{5,6}$-Benzenes / C_1-Tetralins	0.04	0.13
C_1-Naphthalenes	0.03	0.12
C_2-Naphthalenes	<0.01	<0.01
C_3-Naphthalenes / $C_{1,2}$-Fluorenes	<0.01	0.03
Others	0.06	0.11
TOTALS	0.22	0.73

TABLE 32. Concentrations of Total Phenols in Low and High Exposure Solutions During Daphnia magna Static Replacement Tests

Test Concentration mg/L TC	Initial WSF		Water-Leached WSF	
	Initial Phenol (n = 11)	48 Hr Phenol (n = 11)	Initial Phenol (n = 11)	48 Hr Phenol (n = 10)
Low (0.125)	0.43 ± 0.05	0.08 ± 0.06	0.39 ± 0.11	0.22 ± 0.08
High (1.000)	1.21 ± 0.10	0.24 ± 0.16	0.67 ± 0.11	0.47 ± 0.11

TABLE 33. Comparative Toxicity of Initial and Water-Leached Water Soluble Fractions (WSF) of SRC-II on Three Freshwater Invertebrates and a Freshwater Algae[a]

Species	Parameter	Initital WSF			Water-Leached WSF		
		% Dilution	Total C, mg/L	Phenol, mg/L	% Dilution	Total C, mg/L	Phenol, mg/L
Chironomus tentans	growth	0.43	5.1	4.7	0.60	0.60	0.3
Tanytarsus dissimilis	life cycle	0.30	3.4	2.9	0.94	1.1	0.6
Daphnia magna	reproduction	0.02	0.3	0.2	0.43	0.9	0.3
Selenastrum capricornutum	productivity (exposure)	0.42	3.4	4.5	3.95	4.6	2.0
	productivity (recovery)	6.16	60.0	61.5	6.40	7.9	2.4

(a) Data is presented as lowest tested concentration producing a significant difference in test parameter when compared to control.

D. Magna was the most sensitive organism tested. Decreased production of daphnid young occurred at TC concentrations <1 mg/L, or about 5% of the concentration known to cause acute mortality (Bean et al. 1981a). Algae were the least sensitive organism tested. Although productivity was inhibited at TC concentrations near those that caused sublethal effects in other test organisms, recovery of algae occurred after exposures to concentration 12 to 200 times greater than that found in the initial WSF. The most obvious difference in effect between the two WSFs was noted for C. tentans. The water-leached WSF inhibited growth at 0.60 mg/L TC and 0.3 mg/L phenol in contrast to 5.1 mg/L TC and 4.7 mg/L phenol using the initial WSF (Table 33).

At equal TC concentrations, exposure levels over the duration of the test period were actually greater in water-leached WSF test solutions because of the relative stability of the material in solution. Static exposure solutions derived from the initial WSF showed greater losses in phenol concentrations over time than those derived from water-leached WSFs. The rate at which various phenol classes disappeared was variable, with the lower molecular weight compounds exhibiting a greater loss over time. These data suggest that phenolics and hydrocarbons in low concentrations are subjected to rapid and selective degradation.

Since the biological impact of the SRC-II liquid differed as its chemical constituents changed due to leaching and degradation in the aquatic system, a decline in TC or phenol levels would not necessarily indicate a reduced hazard to aquatic life. These tests indicated that the compositional changes in water soluble components toward more stable, heavier molecular weight compounds could result in increased chronic effects at environmental concentrations below those causing acute mortalities.

5.2.4 DEVELOPMENT OF FLOW-THROUGH MIXING AND SEPARATION SYSTEM

Background

A flow-through mixing and separation system (Dauble et al. 1981b) was developed to produce large volumes of a reproducible stock solution for exposing organisms to sublethal concentrations of coal liquid WSF (Figure 25). The WSF obtained in the system was similar in composition to the initial WSF derived from the batch replacement method (Table 34).

Experimental

Reproducibility of the mixing and separation system was evaluated by measuring dye complexable phenol and TC concentrations in the WSF prior to dilution. Separate 14-day and 21-day tests were conducted on newly hatched (7 to 14-day-old) fathead minnow fry (Pimephales promelas) to determine sublethal exposure concentration in flow-through regimes. Phenol levels in test aquaria were analyzed daily to monitor actual organism exposure concentrations.

Results and Discussion

Significant differences in mortality of fathead minnow fry were noted at phenol concentrations equal to 1.5 mg/L TC but not at 1.3 mg/L TC. These concentrations are about 1/10 of the LC50 obtained for adult fathead minnows during acute (96 hour) tests with SRC-II WSFs of similar composition (Section 5.1.2). Significant reduction in growth (length and weight) occurred for 14-day exposures $\geq$1.3 mg/L TC and for 21-day exposures at $\geq$0.4 mg/L (weight only).

Microbial growth in the dilutor system caused problems with maintaining flow rates and consistent exposure levels at low concentrations. River water was used as a diluent and may have provided the microorganisms that in turn used phenolic compounds present in the WSF as a carbon source. DeGraeve et al. (1980) conducted 30-day flow-through bioassays with phenolic compounds and reported no excess of microbial growth. However, they used well water as a diluent.

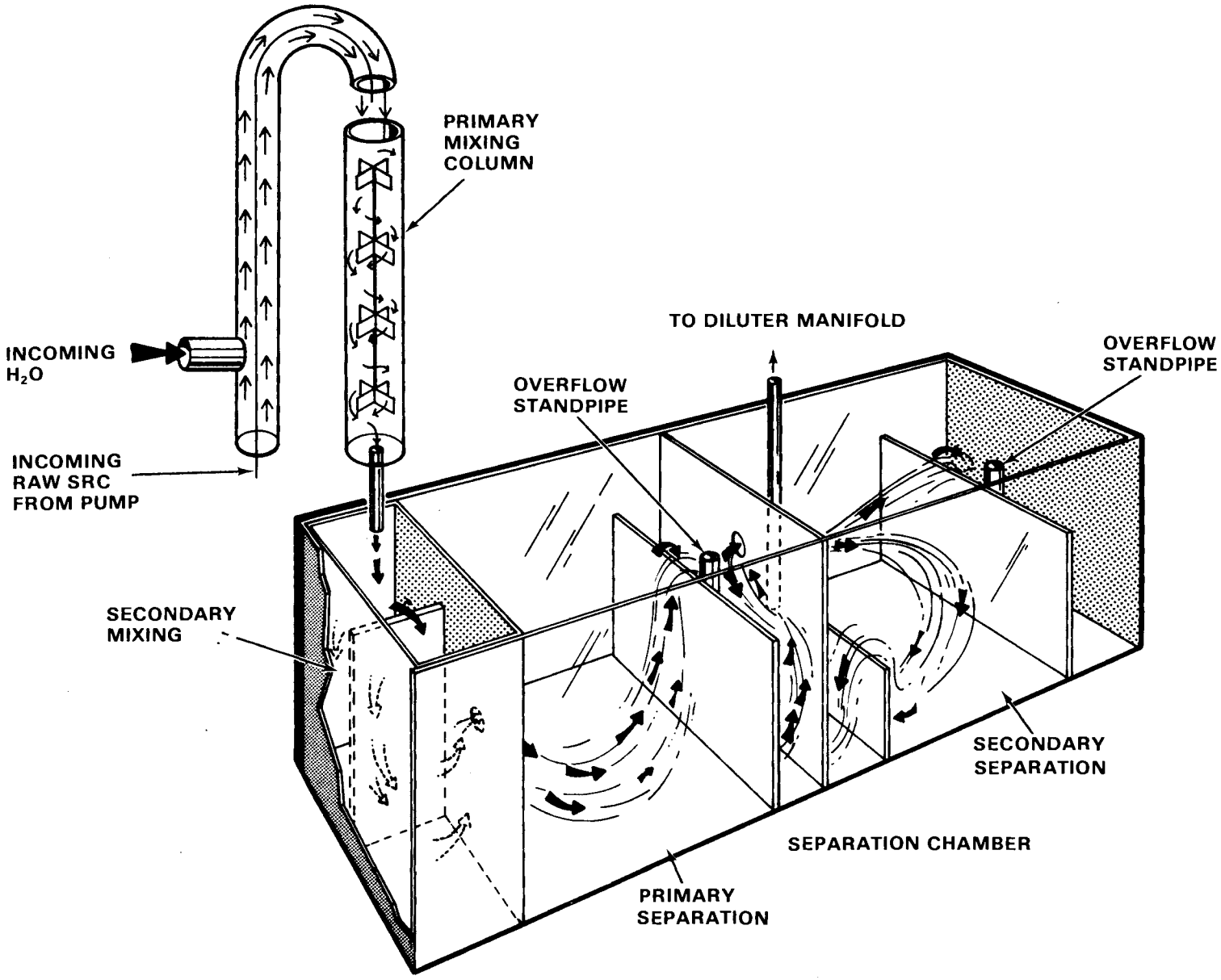

FIGURE 25. Mixing and Separation Apparatus Including Inflow and Outflow Lines and Pattern of Flow Within the System

TABLE 34. Comparison of Phenol Distribution Between a WSF Generated from the Mix-And-Separation Apparatus and a Batch-Prepared WSF

	Flow-through		Batch	
Phenol class	mg/L	% total	mg/L	% total
Phenol	140.3	15.3	162.1	16.4
Cresols	320.5	35.0	367.0	37.1
C_2-Phenols	255.0	27.8	273.8	27.7
C_3-Phenols	84.8	9.3	85.9	8.7
C_4-Phenols	31.1	3.4	14.9	1.5
Indanols	47.3	5.2	50.7	5.1
C_5-Phenols	1.2	0.1	1.3	0.1
C_1-Indanols	26.1	2.9	24.4	2.5
C_2-Indanols	9.6	1.0	7.9	0.8
TOTALS	915.9	100.0	988.0	99.9

5.2.5 FATE AND EFFECTS OF SRC-II CONTAMINATED SEDIMENTS

Background

A sediment exposure system was developed to study the potential for: 1) transformation of SRC-II material in river sediments, 2) the transfer of SRC-II material to the water column, and 3) the effect of key SRC-II compounds on selected test species.

Experimental

Sediments from the Columbia River above McNary Dam, Oregon, were contaminated with SRC-II liquid and subjected to a series of repeated mixing and water replacements or "washouts" to observe retention of phenols and hydrocarbons. Two mixture ratios were used. The high concentration series had 50 µL SRC-II liquid added to 120 g of wet sediment; the low concentration series had 20 µL SRC-II liquid added to 120 g of wet sediment. Adult daphnids (*Daphnia magna*) and chironomid (*Chironomus tentans*) larvae were used as biological indicators of toxicity in the water column and sediment fractions, respectively. Test containers of control and contaminated sediments were characterized after each series of water replacements and at test termination. Dye complexable phenols were determined in the water column. Measurements of total phenols in the sediment fractions were determined by GC.

Results and Discussion

Phenol concentrations in the water column declined with each water replacement (Figure 26). Toxicity or mortality of test organisms after 48-hour exposure also declined with each replacement. Significant mortality of daphnids was noted through three volume replacements at the low concentration series and through six replacements at the high level of sediment contamination (Table 35). Chironomid larvae were more sensitive than adult daphnids to the contaminated sediments. This may be due to larval burrowing habits, whereas daphids come in primary contact with materials suspended in the water column. Significant mortalities were noted for *C. tentans* at both levels of contamination throughout the leaching scheme (Table 35).

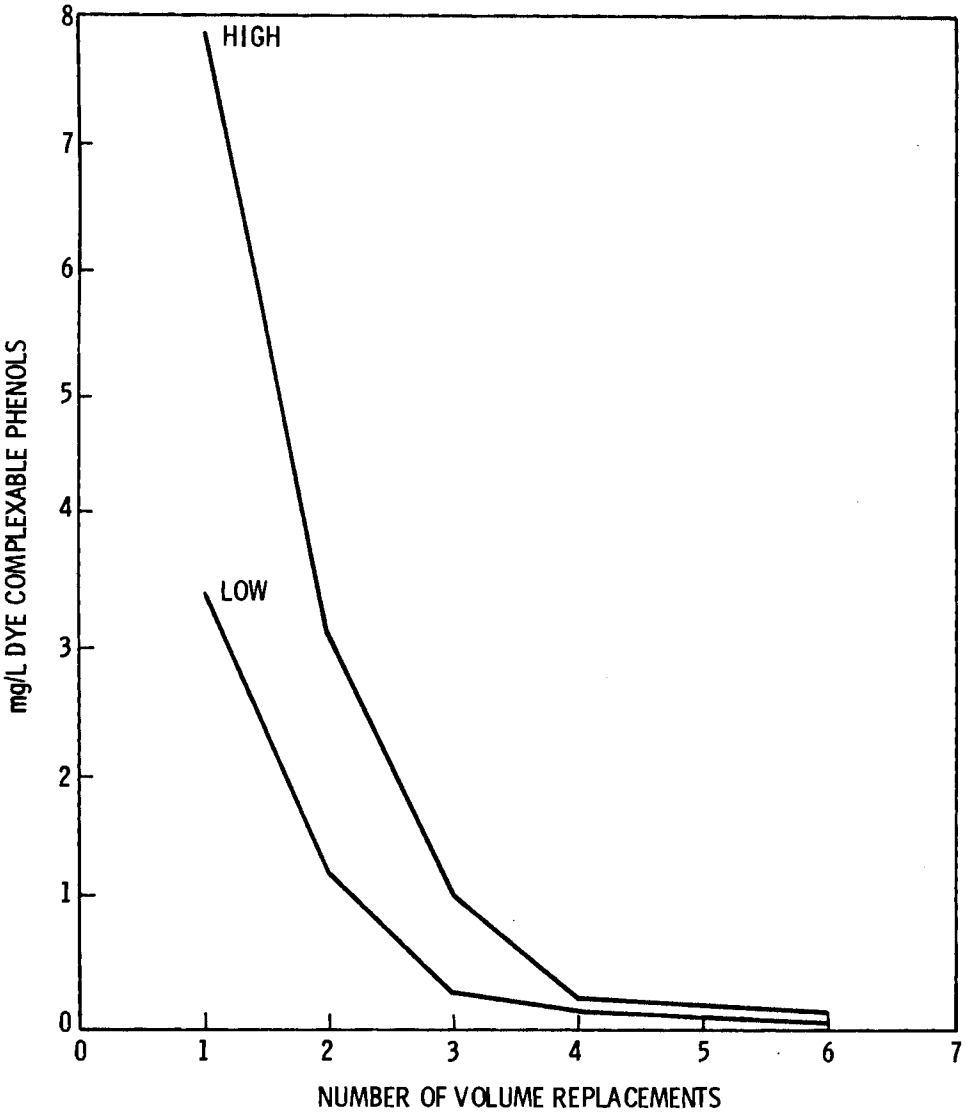

FIGURE 26. Concentrations of Dye Complexable Phenols in the Water Column as SRC-II Contaminated Sediments were Sequentially Extracted with Water

Long-term survival and growth was also monitored for 2nd instar chironomid larvae placed in SRC-II contaminated sediment (20 µL SRC: 120 g wet sediment) over a 17-day period. In contrast to the earlier test series, organisms were added after 6, 8, and 10 water replacements to insure that highly water soluble components were removed. Little growth was observed for the chironomid larvae

TABLE 35. Toxicity of Leached SRC-II Contaminated Sediment Fractions to Adult Daphnids and Larval Chironomids (Data reported as lowest concentration tested causing significantly different mortality than control after 48-hour exposure)

	Low Contamination			High Contamination		
Test Organism	# Volume Replacement	mg/L phenol[(a)] in water	mg/L phenol[(a)] in water	# Volume Replacement	mg/L phenol[(a)] in water	mg/L phenol[(b)] in sediment
Daphnid (*D. magna*)	3	0.28	1.1	6	0.11	1.6
Chironomid (*C. tentans*)	6	0.09	0.3	6	0.11	1.6

(a) Dye complexable phenols.

(b) By gas chromatograph.

residing in either control or contaminated sediment, and there was no difference in growth between treatments. However, there was a significant reduction in organism survival in all contaminated sediments when compared to controls (15% vs. 62%).

The results of the 48-hour exposure test series and the 17-day exposure test series indicated that significant mortality of sediment-dwelling insects still occurred despite removal of readily water soluble components from SRC-II liquids bound to sediments. The level of sensitivity was likely related to the age of the test organism and the duration of exposure. Benthic dwelling organisms (i.e., chironomids) were more sensitive to contaminated sediments than were organisms residing in the water column (i.e., daphnids).

5.3 COMPARATIVE TOXICITY OF SRC-II LIQUID AND REFERENCE MATERIALS

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Quantification of the relative toxicity of synfuel products to aquatic life requires that reference compounds or toxicants[1] be included in testing. Such compounds should include water soluble fractions (WSF) of a crude oil (e.g., Prudhoe Bay Crude), a refined oil (e.g., API standard oil or No. 2 and No. 6 fuel oil) or a specific chemical (e.g., phenol, pentachlorophenol, naphthalene).

1 "Reference" toxicant in the context of this report is considered to be another chemical or chemical mixture, usually a petroleum product, with which the toxicity of a synfuel can be compared. This differs from the common definition of reference toxicant (see Lee 1980).

5.3.1 ACUTE TOXICITY OF SRC-II LIQUID AND PRUDHOE BAY CRUDE OIL

Background

Considerable data are available in the literature on the acute toxicity of hydrocarbon products presently in commerce (Malins 1977). However, direct comparison with literature values are tenuous because of differences in test designs, test organisms, water quality parameters, mixing procedures, analytical precision, and other factors. In the case of crude oil, for example, reliable comparisons can be made only if the stock WSF is prepared similarly to our standard mixing procedure with an oil-to-water ratio of 1 to 99 (see Section 5.1.1). Further, accurate comparison requires use of the dilution index (DI) for few citations are available.

Experimental

Methods used for conducting acute toxicity tests with reference compounds are identical to those used for SRC-II materials, including chemical monitoring and the preparation of WSFs with hydrocarbon products (see Section 5.1.1). Prudhoe Bay Crude oil was obtained from stocks maintained at Battelle Marine Research Laboratory in Sequim Bay, Washington.

Results and Discussion

The components of Prudhoe Bay Crude (PBC) have limited solubility in water compared to the 2.9:1 SRC-II blend. When WSFs were uniformly prepared by mixing 300 mL oil with 29.7 L water, typical concentrations were 1000 mg/L TC and 640 mg/L dye complexable phenol for SRC-II, and 20 mg/L TC and 0.2 mg/L dye complexable phenol for PBC.

The WSF of Prudhoe Bay crude oil produced no mortality in fathead minnow, daphnid, or midge larvae when organisms were exposed to 13.4, 8.0 and 19.0 mg/L TC, respectively, nor was any subacute response detected. These TC levels are usually lethal when the stock is prepared with the 2.9:1 SRC-II blend. Accordingly, the fuel blend is more toxic than Prudhoe Bay crude based on similarly prepared WSFs. It is also likely that the observed difference in toxicity is related to the relative solubility of the two materials in water.

5.3.2. ACUTE AND CHRONIC TOXICITIES OF SRC-II LIQUID AND NO. 2 AND NO. 6 FUEL OILS

Background

In this study, we compared the toxicity and solubility of identically prepared WSFs of SRC-II liquid and two petroleum products (No. 2 diesel fuel and No. 6 bunker feedstock) to *Daphnia magna*.

Experimental

Preparation of Stock Solutions

The SRC-II liquid was the 2.9:1 blend of middle to heavy distillates obtained from the pilot plant at Fort Lewis, Washington. The No. 2 and No. 6 fuel oils were obtained from distributors in the Pacific Northwest and contained none of the preservatives sometimes added during storage. Test solutions of the three fuels were WSFs derived according to a modification of the slow-mix procedure described in Section 5.1.1. One part fuel oil was stirred slowly with 99 parts filtered (5μ) Columbia River water for 4 hours; phases were allowed to separate for 1 hour before siphoning into glass bottles.

Biological Test Procedures

Acute and chronic toxicity tests were conducted under static conditions. Five first-instar *D. magna* were added to 200 mL of test solution in each of eight replicate glass jars for each test concentration and control. Test solutions were replaced from the original water soluble fraction (WSF) three times weekly. Acute tests identified the lowest test concentrations which immobilized a significant number of organisms in 48 hours. Both adult immobilization and number of live offspring produced were recorded during the 21-day chronic tests. Test concentrations were intended to bracket the no-observed-effect and total-observed-effect levels in both acute and chronic test phases. Concentrations producing no apparent acute effects were monitored for an additional 19 days to provide the 21-day chronic test. Acute and chronic tests were started simultaneously and employed adults from the same population source to increase comparability of results. In each experiment, two tests were run

simultaneously: one with SRC-II WSF and one with a reference oil WSF. This paired design maximized comparability between the SRC-II liquid and the reference fuel oils.

Chemical Analyses

Total carbon (TC) in stock WSFs was measured with a Beckman® 915B organic carbon analyzer. Concentrations of major chemical constituents in SRC-II, No. 2 and No. 6 fuel oil stock WSFs were determined by capillary gas chromatography with quantification based on response factors of reference compounds (Bean et al. 1981b).

Statistical Analyses

Chi-square statistics were used to compare the number of D. magna immobilized in exposure groups with that of controls for acute tests. The estimated concentration where the number immobilized was significantly (α= 0.05) greater in exposed groups than in controls is called the Lowest Rejected Concentration Tested (LRCT) (Skalski 1980).

Data on chronic effects were analyzed using a one-way analysis of variance of square root transformed counts of total live young produced per beaker. The LRCTs for chronic effects were established by subsequent comparison of control and exposure group productivity using one-sided (α = 0.05) Fisher's protected least significant difference tests (Fisher 1935).

The DI and RH (see Section 5.1) were applied to results of three paired experiments. Experiment 1 compared toxicity of a SRC-II WSF with that of a No. 6 fuel oil WSF. Experiments 2 and 3 compared the toxicity of a SRC-II WSF with that of a No. 2 fuel oil WSF. However, more detailed chemical analyses were performed in Experiment 3.

Results and Discussion

Acute Effects

Toxicity is the inherent property of a chemical substance to produce harmful effects in an organism after exposure of a certain duration to a specific concentration (Cairns, Dickson and Maki 1978). Selecting the units of measure

for harmful concentrations can be difficult with complex materials. Within our three experiments, the problem was approached by diluting identically prepared stock WSFs to the bracket concentrations at which significant toxic effects were observed (the 48-hour and 21-day LRCTs). Because all test materials had the same opportunity to enter solution, we may directly compare the percent WSF required to achieve the LRCT. Thus, the No. 6 fuel oil WSF was less toxic than the SRC-II WSF because the 48-hour LRCT was achieved with a higher proportion (37.5%) of the No. 6 fuel oil WSF than with SRC-II WSF (0.25%, Table 37). Therefore, certain components of SRC-II must be more soluble, more toxic, or both, than the most toxic of the more soluble components of No. 6 fuel oil. The No. 2 fuel oil WSF caused no mortalities in 48 hours, even at the 100% WSF stock solution.

Some authors fail to express toxicity of complex organic materials as a percent of stock solution and rely instead on the concentration of TC as a measure of toxicity. Based on TC alone, the No. 6 fuel oil WSF was more toxic than either the SRC-II or No. 2 fuel oil WSF (Table 36).

Because toxicity based on TC and percent dilution differs, the DIs and RHs may be used to clarify which material poses the greatest hazard. To generate the same acute toxicity (LRCT), SRC-II WSF was diluted to 1/394 of its original concentration. Thus, under our laboratory test conditions, relative acute hazard to aquatic organisms from the SRC-II WSF was 394 2.6, or 150 times that of No. 6 fuel oil. The acute hazard of SRC-II WSF may exceed 390 times that of No. 2 fuel oil (Table 36).

Chronic Effects

Reduction in population productivity appeared to be a more sensitive indicator of chronic effects than did adult immobilization (Table 37). Based on both TC and percent dilution, SRC-II and No. 6 fuel oil WSFs were more toxic than No. 2 fuel oil WSFs in chronic tests. Based on TC alone, results from Experiment 1 indicate that No. 6 fuel oil was less toxic than SRC-II in chronic tests.

TABLE 36. Relative Acute Hazards of a Coal Liquid and Two Reference Fuel Oil Water Soluble Fractions (WSF) to Daphnia magna

Experiment	SRC-II LRCT[a] as % WSF	SRC-II LRCT[a] as mg/L TC	SRC-II Stock WSF Conc. mg/L TC	SRC-II DI[b]	Fuel Oil LRCT[a] as % WSF	Fuel Oil LRCT[a] as mg/L TC	Fuel Oil Stock WSF Conc. mg/L TC	Fuel Oil DI[b]	RH
1) SRC-II vs No. 6 Fuel Oil	0.25	2.7	1065	390	37.5	1.9	5	2.6	150
2) SRC-II vs No. 2 Fuel Oil	0.25	2.8	1100	390	None observed at 100% WSF		9	<1	>390
3) SRC-II vs No. 2 Fuel Oil	0.50	5.0	1000	200	None observed at 100% WSF		4	<1	>200

(a) Lowest Rejected Concentration Tested = the lowest test concentration causing a statistically significant effect compared to controls.

(b) Dilution Index = $\frac{\text{Concentration of the Stock Solution}}{\text{LRCT}}$.

(c) Relative Hazard = $\frac{DIx}{DIy}$, where x and y are the materials being compared; SRC/reference oil.

TABLE 37. Chronic (21-day) Effects of Coal Liquid and Two Reference Fuel Water Soluble Fractions on *Daphnia magna*

Water Soluble Fraction	Experiment Number	Test Chamber Concentrations as % Stock WSF	Test Chamber Concentrations as mg/L TOC[a]	Immobility (n=40)	Live Young per 40 Adults	% Control Reproduction
Control	1	0.00	0.0	5	1473	100
	2	0.00	0.0	1	1231	100
	3	0.00	0.0	6	1422	100
SRC-II	1	0.03	0.3	0	1163	79
		0.06[b]	0.6[b]	5	544	37
		0.12	1.3	28	70	5
		0.25	2.7	39	2	0
	2	0.03	0.3	10	1025	83
		0.06	0.7	5	1056	86
		0.12[b]	1.3[b]	14	202	16
		0.25	2.7	36	1	0
	3	0.03	0.3	2	1153	81
		0.06[b]	0.6[b]	7	632	44
		0.12	1.2	23	142	10
		0.25	2.5	34	2	0
No. 6 Fuel Oil	1	1.60	0.1	2	2265	154
		3.10	0.2	2	2150	145
		6.20	0.3	5	1730	117
		12.50	0.6	6	2095	142
		25.00[b]	1.2[b]	22	382	26
No. 2 Fuel Oil	1	23.73	2.1	0	2071	168
		31.64	2.9	15	1189	97
		42.19	3.8	5	1072	88
		55.25[b]	5.1[b]	13	814	66
		75.00	6.8	34	239	19
		100.00	9.0	39	10	1
	2	23.73	1.0	1	2206	155
		31.64	1.3	5	1556	109
		56.25	2.3	2	1074	76
		75.00	3.0	4	1399	98
		100.00[b]	4.0[b]	22	586	41

(a) Test concentrations (mg/L TC) were computed from percent dilution of the WSF.
(b) Data represent Lowest Rejected Concentration Tested (LRCT) = the lowest concentration causing a statistically significant effect compared to controls.

To obtain direct, quantitative comparisons of the potential hazard from these materials, we used the DIs (Table 38). The stock SRC-II WSF was diluted up to 1/1667 its original concentration, whereas the stock No. 2 and No. 6 fuel oil WSFs were diluted to no more than one-fourth their original concentrations to achieve the same degree of toxicity (LRCT). Thus, under our laboratory conditions, relative chronic hazard to aquatic organisms of SRC-II WSF was 470 times that of No. 6 fuel oil WSF, and 470 to 1700 times that of No. 2 fuel oil WSF. Results of Experiment 3 may better estimate the RH of SRC-II versus No. 2 fuel oil (RH = 1700) than those of Experiment 2 (RH = 470) because observed toxicity of SRC-II WSF was identical in Experiments 1 and 3, and because the LRCT may give inadequate weight to the higher adult immobilization observed in Experiment 2 (Table 37).

To evaluate the relationship between WSF concentrations and reproduction, the average number of live young per surviving adult on each observation day was averaged over all days of observation. Overall averages were plotted against the percentages of stock solution in each test concentration for both fuel oils (Figure 27) and SRC-II (Figure 28). Reproduction appeared to be enhanced at lower concentrations of No. 2 and No. 6 fuel oils to more than 150% of that in controls. Enhancement may reflect the presence of microorganisms which utilize test solution solutes (Bean et al. 1981a) and serve as food for D. magna. Note in every test with No. 2 and No. 6 fuel oils that although reproduction in dilute exposure solutions exceeded that in controls, production decreased with increasing stock WSF concentrations.

Although absolute reproduction rates differed, the two No. 2 fuel oil tests (Figure 27) showed very similar patterns of average reproduction with test concentration. The difference in magnitude of productivity between the two tests was similar to that between the two controls. The three SRC-II tests were highly reproducible (Figure 28).

Chemical Analyses of Experiment 3 WSF.

Differences in chemical composition of SRC-II and No. 2 fuel oil WSF are given in Table 39. The initial ratio of phenols to aromatic hydrocarbons was about 100:1 in SRC-II WSFs (1000 mg/L TC). After dilution to chronic test

TABLE 38. Relative Chronic Hazards of a Coal Liquid and Two Reference Fuel Oil Water Soluble Fractions (WSF) to Daphnia magna

Experiment	SRC-II				Fuel Oil				
	LRCT[a] as % WSF	LRCT[a] as mg/L TC	Stock WSF Conc., mg/L TC	DI[b]	LRCT[a] % as WSF	LRCT[a] as mg/L TC	Stock WSF Conc., mg/L TC	DI[b]	RH[c]
1) SRC-II vs No. 6 Fuel Oil	0.06	0.6	1065	1800	25.00	1.3	5	3.8	470
2) SRC-II vs. No. 2 Fuel Oil	0.12	1.3	1100	850	56.25	5.1	9	1.8	470
3) SRC-II vs. No. 2 Fuel Oil	0.06	0.6	1000	1700	100.00	4.0	4	1.0	1700

(a) Lowest Rejected Concentration Tested = the lowest test concentration having a statistically significant effect compared to controls.

(b) Dilution Index = $\frac{\text{Concentration of the Stock Solution}}{\text{LRCT}}$.

(c) Relative Hazard = $\frac{DI_x}{DI_y}$, where x and y are the materials being compared; SRC/reference oil.

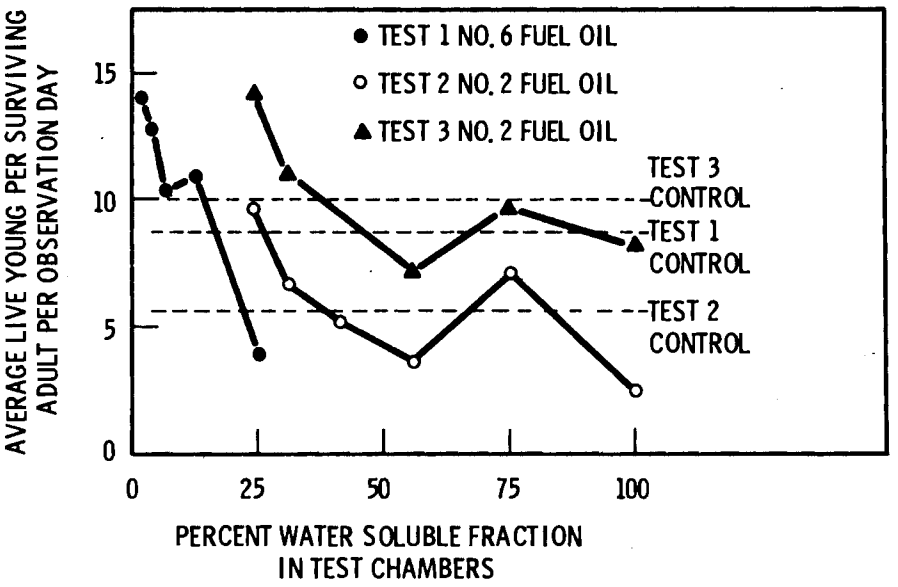

FIGURE 27. Relationships Between Productivity and Test Chamber Concentrations of Water Soluble Fractions for No. 2 and No. 6 Fuel Oils. (Dashed lines represent productivity in control beakers)

concentrations (0.3 to 2.7 mg/L TC), the contribution of aromatic hydrocarbons was insignificant. In contrast, the initial ratio of phenols to aromatic hydrocarbons in No. 2 fuel oil WSFs (4 mg/L TC) was about 3 to 1. After dilution to chronic test concentrations (1.0 to 4.0 mg/L TC), No. 2 fuel oil WSFs still retained significant concentrations of aromatic hydrocarbons. Thus, toxicity of the SRC-II WSF appears mainly attributable to phenolics, whereas toxicity of No. 2 fuel oil may be partly due to aromatic hydrocarbons. Chemical changes in replacement test solutions were greater for No. 2 fuel oil WSFs than for SRC-II WSFs over a 12- to 16-day storage period (Table 39).

Giddings et al. (1980) conducted acute toxicity tests with D. magna using WSFs from a coal liquid and No. 2 and No. 6 fuel oils (sources not specified). However, their method of preparing WSFs differed from ours. Our water-to-oil ratio was 99 to 1 versus their 8 to 1 ratio; our mixing time was 4 hours versus their 16 hours; and we did not filter the WSFs to remove suspended oil droplets. Direct comparison of our results with theirs indicates that the different methods of stock preparation yielded different results. Giddings et al.

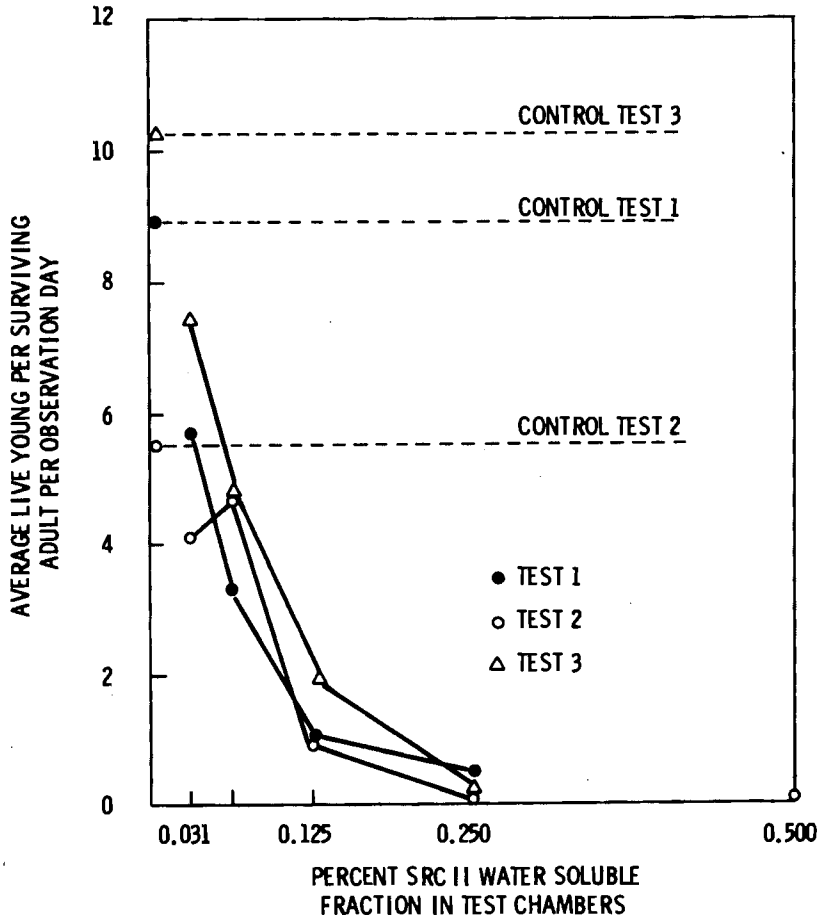

FIGURE 28. Relationships Between Productivity and Test Chamber Concentrations of SRC-II Water Soluble Fractions for 3 Tests. (Dashed lines represent productivity in control beakers)

(1980) obtained a stock coal liquid WSF of 5000 mg/L TC compared to our 1000 mg/L TC and an estimated LC50 (concentration for immobilizing 50% of the daphnid test population) of 15.5 mg/L TC compared to our 3.5 mg/L TC. However, DIs for the two coal liquid WSFs were similar: 320 for Giddings et al. (1980) versus our 290. This suggests that the filterable, highly phenolic chemical composition of a gently-mixed coal liquid aqueous extract may not be greatly influenced by differences in stock preparation.

TABLE 39. Concentration of Major Chemical Constituents of SRC-II and No. 2 Fuel Oil Stock Water Soluble Fractions used in *Daphnia magna* Toxicity Experiment 3

Constituents	SRC Stock Solution, mg/L[a]		No. 2 Fuel Oil Stock Solution, mg/L[a]	
	Day 0	Day 16	Day 0	Day 16
Phenols				
Phenol	183.63	202.85	.03	ND[b]
Cresols	468.49	484.13	.18	ND
C_2-Phenols	385.12	394.60	.72	.29
C_3-Phenols	133.18	133.95	.29	.10
C_4-Phenols	44.33	44.45	.22	.02
Indanols	74.83	78.27	.06	ND
C_1-Indanols	46.42	49.16	.12	.10
Other	24.13	20.49	.09	.09
Total Phenols	1360.13	1407.90	1.71	.60
Aromatic Hydrocarbons				Day 12
C_2-Benzenes	.58	.42	.04	<.01
C_3-Benzenes	.65	.37	.07	ND
Indan	.70	.54	.01	<.01
C_4 Benzenes	1.06	.55	.09	<.01
Tetralin	1.05	.72	.01	<.01
Naphthalene	2.78	2.43	.07	<.01
C_1-Naphthalenes	2.01	1.44	.10	trace
$C_{5,6}$-Benzenes	3.01	1.56	.07	<.01
C_1-Tetralins				
C_2-Naphthalenes	.71	.25	.04	<.01
C_3-Naphthalenes	.92	.25	.02	trace
Other	.31	.08	<.01	<.01
Total Aromatic Hydrocarbons	13.78	8.61	.51	.01

(a) Determined by capillary gas chromatography with quantification based on response factors of reference compounds.
(b) ND = not detected.

The RHs for No. 6 and No. 2 fuel oil WSFs differ between the two studies. Giddings et al. (1980) observed no acute effects at 100% stock WSF (19 mg/L TC) for No. 6 fuel oil and an LC50 of 7.0 mg/L TC for No. 2 fuel oil. Thus, their coal liquid posed over 320 times the hazard of their No. 6 fuel oil and only 21 times that of their No. 2 fuel oil, almost the reverse of our results for SRC-II versus the same fuel oils. This suggests chemical composition, and therefore toxicity, of these primarily lipophilic fuel oil WSFs were influenced more by differences in preparation and treatment than were SRC-II liquid extracts. Thus, both chemical and toxicological factors must be considered in interpreting results of toxicity tests with complex materials. Data from both experiments indicate that coal liquid WSFs pose a greater hazard to daphnids than do WSFs of either fuel oil.

Conclusions

Our results and limited data from the literature indicate that WSFs of the coal liquids tested pose a greater risk of acute and chronic toxicity to daphnids than do identically prepared WSFs of No. 2 and No. 6 fuel oils. However, procedures involved a slow, short-duration mixing regime which generated toxic SRC-II WSFs with high concentrations of water soluble components (primarily phenols) characteristic of coal liquids. The same mixing regime may be insufficient for dispersing more lipophilic components of other fuel oils. Mixing regimens that are more rigorous may reduce differences in toxicity between the three WSFs tested. Moreover, the effects of environmental dilution and degradation must be considered to accurately assign relative environmental hazard to complex materials or their WSFs.

The concepts of DI and RH are presently being applied to different mixing regimes, to fate and effects studies, and to systems-level studies at our laboratories. These studies will contribute to a comprehensive comparison of environmental effects of coal synfuels with other fossil fuels.

5.4 COMPARATIVE TOXICITY TESTING BETWEEN LABORATORIES

T. M. Poston and J. R. Skalski

BACKGROUND

Hazard assessment often necessitates a comparison of sometimes highly variable or conflicting data obtained from different laboratories. Comparisons of toxicity-test data are most effective when a means of calibrating the results is available, and this requires collaboration among participating laboratories.

Several tabulations of aquatic toxicity data are available (McKee and Wolf 1971; Becker and Thatcher 1973; Cushman et al. 1977) that catalogue LC50 values and indicate a wide range of toxic response. The variability in response may be attributed to a number of factors such as test species, species strain, source of toxicant, method of culture, test protocol, photoperiod, definition and interpretation of endpoint, age or life stage of the organism, personnel conducting the tests, and water quality parameters.

Anticipating a need to develop comparative data for complex effluents and materials from a variety of new synthetic fuel technologies, Pacific Northwest Laboratory (PNL) and Oak Ridge National Laboratory (ORNL) collaborated in testing simple compounds (phenol and acridine) on Daphnia magna. We selected D. magna because of its universal use and ease of culturing. Tests with phenol were designed for comparing results generated with the same protocol but without collaboration. The acridine tests were conducted to test two major interlaboratory sources of variability: water quality and laboratory personnel. Other possible sources of variability (mentioned above) between laboratories were resolved by establishing a standardized testing protocol.

EXPERIMENTAL

Phenol Tests

Water

Tests with phenol were performed at each laboratory using respective sources of water [Oak Ridge well water (ORWW) or 5.0μ filtered Columbia River water (FCRW)].

Organisms

Tests performed at both laboratories used a common strain of *D. magna* supplied by ORNL. Brood stocks were maintained under static culture at both laboratories in their respective source waters. Twenty-four-hour-old first instar *Daphnia* were obtained from brood stocks reared in ∿50 mL of diluent in 100 mL beakers.

Brood daphnids at both laboratories were fed processed Purina Trout Chow® supplied by PNL. Two hundred grams of trout chow were liquefied in 1.0 L of glass-distilled water. After the largest particles had settled out (∿1 min), the solution was decanted into plastic bags and frozen at -80°C. The solution was lyophilized, and half was sent to ORNL for feeding their stock. Brood daphnid were fed a screened resuspension of the lyophilized trout chow three times a week at a rate of 2 mg per feeding.

Protocol

Each laboratory used their own stock of phenol (PNL used Baker; ORNL used Mallinckrodt®). A predetermined amount was weighed and dissolved in 2.0 L of source water to obtain the desired high test concentration: 100 mg/L at ORNL and 60 or 36 mg/L at PNL, depending on the test. After mixing, 80 mL of test solution was distributed into each of four 100 mL beakers; a small amount was saved for toxicant analysis using the 4-amino antipyrine dye method (Goss 1975), and the remainder was used for geometric dilution to produce the

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desired concentrations. The dilution factor used by ORNL was 0.6. A 0.75 dilution factor nested within a 0.6 dilution factor was used by PNL in all but its initial screening test, which employed a 0.6 dilution factor throughout.

First instar daphnids (12 ± 12-hr-old) were collected from individual brood beakers pooled prior to loading. Test beakers were loaded in four cycles; each cycle constituted loading six first instars into a beaker at each test concentration in descending order of toxicant concentration. Hence, each test concentration had 24 test organisms evenly distributed among four beakers containing 80 mL of test solution. Each beaker was covered with a watch glass after loading. During the toxicity test, beakers were arranged in rows by concentration. ORNL placed their beakers in fabricated boro-silicate racks for handling ease; PNL used enamel trays.

The culturing of daphnids and the toxicity tests were conducted in environmental chambers at 20°C under a 12:12 light to dark photoperiod. The test duration was 48 hours without feeding. Dissolved oxygen (DO), pH, and toxicant concentrations of the diluent were monitored at the start and end of tests. The experimental endpoint was immobilization, which is defined as the inability of an insulted first instar to swim from the bottom of the test beaker when stimulated by gently rapping the beaker with a watch glass cover. Test beakers were viewed over a dark blue material without the aid of magnifying devices.

Acridine Tests

Water

Source water was exchanged between laboratories in 54 L capacity Nalgene carboys. The shipped (air freight) water was stored at 4°C as soon as it arrived.

Organisms

The ORNL strain of *D. magna* was used in these tests. Fresh cultures were acclimated to exchanged water (ORWW and FCRW at each laboratory) for one complete brood cycle prior to initiating the acridine tests.

Protocol

ORNL provided a common source of acridine (Pfaltz and Bauer) for testing purposes. Both laboratories used a 0.85 dilution factor nested in a 0.7 series dilution factor with a toxicant concentration range of 1.8 to 7.0 mg/L.

RESULTS AND DISCUSSION

Phenol Tests

Point and 95% interval estimates for the LC50 and slope of the concentration response curves (probit analysis) for phenol tests are presented in Table 40. The LC50 values for ORNL tests were 2.3 to 4.6 times greater than LC50 values for PNL tests. Additionally, an F-test with 1 and 4 degrees of freedom inicated a significant ($\alpha<0.01$) difference in the variance in LC50 values between PNL and ORNL with coefficients of variation of 8.1% and 32.5%, respectively. Tests conducted at PNL exhibited a steeper slope, necessitating the higher dilution factor used during toxicant preparation. The steeper slope of the PNL data suggested that PNL test organisms were more sensitive to small changes in toxicant concentration; however, this result may also be an artifact attributed to minor differences in the testing protocol. Slope terms were more variable than the LC50 values.

Phenol concentrations, DO, and pH remained relatively stable at all test concentrations during the initial 24 hours of exposure. However, at 48 hours, phenol concentrations exhibited a concentration-dependent decrease (Table 41) concommitant with decreases in pH (Figure 29) and DO (Figure 30). These phenomena were observed in beakers with and without test organisms.

It is an accepted practice to employ flow-through systems for testing biodegradable compounds such as phenol. The loss of toxicant coupled with changes in pH and depletion of DO in static tests might be expected to increase variation of test results. This may explain the wide range of LC50 values for ORNL results. PNL data did not exhibit such wide fluctuations. Perhaps the key concern is the scheduling of the tests. The PNL tests were conducted with all first instars obtained from the same brood daphnids during a one-week period. The ORNL tests were conducted a month apart; hence, the

TABLE 40. Phenol Toxicity Data (Probit Analysis) from PNL and ORNL Tests

Test Number	LC50	L.F.L.[a]	U.F.L.[a]	Slope	S.D.	Goodness-of-Fit (X^2)
		PNL Tests with Filtered Columbia River Water				
1	7.45	6.44	8.62	5.34	0.8208	2.540
2	7.58	6.63	8.59	4.74	0.6912	6.179
3	7.89	7.10	8.75	6.52	0.9976	2.243
4	6.33	3.03	9.58	3.82	1.0636	18.371[b]
5	7.55	6.62	8.52	4.96	0.7242	7.685
		ORNL Test with Oak Ridge Well Water				
1	28.97	16.51	50.84	4.20	1.6491	32.568[b]
2	18.14	14.93	21.98	3.07	0.3654	7.207

(a) Lower or upper fiducial limits.
(b) Significant at $\alpha = 0.05$

TABLE 41. Loss of Phenol at 48 Hr Among Test Concentrations Used in Static Tests (PNL Phenol Tests 2 through 5)

Phenol T_o hr (n = 4)[b]	Phenol T 24 hr (n = 16)	$\%T_o$ hr	Phenol T 48 hr (n = 16)	$\%T_o$ hr
35.3 ± 1.22[a]	36.8 ± 1.65	104	32.1 ± 2.32	91
21.4 ± 0.81	21.3 ± 0.37	100	15.8 ± 2.82	74
13.1 ± 0.12	12.7 ± 0.14	97	5.5 ± 2.45	42
9.7 ± 0.15	9.2 ± 0.29	95	2.8 ± 1.45	29
7.5 ± 0.18	7.0 ± 0.09	93	1.3 ± 1.02	17
5.5 ± 0.21	5.4 ± 0.17	98	0.5 ± 0.93	9
3.3 ± 0.21	3.4 ± 0.45	103	0.0	0

(a) Mg/L phenol ± 1 standard deviation.
(b) Replicates.

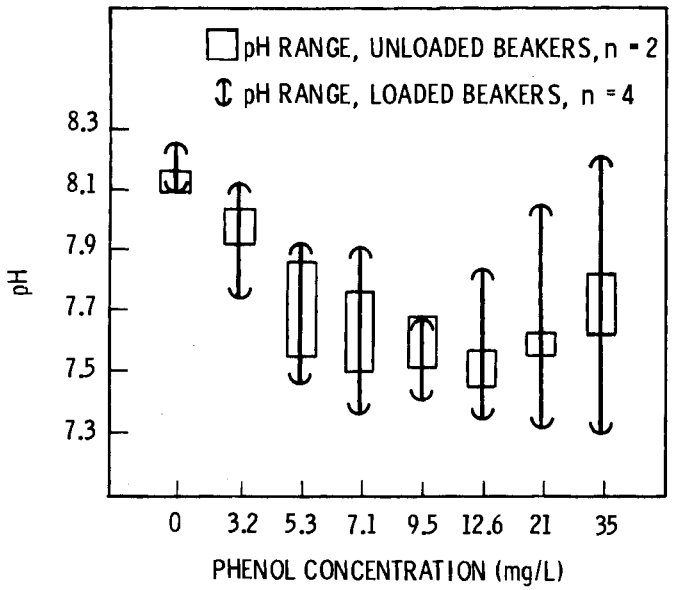

FIGURE 29. Ranges of pH in Loaded (6 Instars/Beaker) and Unloaded Beakers at 48 hr

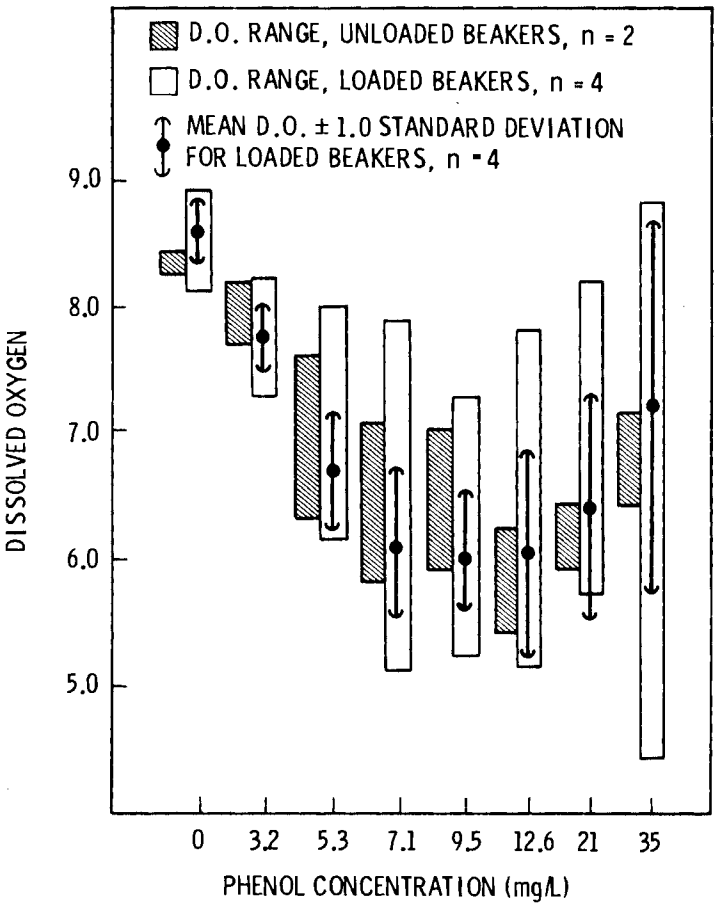

FIGURE 30. Dissolved Oxygen in Loaded (6 Instars/Beaker) and Unloaded Test Beakers at 48 hr. (Dissolved Oxygen at 24 hr was within 93% of Saturation)

first instars from the ORNL tests were obtained from a different batch of brood daphnids. First instars obtained from early broods may respond differently than first instars obtained from later, larger broods. The method of culture used in this study could allow this to happen.

The decrease of DO and pH during phenol tests varied from beaker to beaker within test concentrations. In some cases, DO dropped to lower than recommended concentrations (ASTM 1980) for static tests. We suggest that this high variability in DO resulted from the addition of different volumes of water during the transfer of test organisms to the test beakers. The water in which first instars were pooled was derived in part from brood beakers that contained a healthy population of microbes. Each test beaker received a different "spike" of microbes proportional to the amount of water added during first instar transfer. We speculated that this volume of transferred water could vary from 0.2 mL to 1.0 mL. The decrease in pH probably did not affect the toxicity of phenol, which has a pK of 10.0 and is 99% un-ionized at pH 8.0 and lower.

Acridine Tests

Tests with acridine indicated only trivial differences between laboratories in the toxicology repsonse of Daphnia magna (Table 42). The standard deviations for individual tests were indicative solely of the binomial sampling variance and lack-of-fit to the probit model and was a direct output of the probit analysis. Analysis of variance of the LC50 estimates was used to estimate the intralaboratory variance and tests for possible sources of interlaboratory differences. Analysis of the LC50 (3.85 PNL vs. 2.68 ORNL) and the slope of the concentration-response curves (14.12 PNL vs. 8.12 ORNL) indicated statistically significant main effects for laboratories ($\alpha>0.01$). However the differences are small enough to be without any biological importance. No significant main effects ($\alpha<0.05$) for water source or lab and water interaction were found. Water quality data for the exchange source water are presented in Table 43.

Because of the lack of significance for the main effect for water source or the lab x water interactions, FCRW and ORWW data can be combined at each laboratory and 95% confidence intervals calculated based on the replicated

TABLE 42. Acridine Toxicity Data (Probit Analysis) from PNL and ORNL Tests with Exchanged Water

Test Number	LC50	U.F.L.[a]	L.F.L.[a]	Slope	S.D.	Goodness-of-Fit (X^2)
	ORNL Tests with Oak Ridge Well Water					
1	3.74	4.83	2.90	8.77	2.9209	13.097[b]
2	2.65	3.55	2.00	5.75	1.3177	10.487[b]
3	2.43	2.90	1.80	13.79	4.1206	12.221[b]
Mean	2.94			9.44		
S.D.	0.70			4.06		
	PNL Tests with Oak Ridge Well Water					
1	3.16	3.33	2.99	13.24	1.8707	7.963
2	3.84	4.04	3.65	15.57	2.4238	3.134
3	3.96	6.02	2.61	15.85	5.7967	26.467[b]
Mean	3.65			14.80		
S.D.	0.43			1.35		
	ORNL Tests with Filtered Columbia River Water					
1	2.87	4.48	1.65	8.37	2.2198	11.107[b]
2	2.36	2.58	2.16	6.87	0.9029	8.620
3	2.05	2.38	1.45	5.18	1.377	3.555
Mean	2.43			6.81		
S.D.	0.41			1.60		
	PNL Tests with Filtered Columbia River Water					
1	4.34	4.61	4.13	14.95	2.4735	2.344
2	4.22	4.50	3.93	11.24	1.8542	2.758
3	3.57	3.76	3.39	14.20	2.1492	5.302
Mean	4.04			13.46		
S.D.	0.41			1.96		

(a) Upper or lower fiducial limits.
(b) Significant at $\alpha = 0.05$.

TABLE 43. Water Quality Parameters of Source and Exchanged Water

Parameter	Source[a]		Carboy Water			
	Columbia River	ORNL Well Water	PNL FCRW	PNL ORWW	ORNL FCRW	ORNL ORWW
pH	7.6-8.2	7.8	8.0-8.1	7.8-8.0	7.95[c]	7.5[c]
Alkalinity ($Mg/L\ CaCO_3$)	55-59	119	55-57	118-121	61-63	119-120
Hardness -EDTA ($mg/L\ CaCO_3$)	60-79	140	67-79	122-125	68-71	118-148
Specific Conductivity ($\mu mho\ cm^{-1}$ at 25°C)	---	---	141-149	236-247	140[c]	230[c]
Dissolved oxygen (20°C)	100%[b]	100%	9.6 mg/L	9.4 mg/L	9.4[c]	8.8[c]

(a) Yearly range.
(b) Saturated at the facility.
(c) Composite sample.

tests (Table 44). The standard deviations in LC50 and slope term values estimated the experimental error of the testing protocol and were proper indications of intralaboratory variation. The overall estimate of the experimental error for the LC50 in the acridine tests derived from the analysis of variance was $\sigma = 0.255$ with 8 degrees of freedom.

During the course of the 48-hour test period, acridine concentrations remained constant with slight variations due to evaporation or normal assay variability. Dissolved oxygen and pH were within 5% of the control group for the duration of the exposures. Dissolved oxygen concentrations at the end of the tests were within 89% of saturation at 20°C.

Technical idiosyncrasies probably accounted for the minor observed differences in response beween the two laboratories (e.g. transferring first instars to test beakers, isolating instars from brood mothers).

TABLE 44. Mean (n = 6) and 95% Confidence Intervals (CI) of Acridine Tests Calculated by Combining ORWW and FCRW Data Within Laboratories

Lab	LC50 Mean	LC50 95% C.I.	Slope Mean	Slope 95% C.I.
PNL	3.85	3.44 - 4.26	14.18	12.14 - 16.21
ORNL	2.68	2.27 - 3.10	8.12	6.09 - 10.16

5.5 DEVELOPMENT OF ECOSYSTEM-LEVEL RESPONSE STUDIES

R. M. Emery, R. M. Bean and E. W. Lusty

INTRODUCTION

PNL believes that the potential aquatic effects from SRC-II materials would not be examined completely unless the responses of integrated ecosystems are considered. This "system-level" approach is too frequently overlooked or avoided in the hazards-assessment process. Accordingly, the fundamental objective of our "systems-level" studies is to interpret stress in aquatic ecosystems resulting from exposure to known quantities of SRC-II materials.

EXPERIMENTAL

Work thus far has included an extensive literature search, discussions with peer experts representing a variety of environmental disciplines, the drafting of a planning document, and the design and construction of artificial streams.

RESULTS AND DISCUSSION

The planning document (PNL 1980) describes the recommended strategy for "systems-level" studies. The strategy is to investigate ecosystems response to SRC-II materials by a gradation of inquiries escalating from small "model" ecosystems to large experimental (field) environments. Changes in biomass and system complexity will be observed. Suitable parameters to describe ecosystem form and function, including community structure and energetic dynamics, also will be investigated.

The first stage of our progression towards field testing will involve the use of small experimental streams shown in Figure 31. These streams have been installed and are undergoing preliminary colonization with a continuous supply of Columbia River water. Each of four stream replicates is 34 ft (1036 cm) long, 6 in (15 cm) wide, and 8 in (20 cm) deep. The flow of Columbia River

water can be varied from 0.5 to 50 L/min with depths of 1 to 5 in (3 to 13 cm). Each stream replicate is oriented in a north-south direction to minimize shading. Each replicate contains 1 in (3 cm) of Columbia River sediments.

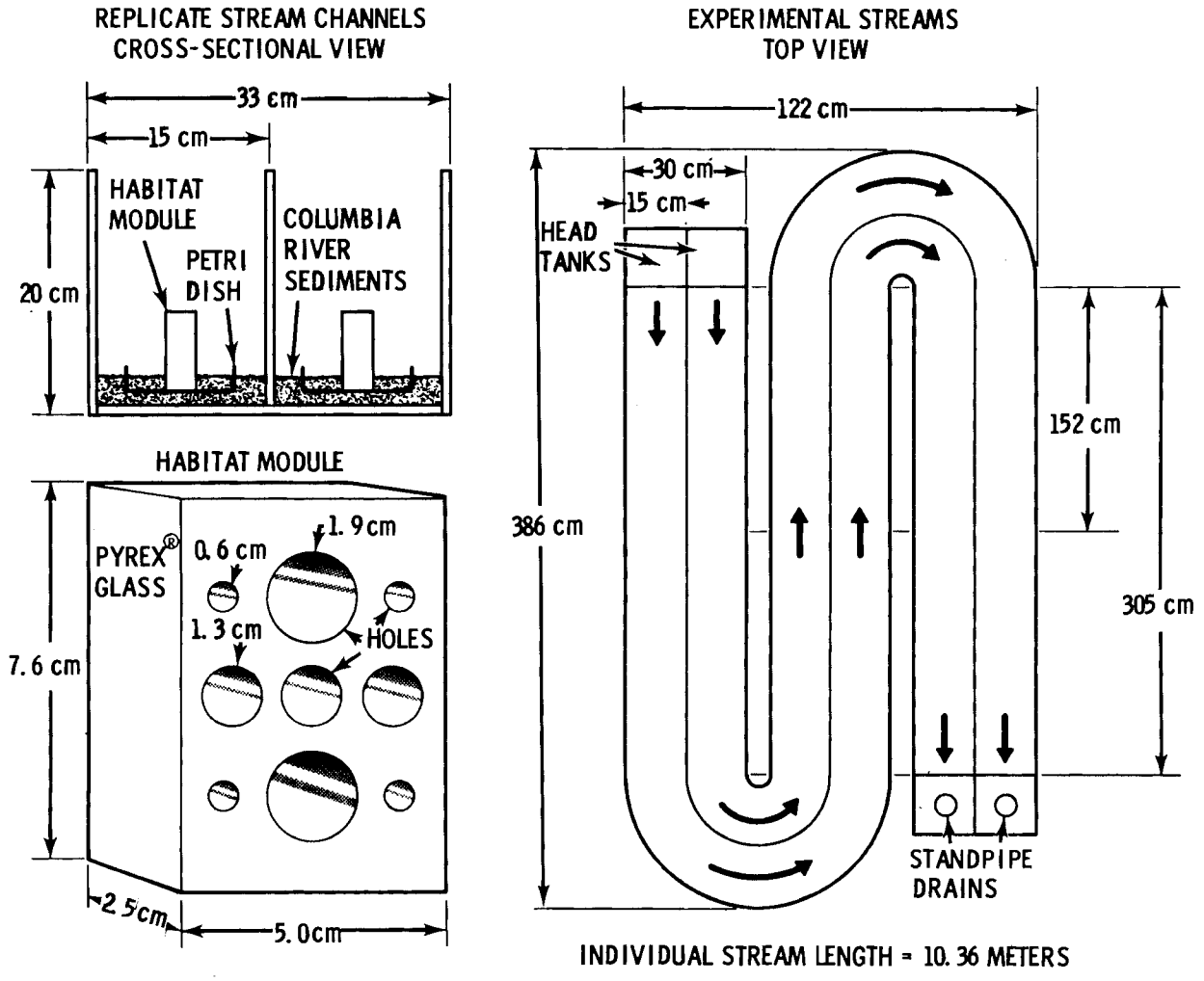

FIGURE 31. Top and Cross-Sectional Views of the Experimental Stream Design. Streams are constructed with PVC materials. Also shown is the glass habitat module, used as a sampling substrate

5.6 GROWTH OF BARLEY EXPOSED TO SRC I AND II MATERIALS ADDED TO SOIL

J. F. Cline, W. H. Rickard and M. E. Thiede

INTRODUCTION

The purpose of this investigation is to examine the growth of barley plants (Hordeum vulgare) grown in Ritzville silt loam (Wildung 1977) treated with solvent refined coal material, SRC solid (SRC-I) and SRC liquid (SRC-II). Although the SRC materials will not be introduced to soil or surface waters in normal uses, they could be spilled during transportation. Such spills could contaminate surface waters and agricultural, rangeland, and forest soils, possibly causing acute or chronic damage to plants. Contamination of soils and water could also provide a way for certain inorganic and organic materials to enter food chains.

EXPERIMENTAL

During the 1979 growing season, pea-sized pellets of SRC-I obtained from the pilot plant at Fort Lewis, Washington, were mixed with soil and placed in special lysimeters (Hinds 1973, 1975; Rickard, Cline and Schreckhise 1979) in two configurations. A 1:1 mixture of soil and pellets by dry weight (2 kg SRC-I/lysimeter) produced a 3-dm surface layer which was superimposed on a 7-dm soil layer creating a soil column 1 m deep. This treatment was replicated six times. In six other lysimeters, a 1-dm layer of pellets (2 kg pellets/lysimeter) was layered over a 6-dm-deep soil column and then the pellet layer was covered with a 3-dm-deep layer of soil. Six other lysimeters were filled with soil alone to serve as controls. Water was added to the lysimeters in amounts approximating field capacity. Barley seeds were planted in the lysimeters in April. Barley seedlings were thinned to two plants per lysimeter. Water was added to each lysimeter as the total weight changed due to water losses via evapotranspiration and then restored to field capacity as determined by frequent weighings.

Two lysimeters from each treatment and two of the control lysimeters were split longitudinally at the end of the first growing season and roots washed

from the soil core as a way to observe root damage. The remaining lysimeters lay fallow over winter and were replanted in April 1980. This was done to determine if weathering and aging produced materials that were deleterious to barley growth.

To test the effect of SRC-II material, three treatments (0.15 L/m^2, 1.48 L/m^2, and 14.84 L/m^2) of undiluted fuel oil (2.9 parts of middle to one part heavy distillate) were added to pairs of lysimeters in a layer 10 cm below the soil surface. The soil column in each lysimeter was watered to restore field capacity and seeded to barley in August 1979 and again in April 1980.

Aboveground plant parts were harvested at maturity, and flowering culm heights and seedhead lengths were measured. This data was compared using the Bonferroni multiple range t-test (Miller 1966). Weights of seed heads, total straw, and unhulled grain were obtained from each lysimeter. The grain was chemically analyzed for nitrogen content using the micro Kjeldahl method.

RESULTS AND DISCUSSION

SRC-I Pellets

1979 Growing Season

Pellets added to soils produced no visible lesions or chlorotic or necrotic tissues in barley plants.

The Bonferroni multiple range t-test indicated a significant decrease ($\alpha = 0.016$) in the height of flowering culms and in the length of seed heads produced in the layered pellet configuration when compared to plants grown in a mixture of pellets and soil or to plants grown in soil alone. The height of flowering culms and the length of seed heads produced from barley plants grown in soil mixed with pellets was not significantly different from plants grown in soil alone (Table 45).

1980 Growing Season

Barley plants grown in mixtures of soil and pellets produced less dry matter than control plants. Barley grown in soil over a layer of pellets also produced less dry matter than plants grown in control lysimeters (Table 46).

TABLE 45. Average Length (cm) ± Standard Error of Flowering Culms and Seed Heads of Barley Plants Grown in Soil Containing SRC-I Pellets (1979 Growing Season) and in Soil Containing SRC-II Liquid (1980 Growing Season)

Parameter	Pellets and Soil Layered		Pellets and Soil, Mixed		Soil Alone		Liquid and Soil Layered ($0.15\ L/m^2$)		Liquid and Soil Layered ($1.48\ L/m^2$)		Liquid and Soil Layered ($14.84\ L/m^2$)	
	$\bar{x}$ SE	n	$\bar{x}$ SE	n	$\bar{x}$ SE	n	$\bar{x}$ SE	n	$\bar{x}$ SE	n	$\bar{x}$ SE	n
Length of Flowering Culms	56.7 ± 2.7	5	71.1 ± 1.1	11	70.8 ± 3.2	17	82.7 ± 2.5	11	40.3 ± 1.9	3	49 ± 6.9	3
	53.4 ± 3.1	10	68.6 ± 1.9	11	77.4 ± 1.5	18	66.6 ± 2.0	8	46.3 ± 0.3	2	0	1
	64.9 ± 2.6	10	73.3 ± 2.1	11	79.8 ± 1.3	14						
	66.1 ± 4.9	8			73.7 ± 2.6	12						
Average	60.5[a] ± 1.9	8	71.0[b] ± 1.0	11	75.4[b] ± 1.2	15	75.9[b] ± 2.5	9	42.7[c] ± 1.8	2	36.8[c] ± 13.2	2
Length of Seed Heads	6.5 ± 0.6		8.4 ± 0.3		7.6 ± 0.4		9.3 ± 0.4		4.8 ± 0.4		4.0 ± 0.6	
	5.7 ± 0.3		7.8 ± 0.3		8.3 ± 0.2		7.3 ± 0.3		4.0 ± 0.0		0	
	6.7 ± 0.3		8.1 ± 0.3		9.1 ± 0.2							
	8.4 ± 0.8				8.2 ± 0.4							
Average	6.7[d] ± 0.3		8.1[e] ± 0.2		8.3[e] ± 0.2		8.4 [e] ± 0.3		4.5[f] ± 0.3		3.0[f] ± 1.1	

Means in each column followed by the same letter are not significantly different ($\alpha = 0.016$).

TABLE 46. Dry Weight of Barley (g) ± Standard Error (SE) Grown in Ritzville Soil in Field Lysimeters with Different Configurations of Pellets (1980 Growing Season)

Barley Part	Soil Mixture: Pellets and Soil, Layered $\bar{x}$ ± SE	n	Pellets and Soil, Mixed $\bar{x}$ ± SE	n	Soil Alone $\bar{x}$ ± SE	n
Total	13.77 ± 4.28	4	24.96 ± 0.99	3	35.33 ± 5.96	4
Straw	6.58 ± 2.21	4	12.48 ± 0.77	3	17.71 ± 2.79	4
Heads	7.19 ± 2.07	4	12.48 ± 0.23	3	17.62 ± 3.25	4
Grain	5.80 ± 1.60	4	10.16 ± 0.30	3	14.53 ± 2.66	4

Barley plants grown in soil with a layer of pellets produced the least amount of dry phytomass and grain. Control plants produced the most dry matter and grain, and plants grown in soil mixed with pellets were intermediate in dry matter production.

Root growth was confined to the soil placed above the solid pellet layer configuration and there were no visible indications that pellets produced damaged root tissues.

SRC-II Fuel Oil

1979 Growing Season

All plants from the August 1979 planting died shortly after germination.

1980 Growing Season

After outdoor weathering through the winter season, the odor of fuel oil could still be detected when handling the lysimeters in the spring of 1980. The high fuel oil treatment (14.84 L/m^2) still inhibited the germinability of barley seeds in the spring of 1980. In one replication, no seedlings established; and when seeds were dug from the soil, the length of the embryonic radicle was only 4 mm long.

Barley plants grown in soil with the medium (1.48 L/m^2) and high (14.84 L/m^2) application rates of fuel oil all developed lesions and chlorotic and necrotic tissue when the plants grew to heights of several centimeters. This suggested that necrosis appeared in the leaf tissue of seedlings when the roots reached the buried oil layers. Significant reduction in height of flowering culms and in length of seed heads was evident in mature barley plants from the medium and high treatments (Table 45). However, the low application rate did not cause a reduction in the length of plant parts when compared to control plants.

Barley plants grown in medium (1.48 L/m^2) and high treatments (14.84 L/m^2) averaged less dry matter production than plants grown at the low application (Table 47). Dry matter production at the low treatment was within the range for barley plants grown in control soil.

Fuel oil applied at medium (1.48 L/m^2) and high (14.84 L/m^2) rates also produced a sharp decrease in grain production (Table 48). Plants grown in the low (0.15 L/m^2) application rate averaged slightly less grain production than controls. Nevertheless, this was an improvement as compared to the August 1979 planting where all plants died shortly after germination. Perhaps, over an extended period of time, the more toxic components of fuel oil are either lost to the air or degraded.

TABLE 47. Average Dry Matter Produced and Sample Range for Barley Plants Grown in SRC-II Liquid and Ritzville Soil (1980 Growing Season)

Soil Treatment	Mean Dry Matter Produced, g	Range	Sample Size
Soil only (control)	35.33	27.26 - 41.62	4
SRC-II liquid applied at 0.15 L/m^2 (low)	24.03	16.97 - 31.08	2
SRC-II liquid applied at 1.48 L/m^2 (medium)	2.50	2.24 - 2.77	2
SRC-II liquid applied at 14.84 L/m^2 (high)	0.40	0 - 0.80	2

TABLE 48. Average Unhulled Grain Weight and Sample Range for Barley Plants Grown in Pellets and Liquid Added to Soil (1980 Growing Season)

Soil Treatment	Mean Grain Weight, g	Range	Sample Size
Soil only	14.53	10.86 - 17.10	4
SRC-I Pellets Mixed with Soil	10.16	9.95 - 10.50	3
SRC-I Pellets Layered in Soil	5.80	3.77 - 7.29	4
SRC-II Liquid Applied at 0.15 L/m^2	9.02	6.81 - 11.23	2
SRC-II Liquid Applied at 1.48 L/m^2	0.59	0.41 - 0.76	2
SRC-II Liquid Applied at 14.84 L/m^2	0.40	0 - 0.80	2

Medium (1.48 L/m^2) and high (14.84 L/m^2) applications of fuel oil produced barley seed with notably high nitrogen content, e.g., 16% as compared to 6.5% for controls (Table 49). The specific nitrogen compounds present in exposed plants were not identified, and the possibility that these are of exogenous origin (WSF) should be established in future studies.

The results of these limited investigations give a first indication of what to expect from severe contamination of soil by SRC-I and SRC-II materials inadvertently added to soil in accidents or spills. Additional studies are required to document the apparent effects suggested by these pilot studies and to test the significance of important environmental variables such as soil type.

Field lysimeters are expected to be useful for the biological screening of the various kinds of waste residues produced in the solvent refined coal

TABLE 49. Percent Nitrogen Content for Barley Grain (1980 Growing Season)

Soil Treatment	Average Nitrogen Content For Barley Grain (%)	Range	Sample Size
Soil only (control)	1.05	1.00 - 1.23	4
SRC-I Pellets Mixed with Soil	1.08	1.08 - 1.10	3
SRC-I Pellets Layered in Soil	1.32	1.21 - 1.50	4
SRC-II Liquid Applied at 0.15 L/m^2	1.12	1.09 - 1.14	2
SRC-II Liquid Applied at 1.48 L/m^2	2.55	2.48 - 2.62	2
SRC-II Liquid Applied at 14.84 L/m^2	1.86	1.86	1

process. This screening can be done using small amounts of materials produced during pilot plant tests. Field lysimeters are especially useful if testing is to be conducted over several growing seasons.

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SECTION 6.0
PUBLICATIONS AND PRESENTATIONS

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