JV TASK 92 – ALCOA/RETEC SFE AND SPME

Final Report

(for the period of October 1, 2007, through March 31, 2009)

Prepared for:

AAD Document Control

U.S. Department of Energy
National Energy Technology Laboratory
PO Box 10940, MS 921-107
Pittsburgh, PA 15236-0940

Cooperative Agreement: DE-FC26-98FT40321
Project Manager: Paula Flenory

Prepared by:

Steven B. Hawthorne

Energy & Environmental Research Center
University of North Dakota
15 North 23rd Street, Stop 9018
Grand Forks, ND 58202-9018

2009-EERC-02-02 February 2009

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government, nor any agency thereof, nor any of their employees makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

This report is available to the public from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161; phone orders accepted at (703) 487-4650.

ACKNOWLEDGMENT

This report was prepared with the support of the U.S. Department of Energy (DOE) National Energy Technology Laboratory Cooperative Agreement No. DE-FC26-98FT40321. However, any opinions, findings, conclusions, or recommendations expressed herein are those of the authors(s) and do not necessarily reflect the views of DOE.

EERC DISCLAIMER

LEGAL NOTICE. This research report was prepared by the Energy & Environmental Research Center (EERC), an agency of the University of North Dakota, as an account of work sponsored by the U.S. Department of Energy. Because of the research nature of the work performed, neither the EERC nor any of its employees makes any warranty, express or implied, or assumes any legal liability or responsibility for the usefulness of any information, apparatus, product, or process disclosed or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement or recommendation by the EERC.

JV TASK 92 – ALCOA/RETEC SFE AND SPME

ABSTRACT

This report summarizes the work performed by the Energy & Environmental Research Center (EERC) under the U.S. Department of Energy Jointly Sponsored Research Program JV Task 92, which is a continuation of JV9. Successful studies performed in 1999 through the end of 2008 demonstrated the potential for using selective supercritical fluid extraction (SFE) and a solid-phase microextraction (SPME) method for measuring sediment pore water polycyclic aromatic hydrocarbons (PAHs) to mimic the bioavailability of PAHs from manufactured gas plant and aluminum smelter soils and sediments both in freshwater and saltwater locations. The studies that the EERC has performed with the commercial partners have continued to generate increased interest in both the regulatory communities and in the industries that have historically produced or utilized coal tar products. Both ASTM International and the U.S. Environmental Protection Agency (EPA) have accepted the pore water method developed at the EERC as standard methods. The studies have demonstrated the effectiveness of our techniques in predicting bioavailability of PAHs from ca. 250 impacted and background field sediments and soils. The field demonstrations from the final years of the project continued to build the foundation data for acceptance of our methods by the regulatory communities. The JV92 studies provide the single largest database in the world that includes measures of PAH bioavailability along with biological end points. These studies clearly demonstrated that present regulatory paradigms based on equilibrium partitioning greatly overpredict bioavailability. These investigations also laid the foundation for present (non-JV) studies being applied to PAHs and polychlorinated biphenyls (PCBs) at EPA Superfund sites, investigations into PAH and PCB bioavailability at U.S Department of Defense sites, and the application of the techniques to investigating the bioavailability of chlorinated dioxins and furans from impacted sediments.

TABLE OF CONTENTS

Continued . . .

TABLE OF CONTENTS (continued)

JV TASK 92 – ALCOA/RETEC SFE AND SPME

EXECUTIVE SUMMARY

Present risk assessment models for polycyclic aromatic hydrocarbon (PAH)-contaminated soils and sediments that are now used by state and federal regulatory agencies frequently require cleaning up of a contaminated site to below ambient background levels. Many recent studies have demonstrated that PAHs become increasingly less available as they age in the environment (especially in the presence of high organic carbon and soot carbon). Application of these ideas in the regulatory framework has been inhibited by the lack of rapid and accurate laboratory tests that can mimic relevant organism uptake upon exposure to PAH-contaminated materials in the environment. This joint venture (JV) task originally proposed that using supercritical carbon dioxide under mild conditions could be used to predict the bioavailability of PAHs in real-world soils and sediments that had been historically contaminated with PAHs. Subsequently, a solid-phase microextraction (SPME) method to measure bioavailable (freely dissolved) pore water concentrations was developed, and the abilities of the supercritical fluid extraction (SFE) and the SPME approaches to predict toxicity to benthic organisms were compared. With an expanded data set of ca. 120 sediments, it was shown that both the SFE and SPME techniques greatly improved toxicity predictions over present regulatory equilibrium partitioning models. However, the SPME approach was significantly better than the SFE model. At the request of regulatory officials, the data base was increased to include ca. 240 sediment samples collected from 20 different sites. These field trials continued to demonstrate the ability of the SPME pore water method to accurately predict the toxicity of PAHs to benthic organisms and to show that present regulatory models greatly overpredict toxicity, with the result of unreasonable and unnecessary cleanup criteria being applied to most sites.

The technical aspects of the project are described in detail in 14 peer-reviewed scientific papers. The results have been positively received by regulators in several states as well as by federal personnel. The SPME method to determine freely dissolved PAHs has been accepted both by ASTM International and the U.S. Environmental Protection Agency (EPA). The technical foundations laid by these investigations are presently being expanded to several (non-JV) studies including predicting the bioavailability of PAHs and polychlorinated biphenyl (PCBs) (freely dissolved concentrations) at EPA Superfund sites, investigations into PAH and PCB bioavailability at U.S. Department of Defense sites, and the application of the techniques to investigating the bioavailability of chlorinated dioxins and furans from impacted industrial sediments. Application of these approaches in the regulatory process to such sites could reduce the quantity of soil or sediment that requires remediation to a small fraction of the amounts required by current regulatory models while remaining fully protective of the environment.

JV TASK 92 – ALCOA/RETEC SFE AND SPME

INTRODUCTION

Present risk assessment models for polycyclic aromatic hydrocarbon (PAH)-contaminated soils and sediments that are now used by state and federal regulatory agencies frequently require cleaning up of a contaminated site to below ambient background levels. This regulatory framework can lead to extensive litigation and inhibit any progress on remediating significantly contaminated soils and sediments. Many recent studies have demonstrated that PAHs become increasingly less available as they age in the environment (especially in the presence of high organic carbon and soot carbon) [1–5]. Application of these ideas in the regulatory framework has been inhibited by the lack of rapid and accurate laboratory tests that can mimic relevant organism uptake upon exposure to PAH-contaminated materials in the environment. The overall purpose of this project was to develop and validate a laboratory test that could improve the prediction of risk from PAH-contaminated soils and sediments to organisms. Based on earlier studies using supercritical fluid extraction (SFE) to demonstrate selective behavior of polychlorinated biphenyls (PCBs) in soils and sediments [6–8], we developed and tested the use of SFE to predict the bioavailability of PAHs from real-world contaminated soils and sediments. The project also involved developing and validating analytical methods for alkyl and parent PAHs including an ultrasensitive method for pore water analysis based on solid-phase microextraction (SPME). The techniques were applied to as many as 240 sediments along with biological testing to determine what improvements in bioavailability predictions could be made over present regulatory models. These investigations also led to an increased understanding of the partitioning chemistry that controls the bioavailability of PAHs in historically contaminated sediments.

OBJECTIVES

The primary objective of these investigations was to develop laboratory tests to improve the prediction of risk from PAH-contaminated soils and sediments. JV92 (a continuation of JV9) increasingly focused on developing and testing a laboratory method to measure freely dissolved sediment pore water PAH concentration including both parent and alkyl PAHs at trace levels and applying these methods to predicting the toxicity of PAHs to benthic organisms.

EXPERIMENTAL

Details of the experimental developments are given in Appendices A–N as peer-reviewed publications. Please see the summary description for these manuscripts below.

RESULTS AND DISCUSSION

The SFE method developed in the earlier years of these investigations was demonstrated to improve the prediction of risk from PAH-contaminated soils and sediments and was shown to have good promise for improving risk assessment based on the several studies described below. As the project progressed, it gained more interest from a broader industrial base and from regulators, with an increasing interest by both parties in focusing on PAH toxicity to benthic organisms in sediments. In our initial studies, the SFE and the SPME methods were equally good at predicting toxicity in contaminated sediments, with both methods being superior to models currently used by state and federal regulators. However, in a larger-scale study of 120 sediments, the SPME method was found to be better than the SFE method for predicting the toxicity to benthic organisms. However, both methods developed in this study were clearly superior to the present regulatory approach based on equilibrium partitioning. Because of the increasing focus on toxicity to benthic organisms in sediments by commercial clients and their regulators, the later years of these investigations focused on the SPME method.

Perhaps the best indication of the project's success is the strong interest from both state and federal authorities and the continually growing list of non-U.S. Department of Energy (DOE) sponsors for these studies. At present, sponsors that have contributed funds either to the joint venture or a similar supporting project include RETEC Inc., ENSR-AECOM, Gas Research Institute, Niagara Mohawk, National Grid, Central Hudson Gas and Electric, NiSource, New York State Electric and Gas (NYSEG), Manitoba Hydro, ALCOA Corporation, U.S. Army Corp of Engineers, U.S. Steel, Test America, CH2MHill, U.S. Department of Defense (Environmental Security Technology Certification Program), and Dow Chemical Company.

Brief descriptions of the results of these investigations are given below. Details can be found in the appendices.

Development of SFE Conditions

The initial development of the mild SFE conditions was conducted to mimic the progress of a bioremediation of PAH-contaminated soil on a football field-sized plot (Appendices A and B). Using the conditions developed in these studies, a 120-minute SFE test correctly predicted the removal of all two- to six-ring PAHs over the 1-year bioremediation. The SFE conditions were then compared on several contaminated soils to water desorption of PAHs from the same soils. In essence, 120 minutes of SFE successfully predicted the desorption of PAHs that occurred with 120 days of water desorption (Appendices B and C). These conditions were also applied to show that lower-molecular-weight aromatics (benzene, toluene, ethylbenzene, and xylenes, or "BTEX") also can show extremely tight binding to soils after decades of aging (Appendix D).

Predicting the Bioavailability of PAHs on Contaminated Soils Using SFE

The first comparison of SFE to true biological systems was for the field bioremediation study described above (Appendices A and B), where 120 minutes of SFE mimicked 1 year of bioremediation. Initial collaborative studies were then conducted that showed SFE may also

predict dermal and ingestion uptake for carcinogenic PAHs (Appendix E). More in-depth studies on several contaminated soils were performed that demonstrated that SFE improved the prediction of PAH uptake in earthworms by a factor of ca. 10 to 100 compared to the "state-of-the-art" equilibrium partitioning model most likely to be used for regulatory risk assessment (Appendix F). A final study with earthworms demonstrated that our mild SFE test removes the actual PAH molecules that cause toxicity while leaving the individual PAH molecules that are too tightly bound to the soil to be available to the worms on the soil sample (Appendix G).

Predicting the Bioavailability of PAHs on Contaminated Sediment Using SFE and Pore Water Measurements

The U.S. Environmental Protection Agency's (EPA's) proposed PAH narcosis model for sediment risk assessment requires measuring both alkyl and parent PAHs (as opposed to all earlier risk assessment that was only based on parent PAHs) [9]. However, we demonstrated that the proposed analytical methods for the alkyl PAHs were not accurate and developed and validated a more accurate method (Appendix H). Proposed methods to measure sediment pore water PAH concentrations were also not adequate for the large number of samples needed for site assessment and would be extremely expensive and laborious because of the need to obtain large volumes of colloid-free pore water. To address these problems, we developed and validated an extremely sensitive and accurate method for both parent and alkyl PAHs that can give the required parts-per-trillion detection limits with 2-mL water samples (Appendix I).

Both the SFE method and the ultratrace pore water method were then compared to the ability of EPA's proposed regulatory model [9, 10] for predicting the toxicity of PAH-contaminated sediments to the aquatic organism, *Hyalella azteca*, for 34 real-world sediment samples. The EPA model grossly overestimated the toxicity of the sediments and predicted that 31 out of the 34 test sediments would be toxic, while only six sediments were actually toxic. In contrast, both the SFE and the pore water method we developed correctly predicted the toxicity (or lack of toxicity) of all 34 sediments (Appendix J). These results clearly demonstrate the abilities of the methods developed to improve risk assessment for PAH-contaminated sites and generated requests (and funding) from several commercial clients and regulators for a larger based of field data—with a special focus on determining which of the two techniques we developed (SFE vs. SPME) gave the best predictions of toxicity to benthic organisms from sediment PAHs. In an expanded study of 120 sediments, both SFE and SPME were much better than the present regulatory models at predicting toxicity. However, SPME measurement of pore water PAHs was the superior method (Appendix K). Therefore, subsequent field demonstrations used the SPME method. At present, we have tested ca. 240 sediments from more than 20 sites, and the superior ability of SPME to predict sediment PAH toxicity remains as good as reported for the 120 sediments in Appendix K.

Because of the success of the SPME method, commercial partners wished to have the method approved by regulatory bodies so that the method could become widely available in contract laboratories. The EERC successfully conducted round-robin studies to gain acceptance by EPA (Method 8272) and by ASTM (Method D 7262-07, Appendix L). It should be noted that EPA method requirements did not allow the full list of PAHs needed for toxicity predictions to be included because no pure standards are possible for the alkylated isomeric clusters of PAHs

that cause the majority of toxicity. However, the ASTM method includes all of the PAHs necessary to predict toxicity and, since EPA has method reciprocity with ASTM, the ASTM method can be used where approved methods are required.

Fundamental Understanding of the Failure of Present Regulatory Models to Predict Bioavailability Compared to SPME Pore Water Measurements

Present regulatory models (and much of the scientific literature) base predictions of bioavailability using the equilibrium partitioning theory. This theory is based on the assumption that each PAH has a constant partitioning coefficient between water and sediment organic carbon (Koc). However, for the ca. 240 sediments we have studied, this assumption has been proven to be dramatically false and can lead to the *overprediction* of PAH effects to benthic organisms by as much as three orders of magnitude. To investigate why this occurs, we measured partitioning coefficients for ca. 120 sediments**,** both to sediment organic carbon (Koc) and to sediment "soot" or "black" carbon (K_{BC}). As shown in Appendices M and N, the assumption that each PAH has constant partitioning coefficients on every sediment (either on an organic carbon or a black carbon basis) is *dramatically* wrong, and leads to overprediction of the bioavailability of PAHs by up to three orders of magnitude. The fundamental partitioning studies reported in Appendices M and N clearly demonstrate why the toxicity predictions using our SPME pore water method are so clearly superior to those from the present regulatory model as shown in Appendices J and K.

CONCLUSIONS

The methods developed in these investigations greatly improve the ability to predict the bioavailability of PAHs compared to state-of-the-art regulatory risk assessment models currently used by state and federal officials. These studies conclusively demonstrate that current risk assessment procedures based on PAH concentrations are not accurate and are unreasonably restrictive. These studies also demonstrate that PAHs on historically contaminated soils and sediments often become so tightly bound that there is no risk to biota. Further demonstration of these techniques and acceptance by regulatory communities is in progress and is expected to greatly reduce the quantities of soils and sediments that require remediation while still being protective of the environment.

REFERENCES

1. Alexander, M. *Environ. Sci. Technol*. **2000**, *34*, 4259–4265.

2. Loehr, R.C.; Webster, M.T. *Pract. Period. Hazard., Toxic, Radioact. Waste Manage*. **2000**, *4*, 118–125.

3. Mackay, A.A.; Gschwend, P.M. *Environ. Sci. Technol*. **2001**, *35*, 1320–1328.

4. Reeves, W.R.; Barhoumi, R.; Burghardt, R.C.; Lemke, S.L.; Mayura, K.; McDonald, T.J.; Phillips, T.D.; Donnelly, K.C. *Environ. Sci. Technol*. **2001**, *35*, 1630–1636.

5. Northcott, G.L.; Jones, K.C. *Environ. Sci. Technol*. **2001**, *35*, 1103–1110.

6. Björklund, E.; Bøwadt, S.; Mathiasson, L.; Hawthorne, S.B. *Environ. Sci. Technol*. **1999**, *33*, 2193–2203.

7. Pilorz, K.; Björklund, E.; Bøwadt, S.; Mathiasson, L.; Hawthorne, S.B. *Environ. Sci. Technol*. **1999**, *33*, 2204–2212.

8. Hawthorne, S.; Björklund, E.; Bøwadt, S.; Mathiasson, L. *Environ. Sci. Technol*. **1999**, *33*, 3152–3159.

9. U.S. Environmental Protection Agency. *Procedures for the Derivation of ESBs for the Protection of Benthic Organisms: PAH Mixtures*; EPA/600/R-02/013, Office of Research and Development, Washington, DC, 2003.

10. U.S. Environmental Protection Agency. *Methods for the Derivation of Site-Specific Equilibrium Partitioning Sediment Guidelines (ESGs) for the Protection of Benthic Organisms: Nonionic Organics*; EPA/822/R/02/042, Office of Science and Technology, Washington, DC, 2004.

APPENDIX A

CORRELATING SELECTIVE SUPERCRITICAL FLUID EXTRACTION WITH BIOREMEDIATION BEHAVIOR OF PAHS IN A FIELD TREATMENT PLOT

PUBLISHED IN *ENVIRONMENTAL SCIENCE AND TECHNOLOGY*

Environ. Sci. Technol. **2000**, *34,* 4103−4110

Correlating Selective Supercritical Fluid Extraction with Bioremediation Behavior of PAHs in a Field Treatment Plot

STEVEN B. HAWTHORNE* AND CAROL B. GRABANSKI

Energy and Environmental Research Center, University of North Dakota, P.O. Box 9018, Grand Forks, North Dakota 58202

Selective supercritical fluid extraction (SFE) behavior of PAHs from manufactured gas plant (MGP) site soils was determined on untreated soil and on soils collected after 1/2 year and 1 year of bioremediation in a field land treatment plot. Sequentially stronger SFE conditions gave selective extraction of PAHs associated with "fast" (or "rapidly desorbing"), "moderate," "slow," and "very slow" sites on the soil collected before and during bioremediation. While all PAHs from the untreated soil showed "stair-step" extraction curves (with molecules in each of the four "fast" to "very slow" SFE fractions), two- and three-ring PAHs were found mostly in the "fast" fraction, while the five- and six-ring PAHs were found almost completely in the "slower" fractions. SFE comparisons of the untreated and bioremediated soils showed that bioremediation only removed PAH molecules which were found in the "fast" fractions by SFE and that remediation for 1 year did not result in the migration of PAHs from "slower" to "faster" sites. One hour SFE of the untreated sample at the mildest condition (120 bar, 50 °C) gave good quantitative agreement with removals achieved after 1 year of bioremediation, and SFE correctly predicted that two- and three-ring PAHs would show ∼90% removals, four-ring PAHs ∼50% removals, and five- and six-ring PAHs <10% removals. Mild SFE reduced the total PAHs on the untreated soil from 6860 mg/kg to 2360 mg/kg (after SFE), which is in excellent agreement with the reduction to 2420 mg/kg achieved following 1 year of bioremediation. The results show that mild SFE may be a rapid and useful test to predict the bioavailability of PAHs on contaminated soil.

Introduction

Understanding the sequestration or binding of organic chemicals which occurs during environmental aging on soils and sediments is important for a broad range of reasons ranging from determining the effect of such pollutants on plant and animal receptors and human health to evaluating the need for and predicting the effectiveness of various remediation and control approaches. A large number of investigations of biological uptake, treatment, and analytical extraction have demonstrated that, in general, longer exposures of persistent organics to a soil or sediment matrix leads to tighter associations with that matrix and, consequently, less availability for transport (e.g., water desorption) and for uptake by biological systems (*1*−*25*). Multisite models and kinetic approaches to explain the sequestration and release of aged organics from soils and sediments are gaining acceptance, as are ideas that aging causes organics such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) to become increasingly associated with soil polymeric organic matter and/or to diffuse further into soil micropores (*1*−*3, 16, 18, 19, 22, 23, 26*−*33*). Regardless of the mechanism (as concluded by Alexander (*3*)), the outcome is the same, i.e., molecules that are sequestered in the soil/sediment matrix are much less available to organisms.

The need to understand the uptake and release of persistent organic pollutants has led to various laboratory approaches for determining the degree of "availability" (whether to water or to biological systems) of organics on soils and sediments. Tests include long-term (several months) water rate-of-release studies (*34*−*36*), biological availability assays such as earthworm uptake (*37*), to chemical assays including solid-phase extraction (*37, 38*), organic solvent extraction with mild solvents (*37, 39, 40*), dialysis (*41*), and pyrolysis (*42*). For the purpose of assessing the risk associated with a chemical in soil or sediment, it would be useful to have a rapid laboratory test capable of predicting the fraction of chemicals that are "available" for biological uptake, treatment, and water transport.

Supercritical fluid extraction (SFE) with pure carbon dioxide (CO_2) has recently been proposed as a potentially rapid method to determine the "availability" of soil- and sediment-bound organics (*43*−*47*). The potential advantage of SFE is that the solubility of target analytes can be varied continuously over several orders of magnitude by controlling the extraction pressure and temperature (*48, 49*). In addition, the kinetics of desorption processes can be enhanced by simply changing the temperature used for extraction. In contrast to organic solvent extractions (which can extract significant fractions of the soil organic matrix), SFE with pure CO_2 can extract hydrophobic pollutants (e.g., PAHs, PCBs) without significantly altering the soil organic matrix (*47*). Although developed independently, the models used to explain the desorption kinetics of organic pollutants from sediments into water and those used to explain SFE behavior of organics are of essentially the same form (*50*−*55*). The similarity of these models and the ability to vary solvent strength over a wide range suggests that SFE could be used as a simple test to investigate the "availability" of organic pollutants.

At present, the relationship between SFE behavior and real-world behavior of organic pollutants is mostly conjectural. However, in a recent series of articles, SFE performed under increasingly stronger conditions showed that PCBs were present in several different types of sites on every soil and sediment tested and that PCBs were associated with sites ranging from "fast" (extracted at the mildest SFE conditions) to "slowly desorbing" sites (*45*−*47*). Furthermore, when the same sediments were exposed to PCBs in water for up to 18 days, the exposure time was only sufficient for the PCBs to sorb to only the "fast" sites, demonstrating that very long exposure times would be needed for the PCBs to gain the "slowly desorbing" sites which were occupied in the original environmentally aged sediments (*47*). In the present study, we extend the SFE conditions developed in the earlier PCB studies to investigate the behavior of PAHs during a 1-year, large-scale field bioremediation of a PAH-impacted soil from a manufactured gas plant (MGP) site.

* Corresponding author phone: (701)777-5256; fax: (701)777-5181; e-mail: shawthorne@eerc.und.nodak.edu.

10.1021/es001178o CCC: $19.00 © 2000 American Chemical Society
Published on Web 08/24/2000

Materials and Methods

Supercritical Fluid Extraction. All extractions were performed with an ISCO model 260D syringe pump (ISCO, Lincoln, NE) filled with SFC-grade CO_2 (Scott Specialty Gases, Plumsteadville, PA) and an ISCO model SFX 2-10 extractor with 10-mL extraction cells and 4 g of the test soil. Extracted PAHs were collected in 22 mL vials containing 15 mL of methylene chloride (CH_2Cl_2) (Fisher Optima grade). Flow rates were controlled at 1 mL/min (measured as compressed CO_2 at the pump) using a variable coaxial restrictor (ISCO) heated to 80 °C. Note that extraction flow rates must be carefully monitored since they may affect the extraction rates of the "fast" PAHs (although changes in flow rate are unlikely to change the extraction rate of the "slow" PAH fractions) as previously described (*56*). In addition, it is important to place the sample at the outlet end of the SFE cell so that cell void volume does not affect the extraction rates.

Kinetic profiles from the different soils were obtained using four sequentially stronger SFE conditions, each applied for either 30 min or for 1 h. The sequential SFE conditions were 120 bar, 50 °C ("rapidly desorbing" or "fast" fraction); 400 bar, 50 °C ("moderate" fraction); 400 bar, 100 °C ("slow" fraction"); and 400 bar, 150 °C ("very slow" fraction). Collection vials were changed at set time intervals during each extraction period (e.g., at 5, 10, 20, and 30 min at each SFE condition) so that the shape of the extraction curve could be determined at each condition. Since adding the PAH concentrations from the multiple fractions collected for the kinetic plots could introduce error in the PAH quantitations, the quantity of each PAH extracted at each SFE condition was further verified by repeating each extraction (in triplicate) and collecting and analyzing the entire fraction for each of the SFE extraction conditions. After the four-step SFE procedure was completed, the soil residue was finally extracted overnight with acetone/CH_2Cl_2 (1:1) to recover any PAHs which were not extracted by SFE. Additional minor procedural details are the same as previously reported (*45*).

Gas Chromatographic Analysis. A Hewlett-Packard model 5890 Series II gas chromatograph (GC) equipped with a flame ionization detector (FID), a split/splitless injection port (300 °C), and a model 7673A auto injector were used for analyzing the extracted fractions. Separations were performed on a 50 m DB-5 column (0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, Rancho Cordova, CA). Injections were at an oven temperature of 80 °C followed by a temperature ramp of 6 °C/min to 320 °C (hold for 5 min). Each fraction was spiked with *n*-undecane as an internal standard. Quantitations were based on calibration curves (PAH peak area versus the internal standard) using pure PAH standards for all major species (at least one PAH for each molecular weight reported). Total PAH concentrations were based on the total FID peak areas (versus the internal standard) of the PAH calibration standards. PAH identifications were confirmed by GC/MS analysis using the same chromatographic conditions.

Samples. Soil samples were collected from an MGP site in the Midwest during biological land treatment in a several hundred m^3 field unit. The bioremediation process essentially involved placing the contaminated soil in a prepared bed land treatment unit ~30 cm deep, supplying water and nutrients, and tilling frequently to supply oxygen for approximately 1 year beginning in May. Detailed descriptions of the process have been previously reported (*36*). During the treatment, the site was divided into 16 separate subplots, and each sampling event consisted of subsamples collected from each subplot which were composited and sieved. The homogenized soils were air-dried and stored at 4 °C until used. Particle sizes of the untreated soil were (1−6 mm) 40 wt %, (0.5−1 mm) 15 wt %, (0.25−0.5 mm) 16 wt %, (0.125−0.25 mm) 17 wt %, and (<0.125 mm) 12 wt %. Carbon, hydrogen, and nitrogen contents of the untreated soil were 4.6, 0.4, and 0.1 wt %, respectively.

Results and Discussion

Effect of Bioremediation on PAH Selective SFE Behavior. Stepwise SFE extraction profiles of representative PAHs from the untreated soil and soils after 1/2 year and 1 year of bioremediation are shown in Figure 1. Each sequentially stronger SFE condition was applied for 30 min to yield the "stair-step" plots showing the PAHs extracted from "fast" sites (0−30 min), "moderate" (30−60 min), "slow" (60−90 min), and "very slow" (90−120 min) sites. (PAH concentrations shown after 120 min are those extracted from the soil residue after SFE by 18 h of Soxhlet extraction.)

Two trends in these plots are worth noting. First, the low molecular weight PAHs (naphthalene, 1- and 2-methylnaphthalene, acenaphthylene, acenaphthene, fluorene, dibenzothiophene, phenanthrene, and anthracene) all show similar behavior, i.e., the majority (~80 to 90%) of each PAH was found in the "fast" (extracted at the mildest SFE condition) fraction. More importantly, as the bioremediation continued to 1/2 and 1 year, *only the molecules which were located in the "fast" fraction show significant removal by the bioremediation process,* while the molecules in the "slower" three SFE fractions show no significant change. Note that the SFE extraction curves for the treated soils are essentially identical to those of the untreated soils in the SFE fractions after the first 30 min (i.e., the "moderate, slow, and very slow" fractions), clearly demonstrating that only the "fast" molecules as defined by SFE are significantly removed by the field bioremediation process.

As the molecular weights of the PAHs increase (4-, 5-, and 6-ring PAHs including fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*e*]pyrene, benzo[*a*]pyrene, perylene, indeno[*1,2,3-cd*]pyrene, dibenz[*a,h*]anthracene, and benzo[*ghi*]perylene), the distribution of the PAHs shifts to the "slower" SFE fractions, and the removals achieved by bioremediation also decrease (Figure 1). Similar to the trend for low molecular weight PAHs, only the fractions of PAHs found in the "faster" two SFE fractions (from 0 to 60 min in Figure 1) show any significant reduction by the bioremediation process. Finally, for the five- and six-ring PAHs, there is no significant reduction in the PAH concentrations over the 1-year bioremediation treatment. (Note that the small changes in concentrations shown for the higher molecular weight PAHs such as benzo[*a*]pyrene and benzo[*ghi*]perylene are likely associated with soil heterogeneity at the field site, since the site was sampled over a 1-year period, and related analytical error.)

When the extraction data are viewed as the percent in each SFE fraction (rather than the PAH concentrations used in Figure 1), the extraction behavior for the high molecular weight PAHs is essentially identical for the untreated and bioremediated soils, as shown for benzo[*a*]pyrene in Figure 2. Therefore, it appears that (in addition to not being removed from the soil by the bioremediation process) the high molecular weight PAHs did not migrate between "slower" and "faster" sites during the 1-year bioremediation period. For the lower molecular weight PAHs, the percentage of molecules shifted toward the "slower" SFE sites (as shown by naphthalene in Figure 2), as would be expected since only the PAH molecules found in the "fast" SFE fraction were removed by the 1-year bioremediation (Figure 1).

Quantitative Comparison of PAH Removals by Bioremediation and SFE. As shown in Figure 1, PAHs found in the first SFE fraction (and possibly the second fraction for higher molecular weight PAHs) appear to best account for the PAH molecules removed during bioremediation, while the "slower" PAHs (those extracted by the strongest two SFE conditions) appeared unaffected by the bioremediation. Therefore,

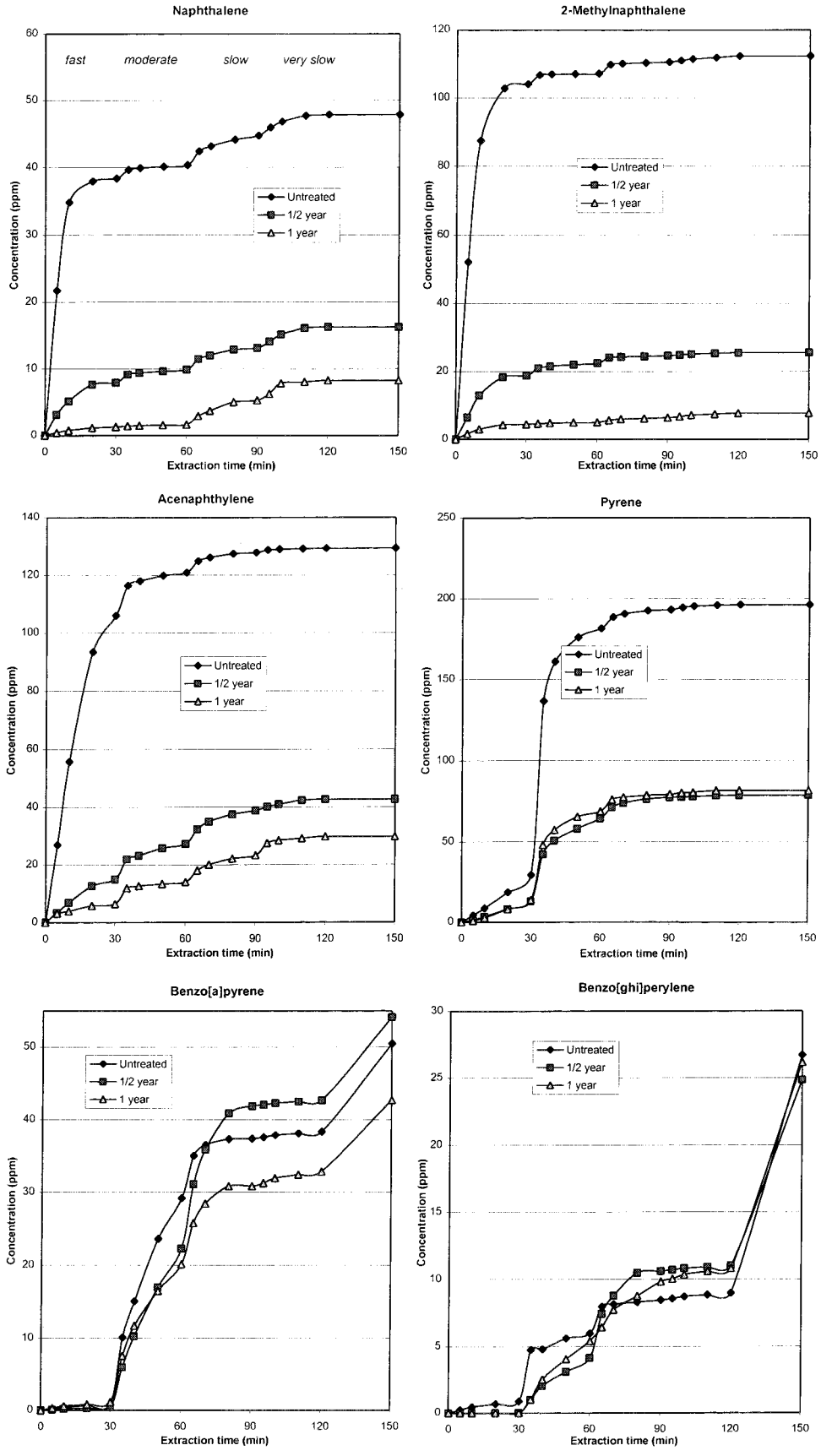


FIGURE 1. Selective SFE removal of representative PAHs from MGP-impacted soil before treatment and after 1/2 year and 1 year of bioremediation in a field site. Sequential SFE was performed with pure CO_2 for 30 min at each condition including 120 bar and 50 °C ("fast" sites), 400 bar and 50 °C ("moderate"), 400 bar and 100 °C ("slow"), and 400 bar and 150 °C ("very slow"). PAHs shown after 120 min are those extracted from the soil residue (after SFE) by 18 h of Soxhlet extraction.

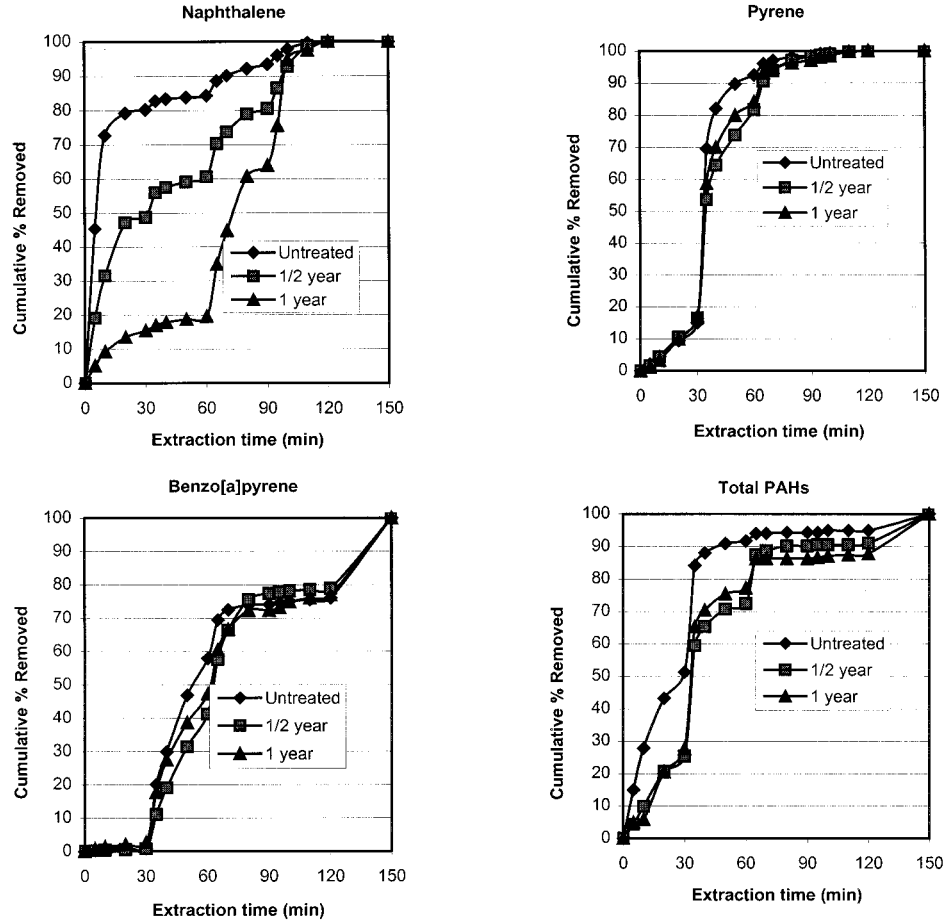


FIGURE 2. Percent removals of representative PAHs from MGP-impacted soil before treatment and after 1/2 year and 1 year of bioremediation in a field site using sequentially stronger SFE conditions. SFE was performed with pure CO_2 for 30 min at each condition including 120 bar and 50 °C ("fast" sites), 400 bar and 50 °C ("moderate"), 400 bar and 100 °C ("slow"), and 400 bar and 150 °C ("very slow"). PAHs shown after 120 min are those extracted from the soil residue (after SFE) by 18 h of Soxhlet extraction.

efforts were made to correlate the quantities of each PAH species removed by the first two SFE conditions (the "fast" and "moderate" molecules) with the quantities of each PAH species removed after 1/2 year and 1 year of bioremediation. Triplicate samples of the untreated soil were extracted sequentially with the mildest (120 bar, 50 °C) and second-mildest (400 bar, 50 °C) SFE conditions to sequentially remove the "fast" and "moderate" PAHs from the untreated soil. Since the 30-min fractions used for Figure 1 were not always sufficient to remove the molecules in a particular fraction (as evidenced by not obtaining a flat extraction curve at the end of each "stair-step" condition), the SFE extraction times were increased to 60 min for each extraction condition. Following extraction at these two SFE conditions, the extracted soil residues were subjected to Soxhlet extraction to determine the unextracted concentrations. The SFE and Soxhlet results were then used to calculate the concentration of each PAH remaining after extraction of the "fast" SFE fractio, and after the extraction of the "fast" and "moderate" SFE fractions, and the results were compared to the concentrations of the same PAHs remaining after 1/2 year and 1 year of bioremediation.

The concentrations of the PAHs found in the field plot after bioremediation and those found after extracting the "fast" and "moderate" PAHs from the untreated soil are shown in Table 1. For the majority of PAHs, the concentrations remaining after the selective SFE removal of both the "fast" and "moderate" PAHs from the untreated soil show good agreement with the concentrations found when the field site was sampled after both 1/2 year and 1 year of bioremediation. The quantitative reproducibility of the selective SFE method was also satisfactory, with the relative standard deviations of replicate SFE experiments (for each fraction) similar to those found for multiple analyses of the bioremediated soils. The generally good agreement obtained between the concentrations predicted by the SFE extraction of the "fast" PAHs and the actual removals achieved by bioremediation are encouraging, especially considering the fact that the predictions were performed with individual PAH concentrations ranging from −10 to over 400 mg/kg, that bioremediation removals of PAHs ranged from − 0% to − 90%, and that PAH molecular weights ranged from 128 to 278 amu (i.e., the entire range of PAHs under regulatory scrutiny).

Figure 3 shows the average percent of PAHs (grouped by ring size) remaining after 1/2 year and 1 year of bioremediation and after the removal of the "fast" and "moderate" PAHs from the untreated soil with SFE. In general, the percent of each group of PAHs removed with the "fast" SFE fraction best agrees with the percent of each group of PAHs removed by bioremediation, regardless of whether high removals were achieved by bioremediation (2- and 3-ring PAHs) or little removal was achieved by bioremediation (5- and 6- ring PAHs). The percent removal for the total PAHs achieved by bioremediation was essentially identical to that removed by the mildest SFE condition (Figure 3). Linear correlation coefficients (r^2) for the percent of each individual PAH (those listed in Table 1) removed by bioremediation and extracted by the mildest SFE condition were 0.93 and 0.92 for 1/2 year and 1 year of bioremediation, respectively.

TABLE 1: Comparison of PAHs Removed by Bioremediation with Those Removed by Selective SFE[d]

	PAH concentration ± SD (mg/kg)				
		after bioremediation[a]		after SFE to extract[b]	
PAHs	untreated soil[a]	1/2 year	1 year	fast fraction	fast and moderate fraction
naphthalene	48 ± 2	16 ± 1	8.2 ± 0.4	17 ± 1	15 ± 1
1-methylnaphthalene	118 ± 4	22 ± 2	6.4 ± 0.4	7 ± 1	4 ± 2
2-methylnaphthalene	112 ± 4	26 ± 3	7.7 ± 0.5	6 ± 1	4 ± 3
acenaphthylene	129 ± 1	43 ± 17	30 ± 6	33 ± 2	25 ± 3
acenaphthene	78 ± 3	16 ± 1	5.7 ± 0.7	4 ± 1	2 ± 2
fluorene	136 ± 6	17 ± 1	12 ± 2	9 ± 2	4 ± 3
dibenzothiophene	70 ± 3	15 ± 2	13 ± 2	7 ± 1	2 ± 1
phenanthrene	434 ± 19	56 ± 1	23 ± 4	52 ± 1	24 ± 10
anthracene	110 ± 7	26 ± 4	14 ± 1	20 ± 5	11 ± 2
fluoranthene	130 ± 6	46 ± 1	41 ± 3	39 ± 8	12 ± 5
pyrene	196 ± 8	79 ± 2	82 ± 5	76 ± 9	21 ± 10
benz[*a*]anthracene	74 ± 2	48 ± 4	46 ± 1	54 ± 3	21 ± 2
chrysene	77 ± 3	52 ± 5	51 ± 1	61 ± 3	28 ± 2
benzo[*b*+*k*]fluoranthene	88 ± 12	73 ± 16	58 ± 10	81 ± 1	43 ± 1
benzo[*e*]pyrene	39 ± 5	39 ± 3	30 ± 2	36 ± 1	25 ± 2
benzo[*a*]pyrene	51 ± 5	54 ± 4	43 ± 3	48 ± 1	33 ± 1
perylene	11 ± 2	11 ± 2	8.3 ± 0.5	11 ± 0	8 ± 1
indeno[*1,2,3-cd*]pyrene	9 ± 2	16 ± 2	14 ± 2	17 ± 1	15 ± 1
dibenz[*a,h*]anthracene	12 ± 1	12 ± 1	12 ± 2	12 ± 0	10 ± 1
benzo[*ghi*]perylene	27 ± 3	25 ± 2	26 ± 3	26 ± 1	23 ± 1
total PAHs by FID[c]	6590 ± 260	2460 ± 240	2420 ± 60	2360 ± 310	1580 ± 180

[a] PAH concentrations and standard deviations based on triplicate Soxhlet extractions of each soil sample. [b]Concentrations remaining after extraction of the fast (60 min at 120 bar, 50 °C) and fast + moderate (previous extraction plus 60 min at 400 bar, 50 °C) fractions from the untreated soil. All extractions were performed in quadruplicate. [c]Total PAH concentrations determined by the sum of individual GC/FID peak areas. [d] Sixty min at each condition.

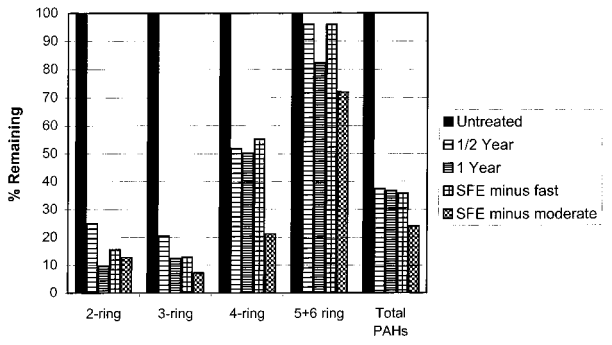


FIGURE 3. Percentage of PAHs (grouped by ring size) remaining on soil after 1/2 year and 1 year of bioremediation, compared to the percentage remaining after 1 h of SFE at 120 bar and 50 °C ("SFE minus fast") and an additional hour of SFE at 400 bar and 50 °C ("SFE minus moderate").

Single Condition SFE Extraction Rates vs Bioremediation Behavior. While the "stepwise" SFE approach described above imitates approaches such as the use of different organic solvents to increase extraction strength (*37, 39, 40*), other approaches to mimic the environmental release of sequestered organics are based on kinetic release curves generated with a single experimental condition. For example, desorption of organics from sediments into water is normally performed for several months, and the desorption rate curves are evaluated to determine "fast" (rapid-desorbing) and "slow" fractions for individual pollutant species (*34, 36*).

In an initial attempt to evaluate the ability of a single SFE condition to mimic field bioremediation behavior of the MGP soil, extractions were conducted using several different pressures and temperatures (ranging from 120 to 400 bar, and 50 to 150 °C) to determine which SFE condition most closely mimicked the actual field bioremediation results. As might be expected based on results of the "stepwise" SFE conditions discussed above, a single SFE condition (200 bar, 50 °C) which was slightly stronger than that used for the "fast" fraction in the stepwise approach (120 bar, 50 °C) appeared to best correlate with the actual bioremediation behavior. This condition was used to extract the untreated MGP soil (in triplicate) for 120 min.

The general shape of the 200 bar (50 °C) SFE curves up to 40 min (right side of Figure 4) show expected trends with the actual removal of PAHs in the field bioremediation treatment (left side of Figure 4), i.e., 1 min of SFE approximates 10 days of bioremediation. Because the bioremediation samples had to be collected over 1 year from a large field site, the jagged nature of the bioremediation curves is most likely a result of soil heterogeneity. However, the agreement between the removal profile by bioremediation over 1 year and the extraction rates by SFE at 200 bar, 50 °C over 40 min are reasonably good for both low and high molecular weight PAHs present in the sample.

Quantitative comparisons of the PAHs removed by bioremediation and the single SFE extraction were also performed by noting that the "fast" fraction of PAHs was generally removed by ~20 min of the SFE process. The quantities of PAHs remaining in the soil after 1/2 and 1 year of bioremediation and after 20 min of SFE at 200 bar, 50 °C (each performed in triplicate) are shown in Figure 5. In general, the concentrations of the PAHs removed by bioremediation and by the single SFE condition agreed well, especially considering that the technique successfully mimics the behavior of PAHs ranging in molecular weight from 128 to 278 amu (two to six rings). Although the selection of 20 min to determine the "fast" fraction in Figure 5 was based only on the soils used in this study, initial extractions of 12 different soils (from different sites) also show similar behavior.

As shown in Figure 4, the extraction rate curves begin to flatten out at longer extraction times, especially for the lower molecular weight PAHs. Two explanations may apply. First, if the extraction rate goes to zero at a certain SFE condition, it could be inferred that the individual PAHs extracted at that condition were in one "compartment," while unextracted PAHs were in a more tightly bound "compartments." However, if the PAHs desorb in a continuum of rates, an infinitely long extraction at a mild SFE condition should

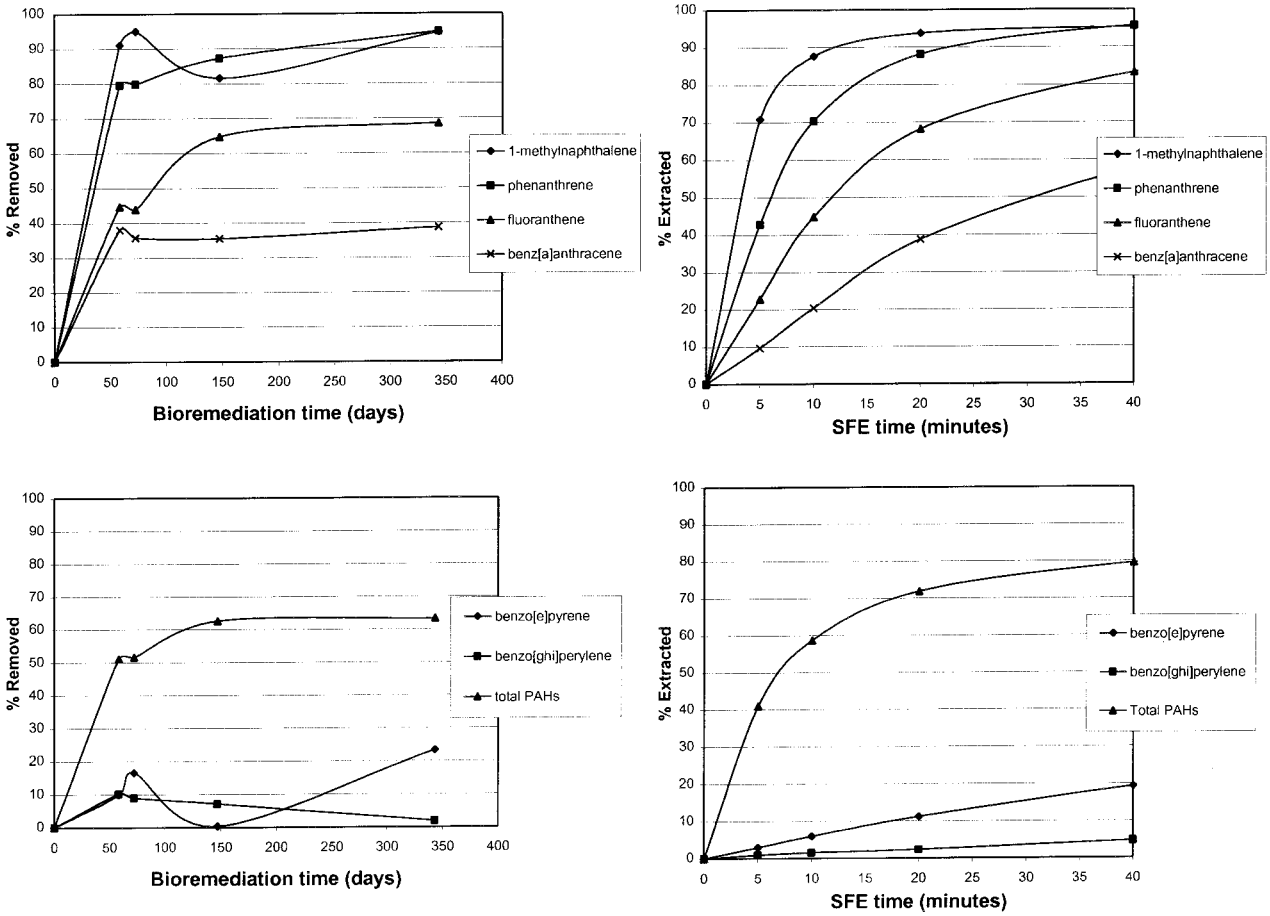


FIGURE 4. Removal of representative PAHs from a historically contaminated soil with bioremediation (left side of figure) and SFE at 200 bar and 50 °C (right side of figure).

remove 100% of the molecules. In an effort to differentiate these mechanisms, additional extractions at 200 bar, 50 °C were conducted on the untreated and the 1-year treated soils for 8 h, with fractions collected every 20 min. For both soils, measurable concentrations of all the PAHs listed in Table 1 were found in all fractions (even the 460−480 min fraction), demonstrating that desorption of the PAHs occurs in a continuum manner, rather than from discrete compartments.

PAH Characteristics, Treatability, and SFE Behavior. The ability to rapidly mimic environmental behavior of PAHs on historically contaminated soils and sediments is complicated by the fact that the physicochemical properties of PAHs vary so greatly. For example, the PAHs commonly associated with MGP sites range from two rings (naphthalene, 128 amu) to six rings (e.g., benzo[*ghi*]perylene, 276 amu), with associated boiling points ranging from 218 to − 500 °C, and water solubilities ranging from 32 to 0.0003 mg/L, respectively (Table 2). Given this wide range of PAH characteristics, the strong correlation between SFE and bioremediation behavior described above may initially seem counterintuitive.

For direct biological uptake (e.g., earthworm ingestion), supercritical CO_2 may appear to be a reasonable solvent, since its polarity is similar to that of biological lipids. However, other biological exposure routes (e.g., microbiological degradation) may require desorption into water prior to biological uptake (*3*, *57*). Even though previous studies with PCBs showed good correlation between SFE and long-term water desorption (*45*, *46*), supercritical CO_2 may initially seem like an unlikely solvent to mimic water-mediated PAH desorption since CO_2 is very nonpolar (similar to hexane), while water is highly polar. Hence, it may seem unlikely that the behavior of PAHs ranging from two to six rings would be similar in water and supercritical CO_2. Surprisingly, when the solubilities of PAHs in ambient water are compared with their solubilities in supercritical CO_2 at the mildest SFE condition (120 bar, 50 °C) previously discussed, the individual PAH solubilities are very similar (Table 2), at least for the PAHs for which data are available. Solubilities are not available for higher molecular weight PAHs at 120 bar and 50 °C, but PAH solubilities at the second strongest SFE condition (400 bar, 50 °C) show excellent correlation with ambient water solubilities (i.e., ~1 order of magnitude higher in CO_2 than water) for PAHs ranging from the two-ring naphthalene to six-ring benzo[*ghi*]perylene. This strong degree of correlation for PAH solubilities between ambient water and supercritical CO_2 is quite striking, especially considering that PAH solubilities vary by five orders-of-magnitude (for naphthalene to benzo[*ghi*]perylene) in both water and CO_2 (Table 2).

TABLE 2: Characteristics of Representative PAHs

PAHs	mol wt (amu)	boiling point (°C)	water solubility (mg/L)[b]	CO_2 solubility (mg/kg)[a] 120 bar, 50 °C	CO_2 solubility (mg/kg)[a] 400 bar, 50 °C
naphthalene	128	218	32	38	116
fluorene	166	297	2	5.7	11
phenanthrene	178	340	1.3	3.2	12
anthracene	178	340	0.073	0.081	0.61
pyrene	202	394	0.14	0.21	1.2
chrysene	228	436	0.002		0.021
perylene	252	498	0.0004		0.005
benzo[*ghi*]perylene	276	500	0.00026		0.002

[a] Adapted from solubilities reported in refs *48* and *49*. [b] Adapted from ref *58*.

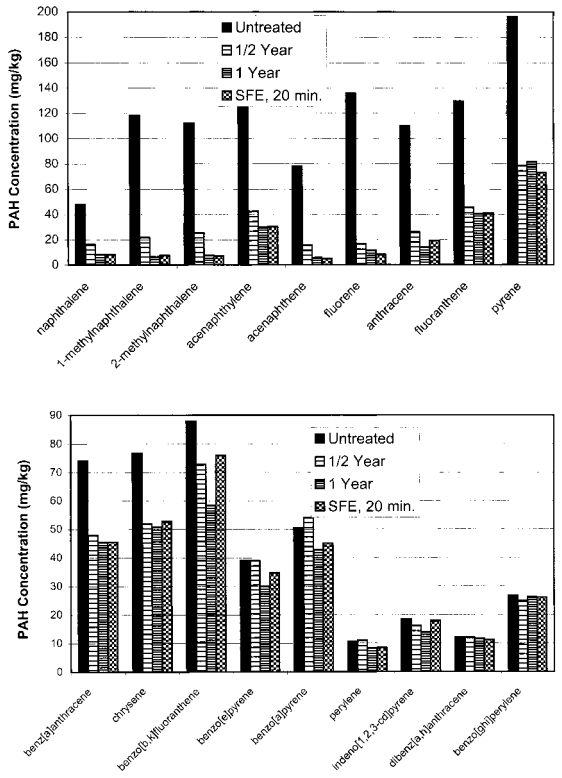


FIGURE 5. Comparison of bioremediation (1/2 and 1 year) and 20 min of SFE at 200 bar (50 °C) on individual PAH concentrations on a historically contaminated soil.

SFE with pure CO_2 also appears to have little or no affect either on the bulk matrix organic material on sediments (in contrast to organic solvents), sediment pH, nor greatly affect their water of hydration (i.e., the 1−2% of water typically left on soils and sediments after air-drying) (*47*). This lack of effect on matrix composition and the correlation between PAH solubilities in water and CO_2 combined with the enhanced mass transfer (faster extraction rates) may contribute to the strong relationship between mild SFE behavior and bioremediation behavior discussed above.

Although the selective SFE approach must be validated by extracting many more soils and sediments and correlating the results with other approaches to determine environmental mobility and bioavailability, the results described above indicate that SFE may be a powerful tool to study the sequestration of PAHs and other persistent organic chemicals and to predict their behavior in the environment. Studies of SFE behavior are planned on several soils and sediments from different MGP processes, and plans include correlating these results with parallel studies on bioremediation behavior, water desorption rates, earthworm toxicity and uptake, and other bioassays.

Acknowledgments

The authors thank John Harju (GRI) and Dave Nakles (ThermoRetec) for helpful discussions and Jim Edwards (ThermoRetec) for soil samples. The financial support of GRI and the U.S. Department of Energy are gratefully acknowledged. This manuscript was prepared with the support of the U.S. Department of Energy, under Cooperative Agreement No. DE-FC26-98FT40321. However, any opinions, findings, conclusions, or recommendations expressed herein are those of the author(s) and do not necessarily reflect the views of the DOE.

Literature Cited

(1) Pignatello, J. J.; Xing, B. *Environ. Sci. Technol.* **1996**, *30*, 1.
(2) Luthy, R. G.; Aiken, G. R.; Brusseau, M. L.; Cunningham, S. D.; Gschwend, P. M.; Pignatello, J. J.; Reinhard, M.; Traina, S. J.; Weber, W. J., Jr.; Westall, J. C. *Environ. Sci. Technol.* **1997**, *31*, 3341.
(3) Alexander, M. *Environ. Sci. Technol.* **1995**, *29*, 2713.
(4) Ball, W. P.; Roberts, P. V. *Environ. Sci. Technol.* **1991**, *25*, 1223.
(5) Ball, W. P.; Roberts, P. V. *Environ. Sci. Technol.* **1991**, *25*, 1237.
(6) Pavlostathis, S. G.; Mathavan, G. N. *Environ. Sci. Technol.* **1992**, *26*, 532.
(7) McGroddy, S. E.; Farrington, J. W. *Environ. Sci. Technol.* **1995**, *29*, 1542.
(8) McGroddy, S. E.; Farrington, J. W.; Gschwend, P. M. *Environ. Sci. Technol.* **1996**, *30*, 172.
(9) Brunk, B. K.; Jirka, G. H.; Lion, L. W. *Environ. Sci. Technol.* **1997**, *31*, 119.
(10) Carmichael, L. M.; Christman, R. F.; Pfaender, F. K. *Environ. Sci. Technol.* **1997**, *31*, 126.
(11) Hatzinger, P. B.; Alexander, M. *Environ. Sci. Technol.* **1995**, *29*, 537.
(12) Pignatello, J. J.; Ferrandino, F. J.; Huang, L. Q. *Environ. Sci. Technol.* **1993**, *27*, 1563.
(13) Nam, K.; Alexander, M. *Environ. Sci. Technol.* **1998**, *32*, 71.
(14) Chung, N.; Alexander, M. *Environ. Sci. Technol.* **1998**, *32*, 855.
(15) Kelsey, J. W.; Kottler, B. D.; Alxander, M. *Environ. Sci. Technol.* **1997**, *31*, 214.
(16) Carroll, K. M.; Harkness, M. R.; Bracco, A. A.; Balcarcel, R. R. *Environ. Sci. Technol.* **1994**, *28*, 253.
(17) Kan, A. T.; Fu, G.; Tomson, M. B. *Environ. Sci. Technol.* **1994**, *28*, 859.
(18) Weber, W. J., Jr.; McGinley, P. M.; Katz, L. E. *Environ. Sci. Technol.* **1992**, *26*, 1955.
(19) Weber, W. J., Jr.; Huang, W. *Environ. Sci. Technol.* **1996**, *30*, 881.
(20) Erickson, D. C.; Loehr, R. C.; Neuhauser, E. F. *Wat. Res.* **1993**, *27*, 911.
(21) Kan, A. T.; Fu, G.; Hunter, M.; Chen, W.; Ward, C. H.; Tomson, M. B. *Environ. Sci. Technol.* **1998**, *32*, 892.
(22) Chiou, C. T.; McGroddy, S. E.; Kile, D. E. *Environ. Sci. Technol.* **1998**, *32*, 264.
(23) Huang, W.; Weber, W. J., Jr. *Environ. Sci. Technol.* **1998**, *32*, 3549.
(24) Kleineidam, S.; Rügner, H.; Ligouis, B.; Grathwohl, P. *Environ. Sci. Technol.* **1999**, *33*, 1637.
(25) Chung, N.; Alexander, M. *Environ. Sci. Technol.* **1999**, *33*, 3605.
(26) Nieman, J. K. C.; Sims, R. C.; Sims, J. L.; Sorensen, D. L.; McLean, J. E.; Rice, J. A. *Environ. Sci. Technol.* **1999**, *33*, 776.
(27) Cornelissen, G.; Van Noort, P. C. M.; Govers, H. A. J. *Environ. Sci. Technol.* **1998**, *32*, 3124.
(28) Ortiz, E.; Kraatz, M.; Luthy, R. G. *Environ. Sci. Technol.* **1999**, *33*, 235.
(29) Schlebaum, W.; Schraa, G.; Van Riemsdijk, W. H. *Environ. Sci. Technol.* **1999**, *33*, 1413.
(30) Rügner, H.; Kleineidam, S.; Grathwohl, P. *Environ. Sci. Technol.* **1999**, *33*, 1645.
(31) Ten Hulscher, Th. E. M.; Vrind, B. A.; Van Den Heuvel, H.; Van Der Velde, L. E.; Van Noort, P. C. M.; Beurskens, J. E. M.; Govers, H. A. J. *Environ. Sci. Technol.* **1999**, *33*, 126.
(32) Ahn, I.-S.; Lion, L. W.; Schuler, M. L. *Environ. Sci. Technol.* **1999**, *33*, 3241.
(33) LeBoeuf, E. J.; Weber, W. J., Jr. *Environ. Sci. Technol.* **1997**, *31*, 1697.
(34) Opdyke, D. R.; Loehr, R. C. *Environ. Sci. Technol.* **1999**, *33*, 1193.
(35) Cornelissen, G.; Van Noort, P. C. M.; Parsons, J. R.; Govers, H. A. J. *Environ. Sci. Technol.* **1997**, *31*, 454.
(36) Loehr, R. C.; Webster, M. T. *Practice of Hazardous, Toxic and Radioactive Waste Management*, accepted for publication.
(37) Tang, J.; Robertson, B. K.; Alexander, M. *Environ. Sci. Technol.* **1999**, *33*, 4346.
(38) Macrae, J. D.; Hall, K. J. *Environ. Sci. Technol.* **1998**, *32*, 3809.
(39) Kelsey, J. W.; Kottler, B. D.; Alexander, M. *Environ. Sci. Technol.* **1997**, *31*, 214.
(40) Tang, J.; Alexander, M. *Environ. Toxicol. Chem*. **1999**, in press.
(41) Woolgar, P. J.; Jones, K. C. *Environ. Sci. Technol.* **1999**, *33*, 2118.
(42) Guthrie, E. A.; Bortiatynski, J. M.; Van Heemst, J. D. H.; Richman, J. E.; Hardy, K. S.; Kovach, E. M.; Hatcher, P. G. *Environ. Sci. Technol.* **1999**, *33*, 119.
(43) Weber, W. J., Jr.; Young, T. M. *Environ. Sci. Technol.* **1997**, *31*, 1686.
(44) Young, T. M.; Weber, W. J., Jr. *Environ. Sci. Technol.* **1997**, *31*, 1692.
(45) Björklund, E.; Bøwadt, S.; Mathiasson, L.; Hawthorne, S. B. *Environ. Sci. Technol.* **1999**, *33*, 2193.

(46) Pilorz, K.; Björklund, E.; Bøwadt, S.; Mathiasson, L.; Hawthorne, S. B. *Environ. Sci. Technol.* **1999**, *33*, 2204.
(47) Hawthorne, S. B.; Björklund, E.; Bøwadt, S.; Mathiasson, L. *Environ. Sci. Technol.* **1999**, *33*, 3152.
(48) Bartle, K. D.; Clifford, A. A.; Jafar, S. A.; Shilstone, G. F. *J. Phys. Chem. Ref. Data* **1991**, *20*, 713.
(49) Miller, D. J.; Hawthorne, S. B.; Clifford, A. A.; Zhu, S. *J. Chem. Eng. Data* **1996**, *41*, 779.
(50) Bartle, K. D.; Clifford, A. A.; Hawthorne, S. B.; Langenfeld, J. J.; Miller, D. J.; Robinson, R. *J. Supercrit. Fluids* **1990**, *3*, 143.
(51) Bartle, K. D.; Boddington, T.; Clifford, A. A.; Hawthorne, S. B. *J. Supercrit. Fluids* **1992**, *5*, 207.
(52) Goto, M.; Sato, M.; Hirose, T. *J. Chem. Eng. Jpn.* **1993**, *26*, 401.
(53) Roy, B. C.; Goto, M.; Hirose, T.; Navaro, O.; Hortacsu, O. *J. Chem. Eng. Jpn.* **1994**, *27*, 768.
(54) Clifford, A. A.; Burford, M. D.; Hawthorne, S. B.; Langenfeld, J. J.; Miller, D. J. *J. Chem. Soc., Faraday Trans.* **1995**, *91*, 1333.
(55) Pawliszyn, J. *J. Chromatogr. Sci.* **1993**, *31*, 31.
(56) Hawthorne, S. B.; Galy, A. B.; Schmitt, V. O., Miller, D. J. *Anal. Chem.* **1995**, *67*, 2723.
(57) Carmichael, L. M.; Christman, R. F.; Pfaender, F. K. *Environ. Sci. Technol.* **1997**, *31*, 126.
(58) MacKay, D.; Shiu, W. Y. *J. Chem. Eng. Data* **1977**, *22*, 399.

Received for review April 12, 2000. Revised manuscript received June 22, 2000. Accepted June 23, 2000.

ES001178O

APPENDIX B

PAH RELEASE DURING WATER DESORPTION, SUPERCRITICAL CARBON DIOXIDE EXTRACTION, AND FIELD BIOREMEDIATION

PUBLISHED IN *ENVIRONMENTAL SCIENCE AND TECHNOLOGY*

Environ. Sci. Technol. **2001**, *35,* 4577−4583

PAH Release during Water Desorption, Supercritical Carbon Dioxide Extraction, and Field Bioremediation

STEVEN B. HAWTHORNE,*,† DUSTIN G. POPPENDIECK,‡ CAROL B. GRABANSKI,† AND RAYMOND C. LOEHR‡

Energy and Environmental Research Center, University of North Dakota, P.O. Box 9018, Grand Forks, North Dakota 58202, and Environmental and Water Resources Engineering Program, College of Engineering, University of Texas, Austin, Texas 78712

Removal rates of polycyclic aromatic hydrocarbons (PAHs) from manufactured gas plant (MGP) soils were determined using water desorption for 120 days and mild supercritical carbon dioxide extraction (SFE) for 200 min. Both techniques were used to compare the changes in desorption rates for individual PAHs from untreated and treated soils that were obtained from a field biotreatment unit after 58, 147, and 343 days. Water desorption profiles (plotted in days) and SFE profiles (plotted in minutes) were very similar regardless of whether a PAH was rapidly or slowly removed. Water and SFE profiles were fit with a simple two-site (fast and slow) model to obtain the fraction of each PAH that was rapidly released (*F*). There was agreement between the *F* values obtained from water desorption and SFE for PAHs ranging from naphthalene to benzo[*a*]-pyrene from all soils, with an overall correlation coefficient (r^2) of 0.81. *F* values from water desorption and SFE also agreed with the actual removal of PAHs obtained after 147 and 343 days of field remediation (r^2 ca. 0.80). The use of shorter desorption times (2−4 days for water and 20−40 min for SFE) allowed *F* values to be estimated for all PAHs and showed excellent agreement with the removal of individual PAHs obtained with 147−343 days of field remediation ($r^2 > 0.9$). The comparisons indicate that short-term SFE can provide a reasonable estimate of the fraction of a PAH that is readily released and available for microbial treatment.

Introduction

For a chemical in a contaminated soil to be of concern to human health and the environment, it must be released from the soil, transported to a receptor, and available to that receptor in concentrations that will cause an adverse effect. Chemical availability for migration and biological uptake are both dependent upon the release of the chemical from soil. Hence, chemical release from soil is a key factor that affects the risk those chemicals pose to human or ecological receptors. The total chemical concentration present in a soil does not indicate the potential for the chemical to be released. Factors that affect chemical release and ultimate receptor impact include chemical hydrophobicity, soil organic carbon content, type and degree of treatment that may have occurred, and weathering of the chemicals in the soil (*1*−*18*).

Methods for evaluating pollutant release and availability have been evolving and include chemical as well as biological methods (*5, 7, 11*−*17, 19*−*28*). The assumption of local equilibrium conditions has been common when describing the dissolution and desorption behavior of chemicals in contaminated soils. This assumption assumes that interactions between an organic compound and the soil particles are rapid. Recent data have indicated that this assumption may be valid for only a few situations, such as chemicals freshly added (spiked) to soils, after a very recent hydrocarbon spill, for chemicals with low hydrophobicity, and for soils with a low organic carbon content. For many other conditions, a nonequilibrium pattern of dissolution/desorption has been observed (*2, 3, 5*−*7, 20, 29*−*31*). Nonequilibrium behavior can be represented using a two-site model in which one fraction of a chemical in the soil is quickly released and another fraction is slowly released (*2*−*5, 7, 9, 20, 22, 29, 30, 32*−*35*). With this model, dissolution/desorption is assumed to occur in two different sites in the soil. These are conceptual "sites" since it is recognized that chemical release undoubtedly occurs from many actual locations in the soil.

The fraction of the total chemical in the soil that is released quickly (*F*) is commonly assumed to be representative of equilibrium release conditions. The fraction of the total contaminant mass that is released slowly ($1 - F$) is considered to be kinetically rate limited. Values of *F* can range from greater than 0.8 for spiked chemicals and unweathered chemicals (*30, 31*) to less than 0.2 for weathered hydrocarbons and for hydrocarbons in treated soils (*3, 5, 29*). On the basis of bioremediation studies (*5*), the readily released fraction (*F*) of the total contaminant mass in a soil is available to interact with the aqueous phase and microorganisms in the soil, while the slowly released fraction is largely unavailable.

The chemical release methods available to determine kinetic parameters frequently take several weeks or months (*5, 11, 29, 30, 32, 34, 35*). While these methods are very appropriate for detailed research studies, methods that obtain such parameters, particularly *F*, more rapidly (and cheaply) are desirable for site investigations and remedial decisions. Supercritical fluid extraction (SFE) methods are more rapid and can be used to determine, for a contaminated soil, whether chemical release is limited by chemical solubility or by kinetics (*22, 23, 27, 28, 36*−*38*). In particular, SFE has been applied to investigating the release of hydrophobic organic compounds such as PAHs and polychlorinated biphenyls (PCBs). The use of pure CO_2 has been suggested since its polarity is similar to biological lipids, and (under proper conditions of temperature and pressure) the solubilities of low and high molecular weight PAHs in water and supercritical CO_2 are similar (*22*).

Presented in this paper are the results of detailed comparative studies that were conducted to determine the correlation between (a) the fraction of a chemical released rapidly (*F*) as determined by long-term, nonequilibrium, water extraction studies and (b) the fraction extracted by SFE using mild extraction conditions. The purpose of these studies was to evaluate the hypothesis that short-term mild SFE extraction conditions can be used to estimate the fast

* Corresponding author e-mail: shawthorne@undeerc.org; phone: (701)777-5256; fax: (701)777-5181.
† University of North Dakota.
‡ University of Texas.

10.1021/es010771i CCC: $20.00 © 2001 American Chemical Society
Published on Web 10/18/2001

release (F) that is obtained by long-term nonequilibrium studies. In addition, the results of the SFE and nonequilibrium studies were compared to the chemical loss that occurred in a field bioremediation unit. This latter comparison evaluated the applicability of the SFE and long-term water desorption results to a field remediation situation.

Materials and Methods

Soils Used. The soil samples used in these studies were obtained from a site in the midwestern United States. At this site, manufactured gas plant (MGP) residuals were discovered in a former creek bed and were excavated in 1997. The excavated and dewatered mixture of MGP residuals and site soil were treated in a prepared bed land treatment unit (LTU) supplied with water, nutrients, and periodic tilling.

Soil samples were obtained on days 0, 58, 147, and 343 of the field LTU operation. The samples contained approximately 35% sand, 40% silt, and 25% clay and had a pH between 7.0 and 7.5, a moisture content between 5.5 and 8.7% (dry weight basis), and an organic carbon content between 3.4 and 4.9%. Additional information about the LTU operation is available (*5, 39*).

Water/XAD_2 Extraction Procedure. A sacrificial batch rate of release (ROR) procedure (*5, 19, 29*) was used to determine the extent and rate of PAHs released from the soil samples to the aqueous phase over long time periods. In the ROR procedure, XAD_2 resin was used in the procedure to maintain a concentration in the aqueous phase of near zero providing a maximum driving force for chemical release. The RORs measured in these studies are considered to be conservative, upper-bound estimates of the rates that might occur under field conditions.

The batch vials contained 2 g of soil, 1.2 g of XAD_2 (Supelpak 2 styrene/divinylbenzene copolymer, Supelco, Bellefonte, PA), and an aqueous solution of 0.02% $HgCl_2$ and 0.01 N $CaCl_2$. At established times, the sacrificed vials were centrifuged to separate the soil, water, and XAD_2. The XAD_2 (which floats on water) was removed from the vial and vacuum-dried. The chemicals were extracted from the soil and XAD_2 with methylene chloride (CH_2Cl_2, Fisher "Optima" grade) and analyzed as described below. The amount of each PAH released at each test time was determined using triplicate vials. Relative standard deviations (RSDs) for the fraction released at each time were typically 5% (*5*).

Supercritical Fluid Extraction. SFE was performed with an ISCO model 260D syringe pump (ISCO, Lincoln, NE) filled with SFC-grade CO_2 (Scott Specialty Gases, Plumsteadville, PA) and an ISCO model SFX 2-10 extractor with 10-mL extraction cells and 4 g of the test soil. Extracted PAHs were collected in 22-mL vials containing 15 mL of CH_2Cl_2. Flow rates were controlled at 1 mL/min (measured as compressed CO_2 at the pump) using a variable coaxial restrictor (ISCO) heated to 80 °C. Kinetic profiles from the different soils were initially obtained at several temperature and pressure conditions (200 bar at 50, 100, and 150 °C; 400 bar at 50, 100, and 150 °C) to determine SFE conditions that yielded extraction curves (in minutes) similar to those obtained from the water desorption (in days). On the basis of these initial results (discussed later in the text), all subsequent extractions were performed at 200 bar and 50 °C for 200 min. Each profile was determined in duplicate. Since adding the PAH concentrations from the multiple fractions collected for the kinetic plots could introduce error in the PAH quantitations, the quantity of each PAH extracted from each soil was further verified by repeating each extraction (in triplicate) and collecting only three fractions, i.e., at 20, 40, and 60 min. After the SFE procedure was completed, the soil residue was extracted overnight with acetone/CH_2Cl_2 (1:1) to recover any PAHs that were not extracted by SFE. Additional minor procedural details are the same as previously reported (*22*). Reproducibilities of the replicate SFE experiments were similar to those shown for individual PAHs in Table 1.

TABLE 1. PAH Concentrations in MGP Soil before and after Treatment Based on Soxhlet Extraction

	PAH concn (mg/kg ± SD[a])			
	day 0	day 58	day 147	day 343
naphthalene	48 ± 2	22 ± 2	16 ± 1	8.2 ± 0.4
1-methylnaphthalene	112 ± 4	17 ± 1	26 ± 3	7.7 ± 0.5
2-methylnaphthalene	118 ± 4	12 ± 2	22 ± 2	6.4 ± 0.4
acenaphthylene	129 ± 1	44 ± 7	43 ± 17	30 ± 6
acenaphthene	78 ± 3	11 ± 2	16 ± 1	5.7 ± 0.7
fluorene	136 ± 6	28 ± 3	17 ± 1	12 ± 2
dibenzothiophene	70 ± 3	17 ± 2	15 ± 2	13 ± 2
phenanthrene	434 ± 19	106 ± 11	56 ± 1	23 ± 4
anthracene	110 ± 7	44 ± 12	26 ± 4	14 ± 1
fluoranthene	130 ± 6	85 ± 8	46 ± 1	41 ± 3
pyrene	196 ± 8	149 ± 15	79 ± 2	82 ± 5
benz[*a*]anthracene	74 ± 2	52 ± 8	48 ± 4	46 ± 1
chrysene	77 ± 3	60 ±6	52 ± 5	51 ± 1
benzo[*b*+*k*]fluoranthene	88 ± 12	66 ± 18	73 ± 16	58 ± 10
benzo[*e*]pyrene	39 ± 5	48 ± 11	39 ± 3	30 ± 2
benzo[*a*]pyrene	51 ± 5	54 ± 10	54 ± 4	43 ± 3
indeno[*1,2,3-cd*]pyrene	19 ± 2	19 ± 4	16 ± 2	14 ± 2
dibenz[*a,h*]anthracene	12 ± 1	7 ± 1	12 ± 1	12 ± 2
benzo[*ghi*]perylene	27 ± 3	31 ± 5	25 ± 2	26 ± 3

[a] PAH concentrations (dry wt) are based on triplicate Soxhlet extractions of each soil.

PAH Analysis. PAH concentrations for the test soils (Table 1) were based on triplicate Soxhlet extractions for 18 h with 1:1 acetone/CH_2Cl_2. Determinations of individual PAH concentrations for all water/XAD_2, SFE, and soil residue fractions were performed using high-resolution gas chromatography with flame ionization detection (GC/FID) with verification by gas chromatography/mass spectrometry (GC/MS) at both the University of Texas (*5*) and the University of North Dakota (*22*).

Data Analysis. The chemical release data were analyzed by normalizing the amount of chemical released from the soil and sorbed onto the XAD_2 by the total chemical concentration in the soil. The data were then modeled using an empirical two-site model, consisting of two first-order expressions (*5, 19*):

$$\frac{S_t}{S_o} = 1 - [Fe^{-k_1 t}] - [(1 - F)e^{-k_2 t}] \qquad (1)$$

where t is time (days); S_t is the mass of chemical on XAD_2 after time t (mg/kg soil dry weight); S_o is the total mass of chemical in soil (mg/kg dry weight); S_t/S_o is the fraction of chemical released after time t; F is the fraction of chemical released quickly; $(1 - F)$ is the fraction of chemical released slowly; k_1 is the first-order rate constant describing the quickly released fraction (day^{-1}); and k_2 is the first-order rate constant describing the slowly released fraction (day^{-1}). The Excel "Solver" regression routine was used to fit release data to eq 1. The parameters of fit were F, k_1, and k_2 as previously described (*5, 40, 41*). The SFE data were analyzed using the same empirical two-site model described in the water/XAD_2 extraction section, except that the units of time were minutes rather than days.

Results and Discussion

Development of SFE Conditions. Previous studies using SFE to investigate PAH and PCB desorption from soils and sediments used sequentially stronger SFE conditions to extract different fractions of PAHs and PCBs (*22, 36*). PAHs and PCBs that extracted at very mild SFE conditions were

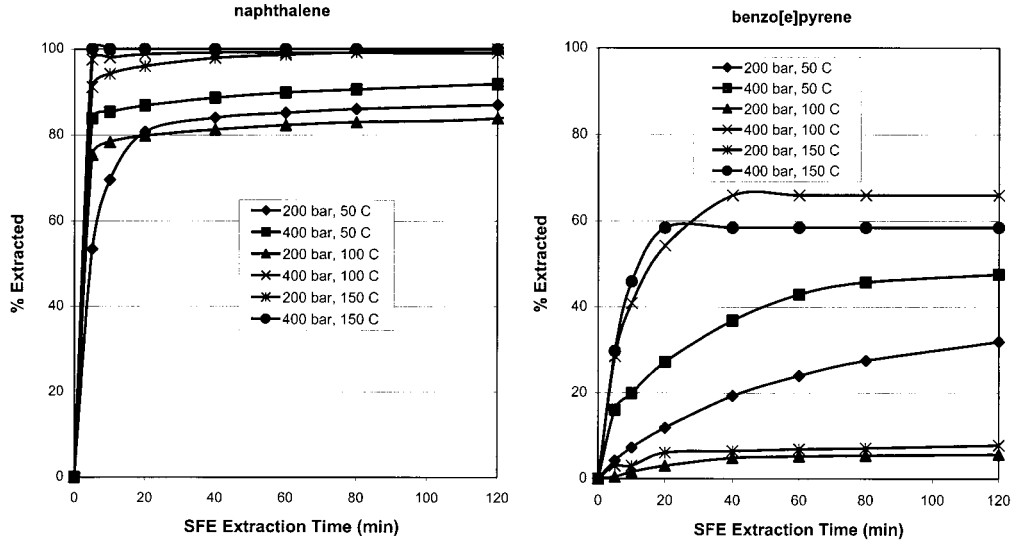


FIGURE 1. Effect of supercritical carbon dioxide temperature and pressure on the extraction rates of representative PAHs from untreated (day 0) MGP soil.

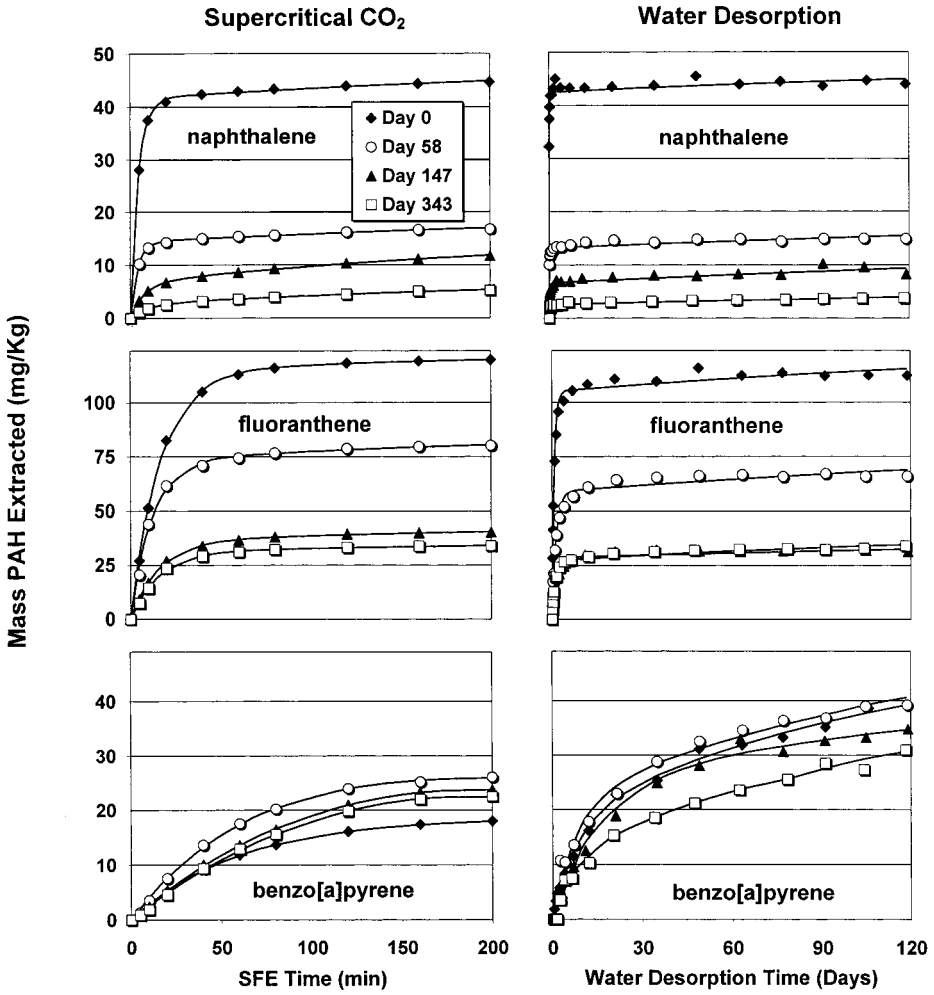


FIGURE 2. Comparison of the concentrations of representative PAHs extracted vs time using SFE (left side, time scale in minutes) and water desorption (right side, time scale in days) from untreated MGP soil, and soils treated in a field unit for 58, 147, and 343 days. The symbols show experimental data, and the lines are generated from the two-site model.

termed "rapidly desorbed" and those that required the strongest SFE conditions were viewed as being tightly sequestered and would be "slowly desorbed" under environmental conditions. Since the goal of the present study was to compare SFE results to water desorption results using the two-site model (which requires a single extraction condition), the stepwise SFE method could not be used. Therefore, initial SFE experiments to remove PAHs from untreated (day 0) and treated soils were performed in an attempt to develop a single SFE condition that could yield

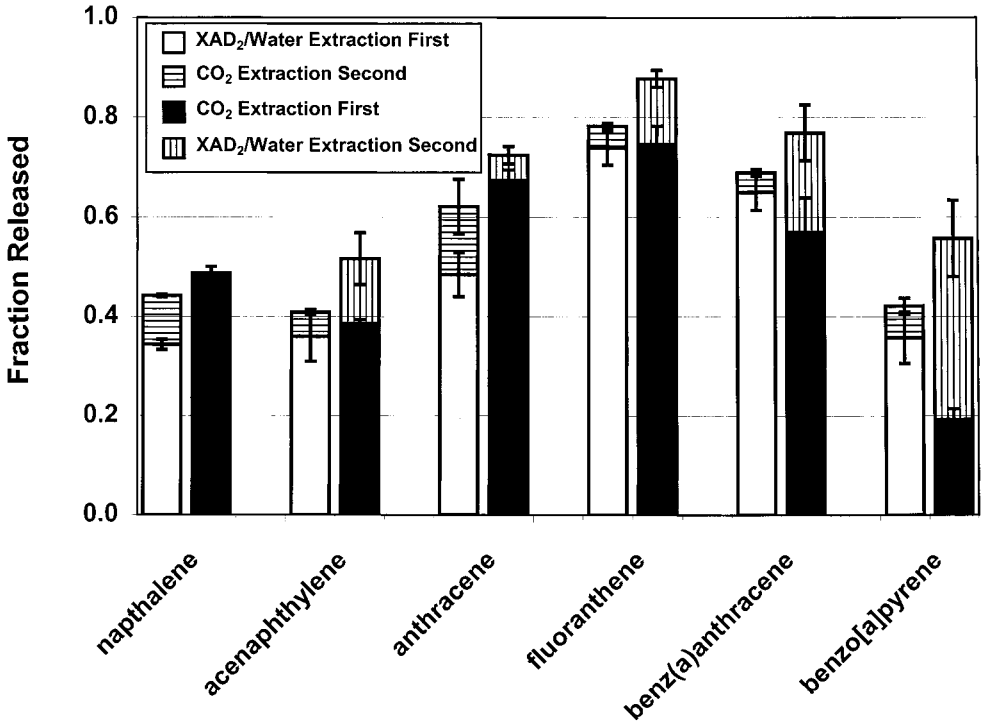


FIGURE 3. Fractions of representative PAHs removed from day 147 soil using sequential 29-day water desorption followed by 40-min supercritical CO_2 extraction (left bar for each PAH) and by supercritical CO_2 extraction followed by water desorption (right bar for each PAH). Error bars represent one standard deviation unit based on triplicate experiments.

desorption profiles similar to those obtained with water desorption.

The effect of SFE conditions on the extraction rates of PAHs from the untreated soil are shown in Figure 1. Since PAH solubility in carbon dioxide is greatly affected by temperature and pressure (*22, 42*) and PAH desorption rates from soils are presumably enhanced by temperature, predicting the effect of SFE conditions on individual PAH removal rates is difficult. As shown in Figure 1, low molecular weight PAHs (such as naphthalene) extract quite rapidly from the untreated soil at all SFE conditions tested. The higher temperature and pressure conditions (400 bar and 150 °C, 400 bar and 100 °C, and 200 bar 150 °C), yielded almost complete removal of naphthalene in a few minutes and did not clearly show the fast and slow fractions that were expected from the water desorption results (discussed below). In contrast, the milder SFE conditions (400 bar and 50 °C, 200 bar and 50 or 100 °C) yielded extraction curves clearly showing the two-site behavior for naphthalene analogous to the water desorption curves.

Higher molecular weight PAHs (e.g., benzo[*e*]pyrene) showed the fastest extraction rates only at the highest pressure (400 bar), with the combination of high pressure and temperature yielding the highest extraction rate. At 200 bar and either 100 or 150 °C, the CO_2 density is too low for efficient solvation of higher molecular weight PAHs (*22, 42*), and thus the extraction rates of higher molecular weight PAHs such as benzo[*e*]pyrene are very slow. Extraction at 50 °C and either 200 or 400 bar gave intermediate extraction rates for the higher molecular weight PAHs (Figure 1). Additional comparisons of the SFE extraction rates (plotted vs minutes) of two- to five-ring PAHs from the untreated and treated soils with the water desorption rates (plotted vs days) demonstrated that the 200 bar, 50 °C condition best mimicked the behavior of PAHs during water desorption. In addition, under these conditions, the solubilities of PAHs in CO_2 are similar to those found in ambient water (*22, 42*). Therefore, all subsequent extractions were performed at 200 bar and 50 °C.

PAH Desorption with Water and SFE. A comparison of PAH desorption curves using water/XAD_2 extraction and SFE are shown in Figure 2 for the untreated (day 0) soil as well as soils after 58, 147, and 343 days of treatment in the land unit. Concentrations of the PAHs in the untreated soil (day 0) and those remaining in the field-treated soils (days 58, 147, and 343) are given in Table 1. The extraction curves obtained using water/XAD_2 and SFE are remarkably similar for all PAHs, regardless of whether the soil was treated or untreated. Figure 2 also illustrates that, under these conditions, CO_2 extractions can be two-phase, exhibiting both fast and slow extraction regions. These two-phase curves are similar to the two-phase release curves exhibited by water/XAD_2 desorption and, therefore, indicate that the two-site model used to determine F should be applicable to the SFE results (discussed below).

Figure 2 also demonstrates the effect of field treatment on the SFE and water/XAD_2 desorption rates for representative PAHs. As expected, for lower molecular weight PAHs (e.g., naphthalene) that are removed by the field remediation process (Table 1), the fast fraction decreases with treatment time for both water/XAD_2 desorption and SFE. For higher molecular weight PAHs such as benzo[*a*]pyrene that were largely unaffected by the field remediation (Table 1), both the SFE and water/XAD_2 desorption curves show no significant change with treatment times.

While the extraction curves from SFE and water/XAD_2 desorption are very similar (Figure 2), similar extraction curves do not dictate that the two extraction methods are removing the same PAH molecules from the soils. To investigate this concern, two sequential extraction studies were performed, i.e., water/XAD_2 desorption followed by SFE and SFE followed by water/XAD_2 desorption. First, treated (day 147) soil was extracted with water/XAD_2 for 29 days. After 29 days, the XAD_2 was removed, and the remaining soil was subjected to SFE for 40 min. The reverse study was also conducted, i.e., day 147 soil was subjected to SFE for 40 min and then to a 29-day water/XAD_2 extraction. After the two sequential treatments, the soil residue was extracted, and

TABLE 2. Fast Released Fraction (*F*) Determined by Water/XAD_2 Desorption and SFE with CO_2[a]

	day 0		day 58		day 147		day 343	
	water	SFE	water	SFE	water	SFE	water	SFE
naphthalene	0.89 (0.87−0.90)[b]	0.86 (0.85−0.88)	0.61	0.66	0.41	0.41	0.31	0.31
1-methylnaphthalene	0.89 (0.87−0.90)	0.97 (0.96−0.98)	0.82	0.81	−[d]	0.87	0.63	0.63
2-methylnaphthalene	0.85 (0.84−0.87)	0.94 (0.93−0.95)	0.81	0.79	−	0.62	0.54	0.38
acenaphthylene	0.68 (0.67−0.69)	0.72 (0.71−0.73)	0.34	0.33	0.29	0.36	0.11	0.18
acenaphthene	0.80 (0.79−0.82)	nd[c]	0.70	nd	−	nd	0.38	nd
fluorene	−	0.97 (0.95−0.99)	0.85	0.83	0.75	0.73	0.47	0.51
dibenzothiophene	nd	0.96 (0.93−0.98)	nd	0.97	nd	0.73	nd	0.51
phenanthrene	0.88 (0.87−0.90)	0.97 (0.93−0.99)	0.73	0.93	0.59	0.76	0.58	0.59
anthracene	0.88 (0.86−0.90)	0.86 (0.85−0.88)	0.52	0.78	0.46	0.53	0.20	−
fluoranthene	0.81(0.79−0.83)	0.89 (0.85−0.92)	0.69	0.85	0.63	0.79	0.66	0.77
pyrene	0.84 (0.81−0.86)	0.92 (0.82−0.97)	0.69	0.83	0.60	0.78	0.64	0.80
benz[*a*]anthracene	0.69 (0.65−0.73)	0.80 (0.71−0.88)	0.60	0.79	0.58	0.69	0.48	0.75
chrysene	0.70 (0.65−0.74)	0.75 (0.67−0.82)	0.55	0.71	0.58	0.71	0.56	0.78
benzo[*b*+*k*]fluoranthene	0.33 (0.21−0.37)	−	0.42	−	0.39	−	0.23	−
benzo[*e*]pyrene	nd	0.44 (0.42−0.46)	nd	0.40	nd	−	nd	−
benzo[*a*]pyrene	0.35 (0.25−0.41)	0.36 (0.31−0.50)	0.39	−	0.45	−	0.25	−
indeno[*1,2,3-cd*]pyrene	nd	0.12 (0.10−0.13)	nd	0.15	nd	−	nd	−
benzo[*ghi*]perylene	nd	0.07 (0.06−0.08)	nd	0.07	nd	−	nd	0.03

[a] *F* values determined by the two-site curve fitting method described in the text based on 120-day water/XAD_2 desorption and 200-min SFE. [b] Confidence intervals (95%) were determined as described in ref *41*. [c] nd, not determined because of chromatographic interferences or because PAH concentrations were below reliable quantitation limits. [d] −, Kinetic curves were generated, but curve fitting did not yield a suitable *F* value based on the criteria that the fast desorption rate constant (k_1) was not at least 10-fold higher than the slow desorption rate constant (k_2).

the fraction of each PAH removed by SFE and water/XAD_2 desorption was determined. As shown in Figure 3, 40 min of SFE after 29-day water/XAD_2 desorption extracted only small amounts of additional PAHs regardless of their molecular weight. Also, 29-day water/XAD_2 desorption performed after 40 min of SFE extracted only small amounts of all of the PAHs except benzo[*a*]pyrene, which might be expected since water/XAD_2 desorption extracted more benzo[*a*]pyrene than SFE from all of the test soils (Figure 2). Note that a small fraction of molecules should be found by the second desorption method since the PAHs continue to desorb at a slower rate, while the first treatment would remove the majority of PAHs that desorb at the faster rate. In any case, the results of these two sequential extraction experiments demonstrate that water/XAD_2 desorption and SFE extract approximately the same molecules in their fast fractions.

Two-Site Model Determination of Fast Fractions. Previous water/XAD_2 desorption studies on PAHs used a simple two-site curve fitting model to determine the fast release fraction (*F*) for each individual PAH from different soil treatment times (*5, 19*). As noted above (Figure 2), the SFE extraction curves also generally show the two-phase behavior, which suggests that the two-site model is appropriate to determine SFE *F* values. Thus, to allow a mathematical comparison of the two methods, the data from each extraction method was curve fit using the methods described earlier to determine *F* (*5, 19*). Table 2 summarizes the *F* data for both extraction methods. As might be expected based on the treatment results shown in Table 1, both water/XAD_2 desorption and SFE show decreasing values of *F* with PAH molecular weight (e.g., larger PAHs are less available). Also, as the field remediation progresses, both methods show that the available fraction, *F*, decreases for PAHs that are removed by treatment. Unfortunately, neither method allows routine determination of *F* for high molecular weight PAHs since the extraction rate for these PAHs by both methods is too slow to allow two-phase behavior to be observed.

The correlation for all *F* values shown in Table 2 generated from water/XAD_2 desorption with those generated from the SFE data is shown in Figure 4. The two methods show good comparability, with linear correlation coefficient (r^2) of 0.81, a slope near unity, and a near-zero intercept.

Comparison of *F* Values to Field Treatment PAH Removals. Previous studies (*5*) have shown that the fast release fraction, *F*, as determined by water/XAD_2 desorption is readily available to microorganisms in the soil and that *F* values are a useful predictor of remediation behavior for individual PAHs. Although an earlier study related SFE behavior to remediation behavior of PAHs (*22*), no kinetic (curve fit) data from SFE has previously been compared to PAH remediation results. Therefore, the relationship among both fast release fractions (water/XAD_2 desorption and SFE) and the extent of PAH removal from the field remediation was investigated. This was done by comparing the *F* values for untreated soil (day 0) obtained from water/XAD_2 desorption and SFE (Table 2) with the actual fraction of each PAH removed as calculated from the day 0, 147, and 343 PAH concentrations (based on Soxhlet extraction) as compared to the untreated soil (Table 1).

Actual PAH removals from the field-treated soil were similar to those previously reported for four MGP soils subjected to biodegradation in stirred reactors (*43*), i.e., high removals of two- and three-ring PAHs, moderate removal of four-ring PAHs, and little or no removal of larger PAHs (Table 1). As indicated for day 343 (Figure 5a), *F* values from both

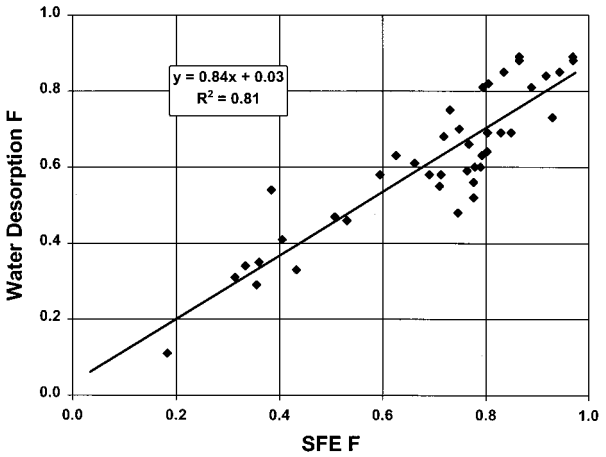


FIGURE 4. Correlation of two-site model fast fractions (*F*) obtained using 120-day water desorption and 200-min supercritical CO_2 extraction. Soils include the untreated MGP soil and those from the field treatment unit after 58, 147, and 343 days. PAHs include those listed in Table 2.

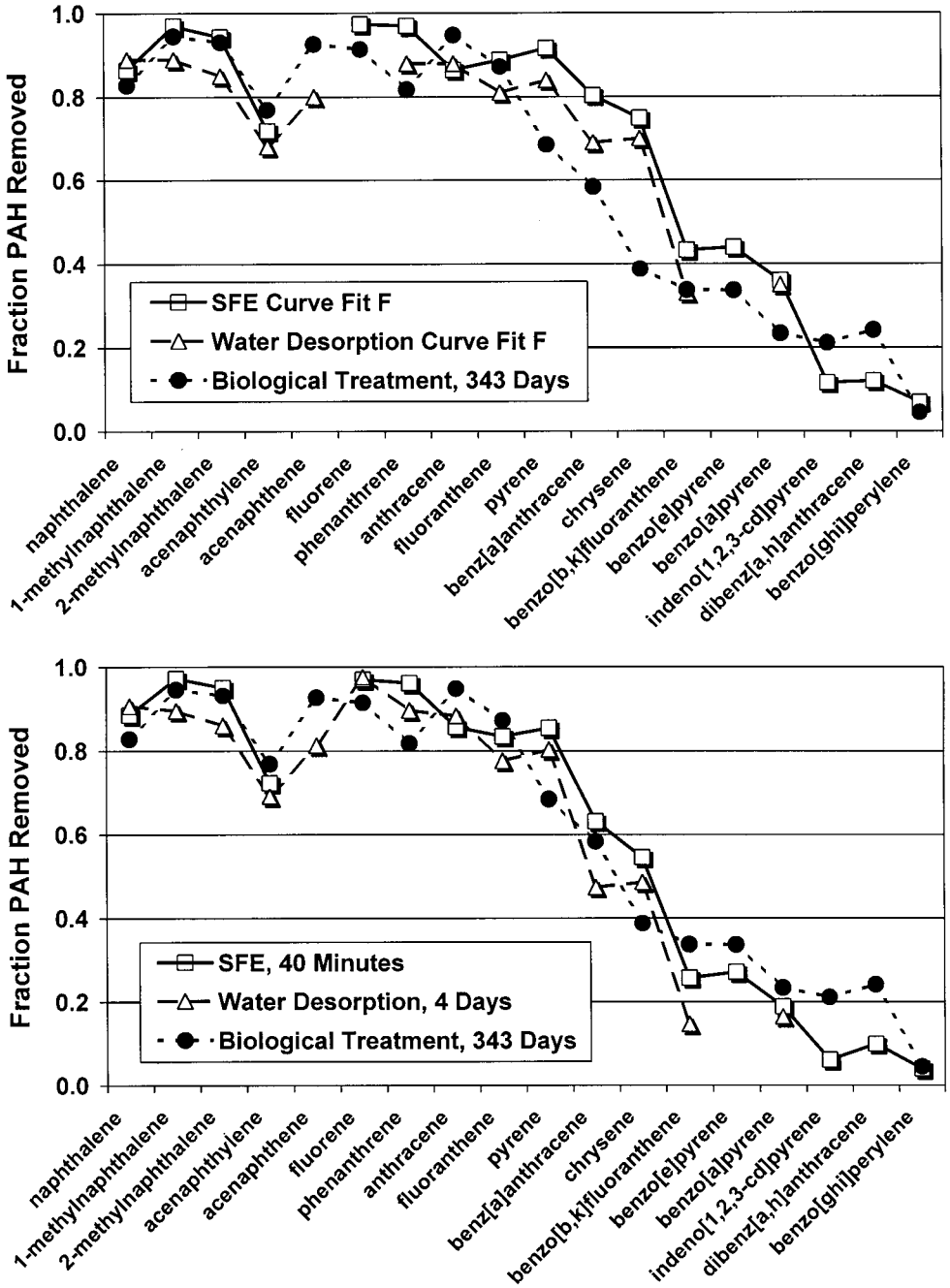


FIGURE 5. Comparison of the fractions of individual PAHs removed after 343 days of field treatment with the fast fractions (*F*) determined based on the two-site model curve fit of the desorption kinetic data from water desorption and supercritical CO_2 extraction (a). *F* values are those determined using the untreated soil (Table 2). Panel b compares the PAH fractions removed by a 40-min SFE desorption and by a 4-day water/XAD_2 desorption of the untreated soil with the fraction of each PAH removed by 343 days of field bioremediation.

extraction methods provided a reasonable prediction of the extent of PAH loss that occurred during field biological treatment, particularly for the lower molecular weight PAHs. For some middle molecular weight PAHs (e.g., pyrene to chrysene), both methods tend to overestimate the release, although the predictive relationship is still fairly strong. For example, correlation coefficients (r^2) for the PAH removals after 147 days were 0.80 and 0.82 for water desorption and SFE, respectively, while for day 343, they were 0.67 and 0.80, respectively. The very slow extraction rates shown by both methods make obtaining curve-fit *F* values difficult for the higher molecular weight PAHs, although the few values obtained from the SFE data agree well with the field remediation results.

Use of a Single Desorption Time. The application of the two-site model required that the kinetic curves be generated. However, generating *F* values requires several months (and a very large number of analyses) for the water/XAD_2 desorption technique. Although SFE reduces the extraction time to a few hours, a large number of fractions still must be analyzed; therefore, a few days are required per sample. In addition, the curve-fit approach cannot generally yield *F* values for the higher molecular weight PAHs because of their slow extraction behavior.

In an initial effort to simplify the approach, the fractions of PAHs extracted after single desorption times (1−20 days for water desorption and 20−60 min for SFE, based on the curve shapes shown in Figure 2) were correlated with the

removal of PAHs after 147 and 343 days of field remediation. For the water/XAD_2 desorption method, comparison of the PAH desorption after 2.2 days agreed best with the PAH removal obtained after 147 days of field remediation ($r^2 = 0.96$), while the 4-day data agreed best with the 343-day treatment ($r^2 = 0.91$) as shown in Figure 5b. Longer water/XAD_2 desorption times overestimated the actual PAH removals obtained in the field. Similarly, the optimal times for SFE were 20 min for the 147-day soil ($r^2 = 0.96$) and 40 min for the 343-day soil ($r^2 = 0.91$) as shown in Figure 5b.

While the predictive abilities of the single time water /XAD_2 desorption and SFE data shown in Figure 5b are attractive, it is important to note that this approach has not been applied to soils and sediments from other sites. In an effort to better generalize the comparison between water/XAD_2 desorption, SFE, and PAH behavior in the environment, analogous studies are currently being performed with 14 different MGP site soils and sediments.

Acknowledgments

Financial support for this research was provided by the Gas Research Institute (GRI). This manuscript was also prepared with the support of the U.S. Department of Energy under Cooperative Agreement DE-FC26-98FT40321. However, any opinions, findings, conclusions, or recommendations expressed herein are those of the author(s) and do not necessarily reflect the views of the DOE or GRI. The authors thank David Nakles, Nadine Gordon, and David Miller for helpful discussions and analytical support.

Literature Cited

(1) Alexander, M. *Environ. Sci. Technol.* **2000**, *34*, 4259−4265.
(2) Morrison, D. E.; Robertson, B. K.; Alexander, M. *Environ. Sci. Technol.* **2000**, *34*, 709−713.
(3) Stroo, H. F.; Jensen, R.; Loehr, R. C.; Nakles, D. V.; Fairbrother, A.; Liban, C. B. *Environ. Sci. Technol.* **2000**, *34*, 3831−3836.
(4) Loehr, R. C.; Webster, M. T.; Smith, J. R. *Pract. Period. Hazard., Toxic, Radioact. Manage.* **2000**, *4*, 53−59.
(5) Loehr, R. C.; Webster, M. T. *Pract. Period. Hazard., Toxic, Radioact. Waste Manage.* **2000**, *4*, 118−125.
(6) Alexander, M. In *Environmentally Acceptable Endpoints in Soils*; Linz, D., Nakles, D., Eds.; American Academy of Environmental Engineering: Annapolis, MD, 1997; pp 43−136.
(7) Beck, A. J.; Wilson, S. C.; Alcock, R. E.; Jones, K. C. *Crit. Rev. Environ. Sci. Technol.* **1995**, *25*, 1−43.
(8) Pignatello, J. L.; Xing, B. *Environ. Sci. Technol.* **1996**, *30*, 1−11.
(9) Smith, J. R.; Egbe, M. E.; Lyman, W. J. In *Bioremediation of Contaminated Soils*; Adriano, D. C., Bollag, J. M., Frankenberg, W. T., Jr., Sims, R. C., Eds.; American Society of Agronomy: Madison, WI, 1999; pp 665−717.
(10) White, J. C.; Kelsey, J. W.; Hatzinger, P. B.; Alexander, M. *Environ. Toxicol. Chem.* **1997**, *16*, 2040−2045.
(11) Bucheli, T. D.; Gustafsson, O. *Environ. Sci. Technol.* **2000**, *34*, 5144−5151.
(12) Macleod, C. J. A.; Semple, K. T. *Environ. Sci. Technol.* **2000**, *34*, 4952−4957.
(13) Bordelon, N. R.; Donnelly, K. C.; King, L. C.; Wolf, D. C.; Reeves, W. R.; George, S. E. *Toxicol. Sci.* **2000**, *56*, 37−48.
(14) Chefetz, B.; Deshmukh, A. P.; Hatcher, P. G.; Guthrie, E. A. *Environ. Sci. Technol.* **2000**, *34*, 2925−2930.
(15) Feng, Y.; Park, J.-H.; Voice, T. C.; Boyd, S. A. *Environ. Sci. Technol.* **2000**, *34*, 1977−1984.
(16) Jonker, M. T. O.; Smedes, F. *Environ. Sci. Technol.* **2000**, *34*, 1620−1626.
(17) Ghosh, U.; Gillette, J. S.; Luthy, R. G.; Zare, R. N. *Environ. Sci. Technol.* **2000**, 34, 1729−1736.
(18) Chiou, C. T.; McGroddy, S. E.; Kile, D. E. *Environ. Sci. Technol.* **1998**, *32*, 264.
(19) Williamson, D. G.; Loehr, R. C.; Kimura, Y *J. Soil Contam.* **1998**, *7*, 543−558.
(20) Zhang, Y.; Wu, R. S. S.; Hong, H.-S.; Poon, K.-F.; Lam, M. H. *Environ. Toxicol. Chem.* **2000**, *19*, 2431−2435.
(21) Saterbak, A.; Toy, R. J.; McMain, B. J.; Williams, M. P.; Dorn, P. B. *Environ. Toxicol. Chem.* **2000**, *19*, 2643−2652.
(22) Hawthorne, S. B.; Grabanski, C. B. *Environ. Sci. Technol.* **2000**, *34*, 4102−4110.
(23) Hawthorne, S. B.; Galy, A. B.; Schmitt, V. O.; Miller, D. J. *Anal. Chem.* **1995**, *67*, 2723−2732.
(24) Werth, C. J.; McMillan, S. A.; Castilla, H. J. *Environ. Sci. Technol.* **2000,** *34*, 2959−2965.
(25) Cuypers, C.; Grotenhuis, T.; Joziasse, J.; Rulkens, W. *Environ. Sci. Technol.* **2000**, *34*, 2057−2063.
(26) Alexander, R. R.; Alexander, M. *Environ. Toxicol. Chem.* **2000,** *34*, 1589−1593.
(27) Young, T. M.; Weber, W. J., Jr. *Environ. Sci. Technol.* **1997**, *31*, 1692.
(28) Johnson, M. D.; Weber, W. J., Jr. *Environ. Toxicol. Chem.* **2001**, *35*, 427−433.
(29) Berg, M. S.; Loehr, R. C.; Webster, M. T *J. Soil Contam.* **1998**, *7*, 675−695.
(30) Cornelissen, G.; Rigterink, H.; Ferdinandy, M. M. A.; Van Noort, P. C. M. *Environ. Sci. Technol.* **1998**, *32*, 966−970.
(31) Loehr, R. C.; Webster, M. T. *J. Soil Contam.* **1996**, *5*, 361−383.
(32) Carmichael, L. M.; Christman, R. F.; Pfaender, F. K. *Environ. Sci. Technol.* **1997**, *31*, 126−132.
(33) Gamerdinger, A. P.; Achin, R. S.; Traxler, R. W. *Soil Sci. Soc. Am. J.* **1997**, *61*, 1618−1626.
(34) Gong, Y.; Depinto, J. V.; Rhee, G.-Y.; Liu, X. *Water Res.* **1998**, *32*, 2507−2517.
(35) Lee, L. A.; Priddy, N. D.; Augustijn, D. C. *Soil and Aquifer Pollution*; Rubin, M. H., Narkis, N., Carberry, J., Eds.; Springer-Verlag: Berlin, 1998; pp 91−108.
(36) Björklund, E.; Bøwadt, S.; Mathiasson, L.; Hawthorne, S. B. *Environ. Sci. Technol.* **1999**, *33*, 2193.
(37) Pilorz, K.; Björklund, E.; Bøwadt, S.; Mathiasson, L. *Environ. Sci. Technol.* **1999**, *33*, 2204.
(38) Hawthorne, S. B.; Björklund, E.; Bøwadt, S.; Mathiasson, L. *Environ. Sci. Technol.* **1999,** *33*, 3152.
(39) Kelly, R. L.; Kayser, K.; Wesolowski, L. L.; Srivastra, V.; Nelson, G. L.; Shipley, S. R.; Golchin, J.; Richard, D.; Hayes, T. *Proceedings of the Gas, Oil and Environmental Biotechnology Symposium*; Institute of Gas Technology: Lake Buena Vista, FL, 1997.
(40) Berthouex, P. M.; Brown, L. C. *Statistics for Environmental Engineers*; Lewis: Boca Raton, FL, 1994.
(41) Opdyke, D. R.; Loehr, R. C. *J. Soil Contam.* **1999**, *8*, 541−558.
(42) Miller, D. J.; Hawthorne, S. B.; Clifford, A. A.; Zhu, S. *J. Chem. Eng. Data* **1996**, *41*, 779.
(43) Haeseler, F.; Blanchet, D.; Druelle, V.; Werner, P.; Vandecasteele, J.-P. *Environ. Sci. Technol.* **1999**, *33*, 4379−4384.

Received for review March 21, 2001. Revised manuscript received August 13, 2001. Accepted August 14, 2001.

ES010771I

APPENDIX C

COMPARING PAH AVAILABILITY FROM MANUFACTURED GAS PLANT SOILS AND SEDIMENTS WITH CHEMICAL AND BIOLOGICAL TESTS

PUBLISHED IN *ENVIRONMENTAL SCIENCE AND TECHNOLOGY*

Environ. Sci. Technol. **2002**, *36,* 4795−4803

Comparing PAH Availability from Manufactured Gas Plant Soils and Sediments with Chemical and Biological Tests. 1. PAH Release during Water Desorption and Supercritical Carbon Dioxide Extraction

STEVEN B. HAWTHORNE,*,† DUSTIN G. POPPENDIECK,‡ CAROL B. GRABANSKI,† AND RAYMOND C. LOEHR‡

Energy and Environmental Research Center, University of North Dakota, P.O. Box 9018, Grand Forks, North Dakota 58202, and Environmental and Water Resources Engineering Program, College of Engineering, University of Texas, Austin, Texas 78712

Soil and sediment samples from oil gas (OG) and coal gas (CG) manufactured gas plant (MGP) sites were selected to represent a range of PAH concentrations (150−40 000 mg/kg) and sample matrix compositions. Samples varied from vegetated soils to lampblack soot and had carbon contents from 3 to 87 wt %. SFE desorption (120 min) and water/XAD_2 desorption (120 days) curves were determined and fit with a simple two-site model to determine the rapid-released fraction (*F*) for PAHs ranging from naphthalene to benzo[*ghi*]perylene. *F* values varied greatly among the samples, from ca. 10% to >90% for the two- and three-ring PAHs and from <1% to ca. 50% for the five- and six-ring PAHs. Release rates did not correlate with sample matrix characteristics including PAH concentrations, elemental composition (C, H, N, S), or "hard" and "soft" organic carbon, indicating that PAH release cannot easily be estimated on the basis of sample matrix composition. *F* values for CG site samples obtained with SFE and water desorption agreed well (linear correlation coefficient, $r^2 = 0.87$, slope = 0.93), but SFE yielded higher *F* values for the OG samples. These behaviors were attributed to the stronger ability of carbon dioxide than water to desorb PAHs from the highly aromatic (hard) carbon of the OG matrixes, while carbon dioxide and water showed similar abilities to desorb PAHs from the more polar (soft) carbon of the CG samples. The combined SFE and water desorption approaches should improve the understanding of PAH sequestration and release from contaminated soils and sediments and provide the basis for subsequent studies using the same samples to compare PAH release with PAH availability to earthworms.

Introduction

Manufactured gas plants (MGPs) produced gas by cracking oil and coal and by releasing light hydrocarbons that were used for lighting and household needs. An estimated 3000−5000 MGP sites exist in the United States, many of which are contaminated with process residues that include tars, sludges, lampblack, light oils, spent oxide wastes, and other hydrocarbons (*1*−*4*). At MGPs that used coal, the residue carbon primarily was pyrolized coal particles. At MGPs that used oil, a finer organic carbon residue similar to lampblack soot was produced. In both cases, the primary organic pollutants of concern are polycyclic aromatic hydrocarbons (PAHs).

Proper management of MGP sites requires better understanding of the mechanisms that control the release of PAHs to the biosphere. Present analytical methods focus on determining total PAH concentrations but ignore whether the pollutants are available to environmental processes (*5*). Changes in the bioavailability and environmental mobility of these organic pollutants that occur with aging and weathering need to be better understood, and simple tools to determine pollutant "availability" to the environment must be developed in order to facilitate appropriate and cost-effective approaches to contaminated field sites (*5*−*17*).

There has been an increasing awareness that laboratory investigations into pollutant availability and sequestration using simple mixtures of known organic pollutants spiked onto well-characterized soils may fail to mimic field situations (*5*, *18*−*22*). The desire to use simpler laboratory-spiked systems is understandable since obtaining, properly characterizing, and performing various chemical and biological experiments with multiple representative field samples is much more difficult (and can yield results that are much harder to interpret) than studies performed with laboratory-generated samples. Comparisons of results among chemical and biological studies performed in different laboratories are also nearly impossible since relevant field samples are largely unavailable to multiple investigators. Thus, despite many investigations utilizing various biological and chemical approaches for studying pollutant availability, interpretation of these results is greatly limited by the lack of a common set of field samples.

To address this lack, a large suite of soils and sediments contaminated with PAHs has been collected from MGP sites, homogenized, and distributed to several laboratories that are investigating a range of chemical and biological approaches to determining pollutant availability. The overall goal of these collaborative studies is to develop an investigative approach that includes measures of chemical bioavailability and that can be used for site-specific risk-based remediation decisions. A key part of such an approach is to have valid short-term laboratory tests to predict the mobility and bioavailability of individual PAHs in field samples.

Part 1 of this series presents sample matrix characterization data, PAH concentrations, supercritical carbon dioxide (SFE) desorption rates, and water/XAD_2 desorption rates from soils and sediments contaminated by residues from oil gas (OG) and coal gas (CG) production. Samples were selected from an initial set of 30 soils and sediments based on their chemical characteristics [individual and total PAH content, elemental analysis, organic carbon, and SFE desorption rates (*9*, *23*)], knowledge of prior MGP activities at the sites, and representation of both OG and CG manufacturing technologies. Part 2 will compare the SFE and water desorption rates of PAHs with earthworm toxicity and uptake (*15*), both in laboratory and field exposure scenarios. These comparisons

* Corresponding author e-mail: shawthorne@undeerc.org; phone: (701)777-5256; fax: (701)777-5181.
† University of North Dakota.
‡ University of Texas.

10.1021/es020626k CCC: $22.00 © 2002 American Chemical Society
Published on Web 10/10/2002

should provide a better understanding of the thermodynamic and kinetic factors leading to PAH sequestration and decreased bioavailability that occurs over decades of field aging.

Experimental Section

Samples. Thirty soil and sediment samples were collected at former MGP sites that had been closed for ca. 50 yr. Upon collection, all samples were placed in sealed containers and cooled for shipment to a central storage facility where approximately 10 kg of each soil was homogenized by ca. 10 cycles of sieving to <6 mm followed by quartering the soil pile and remixing. Sample subsets were then stored in sealed glass containers at 4 °C for shipment to the participating labs. Fifteen samples were then selected for in-depth study on the basis of initial sample characterization including PAH concentrations, SFE behavior, and carbon content. Eleven of these samples were also evaluated using water/XAD_2 desorption.

Sample Characterization. Elemental analyses (C, H, N) were performed using a Leeman Labs model CE440 elemental analyzer. Sulfur was determined by combustion and iodometric titration with a LECO model HF10 sulfur analyzer. "Organic" carbon was determined using the Walkely−Black (ASA 229-3.5.2) and the modified Mebius (ASA 229-3.5.3) procedures, both based on chromic acid digestion applied without and with heat, respectively (*24*). Thermal gravimetric analysis (TGA) was performed as previously described (*17*).

PAH Determinations. PAH concentrations were determined by triplicate Soxhlet extractions (150 mL of 1:1 methylene chloride/acetone for 18 h) on 2-g samples followed by GC with flame ionization detection (GC/FID) using *n*-undecane as an internal standard (*23*). For the samples where the reproducibility in individual PAH concentrations was poor (e.g., typical RSDs > ca. 15% in the triplicate extracts), additional Soxhlet extractions were performed on replicate samples to verify the degree of sample heterogeneity. No concentration data from the multiple extracts were rejected in the subsequent data tables. GC/MS analyses of the same extracts were performed on each sample using identical GC conditions (*23*) on a Hewlett-Packard model 5973 GC/MS. All PAH identities were verified using GC/MS and authentic standards. Concentrations of PAHs determined by GC/FID were also confirmed by independent GC/MS analysis using perdeuterated PAHs as internal standards.

SFE and Water/XAD_2 Desorption. SFE was performed with pure carbon dioxide as previously described (*9*, *23*). All extractions were performed at 200 bar and 50 °C using 1-g samples mixed 1:1 with sodium sulfate. After the SFE was completed, each sample residue was extracted using the Soxhlet method described above to determine residual PAH concentrations. All of the 15 samples were extracted by SFE a minimum of 5 times (*9*, *23*). First, each profile was determined in duplicate by collecting and analyzing separate fractions after 5, 10, 15, 20, 25 30, 40, 50, 60, 80, 100, and 120 min of SFE. The fraction of each PAH extracted was further verified by repeating each SFE extraction and analysis in triplicate with fractions collected at 20, 40, and 60 min. Water/XAD_2 desorption was performed as previously reported (*6*, *23*, *25*).

Desorption Kinetics Data Analysis. SFE and water/XAD_2 kinetic data were analyzed using the simple two-site kinetic model previously described (*23*). In short, the model uses two first-order expressions to describe the fraction (F) of a PAH released by a "fast" process having a first-order rate constant k_1 and the fraction ($1 - F$) released by a "slow" process having a first-order rate constant k_2. The parameters F, k_1, and k_2 were fit to the experimental data as previously described (*6*, *23*).

TABLE 1. Sample Matrix Organic Composition

	C, wt %[a]			molar C/H ratio[b]	thermal gravimetric analysis[c]	
	modified Mebius	Walkely−Black	elemental analysis		total organic wt %	% volatiles
OG-1	13	nd[d]	9.9	2.5	12	38
OG-2	56	2.4	59	6.3	63	14
OG-5	11	0.8	6.9	2.4	9.3	34
OG-10	47	2.6	87	5.0	87	16
OG-13	4.1	1.3	6.5	1.4	8.2	61
OG-14	2.3	0.4	2.9	1.2	4.4	61
OG-17	39	2.1	47	5.7	52	13
OG-18	20	2.5	25	2.5	29	28
CG-2	2.1	1.0	2.6	0.6	4.2	87
CG-3	6.9	2.5	7.5	0.9	12	76
CG-10	2.2	0.8	3.7	1.6	9.8	57
CG-11	13	2.1	29	2.3	45	46
CG-12	7.0	4.0	7.9	1.1	14	58
CG-15	8.9	3.8	24	3.5	26	31
CG-17	7.2	3.8	12	1.8	19	48

[a] Organic carbon was determined using chromic acid oxidation and elemental analysis as described in the text. [b] Molar carbon/hydrogen ratio based on elemental analysis. [c] Thermal gravimetric analysis (TGA) was performed as described in the text. % volatiles is the fraction of organics lost upon heating to 650 °C but before the addition of oxygen as compared to the total organic content determined by TGA. [d] nd, not determined.

Results and Discussion

Sample Characterization. Matrix characteristics of the samples selected for in-depth study are given in Table 1 and Supporting Table S1. CG samples generally had the appearance of sediments (CG-2 and CG-3, both collected from harbors adjacent to MGP sites) or soils (the remaining CG samples, except CG-11). CG-11 was collected at a tar refining site and appeared to contain coal chips, asphaltic tar, and gritty material such as crushed bricks and clinker. The OG samples consisted primarily of lampblack soot (OG-2 and OG-10) or soot mixed with soil (OG-5, OG-14, OG-17, and OG-18). Most of the CG samples, except CG-11, had organic carbon contents typical of soil. In contrast, some of the OG samples were essentially pure lampblack soot and had total carbon contents as high as 87 wt % (Tables 1 and S1).

Conventional methods to measure soil organic carbon [Walkely−Black (WB) and modified Mebius (MM)] rely on chromic acid oxidation without (WB) and with (MM) added heat (*24*). As shown in Table 1, the WB method greatly underestimates the organic carbon in nearly all of the samples, especially for the sootier OG samples. The carbon concentrations from the MM method were substantially lower than those from elemental analysis and TGA for several of the OG samples. Both observations are consistent with soot-based carbon as opposed to typical soil organic carbon. Similarly, the molar ratio of carbon to hydrogen is higher in most of the OG samples than in the majority of CG samples, which indicates that the OG samples have high elemental or aromatic (e.g., very large PAHs) carbon. The high concentrations of aromatic carbon were also confirmed in the OG samples by CPMAS (solid-state cross polarization magic angle spinning) ^{13}C nuclear magnetic resonance (NMR), where only aromatic (not aliphatic) carbon was observed (*26*).

Additional differences in carbon types in the sooty OG samples were shown by TGA, where the proportion of "volatile" organic (the weight loss that occurs during heating under inert gas adjusted for water) is much lower than "fixed" carbon (the weight loss that occurs at 650 °C only after adding oxygen, Table 1). These results are also consistent with a

TABLE 2. Representative PAH Concentrations in 15 MGP Site Samples (mg/kg)

	NAP[a]	PHEN	PYR	B[*a*]A	B[*a*]P	IND	total PAHs EPA[b]	total PAHs FID[c]	weathering index[d]
OG-1	4.4 ± 0.5	8 ± 0	20 ± 2	72 ± 5	30 ± 3	24 ± 1	290 ± 10	850 ± 50	0.04
OG-2	26 ± 5	92 ± 7	1240 ± 90	320 ± 20	830 ± 46	530 ± 30	6190 ± 350	11400 ± 100	0.01
OG-5	120 ±100	110 ± 60	340 ± 70	44 ± 4	170 ± 20	150 ± 20	1730 ± 340	2410 ± 440	0.14
OG-10	1670 ± 350	5110 ± 580	7980 ± 870	1470 ± 170	3050 ± 430	2460 ± 410	39500 ± 4700	54900 ± 6130	0.12
OG-13	140 ± 17	170 ± 20	320 ± 50	44 ± 5	126 ± 13	102 ± 6	1620 ± 150	2830 ± 210	0.23
OG-14	1.5 ± 0.2	2.0 ± 0.6	22 ± 3	4.4± 0.7	22 ± 5	17 ± 4	150 ± 30	390 ± 130	0.02
OG-17	2060 ± 240	2010 ± 280	2620 ± 330	530 ± 70	1250 ± 140	1160 ± 230	16200 ± 2010	22800 ± 2940	0.32
OG-18	6410 ± 600	2560 ± 140	1810 ± 90	365 ± 12	640 ± 30	440 ± 20	17100 ± 1110	23900 ± 1330	2.30
CG-2	4.6 ± 0.6	45 ± 5	48 ± 10	20 ± 4	19 ± 2	9 ± 3	290 ± 40	1210 ± 170	0.14
CG-3	790 ± 60	650 ± 40	344 ± 17	161 ± 9	41 ± 6	80 ± 13	4010 ± 260	12800 ± 1110	2.47
CG-10	5.9 ± 0.7	43 ± 6	74 ± 11	35 ± 5	41 ± 6	32 ± 6	480 ± 70	1560 ± 230	0.07
CG-11	41 ± 4	920 ± 30	1740 ± 69	1350 ± 20	1160 ± 70	890 ± 82	14500 ± 430	29200 ± 810	0.01
CG-12	14 ± 1	560 ± 70	431 ± 45	290 ± 30	250 ± 30	144 ± 27	3620 ± 430	7250 ± 1070	0.02
CG-15	15 ± 2	47 ± 3	151 ± 14	84 ± 5	77 ± 5	56 ± 11	960 ± 38	2910 ± 100	0.06
CG-17	9 ± 2	45 ± 18	71 ± 14	45 ± 10	48 ± 4	32 ± 1	540 ± 70	1490 ± 300	0.06

[a] Compounds listed are naphthalene (NAP), phenanthrene (PHEN), pyrene (PYR), benz[*a*]anthracene (B[*a*]A), benzo[*a*]pyrene (B[*a*]P), and indeno[1,2,3-*cd*]pyrene (IND). Standard deviations are based on 3−5 replicate extractions. Concentrations for all 20 PAHs measured in each sample are given in Supporting Information Table S2. [b] Total concentrations of the 16 PAHs listed by the U.S. EPA. [c] Total PAH concentrations based on GC/FID analysis and verified by GC/MS analysis. [d] Concentration ratio of two-ring PAHs (naphthalene and the two methyl naphthalenes) to the total concentrations of the five- plus six-ring PAHs listed in the text.

highly aromatic matrix. OG-13 and OG-14 appear to have somewhat lower proportions of soot-based aromatic carbon, on the basis of the lower proportion of fixed carbon determined by TGA and by the lower molar C/H ratios (1.4 and 1.2), which are more typical of the CG samples. It should also be noted that the generally good agreement between the elemental carbon values and the "total organic" values determined using TGA demonstrates that carbonate is not significant in these samples. (More complete characterizations including elemental C, H, N, and S concentrations; particle size distribution; and total extractable hydrocarbon content are given in Supporting Information, Table S1.)

PAH Concentrations. The concentrations of representative PAHs for each sample are given in Table 2. (Individual concentrations of the 20 major PAHs in each sample are given in Table S2.) Concentrations of individual and total PAHs vary greatly among the samples, with total PAH concentrations (defined as the sum of the 16 PAHs listed by the U.S. EPA) ranging from ca. 150 to ca. 40 000 mg/kg (4 wt %). All of the OG samples (except OG-18) and several of the CG samples are dominated by the higher molecular weight (four- to six-ring) PAHs (Table 2 and Figure S1). In contrast, some of the samples (notably CG-3 and OG-18) are dominated by the lower molecular weight (two- and three-ring) PAHs. For example, the concentration ratio of the two-ring PAHs (naphthalene and the two methylnaphthalenes) to the five- and six-ring PAHs (including benzo[*b*+*k*]fluoranthene, benzo[*e*]- and benzo[*a*]pyrene, perylene, indeno[1,2,3-*cd*]pyrene, dibenz[*a*,*h*]anthracene, and benzo[*ghi*]perylene) varies from 0.01 to 2.5 for the CG samples and from 0.01 to 2.3 for the OG samples (Table 2). This 200-fold range in relative amounts of the more volatile (and water soluble) lower molecular weight PAHs as compared to the higher molecular weight PAHs indicates that exposure to removal processes (e.g., volatilization, water solubilization, bacterial degradation) was likely very different among the various sites sampled.

PAH Desorption Rates. PAH desorption curves for several samples were obtained so that the PAH release rates obtained using SFE and water desorption could be compared. Although the present work is the first to present SFE desorption data from multiple sample sites, the water/XAD_2 desorption method has previously been used to determine the rapidly desorbed *F* fraction from several soils contaminated with PAHs and related hydrocarbons (*6*, *25*, *27*). The development of the SFE method was described in an earlier report (*23*) in which samples from a single MGP site were extracted with various combinations of temperatures (50−150 °C) and pressures (200 and 400 bar) in an effort to develop SFE conditions that yielded PAH desorption behavior most analogous to water desorption behavior for both low and high molecular weight PAHs. In that study, good agreement between SFE extraction curves generated with 200 bar carbon dioxide at 50 °C and water/XAD_2 curves was obtained for samples from a single CG site during bioremediation (*23*). Therefore, the same SFE conditions were evaluated in the present study to determine if SFE desorption results agree with water/XAD_2 desorption results for a range of samples representative of CG and OG MGP sites.

Figure 1 demonstrates the range of behavior seen among the different samples for representative low (MW = 128) to high molecular weight PAHs (MW = 276) during the SFE desorptions. Interestingly, samples from both OG and CG sites showed a range of very fast to very slow SFE desorption rates. For example, OG-18 and CG-3 both showed very high proportions of fast or available fractions, especially for the two- to four-ring PAHs (MW from 128 to 228). In contrast, samples such as CG-11 and OG-13 showed only small fast fractions for all PAHs, and even the lower molecular weight PAHs showed relatively low availability. The remaining samples showed a range of more intermediate fast fractions as typified by OG-10 and CG-12 (Figure 1).

Similar to the SFE results, water/XAD_2 desorption curves showed a broad range of behaviors for the different samples as illustrated in Figure 2. (Note that the units of time are days for the water/XAD_2 extraction and minutes for the SFE extraction.) Both the water desorption and the SFE curves show the typical two-phase release from the soil matrix, and the curves shown in Figure 2 represent the range of behaviors observed for the individual PAHs from the 11 samples.

Analogous extraction curves were generated for individual PAHs from 15 of the samples using SFE and from 11 of the samples using water/XAD_2 desorption. The two-site curve-fitting model was then applied to each of the data sets to determine values of *F* as well as the fast and slow desorption rate constants, k_1 and k_2. The resultant *F* values for representative PAHs are given in Tables 3 (SFE) and 4 (water). *F* values and representative 95% confidence intervals for all PAHs measured from all samples tested are given in Tables S4 and S5. In general, the simple two-site model yielded curves (solid lines in Figures 1, 2, and S2) that fit the

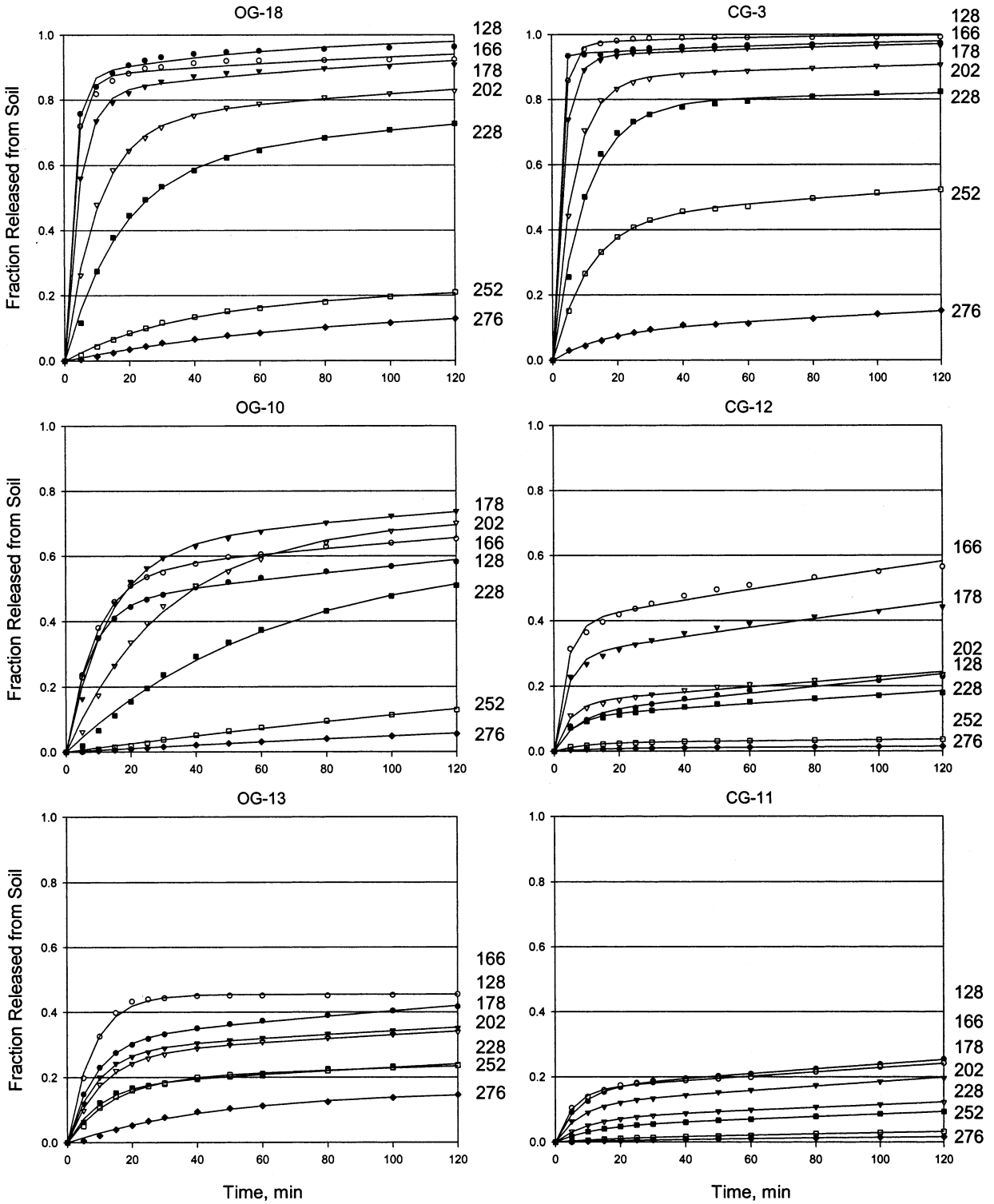


FIGURE 1. SFE desorption rates of representative PAHs from MGP samples showing high, moderate, and low rapidly desorbing fractions (*F*). The solid line is from the two-site model fit, and the individual symbols are experimental data. PAHs are designated by molecular weight and include naphthalene (128), fluorene (166), phenanthrene (178), pyrene (202), benz[*a*]anthracene (228), perylene (252), and benzo-[*ghi*]perylene (276).

experimental data (individual points in each figure) well. The fast desorption rate constants (k_1) were typically 2 orders of magnitude higher (for SFE) or 3 orders of magnitude higher (for water desorption) than the slow desorption rate constants (k_2), regardless of the PAH or sample studied (Table S6). As indicated by the SFE and water desorption curves shown in Figure 2, water desorption rate constants expressed in reciprocal days were similar to SFE rate constants expressed in reciprocal minutes (Table S6). For most samples and for both SFE and water desorption, k_1 values for low molecular weight PAHs (e.g., naphthalene) were typically an order of magnitude higher than for high molecular weight PAHs (e.g., benzo[*a*]pyrene).

F values are reported in Tables 3, 4, S5, and S6. Out of 198 possible *F* values for the water desorption experiments, 127 were obtained by curve fitting the 120-day water desorption data. For the same 11 samples, SFE obtained *F* values for 162 out of 198 possible cases. Low PAH concentrations were most

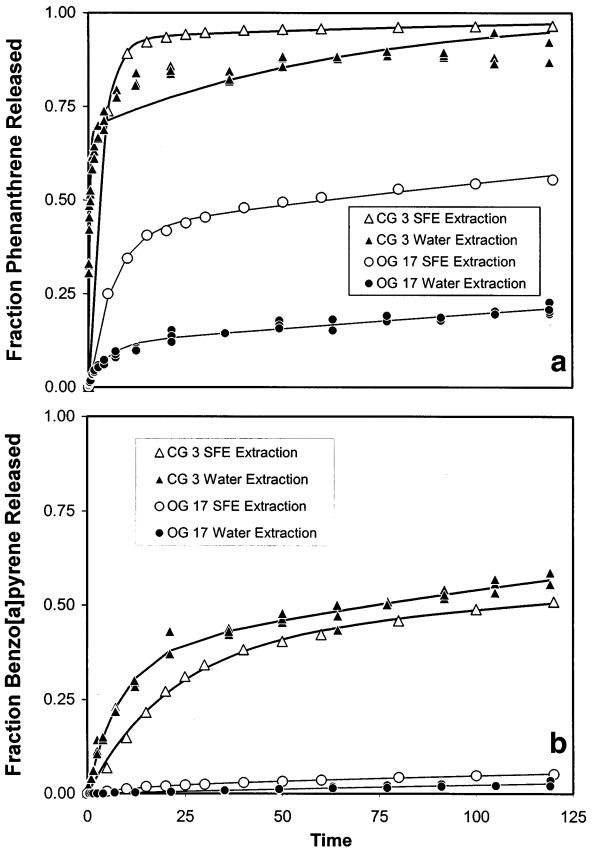


FIGURE 2. SFE and water/XAD$_2$ desorption rates of phenanthrene and benzo[*a*]pyrene from an OG sample (OG-17) and a CG sample (CG-3). Symbols are experimental data, and the lines are calculated from the two-site model. Time units are in minutes for SFE and in days for water/XAD$_2$ desorption.

TABLE 3. SFE Rapidly Desorbing Fractions (*F*) for Representative PAHs Based on the Two-Site Model Curve Fit

	curve fit model *F*[a]					
	NAP[b]	PHEN	PYR	B[*a*]A	B[*a*]P	IND
OG-1	0.22	0.22	0.32	0.05	0.15	*c*
OG-2	0.27	0.34	0.32	*d*	0.06	0.06
OG-5	0.30	0.35	0.74	*d*	*d*	*d*
OG-10	0.46	0.63	0.67	*d*	0.10	0.04
OG-13	0.31	0.28	0.27	0.18	0.15	0.17
OG-14	0.24	0.60	0.78	*c*	0.27	0.15
OG-17	0.63	0.42	0.29	0.12	0.02	0.004
OG-18	0.87	0.82	0.73	0.61	0.22	0.15
CG-1[e]	0.86	0.97	0.92	0.80	0.36	0.12
CG-2	0.22	0.73	0.63	0.50	0.41	*d*
CG-3	0.94	0.93	0.87	0.79	0.40	0.13
CG-10	0.21	0.37	0.30	0.27	0.09	0.02
CG-11	0.17	0.12	0.07	0.05	0.02	0.02
CG-12	0.11	0.29	0.15	0.11	0.05	0.02
CG-15	0.16	0.23	0.41	0.31	*d*	*d*
CG-17	0.27	0.45	0.42	0.35	0.14	0.15

[a] Complete curve fit *F* data and 40-min fractions for the 20 PAHs are given in Tables S4 and S7. [b] Compounds listed are naphthalene (NAP), phenanthrene (PHEN), pyrene (PYR), benz[*a*]anthracene (B[*a*]A), benzo[*a*]pyrene (B[*a*]P), and indeno[1,2,3-*cd*]pyrene (IND). [c] Could not be determined because of analytical detection limits. [d] Did not meet the curve fit criteria described in the text. [e] Data adapted from ref *23*.

often the reason for failing to obtain *F* values. However, in a few cases the water desorption curves did not meet the criteria applied for the two-site model (k_1 was required to be at least 10 times larger than k_2), and a single rate constant was adequate to describe the release curve and no *F* value could be reported (e.g., the benz[*a*]anthracene curve for OG-10 in Figure 1).

TABLE 4. Water/XAD2 Rapidly Desorbing Fraction (*F*) for Representative PAHs Based on the Two-Site Model Curve Fit

	NAP[a]	PHEN	PYR	B[a]A	B[a]P	IND
OG-1	nd[b]	nd	0.01	nd	*c*	nd
OG-2	0.18	0.05	0.02	0.01	<0.01[d]	0.01
OG-5	*c*	0.05	0.04	0.04	0.15	*c*
OG-10	0.24	0.15	0.08	0.03	*c*	<0.01
OG-17	0.46	0.12	0.05	0.01	0.01	nd
OG-18	0.58	0.33	0.26	0.17	0.08	0.05
CG-1	0.89	0.88	0.84	0.69	0.35	nd
CG-2	nd	0.38	0.33	0.24	0.17	0.21
CG-3	0.89	0.67	0.63	0.52	0.37	0.20
CG-11	0.02	*c*	*c*	*c*	0.02	*c*
CG-12	nd	0.01	0.01	0.02	nd	nd

[a] Compounds listed are naphthalene (NAP), phenanthrene (PHEN), pyrene (PYR), benz[*a*]anthracene (B[*a*]A), benzo[*a*]pyrene (B[*a*]P), and indeno[1,2,3-*cd*]pyrene (IND). [b] Not determined. The PAH concentrations were too low, and/or chromatographic interferences were too great for desorption rate curves to be determined. [c] Did not meet the curve fit criteria described in the text. [d] Met curve fit and release criteria; however, the value was too low to be considered accurate to three decimals.

Major variations in the *F* values derived from the two-site model were observed among the various samples by both desorption methods. The fraction of rapidly released naphthalene ranged from as little as 11% to as much as 94% for SFE and from 2 to 89% for water desorption. A similarly wide range of *F* values occurs for the other low and middle molecular weight PAHs. For example, *F* values for pyrene range from 7% to 87% for SFE and from 1 to 84% for water desorption. For higher molecular weight PAHs (five- and six-ring), the range in desorption rates is not quite so large, but even these PAHs show *F* values ranging from 1 to ca. 60% for SFE and from <1 to 37% for water desorption (Tables 3, 4, S5, and S6).

It is interesting to note that neither the SFE nor the water desorption rates of individual PAHs can be predicted by their solubility. For both SFE and water, PAH solubility drops dramatically with increasing PAH size, i.e., by ca. 1 order of magnitude for each additional ring. For example, the solublities of naphthalene and benzo[*ghi*]perylene are 87 and 0.001 mg/g (respectively) in carbon dioxide at the conditions used in this study and are 32 and 0.0003 mg/kg in water (Table S3). Therefore, solubility control would require that naphthalene had the highest desorption rate as compared to all other PAHs in a sample. However, low molecular weight PAHs often show slower desorption than the less soluble higher molecular weight PAHs. As shown in Figure 1 for samples CG-12 and OG-10, naphthalene desorbs more slowly by SFE than higher molecular weight PAHs even as large as pyrene (MW 202). Similarly, water desorption of lower molecular weight PAHs (two- and three-ring) would be expected to show faster rates on the basis of their higher solubilities as compared to larger PAHs (Table S3). However, even low molecular weight PAHs can show slow desorption, as illustrated by phenanthrene desorption from OG-17 (Figure 2a). Higher molecular weight PAHs generally show slower desorption, as would be expected on the basis of their low solubilities (Table S3), but even they can show relatively fast desorption into water and by SFE from some matrixes as illustrated by benzo[*a*]pyrene desorption from CG-3 (Figure 2b). The slow desorption of lower molecular weight PAHs (as compared to their solubility) clearly shows that molecules which have not been removed by weathering have become tightly sequestered in the sample matrix.

It is also important to note that the wide range in desorption rates for the various samples is *not* based on their

PAH concentrations. For example, two of the most concentrated samples (e.g., CG-3 and OG-18) show very fast PAH release during both SFE and water desorption. Therefore, any potential limitations to desorption from PAH saturation or mass transfer limitations if the PAHs were present as a bulk phase do not seem to be significant. One might also suspect that high PAH concentrations could lead to high relative desorption rates since matrix binding sites could be saturated, thus leaving most PAH molecules highly available. However, high concentrations of PAHs do not always result in fast desorption rates as demonstrated by the fact that the most highly contaminated CG sample (CG-11) shows very slow PAH desorption behavior into water and by SFE. Conversely, samples with low contamination levels also show relatively slow (e.g., CG-15) and fast (e.g., OG-14) desorption behavior (Figure S2).

Estimation of *F* Values. Determination of the *F* values using the full kinetic curves requires the generation and analysis of a large number of sample extracts. However, examination of the SFE and water desorption curves indicates that a single time fraction may be sufficient to obtain a valid estimate of *F* since the desorption curves generally show clear transitions between the fast and the slow fractions between 20 and 60 min for SFE and after a few days for water desorption. The possibility of using a single time fraction to estimate *F* was evaluated by performing linear correlations between the kinetic curve *F* values and the fractions desorbed at single times, i.e., at 20, 40, and 60 min for SFE and at 4, 7, 12, and 21 days for water desorption. (Note that the flow rate to sample size ratio for SFE is the same as for the kinetic determinations.) The best correlation for SFE curve *F* data was with the 40-min fraction, which showed $r^2 = 0.977$, a slope of 0.975, and an intercept of −0.002 (for all samples and a total of 220 curve *F* values vs the 40-min fractions. Replicate determinations of the fractions released after 40 min are given in Table S7). Similarly, water/XAD_2 showed the best correlation between the kinetic curve *F* values with the fractions desorbed after 12 days with $r^2 = 0.984$, a slope of 0.930, and an intercept of 0.009 (for the 11 samples used for the water desorption studies with a total of 113 values). Since determining the full desorption curves is very labor intensive, these results support the use of a single desorption time to estimate *F* values for either the SFE or water/XAD_2 methods. (Note that all subsequent discussions in this work use the full-curve *F* values; however, evaluation of the data using the *F* values estimated from 40 min of SFE and after 12 days of water desorption was also performed and showed essentially identical results to those obtained with the full-curve *F* values.)

Correlation of PAH Desorption Behavior with Soil Characteristics. As shown in Tables 1 and S1, the samples used in this study have a very broad range of matrix characteristics representative of MGP sites. To determine whether these matrix characteristics affect PAH mobility, linear correlation coefficients between each sample characteristic measured (Tables 1 and S1) and the curve-fit *F* values for each individual PAH were calculated for both the SFE and the water desorption data sets. Even though organic carbon content has often been discussed in PAH mobility studies, the desorption behavior of the PAHs from these samples showed no relationship to carbon content (r^2 generally less than 0.3), whether the organic carbon content was determined by the WB method, by the MM method, or by TGA, or if the total carbon was determined using elemental analysis. Similarly, the other sample characteristics including elemental N and S, sand/silt/clay content, and total extractable hydrocarbons showed no strong correlation with the *F* values for the individual PAHs, and r^2 values were nearly all under 0.4. This lack of predictive capabilities using common soil parameters is in agreement with recent reports showing that knowledge of simple soil parameters (such as organic carbon) is not sufficient to predict the bioavailability of organic pollutants (*5*, *14*, *16*, *28*−*39*).

Correlation of SFE and Water/XAD_2 Desorption Fast Fractions. Comparisons between the SFE and water desorption *F* values were performed to determine the correlations between the two methods as well as to determine any effect of the sample matrix on such correlations.

Figure 3 shows the correlation of *F* values obtained using water/XAD_2 with those obtained from SFE desorption. When all 11 OG and CG samples were included in one sample set (Figure 3a), the linear correlation between the two methods ($r^2 = 0.71$), while significant, shows a fair amount of difference in *F* values between the two desorption methods. This value was also substantially poorer than the previously reported comparison for four samples from a single CG site ($r^2 = 0.81$) (*23*). Since the SFE conditions were developed on the basis of samples from a CG site, additional correlations were performed separately for the OG and CG site samples. Interestingly, the correlation between SFE and water/XAD_2 *F* values is even less for the OG samples (Figure 3b), and SFE obtains roughly 2−3-fold higher *F* values than water/XAD_2 desorption. However, when the *F* data are compared for the five CG samples (Figure 3c), the correlation is quite high ($r^2 = 0.87$) and compares favorably with the correlation ($r^2 = 0.81$) between SFE *F* values and water/XAD_2 *F* values using the optimized SFE conditions developed in the original study (*23*).

As discussed above, OG and CG samples have significant differences in their matrix compositions that may account for the differences (discussed in more detail below). In any case, the results shown in Figure 3 indicate that SFE can be used to rapidly estimate water desorption behavior for individual PAHs from CG samples, but it appears to be less useful for the prediction of *F* values from OG samples.

Mobile Concentration Comparisons. The results in Tables 3, 4, S4, and S5 address the fraction of a particular PAH that is released rapidly but do not address whether the actual quantity of a particular PAH that is released is large or small. Thus, a sample may have low *F* values but, if highly contaminated, may release higher concentrations of PAHs than a sample with high *F* values and low PAH concentrations. Since the concentrations of individual PAHs on the 11 samples used in the present study range from <1 to several thousand mg/kg, actual PAH concentrations that are "mobile" are important to consider in addition to the fractions that are rapid-released (*F*).

The mobile concentrations of individual PAHs can be obtained by multiplying the concentration of each PAH in a particular sample by its *F* value. Mobile concentrations for PAHs determined using water desorption and SFE are shown in Figure 4 and range from ca. 1 to several thousand mg/kg. As might be expected on the basis of the correlation of *F* values shown in Figure 4, the mobile concentrations predicted by SFE and water desorption agree much better for CG than for OG samples for the samples shown (Tables 3, 4, S5, and S6). When the mobile concentrations are directly compared as concentration (mg/kg) values, agreement between the mobile concentrations determined on the basis of SFE and water *F* values is not strong for the OG samples (Figure 4a). However, for the CG samples, agreement is excellent ($r^2 = 0.98$; Figure 4c). Both plots comparing mobile concentration values have many more data points at low than at high concentration values, which makes the comparisons based on linear correlation coefficients dominated by the PAHs having high mobile concentrations. However, it is clear from Figure 4 that SFE and water desorption agree very well for CG samples on PAH releases that occur at higher concentrations.

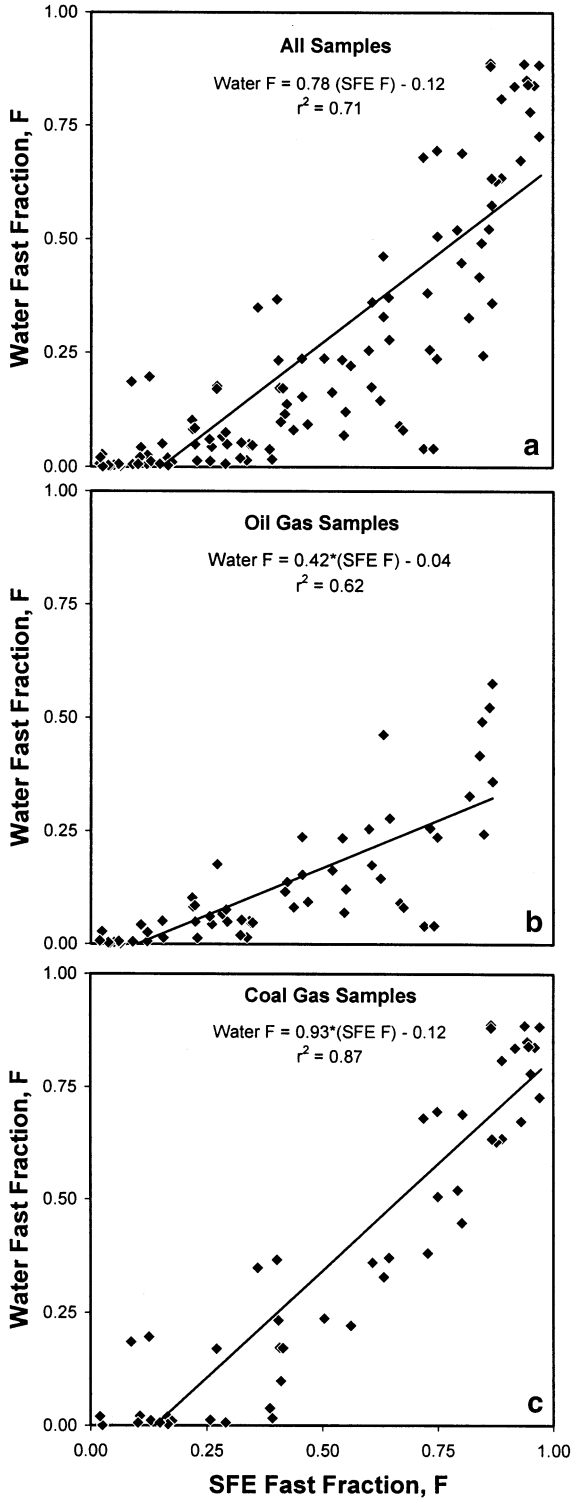


FIGURE 3. Correlation of the two-site model rapidly released fraction *F* values for 17 PAHs from 11 samples determined by SFE and water/XAD_2 desorption.

Plots that have data better distributed for linear correlation comparisons can be obtained by plotting the natural logarithms of the mobile concentrations determined by SFE and water desorption (Figure 4b,d). On this basis, the correlation coefficients for the OG ($r^2 = 0.84$) and the CG ($r^2 = 0.82$) are essentially identical, but it is clear that agreement between mobile concentrations determined by water and SFE desorption is much better for the PAHs that are released at high concentrations from the CG samples.

Effect of PAH and Matrix Organic Composition on Relative SFE and Water Desorption Rates. As described above, PAH mobility measured by 120 min of SFE agreed with 120 days of water desorption for CG samples, but SFE yielded higher mobilities for PAHs from the OG samples. PAH concentrations did not account for this behavior since PAH concentrations varied over a similar range for the OG and CG samples (Tables 2 and S2). Similarly, PAH distribution (i.e., the relative concentrations of low and high molecular weight PAHs or "weathering index") does account for the differences in the behavior of OG and CG samples since both high and low weathering indices were found for OG and CG samples (Table 2).

Since PAHs have been reported to associate with carbon particles in sediments (*14*), it seems reasonable to compare the carbon matrixes in the OG and CG samples. Differences in the degree of aromaticity and polarity might be expected on the basis of the MGP process, with OG samples being more highly aromatic and CG samples having more polarity but a less aromatic nature (*3*, *40*). As discussed above, the OG samples that have high carbon contents look and feel like lampblack soot, a carbon type that has been reported to reduce PAH mobility (*41*). Even the OG samples with lower carbon contents (ca. 10 wt %) appear, under low magnification, to be soot particles distributed in a soil matrix. In contrast to the OG samples, the CG samples have the appearance of normal soils and sediments, except for CG-11, which appears to include particles of coal.

The carbon matrixes in the OG and CG samples can be investigated using different approaches to measuring carbon and organic content (Tables 1 and S1) in order to rank the carbon composition as hard and soft carbon (*17*, *42*−*44*). Hard carbon is characterized by highly condensed aromatic material as evidenced by a high molar C/H ratio, by a low proportion of TGA volatile organic (the organic material that is vaporized in inert atmosphere at <650 °C and before oxygen is introduced), and by a large disagreement between the mild WB and the more rigorous MM oxidation methods.

As shown in Tables 1 and S1, the carbon matrix in the six OG samples is quite different from that in the five CG samples. In the OG samples, the carbon is predominantly present as hard carbon as evidenced by TGA, where 62−87% of the total organic matter is "nonvolatile" or "fixed" organic (corresponding to only 38−13% in volatile organic matter). The hard nature of the OG carbon is also evidenced by the fact that mild oxidation (WB) grossly underdetermines organic carbon as compared to stronger oxidation methods (MM) and as compared to total carbon. In addition, the molar C/H ratios (and molar C/N ratios) of all OG soils are high, which is expected for a highly aromatic matrix material. The aromatic nature of the OG matrix is also supported by solid-state NMR analyses conducted on the OG samples (*26*). All OG sample spectra showed only an aromatic carbon response.

In the CG samples, the type of carbon is generally more typical of soil/sediment organic matter. The carbon present tends to be soft in the CG samples (e.g., the volatile TGA fraction ranges from 46 to 87% of the total organic matter), which is substantially higher than the OG samples (from 13 to 38% volatile organic matter). The volatile TGA fractions from the CG samples also agree well with those determined for three natural sediments and two agricultural soils (70−87%). The organic content values obtained for the CG samples by the mild oxidation WB method also agree better with the results from the more rigorous MM method and total carbon methods. This relative agreement among these oxidation methods of carbon analysis indicates that the carbon in the CG samples is more characteristic of soil organic carbon than of the soot or lampblack material found in the OG samples. In addition, the molar C/H ratios for the CG samples (0.6−

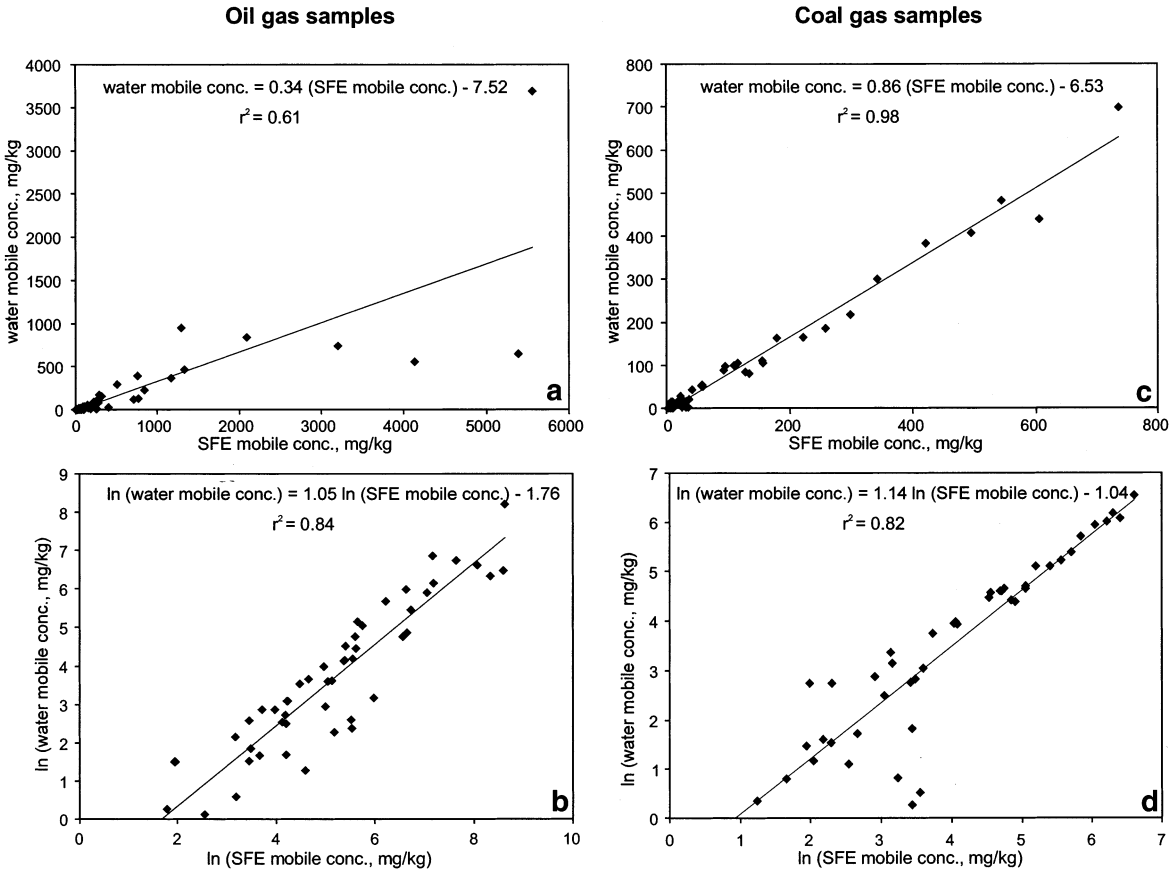


FIGURE 4. Correlation of the mobile concentrations determined by SFE and water/XAD_2 desorption of 17 PAHs from the six OG samples (left side) and five CG samples (right side).

1.1, except for CG-11 which is 2.3, but also contained particles that looked like coal) correspond much better with the five natural sediments and soils (0.6−0.8) than to the much higher ratios for the OG samples (2.4−6.3). Similarly, CG sample molar C/N ratios (22−41) and the five natural soils and sediments (15−25) are substantially lower than those of the OG samples (78−93).

Taken together, these analyses indicate that the aromatic nature of the carbon in the OG samples is much higher than for the CG samples and support the OG and CG carbon structures proposed earlier by others (*3*, *40*). In essence, the OG carbon appears to be predominantly formed by very high molecular weight PAHs with few or no polar functional groups and, as such, bears little relevance to organic matter typically associated with soils and sediments.

This varying carbon matrix for the OG and CG samples helps explain the difference between the results of the SFE and the water desorption for PAH release from the OG matrixes. For both OG and CG samples, it would be expected that, as aromatic hydrocarbons, PAHs would sorb to the sample matrix via nonspecific van der Waals forces. However, for matrixes having more aromatic character, sorption of PAHs would be expected to increasingly occur via more specific aromatic (π) electron associations, much as PAHs strongly adsorb to aromatic resins such as XAD (a styrene−divinylbenzene copolymer). As a very polar solvent, water is very poor at disrupting associations between nonpolar solutes and matrixes. Supercritical carbon dioxide is relatively lipophillic and can more easily displace nonpolar solutes from nonpolar matrixes. Thus, the relative rate of PAH desorption by SFE is expected to be faster than water desorption for the OG samples if this hypothesis of PAH/soot sorption is correct. Supercritical carbon dioxide is also known to swell organic matrixes, which may also enhance its extraction rate from highly aromatic matrixes as compared to water (*45*).

For the CG samples, the analyses discussed above demonstrate that the organic matrix is less aromatic and is more like natural soil or sediment organic carbon. The soil/sediment organic material is also more polar than aromatic soot. This is important for two reasons. First, less aromatic and more polar organic material is a poorer sorbent for PAHs than is a soot matrix. Second, since water is a polar solvent, it can interact with (or "wet") the matrix of the CG samples much better than the matrix of the aromatic OG samples. Since the SFE conditions were developed in our previous study (*23*) to mimic water desorption from a CG matrix, the same conditions are appropriate for the CG samples in this study as well. Thus, the rates of PAH desorption by SFE are expected be proportional to water desorption rates (and the *F* values should be similar) for the CG samples if this hypothesis of PAH/soot sorption is correct. As shown in Figures 3 and 4, this agreement is quite good over CG samples having a very wide range of PAH concentrations, differing molecular weight distributions (as evidenced by the weathering indices), widely varying desorption rates or *F* fractions, and a range of matrix characteristics.

The differences in the results of the two extraction methods, as used with the OG and the CG samples, is not unexpected and can be explained by the differences in the carbon matrixes of the two sets of MGP samples. The results clearly demonstrate that PAH/sample matrix interactions must be considered in any attempt to understand and predict the mobility of PAHs from MGP site soils and sediments. The results also agree with an earlier report showing that PAHs partition from water to diesel soot more strongly than to more "normal" organic carbon (*43*). Differences in desorption mechanisms displayed by carbon dioxide and water may

also be useful in investigating the mechanism of biological uptake of PAHs from MGP samples since supercritical carbon dioxide, as a lipophillic solvent, may more closely mimic lipid-based biological uptake than water desorption (*46*). Part 2 of this series will investigate the uptake of PAHs from OG and CG samples by earthworms and will compare the rates of release found for the same samples by SFE and water desorption in an effort to better understand processes controlling the bioavailability of PAHs.

Acknowledgments

Financial support for this research was provided by the Gas Research Institute (GRI) and the U.S. Department of Energy under Cooperative Agreement DE-F26-98FT40321. However, any opinions, findings, conclusions, or recommendations expressed herein are those of the authors and do not necessarily reflect the views of the DOE or GRI. The authors thank Jim Edwards and Cris Liban for preparing and supplying the samples, Joe Kreitinger for organic carbon determinations and helpful discussions, and Nadine Gordon and David Miller for analytical support.

Supporting Information Available

Complete sample matrix characterization results in Table S1; PAH solubilities in water and in supercritical carbon dioxide in Table S3; additional tables include concentrations, water desorption and SFE *F* values (and related confidence intervals), and representative rate constants for ca. 20 individual PAHs for all samples measured. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) U.S. Environmental Protection Agency. *A Resource for MGP Site Characterization and Remediation*; Solid Waste and Emergency Response; EPA 542-R-00-005; U.S. Goverment Printing Office: Washington, DC, July 2000.

(2) Harkins, S. M.; Truesdale, R. S.; Hill, R.; Hoffman, P.; Winters, S. *U.S. Production of Manufactured Gases*: *Assessment of Past Disposal Practices*; Research Triangle Institute, NC, for U.S. Environmental Protection Agency: Cincinnati, 1987; EPA Contract 68-01-6826 D.O. 35.

(3) Hayes, T. D.; Linz, D. G.; Nakles, D. V.; Leuschner, A. P. *Management of Manufactured Gas Plant Sites, Volume 1*; Amherst Scientific Publishers: Amherst, MA, 1996; p 20.

(4) Mon, G. J. *Land Contam. Reclam.* **1995**, *3*, 1−4.

(5) Alexander, M. *Environ. Sci. Technol.* **2000**, *34*, 4259−4265.

(6) Loehr, R. C.; Webster, M. T. *Pract. Period. Hazard., Toxic, Radioact. Waste Manage.* **2000**, *4*, 118−125.

(7) Cornelissen, G.; Rigterink, H.; Ferdinandy, M. M. A.; Van Noort, P. C. M. *Environ. Sci. Technol.* **1998**, *32*, 966−970.

(8) Awata, H.; Johnson, K. A.; Anderson, T. A. *Toxicol. Environ. Chem.* **1999**, *73*, 25−42.

(9) Hawthorne, S. B.; Grabanski, C. B. *Environ. Sci. Technol.* **2000**, *34*, 4103−4110.

(10) Macleod, C. J. A.; Semple, K. T. *Environ. Sci. Technol.* **2000**, *34*, 4952−5957.

(11) Bordelon, N. R.; Donnelly, K. C.; King, L. C.; Wolf, D. C.; Reeves, W. R.; George, S. E. *Toxicol. Sci.* **2000**, *56*, 37−48.

(12) Cuypers, C.; Grotenhuis, T.; Joziasse, J.; Rulkens, W. *Environ. Sci. Technol.* **2000**, *34*, 2057−2063.

(13) Johnson, M. D.; Weber, W. J., Jr. *Environ. Sci. Technol.* **2001**, *35*, 427−433.

(14) Ghosh, U.; Gillette, J. S.; Luthy, R. G.; Zare, R. N. *Environ. Sci. Technol.* **2000**, *34*, 1729−1736.

(15) Tang, J.; Robertson, B. K.; Alexander, M. *Environ. Sci. Technol.* **1999**, *33*, 4346−4351.

(16) Johnson, M. D.; Huang, W.; Weber, W. J., Jr. *Environ. Sci. Technol.* **2001**, *35*, 1680−1687.

(17) Hawthorne, S. B.; Bjorklund, E.; Bøwadt, S.; Mathiasson, L. *Environ. Sci. Technol.* **1999**, *33*, 3152−3159.

(18) Mackay, A. A.; Gschwend, P. M. *Environ. Sci. Technol.* **2001**, *35*, 1320−1328.

(19) Reeves, W. R.; Barhoumi, R.; Burghardt, R. C.; Lemke, S. L.; Mayura, K.; McDonald, T. J.; Phillips, T. D.; Donnelly, K. C. *Environ. Sci. Technol.* **2001**, *35*, 1630−1636.

(20) Northcott, G. L.; Jones, K. C. *Environ. Sci. Technol.* **2001**, *35*, 1103−1110.

(21) Reeves, W. R.; McDonald, T. J.; Bordelon, N. R.; George, S. E.; Donnelly, K. C. *Environ. Sci. Technol.* **2001**, *35*, 1637−1643.

(22) Kottler, B. D.; White, J. C.; Kelsey, J. W. *Chemosphere* **2001**, *42*, 893−898.

(23) Hawthorne, S. B.; Poppendieck, D. G.; Grabanski, C. B.; Loehr, R. C. *Environ. Sci. Technol*, **2001**, *35*, 4577−4583.

(24) Nelson, D. W.; Sommers, L. E. Total Carbon, Organic Carbon, and Organic Matter. In *Methods of Soil Analysis, Part 2−Chemical and Microbiological Properties*; Page, A. L., Miller, R. H., Keeney, D. R., Eds.; American Society of Agronomy: Madison, WI, 1982; pp 539−594.

(25) Berg, M. S.; Loehr, R. C.; Webster, M. T. *J. Soil Contam.* **1998**, *7*, 675−695.

(26) Ahmad, R.; Kookana, R. S.; Alston, A. M.; Skejemstad, J. O. *Environ. Sci. Technol.* **2001**, *35*, 878−884.

(27) Loehr, R. C.; McMillen, S. J.; Webster, M. T. *Pract. Period. Hazard., Toxic, Radioact. Waste Manage.* **2001**, *5*, 78−87.

(28) Alexander, R. R.; Alexander, M. *Environ. Sci. Technol.* **2000**, *34*, 1589−1593.

(29) Goss, K.-U.; Schwarzenbach, R. P. *Environ. Sci. Technol.* **2001**, *35*, 1−9.

(30) West, C. W.; Kosian, P. A.; Mount, D. R.; Makynen, E. A.; Pasha, M. S.; Sibley, P. K.; Ankley, G. T. *Environ. Toxicol. Chem.* **2001**, *20*, 1104−1111.

(31) McGroddy, S. E.; Farrington, J. W. *Environ. Sci. Technol.* **1995**, *29*, 1542−1550.

(32) Gustafsson, O.; Haghseta, F.; Chan, O.; MacFarlane, J.; Gschwend, P. M. *Environ. Sci. Technol.* **1997**, *31*, 203−209.

(33) Ahmad, R.; Kookana, R. S.; Alston, A. M.; Skjemstad, J. O. *Environ. Sci. Technol.* **2001**, *35*, 878−884.

(34) Karapanagioti, H. K.; Sabatini, D. A. *Environ. Sci. Technol.* **2000**, *34*, 2453−2460.

(35) Castilla, H. J.; Werth, C. J.; McMillan, S. A. *Environ. Sci. Technol.* **2000**, *34*, 2966−2972.

(36) Johnson, M. D.; Keinath, T. M., II; Weber, W. J., Jr. *Environ. Sci. Technol.* **2001**, *35*, 1688−1695.

(37) Bucheli, T. D.; Gustafsson, O. *Environ. Sci. Technol.* **2000**, *34*, 5144−5151.

(38) Chefetz, B.; Deshmukh, A. P.; Hatcher, P. G.; Guthrie, E. A. *Environ. Sci. Technol.* **2000**, *34*, 2925−2930.

(39) Jonker, M. T. O.; Smedes, F. *Environ. Sci. Technol.* **2000**, *34*, 1620−1626.

(40) Akhter, M. S.; Chunghtai, A. R.; Smith, D. M. *Appl. Spectrosc.* **1985**, *39*, 154−167.

(41) Stroo, H. F.; Jensen, R.; Loehr, R. C.; Nakles, D. V.; Fairbrother, A.; Liban, C. B. *Environ. Sci. Technol.* **2000**, *34*, 3831−3836.

(42) Grisi, B.; Grace, C.; Brookes, P. C.; Benedetti, A.; Dell'Abate, M. T. *Soil Biol. Biochem.* **1998**, *30*, 1309−1313.

(43) Accardi-Dey, A.; Gschwend, P. M. *Environ. Sci. Technol.* **2002**, *36*, 21−29.

(44) Gelinas, Y.; Prentice, K.; Baldock, J. A.; Hedges, J. I., *Environ. Sci. Technol.* **2001**, *35*, 3519−3525.

(45) Weber, W. J., Jr.; Young, T. M. *Environ. Sci. Technol.* **1997**, *31*, 1686−1691

(46) Voparil, I. M.; Mayer, L. M. *Environ. Sci. Technol.* **2000**, *34*, 1221−1228.

Received for review March 1, 2002. Revised manuscript received August 21, 2002. Accepted August 27, 2002.

ES020626K

APPENDIX D

EVIDENCE FOR VERY TIGHT SEQUESTRATION OF BTEX COMPOUNDS IN MANUFACTURED GAS PLANT SOILS BASED ON SELECTIVE SUPERCRITICAL FLUID EXTRACTION AND SOIL/WATER PARTITIONING

PUBLISHED IN *ENVIRONMENTAL SCIENCE AND TECHNOLOGY*

Environ. Sci. Technol. **2003**, *37,* 3587−3594

Evidence for Very Tight Sequestration of BTEX Compounds in Manufactured Gas Plant Soils Based on Selective Supercritical Fluid Extraction and Soil/Water Partitioning

STEVEN B. HAWTHORNE* AND DAVID J. MILLER

Energy and Environmental Research Center, University of North Dakota, Campus Box 9018, Grand Forks, North Dakota 58201

Benzene, toluene, ethylbenzene, *o*-, *m*-, and *p*-xylenes (BTEX), and polycyclic aromatic hydrocarbons (PAHs) were extracted from eight manufactured gas plant (MGP) soils from sites that had been abandoned for several decades. Supercritical fluid extraction (SFE) with pure carbon dioxide demonstrated the presence of BTEX compounds that were highly sequestered in both coal gas and oil gas MGP soils and soots. Benzene was generally the slowest compound to extract from all samples and was even more difficult to extract than most two- to five-ring PAHs found on the same samples. Since the solubility of benzene in carbon dioxide is 2−5 orders of magnitude higher than the solubilities of PAHs, these results demonstrate that benzene was more tightly sequestered than toluene, ethylbenzene, xylenes, or the multi-ring PAHs. Additional evidence for very tight binding was based on the fact that BTEX concentrations determined using either SFE or with methylene chloride sonication were much higher than those obtained by the U.S. EPA purge-and-trap method, especially for benzene (whose concentration was underestimated by as much as 1000-fold by the EPA method). However, soil/water desorption showed little benzene mobility, and K_d values for benzene were 1−2 orders of magnitude higher than those calculated based on literature sorption K_{OC} values. These results indicate that environmentally relevant concentrations of benzene may be better represented by mild extraction methods than by methods capable of extracting tightly bound benzene.

Introduction

Benzene is frequently the most important compound in determining risk and remediation criteria from fuel-related releases that may potentially impact groundwater. Soil and sediment concentrations of benzene and the related compounds toluene, ethylbenzene, and the xylenes (BTEX) are routinely determined by U.S. EPA Method 5035/5030, which uses water (or methanol) extraction followed by purge-and-trap analysis. Several investigators have demonstrated that environmental aging of hydrophobic organics can reduce their bioavailability and their rates of desorption into water (*1*−*13*). Studies on aromatic hydrocarbons have focused on multi-ring PAHs since their water solubilities are much lower than BTEX compounds (*7*, *8*, *10*, *11*). However, some initial reports indicate that monocyclic aromatics including BTEX also display reduced availability in aged soils and sediments (*1*−*3*, *5*, *6*).

Since the EPA method is based on desorption of BTEX into water, sequestration of BTEX compounds may be expected to reduce the fraction of these compounds measured by the purge-and trap-method as compared to more aggressive extraction methods. The inability of the EPA method to recover sequestered benzene was previously demonstrated by Askari et al. (*14*), who reported that the purge-and-trap method underestimated benzene concentrations in a field-aged soil by ca. 100-fold and thus demonstrated that the method is not always adequate to determine total benzene concentrations. On the other hand, if BTEX molecules become more highly sequestered from aging, the actual "mobile" fraction of these compounds may be quite low. In such cases, analytical methods determining the actual total concentration may overestimate the environmental significance of these compounds.

Despite the wide-spread impact (and cost) of site remediation needs related to benzene, few studies have investigated the relationships among benzene sequestration, environmental mobility, and analytical methodology. Ideally, analytical methods would be available to measure both "total" benzene concentrations and "mobile" or "bioavailable" concentrations, but such methods are not well developed or accepted.

Previous studies on aromatic hydrocarbons have investigated desorption into supercritical carbon dioxide (SFE) to determine both the "available" and total concentrations of PAHs (*15*−*18*). This approach has recently been applied to historically impacted manufactured gas plant (MGP) samples, where the fractions of individual PAHs extracted at mild SFE conditions in 120 min showed good agreement with those removed by bioremediation in a field treatment unit over 1 yr (*15*) or those removed by water desorption over 120 d (*16*, *17*). Although the SFE approach has not yet been used to investigate benzene sequestration in field samples, one initial study has demonstrated decreased desorption of benzene under mild SFE conditions upon several months of laboratory aging in soil/water columns (*6*).

An interesting result of the previous studies on 15 oil gas (OG) and coal gas (CG) MGP samples was that the lowest molecular weight PAH, naphthalene, frequently showed slower desorption rates by SFE and water desorption than higher molecular weight PAHs (*17*). Slow desorption of naphthalene was initially surprising because, as compared to all the other PAHs in the samples, naphthalene has the highest solubility in both supercritical carbon dioxide and in water. Therefore, naphthalene would be expected to show the fastest desorption rate in either fluid (*17*). The relatively slow desorption rates shown by naphthalene as compared to much less soluble three- and four-ring PAHs was explained by higher proportions of naphthalene being associated with "tightly bound" or "slowly desorbing" sites in the MGP sample matrixes, as compared to the higher molecular weight PAHs. In essence, since naphthalene is the most volatile and water soluble of the PAHs, any naphthalene molecules that remain on the soil or soot matrixes after extensive weathering must be more often associated with "tight" binding sites than higher molecular weight PAHs (which are less volatile and less water soluble) (*17*).

* Corresponding author phone: (701)777-5256; fax: (701)777-5181; e-mail: shawthorne@undeerc.org.

10.1021/es020899f CCC: $25.00 © 2003 American Chemical Society
Published on Web 07/17/2003

TABLE 1. MGP Sample Characteristics

	coal gas samples				oil gas samples			
	CG-3	CG-12	CG-15	CG-17	OG-2	OG-10	OG-17	OG-18
organic carbon (wt %)[a]	6.9	7.0	9.0	7.2	56	47	39	20
molar C/H ratio[b]	0.9	1.1	3.5	1.8	6.3	5.0	5.7	2.5
total EPA PAHs (mg/kg)	4000	3600	960	540	6200	39 500	16 200	17 100

[a] Determined by Modified Mebius Method (*23*). [b] Determined by elemental analysis.

Extending this idea to lower molecular weight aromatic hydrocarbons would indicate that BTEX molecules (and especially benzene) that have survived decades of weathering must be associated with very tight (or slowly desorbing) sites on the MGP sample matrixes. To investigate the possibility that benzene can become very tightly bound under field conditions, the concentrations and SFE desorption behaviors of BTEX from eight MGP OG and CG samples were determined and compared to the desorption behaviors of PAHs from the same samples. BTEX extractions were performed using sequentially stronger SFE conditions and sonication in methylene chloride. These quantitative results are compared to those obtained using the U.S. EPA purge-and-trap Method 5035/5030. BTEX partitioning from soil to water was also determined and related to the BTEX concentrations measured by SFE, sonication, and the EPA method.

Experimental Section

Samples. Fifteen PAH-contaminated soils, sediments, lampblack soot, and soil mixed with soot were the subject of an earlier study determining the behavior of PAHs during SFE desorption, water desorption, and earthworm uptake (*17*). All samples were collected from MGP sites that had been closed for ca. 50 yr. Samples from CG production generally appeared to be coal tar mixed with soil, except for CG-3, which is a sediment sample. Samples from OG production generally appeared to be lampblack soot material either pure or mixed with soil. For the present study, each of these 15 samples was evaluated for BTEX concentrations based on sonication in methylene chloride as described below. Out of the 15 samples, eight had several BTEX compounds with concentrations (based on methylene chloride extraction) greater than 1 mg/kg and were therefore selected as the samples for the present study.

Extractions and Analyses. Sonication extractions were performed in triplicate on 1-g replicates of each sample using 10 mL of methylene chloride placed in a 15-mL screw-top vial with a Teflon-lined cap for 18 h in a bath sonicator. Before sonication, each sample was mixed with an equal weight of sodium sulfate, the methylene chloride was added, and the suspension was spiked with 100 μL of an internal standard solution containing 40−500 μg/mL of perdeuterated benzene-d_6, toluene-d_8, ethylbenzene-d_{10}, *m*-xylene-d_{10}, *o*-xylene-d_{10}, and naphthalene-d_8. Following sonication, the samples were allowed to settle for ca. 2 h, and the extract was removed for GC/MS analysis (described below).

SFE was performed in a manner analogous to that previously reported for PAHs from MGP soils (*15*). That is, the samples were first extracted at mild SFE conditions for 60 min followed by two increasingly rigorous SFE conditions, each for an additional 60 min. Fractions were collected at periodic intervals during all of the SFE extractions. All SFE experiments were repeated in triplicate. Instrumentation and methods are as previously described (*15*), except that the SFE conditions were 400 bar and 50 °C (0−60 min), 400 bar and 100 °C (60−120 min), and finally 400 bar and 150 °C (120−180 min). All SFE extractions were performed with an ISCO model 210 extractor with a model 260 D pump. The flow rate was controlled at 1.0 mL/min (as compressed CO_2 measured at the pump) with a manual variable outlet restrictor heated to 80 °C. Following the SFE extractions, each residue was then mixed with sodium sulfate and sonicated with methylene chloride as described above in an attempt to recover any residual BTEX compounds. Each extract fraction was spiked with perdeuterated internal standards as described above. In addition to the BTEX and naphthalene determinations performed on every extract, selected extracts were also analyzed for higher molecular weight PAHs by GC/MS after spiking with perdeuterated PAHs as internal standards.

SFE and sonication extracts were analyzed by GC/MS (Agilent model 5973) equipped with a 60 m HP-5 MS column (0.25 mm i.d., 0.25 μm film thickness) in the selected ion monitoring mode using the molecular ion for each component and deuterated internal standard. BTEX and PAH compound identities were confirmed by analysis of standard compounds in the same manner as well as by full-scan (50−400 amu) mass spectral data on representative extracts.

BTEX concentrations were also determined on replicate samples by two different contract laboratories which used EPA Method 5035 for sample extraction followed by EPA Method 5030 (purge-and-trap) and GC/PID (Method 8015) for analysis. EPA Method 5035 allows either water or methanol extraction depending on sample characteristics. Lab 1 used water extraction for all samples except OG-18, for which they used methanol extraction. Lab 2 used methanol extraction for all samples. Both laboratories reported that quality control criteria were satisfied.

Soil/Water Partitioning. Soil/water partitioning was performed by mixing 2 g of soil with a weighed amount of HPLC-grade water sufficient to fill a 22-mL glass vial. The contents were mixed by mechanical inversion (ca. 4 rpm) for either 24 h or 28 d. All vials contained 0.02% mercuric chloride to inhibit bacterial growth. Following the equilibration period, the slurries were centrifuged, and 2 mL of the supernatant water was removed for BTEX analysis and transferred to a 2-mL autosampler vial containing a magnetic stir bar. The water was immediately spiked with perdeuterated standards of benzene-d_6, toluene-d_8, ethylbenzene-d_{10}, *m*-xylene-d_{10}, and *o*-xylene-d_{10} (in 4 μL of acetone) and capped. BTEX concentrations in the water were determined by solid-phase microextraction (SPME) using a poly(dimethylsiloxane) (PDMS) fiber with a 7-μm film thickness (*19*, *20*). The fiber was inserted into the 2-mL water sample through the Teflon-lined septum, and the sorption step was performed for 15 min while stirring. BTEX compounds were recovered from the fiber by inserting it into the 300 °C injection port (splitless mode for 0.5 min) of an Agilent model 5973 GC/MS equipped with a 60-m (0.25 μm film thickness, 0.25 mm i.d.) HP-5 MS column (Agilent Technologies) with a GC oven temperature of 10 °C. Following the desorption period, the chromatographic column was heated to 320 °C at 15 °C/min and held for 15 min. Concentrations of the BTEX compounds in the water phase were based on the MS response of the molecular ions as compared to those of the perdeuterated internal standards. Soil/water distribution coefficients (K_d) were calculated as the concentration of the BTEX compound remaining in the soil divided by its concentration in the water

TABLE 2. BTEX Concentrations in MGP Site Samples Determined by SFE, Sonication, and Purge-and-Trap

	concentration (mg/kg) ± SD[a]			
			EPA purge-and-trap	
	SFE	sonication	lab 1[b]	lab 2[b]
Coal Gas				
CG-3				
benzene	4.8 ± 0.2	3.3 ± 0.2	0.02	0.2
toluene	2.9 ± 0.4	1.8 ± 0.04	0.01	0.1
ethylbenzene	9.0 ± 0.5	7.9 ± 0.1	1.8	1.4
m,*p*-xylene	4.7 ± 0.4	3.7 ± 0.1	2.0[c]	1.9
o-xylene	2.6 ± 0.2	2.2 ± 0.1		
naphthalene	450 ± 35	630 ± 23		
CG-12				
benzene	2.6 ± 0.2	1.4 ± 0.1	<0.003	<0.03
toluene	2.8 ± 0.1	2.0 ± 0.1	<0.003	<0.03
ethylbenzene	0.01 ± 0.01	0.1 ± 0.02	<0.003	<0.03
m,*p*-xylene	2.3 ± 0.2	1.6 ± 0.1	<0.01	<0.03
o-xylene	0.2 ± 0.01	0.2 ± 0.02		
naphthalene	23 ± 3	23 ± 10		
CG-15				
benzene	4.1 ± 0.2	1.5 ± 0.04	<0.003	<0.03
toluene	5.0 ± 0.5	2.6 ± 0.1	<0.003	0.04
ethylbenzene	0.9 ± 0.2	0.6 ± 0.02	<0.003	<0.03
m,*p*-xylene	3.3 ± 0.6	2.0 ± 0.1	<0.01	0.09
o-xylene	0.7 ± 0.1	0.5 ± 0.1		
naphthalene	29 ± 5	21 ± 3		
CG-17				
benzene	4.1 ± 0.3	1.4 ± 0.2	<0.003	<0.03
toluene	2.8 ± 0.2	1.5 ± 0.1	<0.003	0.03
ethylbenzene	0.3 ± 0.2	0.3 ± 0.03	<0.003	<0.03
m,*p*-xylene	1.9 ± 0.2	1.2 ± 0.1	<0.01	0.04
o-xylene	0.4 ± 0.1	0.3 ± 0.04		
naphthalene	13 ± 3	9.2 ± 2.2		
Oil Gas				
OG-2				
benzene	18 ± 1.4	4.1 ± 0.5	<0.003	<0.03
toluene	15 ± 2.6	4.2 ± 0.6	<0.003	<0.03
ethylbenzene	2.1 ± 0.4	0.9 ± 0.1	<0.003	<0.03
m,*p*-xylene	6.1 ± 1.0	2.7 ± 0.3	<0.01	<0.03
o-xylene	1.0 ± 0.2	0.5 ± 0.03		
naphthalene	101 ± 12	60 ± 19		
OG-10				
benzene	67 ± 12	19 ± 1.7	0.02	<0.03
toluene	15 ± 3	6.5 ± 0.9	0.01	<0.03
ethylbenzene	6 ± 0.8	4.5 ± 0.2	0.01	0.06
m,*p*-xylene	10 ± 1.2	8.6 ± 0.5	0.02	0.09
o-xylene	1.4 ± 0.2	1.3 ± 0.1		
naphthalene	870 ± 260	760 ± 360		
OG-17				
benzene	53 ± 8	16 ± 0.7	0.08	0.4
toluene	13 ± 1.2	5.7 ± 0.5	0.09	0.5
ethylbenzene	1.7 ± 0.2	1.2 ± 0.1	0.03	0.4
m,*p*-xylene	2.7 ± 0.3	2.2 ± 0.3	0.05	0.9
o-xylene	0.6 ± 0.1	0.5 ± 0.06		
naphthalene	1550 ± 240	1290 ± 180		
OG-18				
benzene	116 ± 52	33 ± 10	<2.7	1.4
toluene	39 ± 14	18 ± 4	<3.1	2.2
ethylbenzene	39 ± 13	26 ± 5	11	11
m,*p*-xylene	23 ± 6	16 ± 4	9.4	13
o-xylene	11 ± 2	9.1 ± 2.3		
naphthalene	3240 ± 3140	4100 ± 960		

[a] Concentrations based on the extraction of triplicate samples except for OG-18 which were based on five replicates. SFE concentrations were based on the sum of the amounts extracted by SFE plus methylene chloride sonication of the SFE residues. However, the methylene chloride extracts of the SFE residues typically contained less than 10% of the total mass of BTEX and naphthalene extracted from each sample. Sonication concentrations were based on 18-h sonication of fresh samples with methylene chloride. [b] Single determinations performed by contract laboratories using EPA Method 5035 (purge-and-trap) and 8021 (GC/PID). [c] Concentrations of all three xylene isomers were reported as a single value by the contract labs.

(after the equilibration period) and have units of milliliter per gram.

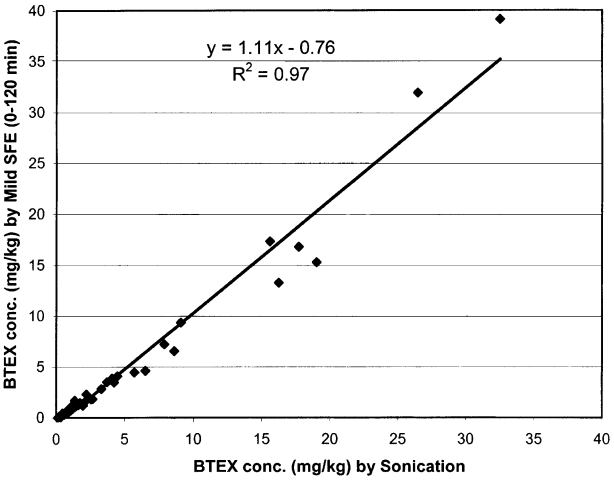


FIGURE 1. Comparison of individual BTEX compound concentrations extracted from eight MGP site samples using methylene chloride sonication vs the sum of the concentrations extracted with the two mildest SFE conditions (sum of 0−120 min).

Results and Discussion

Sample Characteristics. Detailed characterization of the samples including individual PAH concentrations; elemental C, H, N analysis; sample carbon content based on several methods; the proportion of "soft" and "hard" carbon; and sample texture is given in ref *17*. A summary of the characteristics for the samples selected for these BTEX investigations is given in Table 1. CG-3 was a sediment, and the remaining three CG samples were soils. CG samples had organic contents ranging from 7 to 9 wt % and total PAH contents from 540 to 4000 mg/kg. OG samples had the appearance of lampblack soot, carbon contents ranging from 20 to 56 wt %, and total PAH concentrations from 6200 to 39 500 mg/kg. The molar carbon/hydrogen ratio was typically higher in the OG samples, which indicates a more highly aromatic carbon matrix than that found in normal soil organic carbon (*17*).

BTEX Concentrations. BTEX and naphthalene concentrations determined by 18 h of sonication in methylene chloride, by SFE, and by the two contract laboratories based on the EPA purge-and-trap method are given in Table 2. Dramatic differences in the concentrations were found in the BTEX concentrations by the three methods, with SFE yielding the highest concentrations, especially for benzene. Askari et al. earlier reported that the purge-and-trap method underestimated benzene concentrations in a field-aged soil by ca. 100-fold (compared to a hot methanol extraction) (*14*), which is similar to the results shown for the eight field samples shown in Table 2. For these decades-old MGP site samples, benzene concentrations determined by the purge-and-trap method were 2−3 orders of magnitude lower than those determined by SFE or by sonication in methylene chloride (Table 2).

The results also indicate that benzene is present in locations in the soil and soot matrixes that are more accessible to supercritical carbon dioxide than to methylene chloride (i.e., the concentrations of benzene determined by SFE were typically 2−3-fold higher than those determined by methylene chloride sonication. However, the concentrations of the xylenes and naphthalene determined by both methods were quite similar. Regardless of the BTEX compound, the concentrations determined by methylene chloride sonication agreed well with those based on the total amounts extracted during the first two SFE conditions (i.e., from 0 to 120 min), as shown in Figure 1. These results suggest that the milder SFE conditions extract the same BTEX molecules that are accessible to methylene chloride sonication.

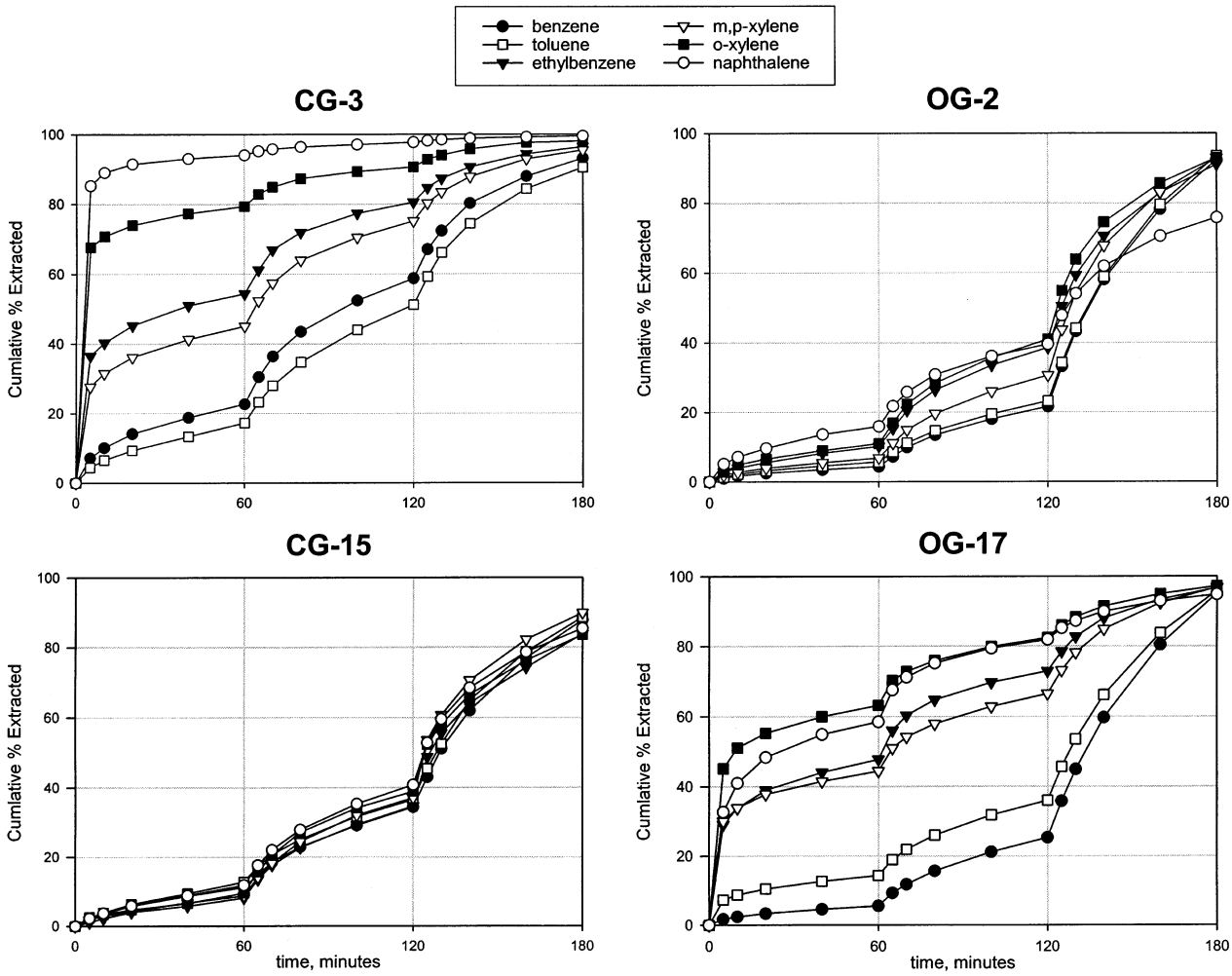


FIGURE 2. SFE extraction rates of BTEX compounds from representative MGP coal gas (CG) and oil gas (OG) site samples with three sequentially stronger SFE conditions. "Fast" molecules were extracted at the mildest SFE conditions from 0 to 60 min, "medium" molecules were extracted from 60 to 120 min, and "slow" molecules were extracted from 120 to 180 min. 100% extracted corresponds to the total amount of each compound recovered from the SFE procedure followed by sonication of the SFE residue in methylene chloride.

It should be noted that the possibility of BTEX contamination in the SFE and methylene chloride extracts was eliminated by performing frequent procedural blanks during these studies. Both the SFE procedure and methylene chloride sonication were performed in a manner identical to the sample extractions, except that no sample was present. None of the procedural blanks that were prepared throughout the study showed detectable levels of BTEX. In addition, all BTEX quantitations were performed by GC/MS, virtually eliminating the influence of any coeluting compounds. The possibility that losses of BTEX occurred between performing the different analyses was also eliminated by performing SFE and methylene chloride extractions both before and after shipping the samples to the contract labs for purge-and-trap analysis. Finally, a different analyst (using separately prepared standards) confirmed the BTEX concentrations by performing independent extractions and analyses on several of the same samples.

SFE Evidence for Tight Sequestration of BTEX Compounds. Representative desorption behaviors of BTEX compounds and naphthalene using the three sequentially stronger SFE conditions are shown in Figure 2. Two general types of behavior were demonstrated for both CG and OG samples. CG-12, CG-15, CG-17, and OG-2 all displayed very similar extraction rates for each BTEX compound and naphthalene (as illustrated in Figure 2 by samples CG-15 and OG-2). In contrast, CG-3, OG-10, OG-17, and OG-18 showed distinct differences in the extraction rates of the individual BTEX compounds, with the lowest molecular weight benzene and toluene desorbing more slowly than the higher molecular weight compounds (as illustrated by CG-3 and OG-17 in Figure 2). Naphthalene generally desorbed more rapidly than the BTEX compounds, and the contrast is especially apparent by comparing the fraction of naphthalene and benzene desorbed under the mildest (0−60 min) conditions (Figures 2 and 3 and Supporting Information Table S1).

The relative desorption rates shown in Figures 2 and 3 and in Table S1 are in direct contrast to expectations based on compound solubility and chromatographic considerations and clearly demonstrate that the BTEX compounds are not present on readily available sorption sites. For example, supercritical fluid chromatography (SFC) separations of these compounds would show an elution order consistent with the molecular weight of the compounds (i.e., benzene would elute first and naphthalene would elute last). If all of the BTEX and naphthalene molecules were present on equally accessible sites on the sample matrix (e.g., all were present on similar surface sites), the same relative desorption rates would be expected (i.e., benzene should show the fastest extraction rate and naphthalene should show the slowest rate). Therefore, the results shown in Figures 2 and 3 and in Table S1 clearly demonstrate that benzene must be present on "tighter" sites than naphthalene.

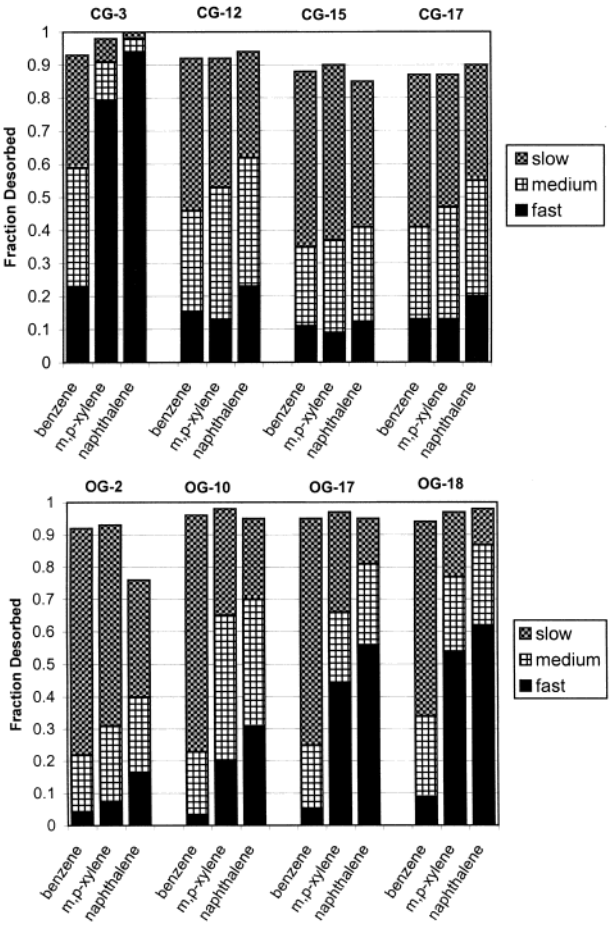


FIGURE 3. "Fast" (0−60 min of SFE), "medium" (60−120 min), and "slow" (120−180 min) fractions of benzene, *m,p*-xylene, and naphthalene from eight MGP site samples (a, coal gas; b, oil gas) determined using the same sequentially stronger SFE conditions as those in Figure 2. 100% extracted corresponds to the total amount of each compound recovered from the SFE procedure followed by sonication of the SFE residue in methylene chloride.

Whether tighter binding is a result of higher binding energies experienced by the sorbate molecule or kinetic limitations on the desorption process cannot yet be proven. Both thermodynamic (stronger binding energies) and kinetic (e.g., diffusion-limited desorption) processes may contribute to the sequestration of hydrophobic organics in soils and sediments, but the relative importance of these mechanisms for reducing desorption rates is not well understood (*13*).

A further demonstration that benzene in these samples is highly sequestered as compared to other aromatic hydrocarbons in the samples is shown in Figure 4 by comparing the extraction behavior of benzene with representative two- to six-ring PAHs present in the same sample extracts. The results of these comparisons are very dramatic, especially when comparing the extraction rates during the first SFE condition (from 0 to 60 min, 400 bar, 50 °C). For all of the samples, benzene desorption was slower than naphthalene, three-ring PAHs (represented by phenanthrene, mol wt = 178), and four-ring PAHs (represented by fluoranthene and benz[*a*]anthracene, mol wt = 202 and 228). In fact, for all of the samples except CG-12, benzene desorbed more slowly than five- and six-ring PAHs (represented by benzo[*a*]pyrene and indeno[*1,2,3-cd*]pyrene, mol wt = 252 and 276).

When the solubilities of PAHs in supercritical carbon dioxide are considered, the relative extraction rates of benzene to PAHs are astoundingly slow since the solubility of a PAH drops dramatically with molecular weight. For example, at the 400 bar and 50 °C conditions used for the first 60 min of the SFE desorptions, benzene is miscible with carbon dioxide (*21*). At the same conditions, naphthalene has a solubility of 116 g/kg, but the six-ring benzo[*ghi*]-perylene (mol wt = 276) only has a solubility of only 0.002 g/kg. Intermediate-sized PAHs have predictably intermediate solubilities as shown in Table 3.

Based simply on solubility considerations, benzene should be by far the most rapidly extracted aromatic hydrocarbon in these samples. The fact that benzene extracts more slowly than PAHs that have solubilities which are several orders of magnitude lower demonstrates that any benzene molecules that remain on these MGP samples after ca. 50 yr of aging must be very tightly sequestered. Although no previous reports could be found which compare desorption rates for a large range of BTEX and PAH compounds, our results are in agreement with previous observations showing that the capacity of a sediment to irreversibly bind toluene was 10-fold higher than its capacity to irreversibly bind naphthalene (*1*), that toluene desorption was slower than xylene desorption from aged soils (*2*), and that water extraction (as in the EPA purge-and-trap method) may extract as little as 1% of the benzene present in soil samples (*14*).

Environmental Relevance of the Results. Table 2 clearly demonstrates the dependence of reported BTEX concentrations on the extraction method. In addition, the results shown in Figure 4 clearly demonstrate that lower molecular weight BTEX compounds are dramatically more resistant to desorption into supercritical carbon dioxide than multi-ring PAHs. Although the relationship between desorption rates into supercritical carbon dioxide and environmental processes such as desorption into water is not initially obvious, previous reports on the similarities of PAH behavior during water desorption, mild SFE, and bioremediation support the use of mild SFE to predict environmental mobility (*15−18*). In addition, a comparison of BTEX and PAH solubilities in supercritical carbon dioxide and ambient water shows a very strong relationship in solubility behavior (i.e., solubilities drop rapidly in both fluids with increasing molecular weight of the aromatic hydrocarbon). Examples of solubility behavior for representative aromatics are given in Table 3. Although the solubilities are much higher in supercritical carbon dioxide (at the 400 bar and 50 °C conditions used for the mildest extraction condition), solubilities in both fluids drop by ca. 6 orders of magnitude from benzene to benzo[*ghi*]-perylene. In fact, the solubilities of BTEX and PAH compounds are almost perfectly related with the solubility of each compound being approximately 10 000-fold higher in supercritical carbon dioxide than in water (Table 3).

From an environmental risk perspective, the mobile concentrations of BTEX compounds (especially benzene) are more important than their total concentrations. Since soil/water partitioning is frequently used to estimate BTEX mobility, the amounts of BTEX desorbed into water (sample/water ratio of 1:10) after 24 h and 28 d were determined, and partitioning coefficients were calculated for two coal gas (CG-3 and CG-15) and two oil gas (OG-2 and OG-17) samples.

The "mobile concentrations" of the BTEX compounds (defined as the mass of each compound desorbed into water per mass of soil or soot sample) are given in Table 4. In general, the amounts of the BTEX compounds desorbed in 24 h or 28 d were quite similar with the exception that significantly more benzene was desorbed from CG-3 and OG-17 after 28 d than after 24 h. The concentrations of "fast" benzene determined by SFE (i.e., the benzene desorbed after 60 min at the mildest SFE condition) agreed fairly well with those determined by water desorption, especially after 28 d. As the molecular weight of the BTEX compounds increases, the fast SFE fraction tends to overestimate the fractions desorbed into water. Thus, for toluene, the fast SFE fraction

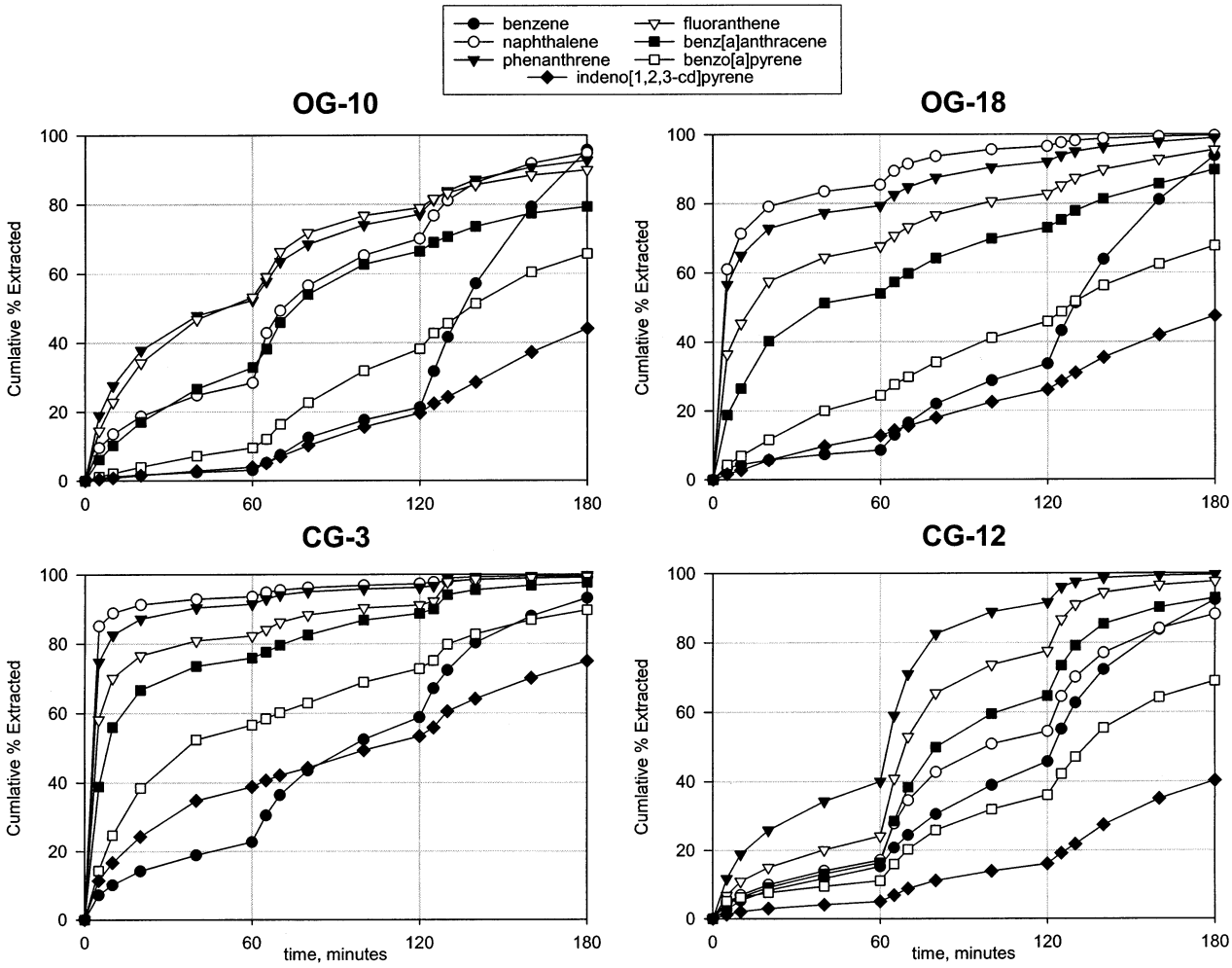


FIGURE 4. SFE extraction rates of representative two- to six-ring PAHs as compared to benzene from MGP site samples using the same SFE conditions as those in Figure 2. 100% extracted corresponds to the total amount of each compound recovered from the SFE procedure followed by sonication of the SFE residue in methylene chloride.

TABLE 3. Solubilities of Benzene and Representative PAHs in Ambient Water and Supercritical Carbon Dioxide (400 bar, 50 °C)

	CO_2 solubility[a] (g/kg)	water solubility[b] (g/kg) (10^4)
benzene	miscible	18 000
naphthalene	120	320
phenanthrene	11	13
pyrene	1.2	1.4
chrysene	0.02	0.02
perylene	0.005	0.004
benzo[*ghi*]perylene	0.002	0.003

[a] Adapted from refs *24* and *25*. [b] Adapted from ref *26*.

is ca. 3-fold higher than the water desorption fractions, while for ethylbenzene and xylenes, the SFE fast fraction is ca. 10-fold higher than the 24-h and 28-d water desorption (Table 4).

Comparison of the water desorption data (Table 4) with the EPA purge-and-trap method data (Table 2) shows that the methanol extraction/purge-and-trap option used by lab 2 generally gave better agreement with the "mobile" BTEX concentrations determined using soil/water partitioning than the concentrations determined by the water desorption/purge-and-trap option used by lab 1. However, benzene concentrations determined with the EPA method were generally much lower than the mobile benzene concentrations determined by soil/water partitioning, and good agreement was only obtained using the methanol extraction (lab 2, Table 2) with the 24-h water desorption data (Table 4) for two of the samples, CG-3 and OG-17. As was the case for the SFE fast fraction, the purge-and-trap method tended to yield higher percent recoveries for the higher molecular weight BTEX compounds, but the results are not as consistent for the different samples as those obtained using the mildest SFE fraction.

Table 4 also shows the soil/water distribution coefficients obtained after 24 h and 28 d along with those predicted by literature organic carbon/water (K_{OC}) partitioning data (*22*) and the organic carbon values shown in Table 1. In general, the 28-d K_d values are similar to or lower than the 24-h K_d values, as would be expected since more time was available for BTEX to partition into the water phase. However, nearly all of the experimental K_d values are substantially higher than those predicted based on literature K_{OC} values. Since the literature K_{OC} values were the average of experimentally determined K_{OC} values reported by several investigators (*22*), the results in Table 4 demonstrate that BTEX compounds (especially benzene) are present in more tightly associated sites in these MGP samples and, therefore, are not readily available for water desorption, either in partitioning studies or for the EPA purge-and-trap method.

It is important to note that, in general, K_d values could not be calculated using the purge-and-trap concentrations because the concentrations of BTEX compounds found in

TABLE 4. Mobile BTEX Concentrations and Soil/Water Distribution Coefficients Based on Water Desorption and SFE Fractions

	total concn (mg/kg of soil)	mobile concn (mg/kg of soil)[a] soil/water desorption 24 h	soil/water desorption 28 d	SFE 0−60 min	soil (soot)/water distribution coeff, K_d (mL/g) predicted[b]	24 h[c]	28 d[c]
benzene							
CG-3	4.8	0.12	1.4	1.1	5	440 ± 12	28 ± 2
CG-15	4.1	0.96	0.67	0.46	6	48 ± 6	52 ± 1
OG-2	18	1.2	1.8	0.78	39	180 ± 10	110 ± 10
OG-17	53	0.23	0.92	2.8	27	2800 ± 590	590 ± 70
toluene							
CG-3	2.9	0.24	0.18	0.50	16	130 ± 20	170 ± 40
CG-15	5.0	0.16	0.14	0.64	20	330 ± 40	400 ± 60
OG-2	15	0.31	0.43	0.86	130	710 ± 120	370 ± 30
OG-17	13	0.57	0.37	1.7	88	290 ± 70	390 ± 50
ethylbenzene							
CG-3	9.0	0.20	0.29	4.9	24	530 ± 160	380 ± 140
CG-15	0.9	0.01	0.01	0.07	31	880 ± 150	880 ± 230
OG-2	2.1	0.02	0.03	0.21	190	1700 ± 70	830 ± 70
OG-17	1.7	0.11	0.10	0.78	130	180 ± 50	190 ± 30
m,*p*-xylene							
CG-3	4.7	0.17	0.19	2.1	23	320 ± 100	300 ± 100
CG-15	3.3	0.04	0.03	0.29	30	1020 ± 150	1020 ± 160
OG-2	6.1	0.06	0.10	0.41	180	1500 ± 240	610 ± 40
OG-17	2.7	0.26	0.18	1.2	130	110 ± 33	170 ± 50
o-xylene							
CG-3	2.6	0.16	0.18	2.0	18	170 ± 18	160 ± 40
CG-15	0.68	0.004	0.01	0.07	24	1940 ± 169	1230 ± 110
OG-2	0.96	0.01	0.01	0.11	150	1500 ± 121	780 ± 130
OG-17	0.55	0.07	0.06	0.35	100	100 ± 5	99 ± 9

[a] Mobile concentrations are defined as the soil concentration that was desorbed into water (10:1 water to soil ratio) after 24 hr and 28 d based on quadruplicate determinations for each time period. Mobile concn = initial soil concentration minus the final soil concentration. Relative standard deviations for the water desorption experiments were benzene (2−14%), toluene (10−20%), and ethylbenzene and xylenes (typically 10−25%). Standard deviations for SFE determinations are given in Supporting Information Table S1. [b] Predicted K_d values were based on the organic carbon contents given in Table 1, and K_{OC} values were from ref *23*. K_{OC} values used were 69 (benzene), 227 (toluene), 348 (ethylbenzene), 330 (*m*,*p*-xylene), and 264 (*o*-xylene). [c] Mean ± SD (standard deviation) from quadruplicate K_d determinations.

water after the water desorptions were generally higher than those obtained by the purge-and-trap methods. Thus, any estimation of K_d values based on water desorption using the purge-and-trap data as the total concentration (as is commonly done) would show that virtually all of the BTEX molecules are mobile. In contrast, using the total BTEX concentrations determined by SFE yields K_d values that are much larger than those normally reported. While the *concentrations* of mobile BTEX compounds are based on the water desorption experiments, the *fraction*s of BTEX compounds that are mobile depend on the method used to determine "correct" BTEX concentrations in the soil.

Authors of an earlier report showing similar low benzene recoveries using the EPA water desorption/purge-and-trap method called for revision of the method to obtain more accurate values (*14*). Our results clearly agree that the purge-and-trap method can greatly underdetermine benzene concentrations, but our results also indicate that, in highly aged samples, only a tiny fraction of the benzene molecules may be available to environmental processes. If the goal is to determine mobile benzene concentrations, the purge-and-trap method using methanol extraction may be more relevant than the true benzene concentrations obtained with more stringent extraction methods. On the basis of these initial water desorption studies, mild SFE gave the best agreement with water desorption of benzene and may be useful for predicting the mobility of benzene. In any case, our results demonstrate that total concentrations have little to do with mobile concentrations of BTEX and that better methods to determine BTEX concentrations that relate to environmental mobility and bioavailability are needed.

Acknowledgments

Financial support for this research was provided by the Gas Technology Institute (GTI) and the U.S. Department of Energy under Cooperative Agreement DE-FC26-98FT40321. However, any opinions, findings, conclusions, or recommendations expressed herein are those of the author and do not necessarily reflect the views of the DOE or GRI. The authors thank David Nakles, Raymond Loehr, and Jerry King for helpful discussions.

Supporting Information Available

Table S1 reports the "fast, medium, and slow" SFE fractions of BTEX and naphthalene for the eight MGP samples along with the standard deviations of triplicate determinations of each fraction. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Kan, A. T.; Fu, G.; Hunter, M.; Chen, W.; Ward, C. H.; Tomson, M. B. *Environ. Sci. Technol.* **1998**, *32*, 892−902.
(2) Pavlostathis, S. G.; Mathavan, G. N. *Environ. Sci. Technol.* **1992**, *26*, 532−538.
(3) Rao, B. H.; Swaminathan, R.; Asolekar, S. R. *J. Air Waste Manage. Assoc.* **2001**, *51*, 1043−1059.
(4) Mackay, A. A.; Chin, Y.-P.; Macfarlane, J. K.; Gschwend, P. M. *Environ. Sci. Technol.* **1996**, *30*, 3223−3231.
(5) Rixey, W. G.; Garg, S.; Murkute, P.; Qu, W. *J. Biorem.* (in press).
(6) Hawthorne, S. B.; Grabanski, C. B.; Miller, D. J.; Rixey, W. G. *Proceedings of the International Petroleum Conference*, Houston, 1999.
(7) Kan, A. T.; Fu, G.; Tomson, M. B. *Environ. Sci. Technol.* **1994**, *28*, 859−867.
(8) Ortiz, E.; Kraatz, M.; Luthy, R. G. *Environ. Sci. Technol.* **1999**, *33*, 235−242.
(9) Farrell, J.; Reinhard, M. *Environ. Sci. Technol.* **1994**, *28*, 63−72.
(10) McGroddy, S. E.; Farrington, J. W.; Gschwend, P. M. *Environ. Sci. Technol.* **1996**, *30*, 172−177.
(11) Huang, W.; Young, T. M.; Schlautman, M. A.; Yu, H.; Weber, W. J., Jr. *Environ. Sci. Technol.* **1997**, *31*, 1703−1710.

(12) Alexander, M. *Environ. Sci. Technol.* **1995**, *29*, 2713−2717.
(13) Pignatello, J. J.; Xing, B. *Environ. Sci. Technol.* **1996**, *30*, 1−11.
(14) Askari, M. D. F.; Maskarinec, M. P.; Smith, S. M.; Beam, P. M.; Travis, C. C. *Anal. Chem.* **1996**, *68*, 3431−3433.
(15) Hawthorne, S. B.; Grabanski, C. B. *Environ. Sci. Technol.* **2000**, *34*, 4103−4110.
(16) Hawthorne, S. B.; Poppendieck, D. G.; Grabanski, C. B.; Loehr, R. C. *Environ. Sci. Technol.* **2001**, *35*, 4577−4583.
(17) Hawthorne, S. B.; Poppendieck, D. G.; Grabanski, C. B.; Loehr, R. C. *Environ. Sci. Technol.* **2002**, *36*, 4795−4803.
(18) Weber, W. J.; Young, T. M. *Environ. Sci. Technol.* **1997**, *31*, 1686−1691.
(19) Sarna, L. P.; Webster, G. R. B.; Friesen-Fischer, M. R.; Ranjan, R. S. *J. Chromatogr. A* **1994**, *677*, 201−205.
(20) Langenfeld, J. J.; Hawthorne, S. B.; Miller, D. J. *Anal. Chem.* **1996**, *68*, 144−155.
(21) Ohgaki, K.; Katayama, T. *J. Chem. Eng. Data* **1976**, *21*, 53−55.
(22) Ma, K. C.; Mackay, D.; Shiu, W. Y. *Physical−Chemical Properties and Environmental Fate Handbook*; Chapman & Hall: London, 1999.
(23) Nelson, D. W.; Sommers, L. E. Total Carbon, Organic Carbon, and Organic Matter. In *Methods of Soil Analysis, Part 2—Chemical and Microbiological Properties*; Page, A. L., Miller, R. H., Keeney, D. R., Eds.; American Society of Agronomy, Inc.: Madison. WI, 1982; pp 539−594.
(24) Bartle, K. D.; Clifford, A. A.; Jafar, S. A.; Shilstone, G. F. *J. Phys. Chem. Ref. Data* **1991**, *20*, 713−756.
(25) Miller, D. J.; Hawthorne, S. B.; Clifford, A. A.; Zhu, S. *J. Chem. Eng. Data* **1996**, *41*, 779−786.
(26) MacKay, D.; Shiu, W. Y. *J. Chem. Eng. Data* **1977**, *22*, 399−402.

Received for review August 26, 2002. Revised manuscript received May 16, 2003. Accepted May 16, 2003.

ES020899F

APPENDIX E

IMPROVING RISK ASSESSMENTS FOR MANUFACTURED GAS PLANT SOILS BY MEASURING PAH AVAILABILITY

PUBLISHED IN *INTEGRATED ENVIRONMENTAL ASSESSMENT AND RISK MANAGEMENT*

Integrated Environmental Assessment and Management — Volume 1, Number 3 — pp. 1–8
© 2005 SETAC

Improving Risk Assessments for Manufactured Gas Plant Soils by Measuring PAH Availability

Hans F. Stroo,† David V. Nakles,† Joseph P. Kreitinger,† Raymond C. Loehr,‡ Steven B. Hawthorne,§ Richard G. Luthy,|| Hoi-Ying Holman,# and Adrienne LaPierre††*

†The RETEC Group, One Monroeville Center, Suite 1015, Monroeville, Pennsylvania 15146, USA
‡Environmental and Water Resources Engineering Program, University of Texas, Austin, Texas 78712, USA
§Energy and Environmental Research Center, University of North Dakota, Campus Box 9018, Grand Forks, North Dakota 58201, USA
||Department of Civil and Environmental Engineering, Stanford University, Stanford, California 93405, USA
#E.O. Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, California 94720, USA
††Iris Environmental, 1615 Broadway, Suite 1003, Oakland, California 94612, USA

(Received 15 February 2005; Accepted 2 March 2005)

Original Research

ABSTRACT

?1 Remediation of soils at oil-gas manufactured gas plant (MGP) sites is driven primarily by the human health risks posed by the carcinogenic polycyclic aromatic hydrocarbons (PAHs), particularly benzo[*a*]pyrene (BaP), that are associated with lampblack residues. Although PAHs on lampblack are tightly sorbed, risk assessments do not account for this reduced availability. A multi-investigator study of 7 oil-gas MGP site soil samples demonstrated that the dermal and ingestion absorption factors are far lower than current default assumptions used in risk assessments. Using these sample-specific absorption factors in standard risk assessment equations increased risk-based cleanup levels by a factor of 72 on average (with a range from 23 to 142 times the default level). The rapidly released fraction of the BaP in each sample, as measured by supercritical fluid extraction, was closely correlated (r^2 = 0.96) to these calculated cleanup levels. The weight of evidence developed during this research indicates that the risks posed by PAHs on lampblack are far less than assumed when using default absorption factors and that a tiered evaluation protocol employing chemical analyses, chemical release data, and in vitro bioassays can be used to establish more realistic site-specific criteria.

Keywords: Bioavailability Bioaccessibility Lampblack Ingestion Dermal absorption

INTRODUCTION

The release of hydrocarbons from contaminated soils and sediments controls the impact that these chemicals may have following contact with biological receptors (DiToro et al. 1991). It is clear that not all the contaminants present in a given soil are equally available, and the risks may be overestimated if chemical release differences are not considered (Alexander 2000). Several studies have documented the extremely low release of polycyclic aromatic hydrocarbons (PAHs) on sooty materials such as lampblack (Bucheli and Gustafsson 2000; Stroo et al. 2000; Jonker and Koelmans 2002).

Prior work has shown that the release of hydrocarbons from such materials to aqueous media occurs in several phases. There is typically an initial phase of relatively rapid release, followed by much slower release of the less available fraction (Gustafsson et al. 1997; Berg et al. 1998; Ghosh et al. 2000). The rapidly released fraction, or F value (Loehr et al. 2003), presumably also dominates the biological uptake during relatively short-term exposures, such as during soil ingestion or dermal contact. Considerable effort has, therefore, been focused on developing rapid and inexpensive chemical assays to measure the F value for use in risk assessments.

Bioavailability is a complex phenomenon, however. The first step in biological uptake is the release from the environmental matrix. The limited release of contaminants from a strongly sorbing matrix such as lampblack can therefore reduce the potential for uptake. This environmental accessibility is a strong determinant of eventual bioavailability, but it is not the only factor impacting the eventual uptake of contaminants from soil. The organism exposed to contaminated soil may have complex uptake and sequestering mechanisms, and the bioavailability may differ significantly between different routes of exposure.

As a result of the complex relationship between accessibility and bioavailability, chemical measures of bioavailability need to be used with care. Organisms do exert some control over total uptake, and chemicals may even be absorbed in some cases without the need for release from the solid matrix (Landrum et al. 1992). Careful validation of chemical extraction tests designed to predict bioavailability assays is therefore essential (National Research Council 2003).

Further, accurately measuring hydrocarbon release and bioavailability and using such information in modifying risk assessments is a difficult task. A suite of tools is needed to develop a credible weight of evidence for any site-specific adjustments, and these tools should yield results that can be directly integrated into risk assessment calculations (Ehlers and Luthy 2003). Ideally, a chemical assay that directly measures the most available fraction of the total hydrocarbon concentration present in a soil could provide a valuable screening-level tool, or a Tier 1 assessment in typical risk-based evaluations (Loehr et al. 2003). In vitro assays targeting specific receptors or pathways could then be used, if needed, for more intensive higher-tier risk assessments.

In response to these needs, a multi-investigator study was initiated to evaluate PAH availability in a series of 7 soil samples from manufactured gas plant (MGP) sites in California. All these sites were impacted by PAHs on

* To whom correspondence may be addressed hstroo@retec.com

lampblack, a residue produced from the pyrolysis of oil to produce gas. Other residual materials were also present at oil-gas MGP sites, including tars and crude oil, but these materials are generally removed along with the concentrated lampblack deposits during cleanup, leaving behind primarily lampblack in thin seams or mixed into native or fill soils.

Lampblack is a sooty, amorphous material composed of highly aromatic carbon that tightly binds PAHs and other aromatic hydrocarbons (Hawthorne et al. 2002; Hawthorne and Miller 2003; Hong et al. 2003). Risk assessments for oil-gas MGP site soils in California are dominated by the human health risks posed by the carcinogenic PAHs (CPAH), and the exposure routes of most concern in these assessments are oral ingestion and dermal contact (California Environmental Protection Agency 1999). The 7 PAHs currently considered carcinogenic by the State of California include benzo[*a*]pyrene (B*a*P), benzo[*a*]anthracene, benzo[*b*]fluranthene, benzo[*k*]fluranthene, chrysene, dibenzo[*a*,*h*]anthracene, and indeno[1,2,3-*cd*]pyrene.

Earlier papers have reported the characteristics of the 7 lampblack samples, including the total and rapid-release PAH concentrations (Hawthorne et al. 2002), as well as the dermal bioavailability of the B*a*P in the samples (Stroo et al. 2004), the availability of PAHs in the samples to earthworms (Kreitinger et al. 2005), and the mechanisms of sorption and the partitioning behavior of the PAHs in these samples (Hong et al. 2003). In addition, in vivo and in vitro oral ingestion studies have been performed (Holman et al., in preparation). Other studies included research on the leachability of hydrocarbons from lampblack and investigations of chemical fingerprinting lampblack residues. All these results are available in a final research report (RETEC 2004).

The purpose of this paper is to present an overview of these research findings and to demonstrate how the results can be incorporated into standard risk assessments to develop site-specific cleanup levels. The results also allow a unique opportunity to compare results from different availability assays on the same samples and to evaluate the ability of a chemical assay of the rapidly released fraction of the PAHs to predict the risk-based criteria developed using the results from in vitro assays.

METHODS

A total of 18 samples (OG-1–OG-18) were obtained from 7 oil-gas MGP sites in California. These samples consisted of mixtures of soil, lampblack residuals, and miscellaneous debris. Approximately 100 kg of each sample were collected. The sampling locations at each site varied with respect to the original MGP operations. For example, some samples were taken very near the locations of former gas plant equipment, such as gas holders or tar storage tanks, while other samples were composites of discrete samples that were taken from general plant process areas. In addition, 5 background samples were also taken from nonimpacted areas that were located near the MGPs. Samples were taken either from the upper 3 feet of soil or from lampblack layers exposed during excavation.

Each sample was collected in the field and placed in large sealed buckets that were then transferred to the RETEC storage facility in Ithaca, New York. The samples were then screened to remove material $>¼$ inch and homogenized by mixing in a rotary mixer. The amount of rejected material ranged from 0 to 30% of the total sample weight. The homogenized samples were then stored at 4°C in airtight 55-gal drums. Subsamples (approximately 5 kg each) were taken from each sample after homogenization. The subsamples were shipped overnight to each of the investigators involved in the research effort. Subsequent analyses (Hawthorne et al. 2002) have shown that the results from the distributed subsamples were comparable and that the mixing yielded highly homogeneous materials in all but 1 case.

The 7 samples selected for further detailed evaluation in separate studies were chosen to represent the range of sample variations based on the following parameters: (1) total PAH concentration and the relative fractions of light (2- and 3-ring) and heavy (4-, 5-, and 6-ring) molecular weight PAHs, (2) the total organic carbon contents, (3) the rapid- and slow-release fractions of PAHs (as determined by supercritical fluid extraction [SFE] for 20 min at 200 bar and 50°C), and (4) the particle size distributions.

Chemical availability was determined using either an aqueous rate of release (ROR) assay (Loehr and Webster 2000) or a previously developed SFE assay (Hawthorne et al. 2001). In brief, the aqueous ROR assay was performed by mixing the test soil with water and an XAD sorbent. After specific time intervals (0–120 d), the concentrations of the PAHs collected on the XAD sorbent and remaining on the soil were determined (Loehr and Webster 2000). The SFE assay was performed by flowing supercritical carbon dioxide through the soil samples and collecting the eluted PAHs at specific time intervals (0–120 min). For both ROR and SFE, the available fraction was determined by fitting a simple 2-site desorption model as previously described (Hawthorne et al. 2002).

Dermal uptake testing was performed using human cadaver skin assays (Roy et al. 1998). That method, which was used in the earlier lampblack analysis (Stroo et al. 2000), was based on measuring the flux of radiolabeled B*a*P added to the matrix of interest immediately before application. To investigate the fluxes of PAHs from MGP site samples, which had been subjected to over 60 y of weathering, the method was modified to allow direct measurement of the release of the native PAHs bound to the soil (Roy and Singh 2001).

The potential for uptake via oral ingestion was evaluated using 2 different tests: an in vitro test and an in vivo feeding study. The in vitro procedure used a simulated gastrointestinal (GI) tract system developed at Lawrence Berkeley National Lab [24]. The in vivo uptake of PAHs by mice was also evaluated by careful mass balance tests in which mice were fed 4 of the lampblack samples. Details are available in the final research report (RETEC 2004). The majority of the PAHs were evidently metabolized within the mouse guts. However, phenanthrene was conserved, allowing this PAH to be used for evaluating in vivo uptake.

Risk-based cleanup levels (RBCLs) were calculated by using the in vitro dermal absorption factors (*DAF*s) for each sample and the in vitro ingestion absorption factors (*IAF*s) for B*a*P in the same samples (values given in Table 1) in standard risk assessment equations set forth in U.S. Environmental Protection Agency (USEPA) and California Environmental Protection Agency (Cal/EPA) risk assessment guidance documents (California EPA 1999; USEPA 2002). These values replace the explicit *DAF* of 0.15 for PAHs (California EPA 1999) and the implicit *IAF* of 1.0 to yield site-specific cleanup levels. For these calculations, the residential exposure scenario was assumed, other default California-specific exposure and

Table 1. Comparison of measures of availability for phenanthrene (Phen) and benzo[*a*]pyrene (B*a*P) across test samples

	ROR[a]		SFE[a]		Earthworm[b]		Dermal[c]	In vitro[d]		In vivo[e]
Sample no.	F_{BaP}	F_{Phen}	F_{BaP}	F_{Phen}	BaP	Phen	BaP	BaP	Phen	Phen
OG-2	0	5	15	33	17	13	0.17	0.5	1.0	0.6
OG-5	15	5	—[f]	35	1.4	0.9	0.59	1.4	15.0	0.7
OG-10	0	15	10	63	17	1.9	0.14	1.3	3.2	1.1
OG-13	NT[g]	NT	15	28	20	0.4	0.36	1.8	8.3	NT
OG-14	NT	NT	27	60	2.3	6.0	1.05	3.0	—[h]	NT
OG-17	1	12	2	42	13	8.5	0.29	0.2	0.8	NT
OG-18	8	33	22	82	—[i]	—[i]	0.25	5.0	11.1	0.6
Mean	4.8	14	15	49	12	5.1	0.41	1.9	6.6	0.75

[a] Represents fast (rapidly available) fractions expressed as a percentage.
[b] Represents percentage of polycyclic aromatic hydrocarbons (PAH) absorbed by earthworm as compared to the predicted uptake based on the equilibrium partitioning model.
[c] Represents percentage of applied dose absorbed across skin section over 24 h.
[d] Represents percentage of polycyclic aromatic hydrocarbons (PAH) solubilized in simulated gastrointestinal (GI) tract.
[e] Represents percentage uptake by mice 6 h after gavage.
[f] Could not be determined: did not fit release curve criteria (apparent F very low).
[g] NT = not tested.
[h] Could not be determined: below detectable limits or chromatographic interferences too large.
[i] Bioavailability could not be determined due to 100% mortality in bioaccumulation tests.

toxicity assumptions were used, and the allowable excess cancer risk was set at 1×10^{-6}. These "risk-based cleanup levels" are provided for illustrative purposes to demonstrate the potential impact of availability measurements on risk-based criteria and are not intended to imply regulatory concurrence at this time.

The equation used for the calculation of RBCLs is

$$\mathrm{RBCL}_{\mathrm{carcinogen}} = \frac{\text{Target Risk Level}}{(\mathrm{CSF}_{\mathrm{oral}})[\mathrm{IF}_{\mathrm{oral}} + \mathrm{IF}_{\mathrm{dermal}}] + (\mathrm{CSF}_{\mathrm{inhalation}})[\mathrm{IF}_{\mathrm{inhalation}}]}$$

?13 where $\mathrm{RBCL}_{\mathrm{carcinogen}}$ is the risk-based cleanup level for carcinogenic effects (mg/kg), Target Risk Level is the target cancer risk level (unitless), IF is the intake factor (a measure of exposure in kg soil/kg body weight/d), and CSF is the cancer slope factor (the toxicity value indicating the carcinogenic potency of a chemical in mg chemical/kg body weight/d).

The equations and exposure parameters for developing the intake factors used in the RBCL equation are presented in ?14 Table 2 and are consistent with values recommended by Cal/EPA. As described previously, the target risk level (i.e., the allowable excess cancer risk) is set at 1×10^{-6}. The oral and inhalation CSF for B*a*P of 12 (mg/kg/d) and 3.9 (mg/kg/d), respectively, established by the Cal/EPA's Office of Environmental Health Hazard Assessment (Cal/EPA OEHHA), were used in the RBCL calculations (California EPA 2004).

RESULTS AND DISCUSSION

The different chemical and biological assay results for each of the samples (Table 1) reveal consistent trends despite the fact that the assays were performed by different investigators, at different times, in different laboratories, and on different subsamples. In general, the PAHs in samples OG-18, OG-14, OG-10, and OG-5 had relatively high F values, while those in OG-2 and OG-17 had lower values. These results generally reflect the strength of binding (Hong et al. 2003). It should be noted that OG-5 consistently exhibited a high degree of heterogeneity and poor reproducibility of results from separate aliquots, while all the other samples appeared to be well homogenized and yielded highly reproducible results from chemical analyses of quadruplicate samples.

The results also show that the in vitro simulated gastrointestinal tract assay, which was originally developed for petroleum hydrocarbons (Holman et al. 2002), consistently overestimated the uptake of phenanthrene as measured in an in vivo uptake test (by a factor of 10 on average for the 4 samples tested by both methods). As mentioned previously, phenanthrene was the only PAH used in the in vivo uptake test because the mass recoveries of the other PAHs in the in vivo test were relatively poor, probably because of partial metabolism in the mouse guts. Nevertheless, the results suggest that the in vitro test is conservative with respect to estimating the actual uptake in vivo.

It is important to note that the in vitro test is intended as a measure of *relative* oral bioavailability and reflects the impact of the specific matrix on the solubilization and, therefore, availability of the PAHs for biological uptake. The use of the results from the in vitro test as an *IAF* in the risk assessment equation represents the bioavailability of the PAHs in the specific soil matrix relative to bioavailability in the toxicological feeding study. The implicit assumption when applying these in vitro measurements directly in the risk assessment equation, therefore, is that the bioavailability of the PAHs in the toxicological feeding study was 100%, but this work is not simply measuring the absorption previously measured in the original feeding studies used to develop cancer slope factors. It is rather an attempt to measure the environmental accessibility, that is, the release of the bound PAHs into a form that can be absorbed, but may not necessarily be absorbed in vivo.

As indicated in Table 1, the *IAF*s for B*a*P were higher than the *DAF*s by roughly a factor of 5 on average. However, the relative impacts of the 2 pathways on the site-specific risk-

Table 2. Intake factor equations and exposure parameters

Intake factor equations	
(1) Oral intake factor (kg/kg-d):	
$$IF_{oral} = \frac{EF_r \times ED_c \times IRS_c \times IAF \times CF}{BW_c \times AT_c} + \frac{EF_r \times ED_a \times IRS_a \times IAF \times CF}{BW_a \times AT_c}$$	
(2) Dermal intake factor (kg/kg-d):	
$$IF_{dermal} = \frac{EF_r \times ED_c \times AF \times DAF \times SA_c \times CF}{BW_c \times AT_c} + \frac{EF_r \times ED_a \times AF \times DAF \times SA_a \times CF}{BW_a \times AT_c}$$	
(3) Inhalation intake factor (kg/kg-d):	
$$InhF_{adj} = \frac{EF_r \times ED_c \times IRA_c}{BW_c \times AT_c \times PEF} + \frac{EF_r \times ED_a \times IRA_a}{BW_a \times AT_c \times PEF}$$	
Exposure parameters[a]	
AF_a	Adherence factor for soil, adult: 0.07 mg/cm^2
AF_c	Adherence factor for soil, child: 0.2 mg/cm^2
AT_c	Averaging time—carcinogens: 25,550 d
BW_a	Body weight, adult: 70 kg
BW_c	Body weight, child: 15 kg
CF	Conversion factor: 0.000001 kg/mg
DAF	Dermal absorption factor: default for PAHs 0.15 (unitless)
ED_a	Exposure duration, adult resident: 24 y
ED_c	Exposure duration, child resident: 6 y
EF_r	Exposure frequency, residential: 350 d/y
IAF	Ingestion absorption factor:[b] default 1.0 (unitless)
IRA_a	Inhalation rate, adult: 20 m^3/d
IRA_c	Inhalation rate, child: 10 m^3/d
IRS_a	Soil ingestion, adult: 100 mg/d
IRS_c	Soil ingestion, child: 200 mg/d
PEF	Particulate emission factor: 1.316 $\times$ 10^9 m^3/kg
SA_a	Exposed surface area for soil/dust, adult: 5,700 cm^2/d
SA_c	Exposed surface area for soil/dust, child: 2,800 cm^2/d

[a] All exposure parameters from U.S. Environmental Protection Agency (2002), with the exception of ABS, which is from State of California Environmental Protection Agency (1999).

[b] The oral ingestion absorption factor (*IAF*), although not always explicitly identified in the risk assessment equation, is by default assumed to be 1.0. A default *IAF* of 1 implies that the oral absorption of the compound evaluated in the risk assessment is assumed to be equivalent to the oral absorption of the compound inherent in the study used to develop the toxicity value (i.e., either the cancer slope factor or the reference dose).

based criteria are actually similar because the default *IAF* (100% relative bioavailability) is 6.7 times the default *DAF* of 15% absorption, which is based on studies using live rhesus monkeys (Wester et al. 1990). The lampblack *IAF*s are lower than those measured for native soils or soils from coal-gas MGP sites. For example, *IAF*s of 37 and 57% were measured for B*a*P in native clay and sandy soils, respectively (Goon 1991). The *IAF*s for B*a*P in soils from coal-gas MGP sites (Weyand et al. 1995) were lower than the native soil values (as low as 11%) but higher than those reported in this study. The results are to be expected because the tarry residuals found at coal-gas MGP sites sorb PAHs more tightly than native soils but less tightly than the lampblack at oil-gas MGP sites (Hawthorne et al. 2002).

Risk-based cleanup levels for B*a*P (Table 3) were calculated by using the site-specific B*a*P *DAF*s and *IAF*s as derived from the in vitro tests described previously. These values were used in the standard California risk assessment calculations for determining cleanup levels for contaminated soil under residential exposure assumptions (California EPA 1999) in place of the default levels for B*a*P that are assumed in the California guidelines. California risk assessment guidelines

Table 3. Calculated risk-based cleanup levels for individual samples

	Sample cleanup level (residential)[a]	Enhancement factor (mg BaP equiv./kg dry soil)[b]
OG-2	5.1	142
OG-5	1.6	44
OG-10	3.0	83
OG-13	1.8	50
OG-14	0.84	23
OG-17	4.8	133
OG-18	0.92	26
Mean	2.6	72
Default	0.036	1

[a] Cleanup levels calculated using 10^{-6} excess cancer risk and California risk assessment guidance, with sample-specific in vitro benzo[*a*]pyrene (BaP) dermal and ingestion absorption factors (Table 1) instead of default assumptions.

[b] Enhancement factor = sample-specific cleanup level divided by the default level.

specifically incorporate "default" *DAF* and *IAF* values but do not provide guidance on how to derive site-specific values and when these site-specific values may be incorporated into risk assessments. Use of in vitro dermal uptake results directly as *DAF*s is consistent with existing guidance (USEPA 2001). Direct use of the in vitro solubilization results as *IAF*s is not yet common practice, but it is reasonable, assuming that the PAHs must first be solubilized prior to uptake.

These risk-based cleanup levels for B*a*P ranged from 0.84 to 5.1 mg/kg. Although these are not final cleanup levels and have not been either submitted to or approved by the State of California, they are the criteria that would result from using the *IAF*s and *DAF*s derived directly from the in vitro assays. The average cleanup level for all 7 samples was roughly 72 times higher than the current default cleanup level (i.e., 0.036 mg B*a*P equivalents/kg soil at a 10^{-6} excess cancer risk level).

Similar increases in risk-based criteria (ranging from 14–107 times default values) were calculated when considering only the dermal contact pathway using these in vitro dermal results to assess the risks to adults exposed to impacted soils (Stroo et al. 2004). The fact that the results from chemical release assays and both the dermal and the oral uptake in vitro assays all yield similar results provides a compelling weight-of-evidence argument that the increases in the risk-based criteria for PAHs on lampblack resulting from this research are both justified and reasonable.

The finding that the soil concentrations calculated to be protective of human health were all at least 1 order of magnitude higher than the default cleanup levels (Table 3)—and in some cases over 2 orders of magnitude higher—emphasizes the value of measuring hydrocarbon availability. Clearly, the default values considered for these risk-based calculations yield unnecessarily and unrealistically conservative values for this particular matrix and perhaps also for similar materials that tightly bind hydrophobic organics. Although this conservatism has been recognized for several years (Stroo et al. 2000), the use of chemical release and in vitro bioavailability measurements provides a quantitative method to modify the risk assessment parameters to yield more realistic but still protective cleanup levels.

The use of only the B*a*P F values and absorption factors to calculate cleanup levels for the total CPAH is justified for 3 reasons. First, the assumed carcinogenicity of B*a*P is considered to be 3 to 100 times greater than the other 6 carcinogenic PAHs, and thus the criteria for these 7 heavy (4-, 5-, and 6-ring) and nonvolatile CPAHs are typically expressed in terms of B*a*P equivalents. Second, the total concentrations of B*a*P on lampblack are greater than the concentrations of any of the other CPAHs (Hawthorne et al. 2002; Stroo et al. 2000). Finally, the F values for all of the CPAHs were similar to those measured for B*a*P (Hawthorne et al. 2002). However, situations may well exist with other materials or other regulatory environments where the use of only B*a*P may be misleading.

For each soil sample, the available B*a*P concentration was calculated by multiplying the total B*a*P concentration by the F value as determined by the SFE analyses. For each of the 6 samples for which the available B*a*P could be calculated (i.e., the samples with a measurable F value; see Table 1), the available B*a*P was closely correlated (r^2 = 0.924) to the solubilization of B*a*P during the in vitro oral uptake assay (Figure 1). In contrast, the correlation between the in vitro solubilization and total B*a*P concentration was only 0.629 (Table 4).

In fact, the correlations between the in vitro solubilization and the available concentrations were better than the correlations between solubilization and total concentrations for all 14 PAHs present at detectable levels (Table 4). Because oral ingestion is so important in the risk assessment calculations for oil-gas MGP site soils, these results suggest that the rapidly released fraction may largely determine the actual risk and that measurements of the F values can provide useful predictions of the eventual site-specific cleanup levels.

The predictive value of chemical availability assays was evaluated by comparing the calculated site-specific cleanup levels with the rapidly released fractions (F values) of the total B*a*P as determined by SFE (Figure 2). Only 6 of the samples could be used in this comparison because the F value for OG-5 could not be measured. The results demonstrate that a close correlation (r^2 = 0.96) exists between the F values and the cleanup levels, even though the dermal uptake, oral

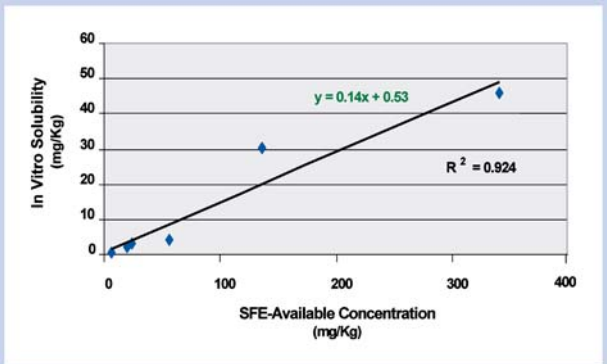


Figure 1. Relationship between the amounts of benzo[*a*]pyrene solubilized during the in vitro simulated gastrointestinal tract assay and the total amounts of "available" benzo[*a*]pyrene (total concentration multiplied by the F value).

Table 4. Correlations of in vitro uptake to total and SFE-derived available concentrations

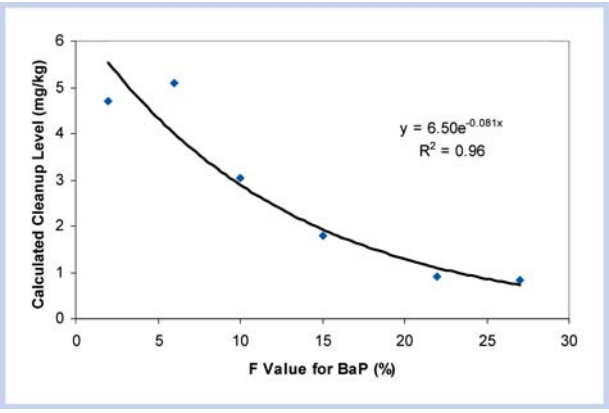
	r^2 values	
PAH[a] compound	Available	Total
Naphthalene	0.939	0.866
Acenaphthene	0.891	0.354
Fluorene	0.984	0.747
Phenanthrene	0.647	0.434
Anthracene	0.870	0.529
Fluoranthene	0.728	0.678
Pyrene	0.760	0.707
Benz[*a*]anthracene	0.786	0.435
Chrysene	0.900	0.626
Benzo[*b,k*]fluorene	0.986	0.454
Benzo[*a*]pyrene	0.924	0.629
Dibenz[*a,h*]anthracene	0.680	0.007
Benzo[*g,h,i*]perylene	0.884	0.442
Indeno[1,2,3-*cd*]pyrene	0.883	0.348

[a] PAH = polycyclic aromatic hydrocarbon.

uptake, and SFE analyses were performed by 3 separate research groups, using separate subsamples of the same homogenized initial site samples. Over the range of availabilities measured, the best-fit linear relationship (not shown) was y = 5.23 – 0.18x, which also provided a close correlation (r^2 = 0.89).

The total B*a*P concentrations in the samples had no relationship to the calculated cleanup levels (r^2 values of only 0.19 and 0.26 for the best-fit linear and exponential relationships, respectively). Similarly, the relative abundance the other CPAH compounds did not exhibit significant correlations with the calculated cleanup levels.

These results suggest that the strong sorption of PAHs to the lampblack matrix has a large effect on the ability of organisms to absorb the PAHs as well as on the availability of the PAHs for release under the relatively mild extraction conditions used in the SFE or ROR analysis. Further, the F values and the individual bioavailability results were closely correlated, suggesting that biological uptake in fact occurs primarily from the fraction of the total concentration that is available for rapid desorption.



?16 **Figure 2.** Relationship between the risk-based cleanup levels for carcinogenic polycyclic aromatic hydrocarbon (PAH), expressed as benzo[*a*]pyrene (B*a*P) equivalents and calculated using the sample-specific dermal and ingestion absorption factor and the percentage of B*a*P in the fast-release fraction (F value) for each sample as determined by supercritical fluid extraction.

Related work on these samples indicates that there are essentially 2 pools of PAHs on lampblack: PAHs bound directly to the lampblack matrix and PAHs present as a separate phase that cannot bind directly to the lampblack because the surface area available for direct binding is saturated (Hong et al. 2003). For example, the sample with the highest PAH:total organic carbon (TOC) ratio (and therefore presumably the largest fractions of "free-phase" PAHs) also had the highest F value and the lowest calculated cleanup level (i.e., OG-18). Consistent with this hypothesis, OG-2 had the lowest PAH:TOC ratio and also a low F value and the highest cleanup level.

The interrelated research done on this set of oil-gas MGP site samples represents the first such integrated study of chemical release and bioavailability on a common solid matrix. The results support the overall hypothesis of this work, which is that the risks of hydrocarbons to human health are strongly affected by the strength with which the hydrocarbons are bound to the solid matrix and that the risks can be more reasonably predicted by chemical release and bioavailability assays. The weight of evidence developed by this multi-investigator research effort indicates that the risks posed by PAHs on lampblack are far less than assumed when using default absorption factors and that a tiered evaluation protocol employing chemical assays, chemical release data (F values), and in vitro assays can be used to establish more realistic site-specific criteria.

In this case, a simple 40-min SFE test under defined conditions provided an exceptional correlation to the risk-based cleanup levels derived from much more costly and time consuming in vitro bioavailability assays. That relationship spans a range of rapidly released fractions from 2 to 27%, using a series of samples of lampblack-impacted soils from several oil-gas MGP sites in California.

These results are not necessarily limited to the human health risks of PAHs on lampblack or to the use of SFE as an analytical method. Prior work with these and other samples has shown that the rapidly released fractions measured by SFE extraction are closely correlated to those measured by the aqueous ROR assay (Hawthorne et al. 2002). Further, rates of release have been measured for a wide range of hydrocarbons in 40 different field samples, leading to a calibrated 7-d batch desorption test that accurately estimates sample-specific F values without the need for SFE equipment or expertise [9]. Finally, the F values as determined by ?5 SFE for these oil-gas MGP site samples and a similar set of samples from coal-gas MGP sites have been shown to correlate closely to the uptake of PAHs by earthworms (Kreitinger et al. 2005). These results indicate that chemical availability assays may also be useful in evaluating ecological risks.

In a practical sense, the proposed approach to developing risk-based criteria requires careful measurement of the B*a*P release and/or in vitro absorption measurements. Given the importance of B*a*P in setting criteria for oil-gas MGP site residuals, this approach is justified, but rigorous QA/QC and

use of multiple replicates and site samples will probably be needed to reduce the inherent uncertainty.

Additionally, this work has deliberately used samples containing relatively high PAH concentrations because of the relatively high detection limits for PAHs by conventional analytical methods, which are particularly problematic when attempting to measure release or uptake from materials that sorb hydrocarbon so tightly. Analytical refinements will be needed to ensure reliable measurements of the rapidly released fractions from lower-concentration soil samples that are closer to the estimated risk-based criteria. The approach used in this work is predicated on the assumption that the PAHs in the lower-concentration samples are associated with lampblack particles but that these particles are simply more dispersed throughout the soil matrix. Evidence from examinations of these samples indicates that this assumption is valid because virtually all the PAHs were found on the lampblack particles and not on the separated "soil" fraction (Hong et al. 2003).

CONCLUSIONS

This integrated study of 7 oil-gas MGP site samples included a series of chemical and biological assays designed to develop a weight-of-evidence approach for measuring contaminant bioavailability that is consistent with the guidance developed by the National Research Council Committee on Bioavailability (2003). Several investigators using different assays have all shown that the availability of PAHs on lampblack is far lower than is assumed in the standard risk assessment guidance used in California (California EPA 1999) or by the USEPA (1991). Further, a protocol based on the use of in vitro assays for both dermal and oral ingestion of B*a*P (by far the most important PAH in determining risk-based criteria for oil-gas MGP site soils) yielded calculated risk-based cleanup criteria that are 23 to 142 times higher than the default criteria (72 times higher on average).

Finally, the F values for B*a*P in these oil-gas MGP site samples has been demonstrated to be closely correlated to the in vitro results. This relationship suggests that most of the biological uptake of B*a*P occurs from the fraction that is available for rapid release. The SFE, under conditions previously developed to measure the rapidly released fraction of the total PAH concentration in a soil sample (Hawthorne et al. 2001), provided an exceptionally accurate prediction of the uptake during the in vitro tests and therefore of the calculated risk-based criteria. The SFE, or the closely correlated batch desorption method to estimate F values (Loehr et al. 2003), can therefore provide rapid and inexpensive screening-level assessments of the chemical release, bioavailability, and associated risks of PAHs in a particular oil-gas MGP site sample.

Acknowledgement—This work was funded by the Gas Technology Institute, the Southern California Gas Company, Southern California Edison, and Pacific Gas & Electric. The advice and guidance of Ron Jensen, Anita Bohrnerud, Robert Doss, Dianne Saber, and Steve DiZio are deeply appreciated. SBH also acknowledges the partial financial support of the U.S. Department of Energy under Cooperative Agreement DE-FC26-98FT40321. However, any opinions, findings, conclusions, or recommendations expressed herein are those of the authors and do not necessarily reflect the views of DOE or any of the funding organizations.

REFERENCES

Alexander MA. 2000. Aging, bioavailability, and overestimation of risk from environmental pollutants. *Environ Sci Technol* 34:4259–4265.

Berg, MS, Loehr RC, Webster MT. 1998. Release of petroleum hydrocarbons from bioremediated soils. *J Soil Contam* 22:675–695.

Bucheli TD, Gustafsson O. 2000. Quantification of the soot-water distribution coefficient of PAHs provides mechanistic basis for enhanced sorption observations. *Environ Sci Technol* 34:5144–5151.

California Environmental Protection Agency, Department of Toxic Substances Control. 1999. Preliminary endangerment assessment guidance manual: A guidance manual for evaluating hazardous substance release sites. 2nd ed. ?6

California Environmental Protection Agency. 2004. Toxicity criteria database: Cancer potency factor list. www.oehha.ca.gov/risk/ChemicalDB/index.asp. Accessed 3 June 2004.

Di Toro DM, Zarba CS, Hansen DJ, Berry WJ, Swartz RC, Cowan CE, Pavlou SP, Allan HE, Thomas NA, Paquin PR. 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environ Toxicol Chem* 10:1541–1584.

Ehlers LJ, Luthy RG. 2003. Contaminant bioavailability in soil and sediment. *Environ Sci Technol* 37:295A–302A.

Ghosh U, Gillette JS, Luthy RG, Zare RN. 2000. Microscale location, characterization and association of polycyclic aromatic hydrocarbons on harbor sediment particles. *Environ Sci Technol* 34:1729–1736.

Goon D. 1991. Oral bioavailability of aged, soil-adsorbed benzo(a)pyrene (BaP) in rats. Society of Toxicology Annual Meeting Abstracts #1356. ?7

Gustafsson O, Haghseta R, Chan C, MacFarlane J, Gschwend PM. 1997. Quantification of the dilute sedimentary soot phase: Implications for PAH speciation and bioavailability. *Environ Sci Technol* 31:203–209.

Hawthorne SB, Poppendieck DG, Grabanski CB, Loehr RC. 2001. PAH release during water desorption, supercritical carbon dioxide extraction and field bioremediation. *Environ Sci Technol* 35:4577–4583.

Hawthorne SB, Poppendieck DG, Grabanski CB, Loehr RC. 2002. Comparing PAH availability from manufactured gas plant soils and sediments with chemical and biological tests. Part 1—PAH release during water desorption and supercritical carbon dioxide extraction. *Environ Sci Technol* 36:4795–4803.

Hawthorne SB, Miller DJ. 2003. Evidence for very tight sequestration of BTEX compounds in manufactured gas plant soils based on supercritical fluid extraction and soil/water partitioning. *Environ Sci Technol* 37:3587–3594.

Holman HN, Goth-Goldstein R, Aston D, Yun M, Kengsoontra J. 2002. Evaluation of gastrointestinal solubilization of petroleum hydrocarbon residues in soil using an in vitro physiologically based model. *Environ Sci Technol* 36:1281–1286.

Hong L, Ghosh U, Mahajan T, Zare RN, Luthy RG. 2003. PAH sorption mechanism and partitioning behavior in lampblack-impacted soils from former oil-gas plant sites. *Environ Sci Technol* 37:3625–3634.

Jonker MTO, Koelmans AA. 2002. Sorption of polycyclic aromatic hydrocarbons and polychlorinated biphenyls to soot and soot-like materials in the aqueous environment: Mechanistic considerations. *Environ Sci Technol* 36:3725–3734.

Kreitinger JP, Quinones-Rivera A, Neuhauser EF, Alexander M, Hawthorne SB. 2005. Evidence for greatly reduced toxicity and bioavailability of PAHs in soils from manufactured gas plant sites. *Environ Toxicol Chem* (in press). ?8

Landrum PF, Lee H, Lydy MJ. 1992. Toxicokinetics in aquatic systems: Model comparisons and use in hazard assessment. *Environ Toxicol Chem* 11:1709–1725.

Loehr RC, Webster MT. 2000. Decreased release of PAHs from soils as a result of field bioremediation. *Practice Periodical of Hazardous, Toxic and Radioactive Waste Management* 4:118–125.

Loehr RC, Lamar MR, Poppendieck DG. 2003. A protocol to estimate the release of anthropogenic hydrocarbons from contaminated soils. *Environ Toxicol Chem* 22:2202–2208.

National Research Council. 2003. Bioavailability of contaminants in soils and sediments: Processes tools and applications. Washington (DC): National Academy Press. ?9

RETEC. 2004. Final report: Environmentally acceptable endpoints for lampblack and lampblack-impacted soils. Chicago (IL), USA: Gas Technology Institute.

Roy TA, Krueger AJ, Taylor BB, Mauro DM, Goldstein LS. 1998. Studies estimating the dermal bioavailability of polynuclear aromatic hydrocarbons from manufactured gas plant tar-contaminated soils. *Environ Sci Technol* 32:3113–3117.

Roy TA, Singh R. 2001. Effect of soil loading and soil sequestration on dermal bioavailability of polynuclear aromatic hydrocarbons. *Bull Environ Contam Toxicol* 67:324–331.

Stroo HF, Roy TA, Liban CB, Kreitinger JP. 2004. Dermal bioavailability of PAHs on ?10 lampblack: Implications for risk assessment. *Environ Toxicol Chem* (in press).

Stroo HF, Jensen RD, Nakles DV, Fairbrother A, Loehr RC, Liban CB. 2000. Environmentally acceptable endpoints for PAHs at a manufactured gas plant site. *Environ Sci Technol* 34:2831–3836.

[USEPA] U.S. Environmental Protection Agency. 1991. Human health evaluation manual, supplemental guidance: Standard default exposure factors, March 25, 1991. OSWER Directive 9285.6-03. Washington (DC): Office of Solid Waste and Emergency Response.

[USEPA] U.S. Environmental Protection Agency. 2001. Risk assessment guidance for Superfund: Human health evaluation manual (Part E, Supplemental guidance for dermal risk assessment) interim, EPA 540/R/99/005. OSWER Directive 9285.7-02EP. Washington (DC): Office of Solid Waste and Emergency Response.

[USEPA] U.S. Environmental Protection Agency. 2002. Region 9 preliminary remediation goals (PRGs): User guide/technical background document, table 2002 update, October 1, 2002. www.epa.gov/region09/waste/sfund/prg/index.htm. Washington (DC). ?11

Wester RC, Maibach HI, Bucks DAW, Sedik L, Melendres J, Lioa C, Dizio S. 1990. Percutaneous absorption of ^{14}C DDT and ^{14}C benzo[a]pyrene from soil. *Fundam Appl Toxicol* 15:510–516.

Weyand EH, Chen Y-C, Wu Y, Koganti A, Dunsford HA, Rodriguez LV. 1995. Differences in the tumorigenic activity of a pure hydrocarbon and a complex mix following ingestion: B(a)P vs. manufactured gas plant residue. *Chem Res Toxicol* 8:949–954.

APPENDIX F

SUPERCRITICAL CARBON DIOXIDE EXTRACTION AS A PREDICTOR OF POLYCYCLIC AROMATIC HYDROCARBON BIOACCUMULATION AND TOXICITY BY EARTHWORMS IN MANUFACTURED-GAS PLANT SITE SOILS

PUBLISHED IN *ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY*

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 26, No. 9, pp. 1809–1817, 2007
© 2007 SETAC
Printed in the USA
0730-7268/07 $12.00 + .00

Environmental Toxicology

SUPERCRITICAL CARBON DIOXIDE EXTRACTION AS A PREDICTOR OF POLYCYCLIC AROMATIC HYDROCARBON BIOACCUMULATION AND TOXICITY BY EARTHWORMS IN MANUFACTURED-GAS PLANT SITE SOILS

JOSEPH P. KREITINGER,*† ANTONIO QUIÑONES-RIVERA,† EDWARD F. NEUHAUSER,‡ MARTIN ALEXANDER,† and STEVEN B. HAWTHORNE§

†Institute for Comparative and Environmental Toxicology and Department of Soil, Crop and Atmospheric Sciences, Cornell University, Ithaca, New York 14850, USA

‡National Grid, 300 Erie Boulevard West, Syracuse, New York 13202, USA

§Energy and Environmental Research Center, University of North Dakota, Box 9018, Grand Forks, North Dakota 58202, USA

(*Received* 4 *December* 2006; *Accepted* 16 *February* 2007)

Abstract—The toxicity and uptake of polycyclic aromatic hydrocarbons (PAHs) by earthworms were measured in soil samples collected from manufactured-gas plant sites having a wide range in PAH concentrations (170–42,000 mg/kg) and soil characteristics. Samples varied from vegetated soils to pure lampblack soot and had total organic carbon contents ranging from 3 to 87%. The biota–soil accumulation factors (BSAFs) observed for individual PAHs in field-collected earthworms (*Aporrectodea caliginosa*) were up to 50-fold lower than the BSAFs predicted using equilibrium-partitioning theory. Acute toxicity to the earthworm *Eisenia fetida* was unrelated to total PAH concentration: Mortality was not observed in some soils having high concentrations of total PAHs (>42,000 mg/kg), whereas 100% mortality was observed in other soils having much lower concentrations of total PAHs (1,520 mg/kg). Instead, toxicity appeared to be related to the rapidly released fraction of PAHs determined by mild supercritical CO_2 extraction (SFE). The results demonstrate that soils having approximately 16,000 mg rapidly released total PAH/kg organic carbon can be acutely toxic to earthworms and that the concentration of PAHs in soil that is rapidly released by SFE can estimate toxicity to soil invertebrates.

Keywords—Polycyclic aromatic hydrocarbon Manufactured-gas plant Biota–soil accumulation factor *Eisenia fetida* *Aporrectodea caliginosa*

INTRODUCTION

When bioaccumulation and toxicity test results are compared from experiments performed with different sediments or soils, essentially no relationship is observed between the total concentration of hydrophobic organic compounds and biological effects [1,2]. A correlation between chemical concentration in soils or sediments and biological effects becomes apparent, however, when the concentration of chemical that is biologically available is estimated using equilibrium-partitioning theory and the assumption that it is sorbed to natural organic carbon [1]. The equilibrium-partitioning theory was developed for predicting bioaccumulation by organisms living in aquatic environments and was subsequently extended to predict bioaccumulation by terrestrial invertebrates [3–6]. Often, however, it is observed that the predicted bioaccumulation of hydrophobic chemicals by aquatic and terrestrial invertebrates exceeds the measured value by an order of magnitude or more [7–11]. A number of biotic and abiotic mechanisms, including strong sorption to anthropogenic carbon phases, may be responsible for the apparently reduced bioavailability of polycyclic aromatic hydrocarbons (PAHs) in soils and sediments [7,12–14].

Although the toxicity and uptake of nonpolar organic compounds from soils by invertebrates often is unrelated to the total concentration in soil, they have been directly correlated to the concentration of chemical in soil pore water [3,6,15]. Soils collected in urban and industrial environments often have anthropogenic sources of hard or black carbon (e.g., charcoal, coal, coal tar pitch, coke, and soot) that strongly sorb and reduce the concentration of nonpolar organic compounds in pore water [12,16]. The aqueous partitioning of PAHs from anthropogenic carbon often is 1,000-fold less than the partitioning observed for natural organic matter [13,14,17]. Particle-scale analyses of aquatic sediments collected from several urban waterways shows that most of the PAHs are associated with natural and anthropogenic organic particles [18,19]. Although anthropogenic organic particles generally account for a very small fraction of the sediment, they appear to control the release and bioavailability of hydrophobic compounds, such as PAHs [18]. The presence of black carbon in soils and sediments has been used to explain a lack of observed chemical uptake by aquatic and soil organisms [8–10,16,20].

The large variation in the bioavailability and toxicity of PAHs observed in different soils and sediments has resulted in a search for a chemical method that can be used to estimate the bioavailability of PAHs. For example, Krauss and Wilcke [17] used a 15-d extraction procedure with silica disks to estimate the concentration of bioavailable PAHs and the biota–soil accumulation factors (BSAFs) for *Lumbricus terrestris* in field-contaminated urban soils. Van der Wal et al. [21] demonstrated that a 20-d extraction with polydimethylsiloxane solid-phase microextraction fibers could be used to measure the concentration of nonpolar chlorinated hydrocarbons freely dissolved in soil pore water and to predict uptake of the chemicals by the earthworms *Eisenia andrei* and *Aporrectodea caligi-*

* To whom correspondence may be addressed (jkreitinger@retec.com).

nosa. Kraaij et al. [22] demonstrated that much of the variability in BSAFs of field-contaminated and spiked PAHs observed for an aquatic deposit-feeding amphipod (*Corophium volutator*) could be explained by the rapidly desorbing fraction measured using Tenax TA resin in 25-d extraction tests.

Mild supercritical-fluid extraction (SFE) using pure CO_2 also has been suggested as a chemical method for predicting the bioavailability of PAHs in soils. Mild SFE is an attractive method, because the solubility of individual PAHs in CO_2 at 200 bar and 50°C is proportional to their solubility in water and the desorption profiles of PAHs into water and CO_2 are highly correlated [23,24]. In addition, the polarity of CO_2 at mild supercritical temperatures and pressures (200 bar and 50°C) is similar to that of biological lipids, and the extraction procedure has little effect on the organic carbon matrix of soils [24,25]. In earlier studies, 16 soils having a wide range of PAH concentrations and soil carbon contents were collected from historic manufactured-gas plant (MGP) sites, and the rapidly released fractions (*F* values) of PAHs were determined using SFE and solid-phase aqueous desorption with XAD-2 resin [24,26]. In the present study, the bioavailability of PAHs to earthworms was evaluated and compared to the rapidly released fractions previously determined by SFE [23,25]. Initial tests showed a wide range in toxicity that was unrelated to the concentration of PAHs in soil. Therefore, we evaluated whether chemical availability as measured by mild SFE is related to the acute toxicity and accumulation of PAHs by earthworms.

MATERIALS AND METHODS

Soil samples

Sixteen soil samples were collected from coal gas (CG) and oil gas (OG) MGP sites that had been closed for approximately 50 years. All the samples have been described in earlier reports [24,26].

Soil PAH and SFE rapidly released fractions

Methods to determine the total PAH concentration in soil and the rapidly released fraction of PAHs have been previously described [26]. Briefly, the rapidly released fraction was estimated from the concentration of PAHs extracted using 40 min of mild SFE (200 bar and 50°C) compared to their total concentrations in soil [24,26]. The rapidly released fraction of total PAHs is the sum of the individual molar PAH concentrations measured by extraction using SFE divided by the sum of the individual molar PAH concentrations measured using Soxhlet extraction.

Soil spiking

To evaluate the bioavailability of individual PAHs, a spiking solution that contained two- to five-ring, uniformly labeled perdeuterated PAHs (d-PAHs) was added to four MGP site soils (OG2, CG12, CG15, and CG17), and the BSAF for the spiked and field-contaminated PAHs were compared. All d-PAH isotopic purities were greater than 98% as determined by the manufacturer. The d-PAHs were added at 3 to 65% of the concentration of the field-contaminated PAH, and BSAFs were calculated based on the concentration of PAHs determined in soils and earthworms at the end of the 14-d bioassay. A 200-μl portion of solution containing d-PAHs in methylene chloride was added to 20 g of air-dried soil contained in 60-ml glass jars. The solution of d-PAHs was thoroughly mixed with the soil, and the soil moisture was adjusted to 80% of water-holding capacity (0.33 bar). Each jar was fitted with a polyurethane foam (PUF) plug and allowed to stand at room temperature for 24 h before addition of the earthworms.

Analytical internal standards

Perdeuterated PAHs also were used as analytical internal standards to aid in the analysis of all soil, earthworm, SFE, and PUF foam extracts. The d-PAH internal standards for extracts generated from the spiked soil samples (discussed above) included 1-methylnaphthalene-d_{10}, acenaphthene-d_{10}, phenanthrene-d_{10}, fluoranthene-d_{10}, chrysene-d_{12}, perylene-d_{12}, and benzo[*ghi*]perylene-d_{12}. Note that none of these d-PAHs were in the soil spiking d-PAH mixture, thus allowing their use as analytical internal standards for both the native and the d-PAHs used to spike the soils. For samples that did not include the d-PAHs as spiked compounds, the d-PAH analytical internal standards were naphthalene-d_8, fluorene-d_{10}, anthracene-d_{10}, pyrene-d_{10}, benz[*a*]anthracene-d_{12}, and benzo[*a*]pyrene-d_{12}.

Determination of PAH losses to the headspace

Loss of PAHs by vaporization from the bioassay jars was determined according to the method described by Hawthorne and Grabanski [27]. In brief, each jar containing soil and earthworms was plugged with a PUF sorbent plug (diameter, 4 cm; length, 5 cm) previously prepared by sonication in acetone. The PUF plug sealed the airspace in the bioassay jar so that vaporized PAHs could not exit from the flask without passing through the sorbent plug. The PAHs collected on the PUF sorbent plugs were extracted with 50 ml of acetone aided by sonication for 8 h after adding the d-PAH internal standards discussed above.

Earthworm cultures

Earthworms (*E. fetida*) were purchased from Connecticut Worm Farm (Enfield, CT, USA). Earthworms of the same age were prepared for bioassays by placing 150 adult earthworms in a 5.5-L, polyethylene box containing 2.5 L of moistened commercial worm bedding (Magic Worm Products, Amherst Junction, WI, USA). Earthworms were cultured at 21 ± 2°C and fed ad libitum using commercial earthworm food (Magic Worm Products). Adult earthworms were allowed to produce cocoons for two to three weeks. The adults were then removed for testing. Juvenile earthworms would emerge from cocoons after two to three weeks. To reduce the density of earthworms, the juvenile earthworms and bedding were thinned after four weeks of growth. The earthworms were then allowed to mature, and after 12 weeks, 150 adult earthworms were removed to start a new colony.

Earthworm bioassays

Earthworm survival and bioaccumulation was evaluated in laboratory tests conducted using *E. fetida* as described by Tang et al. [28]. Five adult earthworms were placed in approximately 20 g (dry wt) of soil contained in 60-ml, wide-mouth jars (Qorpak, Bridgeville, PA, USA). Adult earthworms averaged 0.5 g wet weight per individual, and the initial wet weight of five earthworms used in bioassays ranged from 1.71 to 3.84 g wet weight. The average lipid content following exposure to soils was 1.8% wet weight (range, 1.2–3.5%). Earthworms were not fed during tests and lost weight during the 14-d bioassays. Control earthworms exposed to an uncontaminated agricultural soil (Lima Loam, 1.6% total organic carbon) were

observed to lose, on average, 12.5% (range, 8.3–15.6%) of their wet weight during 14-d tests. Soils were moistened with deionized water to 80% of water-holding capacity 24 h before conducting a test. The water content of the soils was maintained by periodically adding deionized water. Earthworms were assigned randomly to each treatment and jar. Tests were conducted in triplicate or quadruplicate for 7, 14, or 28 d. Bioassays were conducted at 20 ± 1°C with constant overhead illumination. At the end of the exposure period, the earthworms were removed from the soil, rinsed twice with deionized water, and placed in a 9-cm, glass Petri dish containing a wetted Whatman no. 2 filter paper (Whatman, Florham Park, NJ, USA). The earthworms were then allowed to depurate for 24 h and rinsed twice with deionized water, blotted dry on a moist paper towel, placed into a 20-ml glass vial with a Teflon®-lined cap, and weighed. The vial was then tightly sealed and frozen (−40°C) until the earthworm tissue was prepared for extraction. Two species of earthworms, *A. caliginosa* and *Lumbricus rubellus*, were observed in soils CG12, CG15, and CG17 during sample collection. Triplicate samples of the most commonly observed earthworm, *A. caliginosa*, were collected from each soil, prepared for extraction, and analyzed for PAH residues as described below.

Determination of PAHs in earthworm tissue

The concentration of PAHs in earthworm tissue was determined in triplicate or quadruplicate following Soxhlet extraction (100 ml of *n*-hexane for 4 h) of 2 to 3 g (wet wt) samples. Frozen tissue was ground with 20 g of anhydrous Na_2SO_4 in a mortar and pestle until the sample appeared to be dry and free-flowing. Perdeuterated PAHs (listed above) were then added to the sample before Soxhlet extraction with hexane. Concentrated extracts of earthworm tissue were divided for analysis of PAHs and gravimetric determination of lipids. Extracts for determination of PAHs were passed through silica gel to remove cholesterol and other interfering compounds before chromatographic analysis using a modification of U.S. Environmental Protection Agency method 3036C [29]. The tissue extraction and cleanup procedure provided 83 to 112% recovery for individual PAHs (molecular wt, 128–276 g/mole) with a relative standard deviation of less than 5%.

Gas chromatographic separations were performed using split or splitless injection into a Hewlett-Packard model 5973 gas chromatography/mass spectrometry (GC/MS; Agilent Technologies, Santa Clara, CA, USA) equipped with a DB-5 capillary column (length, 60 m; inner diameter, 25 μm; film thickness, 250 μm; J&W Scientific, Rancho Cordova, CA, USA). The oven temperature (80°C) at injection was held for 10 min, followed by an increase of 6°C/min to 320°C. Polycyclic aromatic hydrocarbon identities were verified using GC/MS, and PAH concentrations were determined by comparison to the perdeuterated internal standards. Statistical comparisons of data were performed using analysis of variance with comparison of treatment means performed using Tukey's method of multiple comparisons.

Predicting PAH uptake using mild SFE

The concentration of PAHs in field-collected earthworms was predicted using equilibrium-partitioning theory, the organic carbon–normalized PAH concentration in soil, and the rapidly released PAH fraction (*F*) as determined by SFE. The model used for predicting earthworm PAH concentrations is described by the following equation:

$$\text{Worm lipid PAH concn.} = \frac{\text{Worm PAH concn.}}{\text{Wt \% lipid}} = \frac{\text{Soil PAH concn.}}{\text{Wt \% total soil carbon}} \cdot K_{OW}^{-0.038} \cdot F \quad (1)$$

Except for the final factor, *F*, Equation 1 is the standard equilibrium-partitioning model with the default lipid–organic carbon partitioning coefficient proposed by Di Toro and McGrath (i.e., the octanol–water partitioning coefficient raised to the −0.038 power) [30]. The factor of the rapidly released fraction (*F*) proportionally adjusts the model for the rapidly released PAH fraction measured by SFE. Prediction of PAH uptake by earthworms using *F* assumes that the bioavailable fraction of PAHs in soil is the same as the rapidly released fraction as measured by SFE.

RESULTS

Toxicity of PAHs in MGP soils

The 16 soils had a wide range in total parent PAH concentrations (168–42,100 mg/kg), total carbon concentration (2.6–87%), and molar carbon to hydrogen ratio (0.6–6.3) (Table 1). Several soils (e.g., OG10 and OG2) were primarily soot, as evidenced by the high total carbon content and high molar carbon to hydrogen ratio. Despite the wide range in the concentrations of total PAHs in the soils, the acute toxicity to *E. fetida* in 14-d assays was not related to the total PAH concentration expressed as dry weight of soil or carbon content (Table 1). For example, no earthworms survived in soil CG1, with a total PAH concentration of 1,520 mg/kg, whereas all the earthworms exposed to soil OG10 (total PAH concentration, 42,100 mg/kg) survived. Several highly contaminated soils (OG10, OG17, CG11, OG2, and CG12) were not acutely toxic despite the concentration of PAHs ranging from 1 to 5% of the total carbon in the soil.

BSAFs in field-collected earthworms

The BSAF is the PAH concentration in biota lipids divided by the PAH concentration in soil organic carbon. The BSAFs for individual PAH compounds were determined for the native earthworm species (*A. caliginosa*) collected from soils CG12, CG15, and CG17. The PAH BSAFs observed in earthworms collected from CG12 were nearly fivefold lower (range of BSAFs, 0.01–0.14) than the BSAFs observed in earthworms collected from soil CG15 and CG17 (range of BSAFs, 0.04–0.49 and 0.04–0.59, respectively). It is interesting to note that lower BSAFs were observed in soil CG12, which had individual soil PAH concentrations three- to sevenfold higher (total PAH concentration, 3,790 mg/kg) than the individual PAH concentrations observed in soils CG15 and CG17 (total PAH concentrations, 1,020 and 577 mg/kg, respectively) (Fig. 1 and Table S1 [http://dx.doi.org/10.1897/06-608.S1]). Surprisingly, the BSAFs were approximately 4- to 50-fold lower than predicted based on the assumed partitioning between natural organic carbon and biota lipids [30]. Given the lack of toxicity observed in soils having high PAH concentrations and the low BSAFs observed in field-collected earthworms, tests were conducted to evaluate the PAH bioaccumulation by *E. fetida* under laboratory conditions using several of the soils.

Comparison of PAH uptake by E. fetida *and* A. caliginosa

To evaluate the utility of laboratory bioassays using *E. fetida* to predict PAH bioavailability to earthworms in the field,

Table 1. Soil properties, polycyclic aromatic hydrocarbon (PAH) concentrations and worm survival after 14-d bioassays

Soil	Total PAH concn.[a] (μg/g soil)	Total carbon (%)	Molar C:H ratio[b]	Total PAH concn., organic C normalized (μg/g C)	SFE rapidly released fraction[c]	Worm survival (%)
OG10	42,100	86.6	5.0	48,600	0.37	100
OG18	17,300	25.5	2.5	67,900	0.80	0
OG17	17,200	47.3	5.7	36,400	0.32	100
CG11	15,600	29.3	2.3	53,200	0.07	100
OG2	6,760	59.4	6.3	11,400	0.22	100
CG3	4,100	7.5	0.9	54,700	0.87	0
CG12	3,790	7.9	1.1	48,000	0.18	100[d]
OG5	1,870	6.9	2.4	27,100	0.43	100
OG13	1,700	6.5	1.4	26,200	0.27	100
CG1	1,520	4.6	1.0	33,000	0.81	0
CG15	1,020	24.1	3.5	4,200	0.25	100[d]
CG17	577	12.5	1.8	4,620	0.32	100[d]
CG18	554	4.6	1.0	12,000	0.56	100
CG10	521	3.7	1.6	14,100	0.25	25
CG2	307	2.6	0.6	11,800	0.59	100
OG14	168	2.9	1.2	5,790	0.49	100

[a] Sum of 16 compounds (naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*+*k*]fluoranthene, benzo[*e*]pyrene, benzo[*a*]pyrene, perylene, indeno[1,2,3-*cd*]pyrene, dibenz[*a,h*]anthracene, and benzo[*ghi*]perylene).
[b] Molar carbon to hydrogen ratio.
[c] Rapidly released fraction of total PAHs determined by mild supercritical CO_2 extraction (SFE).
[d] *Aporrectodea caliginosa* and *Lumbricus rubellus* observed in soil at the time of sample collection.

PAH residues in *E. fetida* following a 14-d laboratory test were compared to PAH residues in field-collected *A. caliginosa*. Initial uptake tests using *E. fetida* confirmed previous reports that PAH uptake was near equilibrium following a 14-d exposure to soil [8,21] (Figs. S1 and S2 [http://dx.doi.org/10.1897/06-608.S2]). *Eisenia fetida* exposed to soils in the laboratory bioassays accumulated, on average, 0.06% (range, <0.01–0.17%) of the Soxhlet-extractable PAH mass present in 20 g of soil, which represented 0.6% (range, 0.017–5.6%) of the rapidly released mass of PAHs determined by SFE. Although somewhat lower, the lipid-normalized total PAH concentrations measured in *E. fetida* after exposure for 14 d in the laboratory (CG12, 1,390 ± 364 mg/kg; CG15, 335 ± 109 mg/kg; CG17, 419 ± 176 mg/kg) were not significantly different ($p < 0.05$) than the lipid-normalized total PAH concentrations measured in *A. caliginosa* collected in the field (CG12, 2,556 ± 848 mg/kg lipid; CG15, 901 ± 439 mg/kg lipid; CG17, 1,042 ± 408 mg/kg lipid) (Fig. 2). The concentration of total PAHs in laboratory-exposed earthworms were 37 to 54% of the concentration measured in field-collected earthworms despite differences in earthworm species, ecological niche and feeding behavior, and inherent spatial variability in the concentration of PAHs in the field.

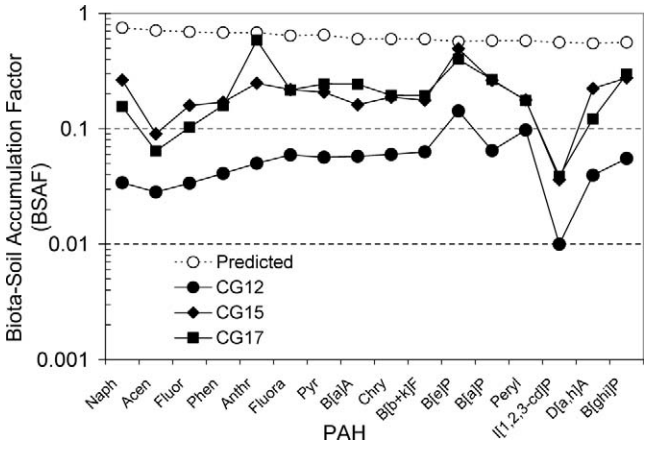


Fig. 1. Biota–soil accumulation factors measured in field-collected *Aporrectodea caliginosa*. Naph = naphthalene; Acen = acenaphthene; Fluor = fluorene; Phen = phenanthrene; Anthr = anthracene; Fluora = fluoranthene; Pyr = pyrene; B*a*A = benzo[*a*]anthracene; Chry = chrysene; B*b*+*k*F = benzo[*b*+*k*]fluoranthene; B*e*P = benzo[*e*]pyrene; B*a*P = benzo[*a*]pyrene; Peryl = perylene; I1,2,3-*cd*P = indeno[1,2,3-*cd*]pyrene; D*ah*A = dibenz[*a,h*]anthracene; B*ghi*P = benzo[*ghi*]perylene; ○ = predicted using equilibrium-partitioning model with model estimates of organic carbon–lipid partitioning coefficients based on natural organic carbon [40].

Bioavailability of spiked and field-contaminated PAHs

The bioavailability of freshly spiked d-PAHs and field-contaminated PAHs were evaluated in soils OG2, CG12, CG15, and CG17. Deuterated PAHs were not added to the more highly contaminated nontoxic samples, because the addition of the d-PAHs at such high concentrations resulted in toxicity. A comparison of the uptake of PAHs by earthworms exposed to unamended control soils and amended soils demonstrated that the addition of the d-PAHs had no significant effect on the bioavailability of the field-contaminated PAHs. The field-contaminated and spiked d-PAHs were not detected (<50 ng/plug) in PUF plugs, demonstrating that significant concentrations of d-PAHs did not volatilize from the soil.

The BSAFs for added d-PAHs were significantly higher than those observed for field-contaminated PAHs (Table 2). Although all the added d-PAHs had higher BSAFs than field-contaminated PAHs, the uptake of the d-PAHs compared to the field-contaminated PAHs increased dramatically as the molecular weight increased. The uptake of the lowest-molecular-weight PAH added to soil, naphthalene-d_8, was 1.8- to 5.7-fold greater than that of the corresponding field-contaminated naphthalene (Table S2 [http://dx.doi.org/10.1897/06-608.S1]). In contrast, the BSAF for added benzo[*a*]pyrene-d_{12} was 12- to 50-fold greater than the corresponding field-contaminated benzo[*a*]pyrene.

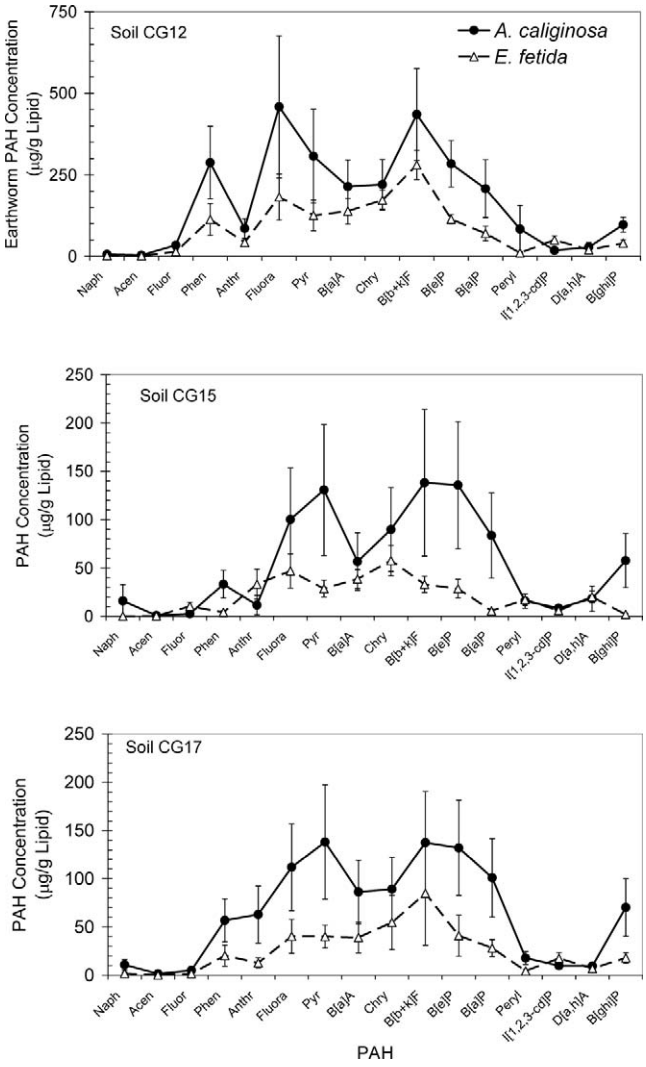


Fig. 2. Earthworm polycyclic aromatic hydrocarbon (PAH) concentrations in field-collected *Aporrectodea caliginosa* and laboratory-tested *Eisenia fetida*. Error bars show one standard deviation for analysis of three separate extracts of field-collected *A. caliginosa* (approximately 2 worms/extract) or four separate extracts of *E. fetida* (approximately five worms/extract) following laboratory 14-d bioassays. See Figure 1 for definitions of the PAH abbreviations.

Predicting PAH uptake using mild SFE

The equilibrium-partitioning model with no adjustment for the SFE rapidly released fraction overpredicted the concentration of total PAHs in the tissue of field-collected earthworms by 2.7- to 11-fold (Fig. 3). However, adjustment of the equilibrium-partitioning model for the SFE rapidly released fraction (Eqn. 1) provided a much better prediction of PAH concentrations in earthworm lipids. The predicted concentration of total PAHs using the rapidly released fraction of PAHs was 0.8- to 2.1-fold the concentration of PAHs observed in field-collected *A. caliginosa*.

Predicting toxicity using mild SFE

Although no relationship between earthworm toxicity and the concentration of total PAHs using exhaustive extraction (Soxhlet) is evident, as shown in Table 1, the concentration of the rapidly released total PAHs determined by mild SFE expressed per gram of organic carbon in soil was related to acute toxicity (Fig. 4). Except for soil sample CG10, earthworm toxicity was only observed in soils having more than 16,200 mg rapidly released total PAHs/kg organic carbon.

DISCUSSION

The low BSAFs for PAHs in field-collected earthworms and the low uptake of PAHs by earthworms in laboratory tests provide strong evidence for greatly reduced toxicity and bioavailability of PAHs in soils from MGP sites compared to the toxicity and bioavailability of PAHs observed in laboratory tests using uncontaminated soils spiked with PAHs. Soils at MGP sites commonly include the industrial residuals from the gas manufacturing process. In addition to coal ash and cinders, soils at MGP sites often have significant quantities of fine particles of coal, coke, lampblack, soot, pitch, and tar [31]. The soils used in the present study possessed a wide range in their content of anthropogenic carbon as evidenced by the range in total carbon content and molar carbon to hydrogen ratio. The molar carbon to hydrogen ratio of natural organic matter ranges from 0.6 to 0.8 [24]. In contrast, seven soils collected from MGP sites in the present study had molar carbon to hydrogen ratios greater than 2.0, with some soils having a molar carbon to hydrogen ratios exceeding 5.0. These data demonstrate that anthropogenic carbon is the predominant form of organic carbon in some MGP site soils. The low bioavailability and toxicity of PAHs in some of the MGP site soils is likely the result of the stronger sorption of PAHs to anthropogenic carbon and the extensive weathering that has occurred during the past 50 to 100 years [12,26]. The low levels of PAH bioaccumulation in field-collected earthworms and the low uptake of PAHs in laboratory bioassays indicate that the weathering of the hydrocarbons has apparently removed the more readily available molecules, resulting in an enrichment of tightly bound PAHs that are not readily available to soil invertebrates.

Reduced toxicity

Previous studies investigating the toxicity of PAHs to soil invertebrates have identified soil PAH concentrations that are expected to result in acute toxicities that are significantly lower than the PAH concentrations observed in our soils that had no effect on *E. fetida* survival. For example, soil pyrene concentrations exceeding 218 mg/kg are expected to result in 50% mortality [32]. Quite unexpectedly, however, mortality was not observed in MGP soils having up to 7,980 mg/kg of pyrene and more than 42,000 mg/kg of total PAHs. Seven of 10 soils tested having pyrene concentrations exceeding 218 mg/kg were observed to have 100% survival. Previous tests measuring water desorption and mild SFE extraction showed that these MGP site soils have a wide range in the PAH fraction that is rapidly released by mild SFE extraction and that this rapidly released fraction is highly correlated with aqueous desorption [24,26]. Earthworm toxicity has been previously shown to be predicted by the aqueous concentration of hydrophobic chemicals [7]. The toxicity of soil CG10 appears to be unrelated to total concentration or the rapidly released concentration of PAHs as a number of soils tested with higher PAH concentrations were not toxic.

Field BSAFs

The BSAFs for earthworms collected in the field provide additional evidence for the reduced bioavailability of PAHs. The BSAFs are similar to the values previously observed in earthworms (*E. andrei* and *L. terrestris*) exposed to field-

Table 2. Biota-soil accumulations factors (BSAFs) for freshly spiked and field-aged polycyclic aromatic hydrocarbons (PAHs)

Soil	PAH	Biota–soil accumulation factor[a]		Ratio added to field BSAF
		Field	d-PAH	
OG2	Naphthalene	0.013 ± 0.012	0.068 ± 0.025	3.0
	Fluorene	0.023 ± 0.014	0.142 ± 0.042	2.8[b]
	Anthracene	0.020 ± 0.004	0.39 ± 0.12	15[b]
	Pyrene	0.091 ± 0.005	1.75 ± 0.54	16[b]
	Benz[*a*]anthracene	0.102 ± 0.009	2.48 ± 0.61	21[b]
	Benzo[*a*]pyrene	0.090 ± 0.005	1.44 ± 0.36	14[b]
CG15	Naphthalene	0.014 ± 0.008	0.049 ± 0.033	2.5[b]
	Fluorene	0.015 ± 0.011	0.076 ± 0.030	4.2[b]
	Anthracene	0.023 ± 0.009	0.54 ± 0.12	22[b]
	Pyrene	0.091 ± 0.032	2.07 ± 0.22	22[b]
	Benz[*a*]anthracene	0.140 ± 0.047	3.07 ± 0.26	21[b]
	Benzo[*a*]pyrene	0.130 ± 0.052	1.64 ± 0.34	12[b]
CG12	Naphthalene	0.004 ± 0.000	0.025 ± 0.003	5.7[b]
	Fluorene	0.013 ± 0.002	0.273 ± 0.093	26[b]
	Anthracene	0.023 ± 0.002	1.21 ± 0.20	66[b]
	Pyrene	0.051 ± 0.017	1.93 ± 0.22	49[b]
	Benz[*a*]anthracene	0.054 ± 0.012	1.16 ± 1.33	41
	Benzo[*a*]pyrene	0.037 ± 0.007	1.44 ± 0.06	49[b]
CG17	Naphthalene	0.019 ± 0.008	0.050 ± 0.025	1.8[b]
	Fluorene	0.016 ± 0.005	0.138 ± 0.040	7.6[b]
	Anthracene	0.060 ± 0.011	0.766 ± 0.151	12[b]
	Pyrene	0.129 ± 0.046	3.44 ± 0.56	25[b]
	Benz[*a*]anthracene	0.227 ± 0.093	5.14 ± 0.82	21[b]
	Benzo[*a*]pyrene	0.196 ± 0.083	3.02 ± 0.57	14[b]

[a] Values are presented as the mean ± standard deviation. d-PAH = perdeuterated PAHs.
[b] Uptake of added d-PAH was significantly greater than that of field-aged PAH ($p > 0.95$).

contaminated soils in laboratory tests [8,33] and the BSAFs in field-collected earthworms (*L. rubellus*) from other industrial sites [34]. The field-collected earthworms in the present study, however, were observed in soils having PAH concentrations two to three orders of magnitude higher than those previously reported. In the most highly contaminated field soil (CG12) from which earthworms were collected, the molar concentration of total PAHs in earthworm tissue was 11-fold lower then expected based on the assumed partitioning between natural organic carbon and biota. The molar concentration of total PAHs in earthworms collected from soils CG15 and CG17, however, was only 2.7- and 2.9-fold lower than expected based on the assumed partitioning between natural organic carbon and biota.

PAH uptake in laboratory bioassays

Laboratory bioassays are necessary for the measurement of PAH bioavailability in soils that do not have native earthworms. The concentration of PAHs in *E. fetida* following 14-d uptake tests provides a good estimate of the PAH concentration measured in field-collected *A. caliginosa*, validating the use of laboratory bioassays for estimating the bioavailability of PAHs in the field. The uptake of PAHs by *E. fetida* was 25 to 42% of the concentration of PAHs in field-collected *A. caliginosa*. The uptake of nonpolar organic compounds by the two earthworm species *A. caliginosa* and *E. andrei* have been shown to be similar in laboratory bioassays as well as the uptake by *A. caliginosa* in laboratory assays with residues measured in field-collected earthworms (mainly *L. castaneus*) [11,35]. A good correlation also has been previously reported in the uptake of PAHs by *E. andrei* and *L. rubellus* in laboratory bioassays using field-contaminated soils [11].

Laboratory bioassays in the present study demonstrated that the PAHs in field-contaminated soils were 5- to 50-fold less available than the same PAHs freshly added to soils. Others have observed a wide range in BSAFs for field-contaminated PAHs that are significantly lower than the same compounds added to artificial soil [36]. The bioavailability of PAHs to aquatic amphipods in field-contaminated and amended sediments has been observed to be only 1.4- to 3.3-fold higher than that of the field-contaminated compounds [22]. In the present study, tests with d-PAHs added to field-contaminated soils demonstrated a large difference in the bioavailability of freshly added compared to field-contaminated compounds. Other researchers have added PAHs to uncontaminated soil, allowed them to age for varying periods of time, and then observed up to 65% reduction in uptake by earthworms compared to the freshly amended soils [28,37–39]. The results of these laboratory amendment and aging experiments, however, do not fully explain the lack of toxicity of highly contaminated soils such as OG10, OG17, and CG11, which were observed to have 42,000, 17,200, and 15,600 mg/kg of total PAHs, respectively, or the large reduction in the bioavailability (up to 50-fold) of field-contaminant, high-molecular-weight PAHs compared to added d-PAHs. The data presented here confirm previous reports that the PAHs in some soils at MGP sites are in an advanced state of sequestration compared to freshly added d-PAHs [14]. In contrast to other studies in which PAHs were added to soils, the soils used in the present study represent a very wide range in total organic carbon (2.6–85%) and a wide range in soot content (molar carbon to hydrogen ratio, 0.6–6.3). The soot-like matrix of the organic carbon present in some of these soils and the removal of the more mobile fraction because of 50 to 100 years of weathering may explain the very low availability of PAHs observed in some MGP site soils.

SFE rapidly released fractions

The observed low values for the rapidly released fraction of total PAHs determined using mild SFE provides additional

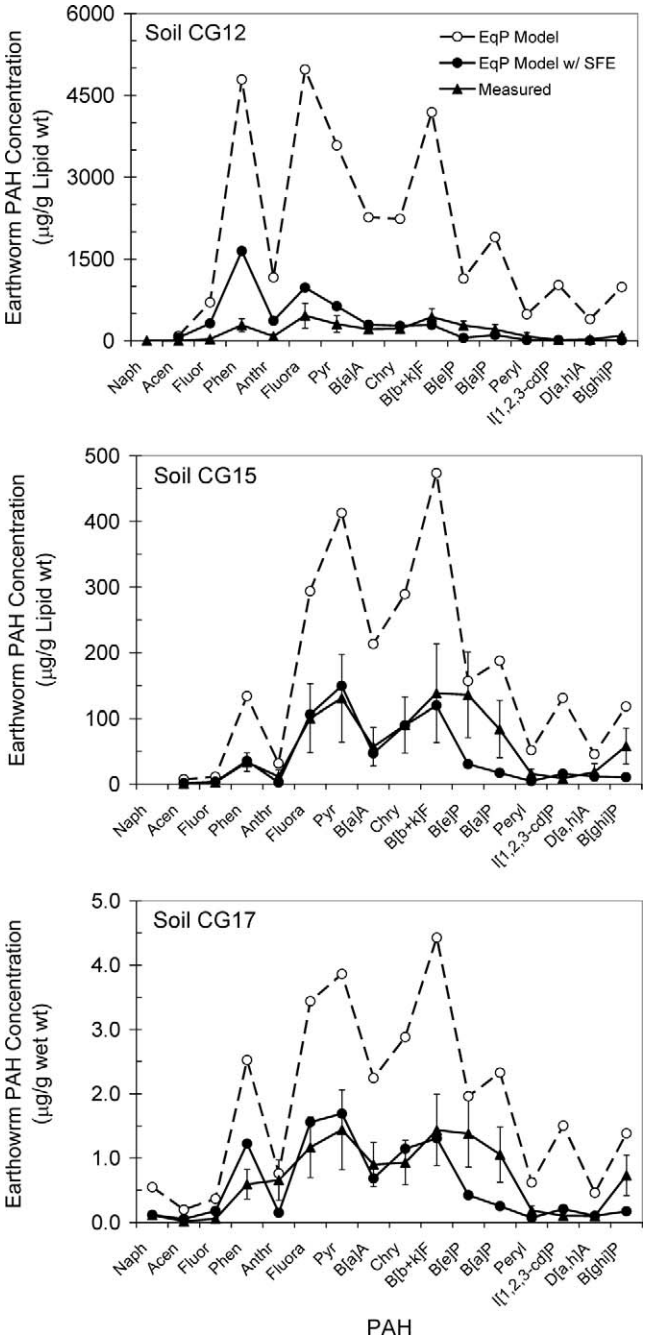


Fig. 3. Measured and predicted polycyclic aromatic hydrocarbon (PAH) in field-collected *Aporrectodea caliginosa*. Error bars show one standard deviation of triplicate extracts of field-collected *A. caliginosa* (approximately two worms/extract). ○ = predicted using equilibrium partitioning model (EqP); ● = predicted using equilibrium-partitioning model adjusted for the rapidly released PAH fraction determined by supercritical carbon dioxide extraction (SFE); ▲ = measured concentration of PAHs in *A. caliginosa*. See Figure 1 for definitions of the PAH abbreviations.

evidence for the greatly reduced availability of PAHs in some soils from MGP sites. In addition, the concentration of rapidly released PAHs appears to be predictive of earthworm toxicity and may be useful for evaluating bioavailability and understanding the variability in PAH toxicity observed in different soils. When Equation 1 is used to predict the internal PAH concentration of *E. fetida* exposed to soils OG10 and CG1

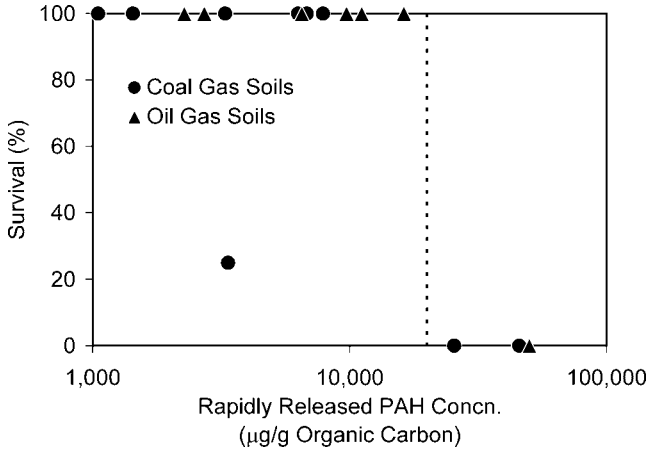


Fig. 4. Survival of *Eisenia fetida* compared to the rapidly released concentration of total polycyclic aromatic hydrocarbons (PAHs; sample CG10, 3,360 μg PAH/g organic carbon, 25% survival).

(16,200 and 23,900 mg/kg OC, respectively), an internal molar concentration of 40 and 86 μmol/g lipid, respectively, is predicted. This is in the same range as the genus mean acute values developed for freshwater aquatic annelids using the hydrocarbon narcosis model [40]. The genus mean acute values for the freshwater oliogchaete *Lumbriculus variegates* is estimated to be more than 53 μmol/g lipid, and the genus mean acute values for the saltwater polychaete *Neanthes arenaceodentata* is estimated to be 76 μmol/g lipid. These values compare favorably to the predicted internal PAH concentrations for *E. fetida* at which survival was reduced when exposed to MGP soils (Fig. 4).

CONCLUSION

Two surprising observations were made in the present study. Acute toxicity was not observed in some soils from MGP sites having up to 42,000 mg/kg of total PAHs, and several soils were observed to have native populations of worms despite total PAH concentrations up to 3,700 mg/kg soil. Polycyclic aromatic hydrocarbon residues observed in field-collected worms and PAH uptake in laboratory bioassays clearly demonstrated the low bioavailability of PAHs in soils in which the organic carbon was chiefly anthropogenic. The bioavailability of PAHs in MGP site soils that contain many sources of anthropogenic carbon and have had more than 50 years for weathering of the hydrocarbon mixture was 5- to 50-fold lower than the bioavailability of freshly added PAHs. A comparison of the rapidly released fraction of PAHs extracted using mild SFE with worm survival indicated that SFE may be a useful tool for predicting PAH uptake and toxicity to terrestrial worms.

SUPPORTING INFORMATION

Table S1. Biota–sediment accumulation factors (BSAF) in field-collected worms.

Table S2. Measured amended and native polycyclic aromatic hydrocarbon (PAH) concentrations in soil and worms after 14-d exposure.

Both tables found at DOI: 10.1897/06-608.S1 (85 KB PDF)

Figure S1. Earthworm polycyclic aromatic hydrocarbon (PAH) concentrations and biota–soil accumulation factors for *Eisenia fetida* exposed for 7, 14, and 28 d to soil HP2. Error

bars show one standard deviation of triplicate extracts of *E. fetida*.

Figure S2. Earthworm polycyclic aromatic hydrocarbon (PAH) concentrations and biota–soil accumulation factors for *Eisenia fetida* exposed for 7, 14, and 28 d to soil HP12. Error bars show one standard deviation of triplicate extracts of *E. fetida*.

Both figures found at DOI: 10.1897/06-608.S2 (49 KB PDF)

Acknowledgement—The authors thank James Edwards, Carol Grabanski, and David Miller for sample collection and analytical support and Roman Lanno, Raymond Loehr, Charlie Menzie, and David Nakles for helpful discussions and encouragement. Financial support for this research was provided by the Gas Research Institute, National Grid, and the U.S. Department of Energy under Cooperative agreement DE-F26-98FT40321. However, any opinions, findings, conclusions, or recommendations expressed herein are those of the authors and do not necessarily reflect the views of the U.S. Department of Energy, Gas Research Institute or National Grid.

REFERENCE

1. Di Toro DM, Zarba CS, Hansen DJ, Berry WJ, Swartz RC, Cowan CE, Pavlou SP, Allen HE, Thomas NA, Paquin PR. 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environ Toxicol Chem* 10:1541–1584.
2. Alexander M. 1995. How toxic are toxic chemicals in soil? *Environ Sci Technol* 29:2713–2717.
3. Van Gestel CAM, Ma WC. 1990. An approach to quantitative structure–activity relationships QSARs in earthworm toxicity studies. *Chemosphere* 21:1023–1034.
4. Connell DW, Markwell RD. 1990. Bioaccumulation in the soil to earthworm system. *Chemosphere* 20:91–100.
5. Belfroid AC, Seinen W, Van Gestel KCAM, Hermens JLM, Van Leeuwen KJ. 1995. Modeling the accumulation of hydrophobic organic chemicals in earthworms: Application of the equilibrium partitioning theory. *Environ Sci Pollut Res* 2:5–15.
6. Jager T. 1998. Mechanistic approach for estimating bioconcentration of organic chemicals in earthworms (Oligochaeta). *Environ Toxicol Chem* 17:1080–1090.
7. Belfroid AC. 1996. Toxicokinetics of hydrophobic chemicals in earthworms: Validation of equilibrium partitioning theory. PhD thesis. Utrecht University, Utrecht, The Netherlands.
8. Krauss M, Wilcke W, Zech W. 2000. Availability of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) to earthworms in urban soils. *Environ Sci Technol* 34: 4335–4340.
9. Paine MD, Chapman PM, Allard PJ, Murdoch MH, Minifie D. 1996. Limited bioavailability of sediment PAH near an aluminum smelter: Contamination does not equal effects. *Environ Toxicol Chem* 15:2003–2018.
10. West CW, Kosian PA, Mount DR, Makynen EA, Pasha MS. 2001. Amendment of sediments with a carbonaceous resin reduces bioavailability of polycyclic aromatic hydrocarbons. *Environ Toxicol Chem* 20:1104–1111.
11. Jager T, Baerselman R, Dijkman E, De Groot AC, Hogendoorn EA, De Jong A, Kruitbosch JAW, Peijnenburg W. 2003. Availability of polycyclic aromatic hydrocarbons to earthworms (*Eisenia andrei*, Oligochaeta) in field-polluted soils and soil–sediment mixtures. *Environ Toxicol Chem* 22:767–775.
12. Luthy RG, Aiken GR, Brusseau ML, Cunningham SD, Gschwend PM, Pignatello JJ, Reinhard M, Traina SJ, Weber WJ Jr, Westall JC. 1997. Sequestration of hydrophobic organic contaminants by geosorbents. *Environ Sci Technol* 31:3341–3347.
13. Gustafsson O, Haghseta F, Chan C, Macfarlane J, Gschwend PM. 1997. Quantification of the dilute sedimentary soot phase: Implications for PAH speciation and bioavailability. *Environ Sci Technol* 31:203–209.
14. Jonker MTO, Koelmans AA. 2002. Sorption of polycyclic aromatic hydrocarbons and polychlorinated biphenyls to soot and soot-like materials in the aqueous environment: Mechanistic considerations. *Environ Sci Technol* 36:3725–3734.
15. Van Gestel CAM, Ma WC. 1988. Toxicity and bioaccumulation of chlorophenols in earthworms in relation to bioavailability in soil. *Ecotoxicol Environ Saf* 15:289–297.
16. Koelmans AA, Jonker MT, Cornelissen G, Bucheli TD, Van Noort PC, Gustafsson O. 2006. Black carbon: The reverse of its dark side. *Chemosphere* 63:365–377.
17. Krauss M, Wilcke W. 2001. Biomimetic extraction of PAHs and PCBs from soil with octadecyl-modified silica disks to predict their availability to earthworms. *Environ Sci Technol* 35:3931–3935.
18. Ghosh U, Zimmerman JR, Luthy RG. 2003. PCB and PAH speciation among particle types in contaminated harbor sediments and effects on PAH bioavailability. *Environ Sci Technol* 37: 2209–2217.
19. Rockne KJ, Shor LM, Young LY, Taghon GL, Kosson DS. 2002. Distributed sequestration and release of PAHs in weathered sediment: The role of sediment structure and organic carbon properties. *Environ Sci Technol* 36:2636–2644.
20. Belfroid AC, Sijm DTHM, Van Gestel CAM. 1996. Bioavailability and toxicokinetics of hydrophobic aromatic compounds in benthic and terrestrial invertebrates. *Environ Rev* 4:276–299.
21. van der Wal L, Jager T, Fleuren RHLJ, Barendregt A, Sinnige TL, vanGestel CAM, Hermens JLM. 2004. Solid-phase microextraction to predict bioavailability and accumulation of organic micropollutants in terrestrial organisms after exposure to a field-contaminated soil. *Environ Sci Technol* 38:4842–4848.
22. Kraaij RH, Ciarelli S, Tolls J, Kater BJ, Belfroid A. 2001. Bioavailability of lab-contaminated and native polycyclic aromatic hydrocarbons to the amphipod *Corophium volutator* relates to chemical desorption. *Environ Toxicol Chem* 20:1716–1724.
23. Hawthorne SB, Poppendieck DG, Grabanski CB, Loehr RC. 2002. Comparing PAH availability from manufactured-gas plant soils and sediments with chemical and biological tests. 1. PAH release during water desorption and supercritical carbon dioxide extraction. *Environ Sci Technol* 36:4795–4803.
24. Hawthorne SB, Poppendieck DG, Grabanski CB, Loehr RC. 2001. PAH release during water desorption, supercritical carbon dioxide extraction, and field bioremediation. *Environ Sci Technol* 35:4577–4583.
25. Hawthorne SB, Bjorklund E, Bowadt S, Mathiasson L. 1999. Determining PCB sorption/desorption behavior on sediments using selective supercritical fluid extraction. 3. Sorption from water. *Environ Sci Technol* 33:3152–3159.
26. Hawthorne SB, Grabanski CB. 2000. Correlating selective supercritical fluid extraction with bioremediation behavior of PAHs in a field treatment plot. *Environ Sci Technol* 34:4103–4110.
27. Hawthorne SB, Grabanski CB. 2000. Vaporization of polycyclic aromatic hydrocarbons (PAHs) from sediments at ambient conditions. *Environ Sci Technol* 34:4348–4353.
28. Tang J, Carroquino MJ, Robertson BK, Alexander M. 1998. Combined effect of sequestration and bioremediation in reducing the bioavailability of polycyclic aromatic hydrocarbons in soil. *Environ Sci Technol* 32:3586–3590.
29. U.S. Environmental Protection Agency. 1996. EPA Test Methods for Evaluating Solid Waste, 3rd ed. SW-846. Final Update. National Technical Information Service, Springfield, VA.
30. Di Toro DM, McGrath JA. 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. II. Mixtures and sediments. *Environ Toxicol Chem* 18:1971–1982.
31. Hayes TD, Linz DG, Nakles DV, Leuschner AP. 1996. *Management of Manufactured Gas Plant Sites.* Amherst Scientific, Amherst, MA, USA.
32. Sverdrup LE, Krogh PH, Nielsen T, Stenersen J. 2002. Relative sensitivity of three terrestrial invertebrate tests to polycyclic aromatic compounds. *Environ Toxicol Chem* 21:1927–1933.
33. Matscheko N, Lundstedt S, Svensson L, Harju M, Tysklind M. 2002. Accumulation and elimination of 16 polycyclic aromatic compounds in the earthworm (*Eisenia fetida*). *Environ Toxicol Chem* 21:1724–1729.
34. Ma WC, Van Kleunen A, Immerzeel J, De Maagd PGJ. 1998. Bioaccumulation of polycyclic aromatic hydrocarbons by earthworms: Assessment of equilibrium partitioning theory in in situ studies and water experiments. *Environ Toxicol Chem* 17:1730–1737.
35. Jager T, van der Wal L, Fleuren RHLJ, Barendregt A, Hermens JLM. 2005. Bioaccumulation of organic chemicals in contaminated soils: Evaluation of bioassays with earthworms. *Environ Sci Technol* 39:293–298.

36. Jager T, Baerselman R, Dijkman E, de Groot AC, Hogendoorn EA, de Jong A, Kruitbosch JAW, Peijnenburg WJGM. 2003. Availability of polycyclic aromatic hydrocarbons to earthworms (*Eisenia andrei*, oligochaeta) in field-polluted soils and soil–sediment mixtures. *Environ Toxicol Chem* 22:767–775.
37. Kelsey JW, Kottler BD, Alexander M. 1997. Selective chemical extractants to predict bioavailability of soil-aged organic chemicals. *Environ Sci Technol* 31:214–217.
38. Kelsey JW, Alexander M. 1997. Declining bioavailability and inappropriate estimation of risk of persistent compounds. *Environ Toxicol Chem* 16:582–585.
39. Chung N, Alexander M. 1999. Effect of concentration on sequestration and bioavailability of two polycyclic aromatic hydrocarbons. *Environ Sci Technol* 33:3605–3608.
40. U.S. Environmental Protection Agency. 2003. Procedures for derivation of equilibrium sediment benchmarks (ESBs) for protection of benthic organisms: PAH mixtures. EPA 600/R-02/013. Final Technical Report. Naragansett, RI.

APPENDIX G

REDUCTION IN ACUTE TOXICITY OF SOILS TO TERRESTRIAL OLIGOCHAETES FOLLOWING THE REMOVAL OF BIOAVAILABLE POLYCYCLIC AROMATIC HYDROCARBONS WITH MILD SUPERCRITICAL CARBON DIOXIDE EXTRACTION

PUBLISHED IN *ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY*

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 24, No. 8, pp. 1893–1895, 2005
© 2005 SETAC
Printed in the USA
0730-7268/05 $12.00 + .00

Short Communication

REDUCTION IN ACUTE TOXICITY OF SOILS TO TERRESTRIAL OLIGOCHAETES FOLLOWING THE REMOVAL OF BIOAVAILABLE POLYCYCLIC AROMATIC HYDROCARBONS WITH MILD SUPERCRITICAL CARBON DIOXIDE EXTRACTION

STEVEN B. HAWTHORNE,*† ROMAN LANNO,‡ and JOSEPH P. KREITINGER§
†Energy and Environmental Research Center, University of North Dakota, Box 9018, Grand Forks, North Dakota 58202, USA
‡Department of Entomology, Ohio State University, Columbus, Ohio 43210, USA
§Institute for Comparative and Environmental Toxicology and Department of Soil, Crop and Atmospheric Sciences, Cornell University, Ithaca, New York 14850, USA

(*Received* 28 *October* 2004; *Accepted* 28 *January* 2005)

Abstract—Three soil samples contaminated with polycyclic aromatic hydrocarbons (PAHs) that caused 100% mortality to terrestrial oligochaetes were extracted with supercritical carbon dioxide to remove the bioavailable fraction of PAHs. Although the remaining PAH concentrations were high after supercritical fluid extraction (SFE), 650 to 8,000 mg/kg, acute toxicity to *Eisenia fetida* and *Enchytraeus albidus* essentially was eliminated. These results demonstrate that mild SFE with pure carbon dioxide preferentially extracts PAH molecules that are bioavailable toxicologically to the oligochaetes, although biologically unavailable PAHs are not extracted, suggesting that SFE could be used for the removal of toxicity due to hydrophobic organic chemicals in soils during toxicity identification evaluations.

Keywords—Oligochaetes Supercritical fluid extraction Polycyclic aromatic hydrocarbons Toxicity identification evaluations Bioavailability

INTRODUCTION

Supercritical fluid extraction (SFE) under mild conditions using pure carbon dioxide has been proposed as a rapid chemical test to determine the bioavailable concentrations of hydrophobic organic chemicals (HOCs), including polycyclic aromatic hydrocarbons (PAHs) [1–5] and polychlorinated biphenyls [6–8]. The majority of investigations have focused on using mild SFE to predict bioavailability of PAHs from manufactured gas plant (MGP) sites that have been abandoned for several decades [1–3]. The available (or fast) fractions of 20 individual PAHs from 11 MGP soils, soots, and sediments recently were determined with 120 d of water desorption and compared to 120 min of SFE. Both techniques showed good agreement for 2- to 6-ring PAHs [3]. Mild SFE also has been used to predict the removal of individual and total PAHs achieved with one year of bioremediation in a field treatment study [1,2] and to increase substantially the accuracy of estimating PAH uptake by earthworms over the standard equilibrium partitioning model [4].

Although water desorption, mild organic solvent extraction, and mild SFE have been used to study the chemical availability of PAHs [4–8], there is little evidence that the individual PAH molecules removed by these extraction methods are actually the same molecules that affect organisms. Macroorganism/SFE relationships for HOCs (e.g., PAHs, polychlorinated biphenyls) are limited to bioaccumulation data, not whole organism response data (i.e., toxicity) [4,9]. Therefore, the purpose of the present study was to determine whether the PAH molecules that are extracted by mild SFE actually are the same PAH molecules that cause biological effects. Supercritical fluid extraction particularly is suited to investigating the effects of PAHs, because pure carbon dioxide only extracts nonpolar organics and does not affect other soil components. In contrast to water desorption, which can leach natural organic matter, metals, and salts, SFE with pure carbon dioxide does not extract metals and inorganic salts and does not measurably affect the soil organic matter composition [7]. In addition, SFE does not leave any organic solvent residue and associated solvent toxicity. These characteristics of SFE are important from the perspective of conducting toxicity identification evaluations (TIEs) of contaminated soils. Although methods are readily available for conducting TIEs with aqueous samples [10], only a few techniques for the selective removal of HOCs from sediments are available [11,12]. Although these techniques do not leave residual toxicity, they may alter sediment organic carbon structure. Techniques for the selective removal of HOCs in soil are not available, and SFE may prove to be a valuable technique for the development of TIEs in soil.

To investigate the relevance of SFE to toxicologically important PAHs, three soil samples from MGP sites that were found to be toxic to earthworms were selected for extraction with mild SFE to remove the available PAH molecules. Individual and total PAH concentrations were determined before and after mild SFE. Sufficient quantity of each of the three soils was prepared to allow toxicity testing with two oligochaete species, *Eisenia fetida* (Savigny 1826) and *Enchytraeus albidus* (Henle 1837).

MATERIALS AND METHODS

Soil samples

Three soil samples that caused 100% mortality to *E. fetida* were selected from 16 MGP site soils reported in an earlier study [2,3]. In-depth descriptions of PAH composition, PAH fast fractions determined with SFE and water desorption, and

* To whom correspondence may be addressed (shawthorne@undeerc.org).

the soil matrix compositions have been reported previously [2,3].

PAH determinations and SFE extractions

Soil PAH concentrations were determined using gas chromatography/mass spectrometry with the aid of perdeuterated PAH internal standards as described previously [3]. Removal of the SFE-available PAHs from the soils for the toxicity tests was performed under identical conditions to those previously used to determine the SFE available (or fast) fraction for the same samples [3]. In short, approximately 8-g soil samples were loaded as received into the SFE cell and extracted at 200 atmospheres and 50°C for 40 min at a flow rate of 0.5 ml/min/g sample (4 ml/min for the 8-g samples). This was repeated until approximately 150 g of each soil was prepared for the oligochaete toxicity tests. Each bulk soil sample then was mixed thoroughly, and quadruplicate subsamples (2 g) were removed to determine the PAH concentrations after the SFE extraction.

Oligochaete bioassays

Acute toxicity tests were conducted using *E. fetida* and *E. albidus* as described previously [9,13]. For *E. fetida*, five adult worms were placed in approximately 20 g (dry wt) of soil that had been moistened with deionized water to 100% of water-holding capacity. Tests were conducted in quadruplicate for 14 d. For *E. albidus*, 15 g (dry wt) of soil was moistened to 75% of water-holding capacity, and 10 adults were placed in each of the triplicate jars. Survival was determined at 21 d.

RESULTS AND DISCUSSION

The PAH concentrations of the three soil samples before and after removal of the SFE-available PAH fractions are summarized in Table 1. Individual PAH and reproducibility data are given in ETC Supplementary Information Supporting Table S1 [SETAC Supplemental Data Archive, Item ETC-24-08-001; http://etc.allenpress.com]. The fractions of the individual and total PAHs removed from each of the three samples during the SFE preparation of the bulk sample for the toxicity testing agrees well with those values previously reported for the SFE available or fast fraction [2,3]. Therefore, the use of the bulk sample to test whether the SFE-available PAH molecules actually are the bioavailable molecules was validated.

As shown in Table 1, the PAHs with the lowest molecular weights are the most available to removal by mild SFE, with approximately 90% of the 2-ring naphthalene being extracted from all three samples. However, the higher molecular weight 5- and 6-ring PAHs showed little or no change in concentration after the mild SFE, as is consistent with the low availability values previously reported for these PAHs from the same samples using both SFE and water desorption [3]. The total PAH concentration (based on the sum of the U.S. Environmental Protection Agency 16 priority pollutant PAHs) was reduced by 60 to 82% by the mild SFE. However, all three soils still had PAH concentrations >650 mg/kg that would be expected to result in complete and rapid (96-h) mortality based on earlier work by Wells and Lanno [13], and the OG18 soil had a very high total PAH concentration (7,533 mg/kg) even after removal of the SFE-available fraction.

The effect of removing the SFE-available PAHs from the three soils on oligochaete mortality is shown in Table 2. Prior to removal of the available PAHs, all three soils were acutely toxic to both *E. fetida* and *E. albidus*, with 100% mortality normally occurring within the first few days of the test. In contrast, *E. fetida* mortality did not differ from control treatments, showing essentially no mortality over 14 d in the three soils after removing the SFE-available fraction of the PAHs. Similarly, *E. albidus* showed no mortality compared to the reference soils for soils CG1 and CG3, and greatly reduced mortality for the highly contaminated soil, OG18 (Table 2). In contrast to the original OG18 soil, in which *E. albidus*

Table 1. Polycyclic aromatic hydrocarbon (PAH) concentrations before and after mild supercritical fluid extraction (SFE) to remove the available PAH fraction

	Initial (μg/g soil)	Post-SFE extraction (μg/g soil)	Mass extracted (μg/g soil)	% Removed
Soil CG1 (4.6 wt. % C)[a] C/H = 1.0[b]				
2-Ring[c]	53	8	45	86
3-Ring	946	126	820	87
4-Ring	679	302	376	55
5-Ring	165	150	15	9
6-Ring	93	87	6	7
Total	1,936	673	1,263	65
Soil CG3 (7.5 wt. % C) C/H = 0.9				
2-Ring	746	26	720	97
3-Ring	1,397	74	1,323	95
4-Ring	979	171	808	83
5-Ring	300	164	136	45
6-Ring	189	164	25	13
Total	3,611	600	3,011	83
Soil OG18 (25 wt. % C) C/H = 2.5				
2-Ring	6,078	408	5,670	93
3-Ring	4,850	996	3,854	79
4-Ring	5,740	2,914	2,827	49
5-Ring	1,814	1,679	135	7
6-Ring	1,503	1,537	NC[d]	NC
Total	19,985	7,533	12,451	62

[a] OG and CG refer to soils collected at former manufactured gas plant sites that produced oil gas (from petroleum) and coal gas (from coal), respectively.
[b] Molar carbon to hydrogen ratio.
[c] 2-Ring = naphthalene; 3-ring = sum of acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene; 4-ring = sum of fluoranthene, pyrene, benz[*a*]anthracene, chrysene; 5-ring = sum of benzo[b+k]fluoranthene, benzo[*a*]pyrene; 6-ring = sum of indeno[1,2,3-*cd*]pyrene, dibenz[*a,h*]anthracene, benzo[*ghi*]perylene.
[d] NC = no measurable change in concentration.

Table 2. Earthworm survival before and after removal of the available polycyclic aromatic hydrocarbon fraction with mild supercritical fluid extraction (SFE)[a]

	% Survival			
	Eisenia fetida[b]		*Enchytraeus albidus*[c]	
	Initial soil	After SFE	Initial soil	After SFE
Soil CG1	0 ± 0	100 ± 0	0 ± 0	97 ± 6
Soil CG3	0 ± 0	100 ± 0	0 ± 0	83 ± 15
Soil OG18	0 ± 0	95 ± 10	0 ± 0	40 ± 10
Reference soil 1	100 ± 0		97 ± 6	
Reference soil 2	100 ± 0		80 ± 10	

[a] OG and CG refer to soils collected at former manufactured gas plant sites that produced oil gas (from petroleum) and coal gas (from coal), respectively.
[b] Survival and standard deviations based on five worms in each of four replicate jars.
[c] Survival and standard deviations based on 10 worms in each of three replicate jars.

mortality occurred in the first 2 or 3 d, survival was nearly 100% in the SFE-extracted OG18 until the last few days of the assay.

Considering the very high PAH concentrations, the relative lack of toxicity in the SFE-treated OG18 sample is consistent with a previous study that concluded that soot (or black carbon) can bind PAHs more strongly than soil organic carbon and, therefore, cause slow release (and, thus, lower bioavailability) of PAHs that are associated with soot particles [3]. As shown in Table 1, OG18 does have much higher organic carbon (25% by weight) than the other two soil samples (4.6 and 7.5% by weight). It also has the appearance of lampblack soot, while CG1 and CG3 clearly are soils. Finally, OG18 has a molar C/H ratio of 2.5, which indicates a highly aromatic carbon soot matrix, although the two soils have molar C/H ratios of approximately one, a ratio consistent with normal soil organic matter [3]. Thus, the oligochaete mortality data and PAH concentration data support earlier conclusions that soot carbon has a much greater capacity than natural soil organic carbon to reduce the bioavailability of PAHs.

The results in Table 1 and Table 2 also demonstrate that the lower molecular weight PAHs are the chemicals most responsible for acute oligochaete toxicity. However, it is important to note that the soil concentration of the lower molecular weight PAHs is not the factor determining their toxicity. For example, the concentration of naphthalene in soil CG1 was 53 mg/kg in the original soil that caused 100% mortality of both oligochaete species (i.e., before SFE). In contrast, even though the concentration of naphthalene in OG18 after SFE still was very high at 408 mg/kg, the sample showed little or no toxicity. Similarly, the total PAH concentration has no apparent relationship to oligochaete mortality. For example, after SFE, OG18 soil had 7,533 mg/kg total PAH concentration, but displayed no toxicity to *E. fetida* and greatly reduced toxicity to *E. albidus*. In contrast, the original CG1 soil caused 100% mortality of both oligochaete species, even though CG1 had only 1,936 mg/kg total PAHs and had substantially lower concentrations of all 2- to 6-ring PAHs than OG18 after SFE (Table 1 and ETC Supplementary Data Table S1 [SETAC Supplemental Data Archive, Item ETC-24-08-001; http://etc.allenpress.com]).

Although previous studies have shown a strong correlation between the fraction of PAHs extracted by mild SFE and the fraction of PAHs available for bioremediation, water desorption, and earthworm uptake, they have not demonstrated that the molecules extracted by SFE are involved in toxicity [1–4]. However, the results shown in Table 2 clearly demonstrate that the PAH molecules removed by the mild SFE technique are indeed the same PAH molecules that cause toxicity to oligochaetes, suggesting that mild SFE would be a useful technique for the removal of HOCs from soils in the development of soil TIE procedures.

CONCLUSION

Oligochaete mortality in PAH-contaminated soils clearly cannot be predicted by either individual or total PAH concentrations, but should be based on a measurement of available concentrations. The reduction or elimination of acute toxicity to *E. fetida* and *E. albidus* by mild SFE demonstrates that SFE does extract biologically relevant PAHs and may provide a means for the removal of toxicity due to HOCs in soils during the performance of TIEs.

Acknowledgement—The financial support of the Gas Research Institute (GRI), the RETEC Group, and the U.S. Department of Energy (DOE) under Cooperative Agreement DE-FC26–98FT40321 gratefully is acknowledged. However, any opinions, findings, conclusions, or recommendations expressed herein are those of the authors and do not necessarily reflect the views of the GRI or DOE. The authors thank C. Grabanski for technical assistance.

REFERENCES

1. Hawthorne SB, Grabanski CB. 2000. Correlating selective supercritical fluid extraction with bioremediation behavior of PAHs in a field treatment plot. *Environ Sci Technol* 34:4103–4110.
2. Hawthorne SB, Poppendieck DG, Grabanski CB, Loehr RC. 2001. PAH release during water desorption, supercritical carbon dioxide extraction, and field bioremediation. *Environ Sci Technol* 35: 4577–4583.
3. Hawthorne SB, Poppendieck DG, Grabanski CB, Loehr RC. 2002. Comparing PAH availability from manufactured gas plant soils and sediments with chemical and biological tests. 1. PAH release during water desorption and supercritical carbon dioxide extraction. *Environ Sci Technol* 36:4795–4803.
4. Hawthorne SB, Kreitinger J, Grabanski CB, Miller DJ. 2004. Predicting PAH uptake in terrestrial worms using selective supercritical fluid extraction and carbon matrix characteristics. *Abstracts*, 14th Annual Meeting, SETAC Europe, Prague, Czech Republic, April 18–22, p 81.
5. Weber WJ, Young TM. 1997. A distributive reactivity model for sorption by soils and sediments. 6. Mechanistic implications of desorption under supercritical fluid conditions. *Environ Sci Technol* 31:1686–1691.
6. Björklund E, Nilsson T, Bøwadt S, Pilorz K, Mathiasson L, Hawthorne SB. 2000. Introducing selective supercritical fluid extraction as a new tool for determining sorption/desorption behavior and bioavailability of persistent organic pollutants in sediment. *J Biochem Biophys Methods* 43:295–311.
7. Hawthorne SB, Björklund E, Bøwadt S, Mathiasson L. 1999. Determining PCB sorption/desorption behavior on sediments using selective supercritical fluid extraction. 3. Sorption from water. *Environ Sci Technol* 33:3152–3159.
8. Nilsson T, Sporring S, Björklund E. 2003. Selective supercritical fluid extraction to estimate the fraction of PCB that is bioavailable to a benthic organism in naturally contaminated sediment. *Chemosphere* 53:1049–1052.
9. Tang J, Carroquino MJ, Robertson BK, Alexander M. 1998. Combined effect of sequestration and bioremediation in reducing the bioavailability of polycyclic aromatic hydrocarbons in soil. *Environ Sci Technol* 32:3586–3590.
10. U.S. Environmental Protection Agency. 1991. *Methods for Aquatic Toxicity Identification Evaluations: Phase Characterization Procedures, Phase I-Toxicity Procedures,* 2nd ed. EPA/600/6–91/003. Environmental Research Laboratory, Duluth, MN.
11. Ho KT, Burgess RM, Pelletier MC, Serbst JR, Cook H, Cantwell MG, Ryba SA, Perron MM. 2004. Use of powered coconut charcoal as a TIE manipulation for organic toxicants in marine sediments. *Environ Toxicol Chem* 23:2124–2131.
12. Heinis LJ, Highland TL, Mount DR. 2004. Method for testing the aquatic toxicity of sediment extracts for use in identifying organic toxicants in sediments. *Environ Sci Technol* 38:6256–6262.
13. Wells JB, Lanno RP. 2001. Passive sampling devices (PSDs) as biological surrogates for estimating the bioavailability of organic chemicals in soil. In Greenberg BM, Hull RN, Roberts MH Jr., Gensemer RW, eds, *Environmental Toxicology and Risk Assessment: Science, Policy, and Standardization—Implications for Environmental Decisions,* Vol 10. STP 1403. American Society for Testing and Materials, Philadelphia, PA, pp 253–270.

APPENDIX H

MEASUREMENT OF TOTAL POLYCYCLIC AROMATIC HYDROCARBON CONCENTRATIONS IN SEDIMENTS AND TOXIC UNITS USED FOR ESTIMATING RISK TO BENTHIC INVERTEBRATES AT MANUFACTURED GAS PLANT SITES

PUBLISHED IN *ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY*

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 25, No. 1, pp. 287–296, 2006
© 2006 SETAC
Printed in the USA
0730-7268/06 $12.00 + .00

MEASUREMENT OF TOTAL POLYCYCLIC AROMATIC HYDROCARBON CONCENTRATIONS IN SEDIMENTS AND TOXIC UNITS USED FOR ESTIMATING RISK TO BENTHIC INVERTEBRATES AT MANUFACTURED GAS PLANT SITES

STEVEN B. HAWTHORNE,*† DAVID J. MILLER,† and JOSEPH P. KREITINGER‡
†Energy and Environmental Research Center, University of North Dakota,, Box 9018, Grand Forks, North Dakota 58202, USA
‡The RETEC Group, Ithaca, New York 14850, USA

(*Received* 16 *February* 2005; *Accepted* 7 *July* 2005)

Abstract—The U.S. Environmental Protection Agency's (U.S. EPA) narcosis model requires the measurement of 18 parent and 16 groups of alkyl polycyclic aromatic hydrocarbons (PAHs) (so-called 34 PAHs) in sediments to calculate the number of PAH toxic units (TU) available to benthic organisms. If data for the 34 PAHs are not available, the U.S. EPA proposes estimating the risk by multiplying the TU for 13 parent PAHs by 11.5 (95% confidence interval) based on data from 488 sediments. This estimate is overly conservative for PAHs from pyrogenic manufactured gas plant (MGP) processes based on the analysis of 45 sediments from six sites. Parent PAHs contributed approximately 40% of the total concentrations and TU for MGP sediments. In contrast, parent PAHs from diesel fuel and petroleum crude oil contributed only 2 and 1%, respectively, of the PAH concentrations and TU, compared to approximately 98 to 99% contributed by the alkyl PAHs. Statistical comparison of the TU based on the measured 34 alkyl and parent PAHs and those based on only 13 parent PAHs demonstrated that a factor of 4.2 (rather than 11.5) is sufficient to estimate total TU within a 95% confidence level for MGP sites. Similarly, measurement of parent PAHs is sufficient to accurately estimate the total 34 alkyl and parent PAH concentrations for MGP-impacted sediments.

Keywords—Manufactured gas plant Polycyclic aromatic hydrocarbons Sediment Hydrocarbon narcosis Toxic units

INTRODUCTION

The U.S. Environmental Protection Agency's (U.S. EPA) guidelines for protecting benthic organisms in polycyclic aromatic hydrocarbon (PAH)–contaminated sediments are based on the calculation of benchmark values for complex mixtures of PAHs that estimate risk using the hydrocarbon narcosis and equilibrium partitioning models [1–5]. The hydrocarbon narcosis risk model requires the measurement on sediment of 18 parent PAHs and 16 groups of prominent C1 to C4 alkyl PAH derivatives (so-called 34 PAHs) rather than the historically measured 16 priority pollutant parent PAHs determined using U.S. EPA Method 8310 (http://www.epa.gov/SW-846/pdfs/8310.pdf) or the 13 (or 23) PAHs reported in the majority of sediment monitoring programs in the United States [2]. Sediment concentrations of the 34 PAHs are used along with their expected sediment/water/lipid partitioning behavior to calculate a hazard quotient, referred to as a toxic unit (TU), that is used as a benchmark for predicting the toxicity of PAHs to benthic invertebrates. Because the typical sample contains hundreds of alkyl PAH isomers and because few alkyl PAH standards are available, accurately determining the total 34 PAH concentrations can be difficult and expensive. In addition, nearly all previous investigations have relied only on parent PAH measurements to determine total PAH concentrations, which makes the use of historical data problematic.

In an effort to address these problems, the U.S. EPA protocol suggests the estimation of TUs based on the sediment concentrations of 13 parent PAHs multiplied by a factor of 11.5 (95% confidence interval) [2]. However, the use of this correlation is discouraged since petrogenic (petroleum-derived) and pyrogenic PAHs (e.g., produced under pyrolysis conditions such as those used for manufactured gas plant [MGP] processes) have dramatically different relative proportions of parent versus alkyl PAHs. Petrogenic PAHs are dominated by alkyl PAH derivatives, while pyrogenic MGP PAHs have much lower proportions of alkyl derivatives [6–8]. Thus, any correlations used to estimate total 34 parent and alkyl PAH concentrations based on parent PAH concentrations will be more accurate if they are based on the specific industrial process or site-specific PAH distributions that occur at the sites in question.

The goal of the present study was to evaluate and improve the accuracy of the proposed methods to determine the 34 PAHs on sediment samples and to investigate the effects of common analytical approaches for both pyrogenic and petrogenic PAHs on the TU predicted using the proposed U.S. EPA narcosis risk model. The total 34 alkyl and parent PAH concentrations are reported for 45 sediment samples collected at six different sites impacted by MGP processes. The number of alkyl PAH standards is increased over those in the proposed 34 PAH method [2,9] in an effort to more accurately determine alkyl isomer concentrations. The relative proportions of parent and alkyl PAHs from these pyrogenic sources are compared to those from petrogenic sources (diesel fuel from a local supplier and petroleum crude oil from the National Institute of Standards and Technology [NIST], Gaithersburg, MD, USA, SRM 1582). Manufactured gas plant site-specific factors for estimating the total 34 PAH concentrations and PAH TU based on parent PAH concentrations are presented.

EXPERIMENTAL

Sediment samples

More than 100 sediment samples were collected using a Ponar grab sampler (Forestry Suppliers, Jackson, MS, USA)

* To whom correspondence may be addressed (shawthorne@undeerc.org).

at six different sites thought to be impacted by former MGP activities. Approximately 15 L each of sediment/water slurry were immediately transferred to a 20-L bucket, sieved through a 2-mm screen to remove debris, and briefly mixed before subsampling into new glass jars. Samples (~200 g) were then cooled on ice in the dark and shipped to the laboratory by overnight air delivery. (The bulk of each sample was stored separately for subsequent biological testing.) Storage was at 4°C in the dark. The sediment samples typically had approximately 50 wt % water as stored. Sediment collection and storage procedures were based on previous recommendations [10,11].

A preliminary estimate of PAH concentrations on each sediment was performed by mixing 2 g of the wet sediment with 2 g of sodium sulfate and extracting with 20 ml of 1:1 acetone/methylene chloride for 18 h in a bath sonicator and analyzing the extracts as described here for the Soxhlet extracts. Based on these initial estimates of PAH concentrations, 45 sediments were selected for additional study to represent the range of PAH concentrations (from background to highly contaminated) found at the six MGP sites.

Sediment preparation and analysis

All sediment analyses were performed within 28 d of sample collection. Sediment samples were prepared fresh daily as suggested by the U.S. EPA [10] by transferring approximately 40 ml of the sediment/water slurry to a certified clean 40-ml glass VOA vial and centrifuging for 30 min at 1,000 *g*. (Higher speed caused the glass vials to break.) This typically resulted in 10 to 20 ml of pore water that could be removed with a pipette. The remaining wet sediment was recovered, quadruplicate 2-g samples of the sediment were mixed with an equal weight of sodium sulfate, and each replicate was extracted for 18 h in a Soxhlet apparatus with 150 ml of 1:1 acetone:methylene chloride. Each extract was then spiked with 5 μl of a mixture of two- to six-ring perdeuterated PAHs (*d*-PAHs) as internal standards and analyzed as described here. More dilute extracts were concentrated under a gentle stream of clean nitrogen before gas chromatography/mass spectrometry (GC/MS) analysis. No samples were air-dried before extraction in order to avoid any losses of the more volatile PAHs. However, replicate portions of each sediment were dried overnight at 80°C to allow their moisture content to be determined and to allow presentation of the concentration data on a dry-weight basis.

The 34 parent and alkyl PAH sediment concentrations were determined in a manner analogous to the method proposed by the U.S. EPA, based on the previous work by Denoux et al. [2,9]. However, the method was modified to increase the number of *d*-PAH internal standards and the number of alkyl PAH calibration standards (Table 1). The *d*-PAH internal standards included naphthalene-d_8 (0.97 mg/ml), acenaphthene-d_{10} (0.95 mg/ml), fluorene-d_{10} (1.00 mg/ml), phenanthrene-d_{10} (0.90 mg/ml), fluoranthene-d_{10} (1.00 mg/ml), pyrene-d_{10} (0.89 mg/ml), benz[*a*]anthracene-d_{12} (0.73 mg/ml), chrysene-d_{12} (0.73 mg/ml), benzo[*a*]pyrene-d_{12} (0.24 mg/ml), perylene-d_{12} (0.54 mg/ml), and benzo[*ghi*]perylene-d_{12} (0.91 mg/ml). When no deuterated analog of a PAH was available, the *d*-PAH with the closest molecular structure was used (e.g., benzo[*a*]pyrene-d_{12} was used as the internal standard for benzo[*e*]pyrene, the parent *d*-PAH was used as the internal standard for the related alkyl PAHs).

While most parent PAHs (and many of their perdeuterated analogs) are available as pure standards, few alkyl PAHs are available, so their response must be estimated rather than measured in many cases. The calibration and quantitation of the more highly alkylated PAHs is increasingly complicated by the fact that a single group can have multiple (even hundreds for C_3- and C_4-alkyl PAHs) of alkylated isomers that are listed as a single PAH in the total 34 PAH list. Finally, the MS response of different isomers will vary. In an effort to best determine and estimate the alkyl PAH response factors, we determined the GC/MS response (vs the appropriate parent *d*-PAH) of every alkyl PAH available as pure standards from commercial sources (Table 1). This attempt was complicated by the fact that stated purities of several of the alkyl PAHs were not accurate. Therefore, each of the alkyl PAHs we obtained from commercial sources was analyzed by GC with flame ionization detection to determine its purity and by GC/MS to determine if its mass spectrum was consistent with its reported identity. The chemical purity of the 22 alkyl PAHs tested ranged from approximately 70 to 98%, and these purities were used to correct their response factors. When no standard was available for a particular group of alkyl isomers, the response factor was estimated on the basis of the closest analogous isomers (Table 1).

All GC/MS quantitations of the sediment PAH concentrations were based on daily three-point calibration curves containing the parent and alkyl PAH standard compounds. All analyses were performed with an Agilent model 5973 GC/MS (Agilent Technologies, Wilmington, DE, USA) operated in the selected ion mode for the molecular ions of the target PAHs and *d*-PAHs and equipped with a 60-m Agilent HP-5 MS column (0.25-μm film thickness, 250-μm i.d.). The oven temperature was held at 100°C during the injection, then programmed at 6°C per min to 320°C (hold for 10 min). The possible presence of petroleum contaminants was routinely evaluated by monitoring an ion (m/z = 85) that is characteristic for petroleum alkanes.

Toxic unit calculations

According to the U.S. EPA protocol [1,2], risk from sediment PAHs to benthic organisms is based on the number of PAH TU freely dissolved in the sediment pore water. All PAHs are assumed to have the same toxicity (on a molar basis) in this model and differ only in their tendency to partition from sediment to pore water and from pore water to the organism. For sediment PAHs, K_{oc} and K_{lipid} partitioning coefficients (estimated from K_{ow} values as described by Di Toro et al. [2–4]) can be used to calculate the concentration of each PAH that represents one toxic unit (details are given in U.S. EPA [2]). The sediment concentrations (on a sediment organic carbon basis) of each PAH that represents one toxic unit are given in the Appendix and are fairly similar for all the PAHs. Toxic units are calculated simply by dividing each sediment PAH concentration by the related concentration that represents one toxic unit (Appendix) and by the organic carbon content of the sediment.

For the sediment samples, the concentrations of individual PAHs that represent one toxic unit are quite similar and range only from approximately 400 μg/g for lower-molecular-weight PAHs to approximately 1,100 μg/g for higher-molecular-weight PAHs (Appendix). Also, little change exists in the values for alkyl PAHs versus their parent PAH. In essence, the values for sediment PAHs are similar since the K_{oc} and K_{ow} values used to estimate the partitioning behavior of a particular

Table 1. Relative contributions of parent and alkyl polycyclic aromatic hydrocarbons (PAHs) from petroleum crude oil, diesel fuel, and manufactured gas plant (MGP)–contaminated sediments to total PAH concentrations

	PAH concn. (μg/g)					
			MGP-contaminated sediments			GC/MS RRF[b] vs parent
	Crude oil[a]	Diesel fuel[a]	HD-5	HD-10	HD-22	
Naphthalene	3.52	6.04	135	8.1	0.24	1.00
2-Methylnaphthalene	27.18	20.75	86	7.6	0.14	0.55
1-Methylnaphthalene	10.29	16.90	50	4.4	0.07	0.54
C2 Naphthalenes	96.94	154.64	134	10.0	0.56	0.34
C3 Naphthalenes	100.21	222.40	61	4.6	0.46	0.31
C4 Naphthalenes	100.23	160.59	23	2.2	0.37	0.25
Acenaphthylene	0.48	0.32	16	2.4	0.28	1.00
Acenaphthene	0.43	0.22	63	5.8	0.07	1.00
Fluorene	1.04	4.29	37	4.1	0.09	1.00
C1 Fluorenes	8.47	21.23	42	4.3	0.44	0.49
C2 Fluorenes	23.79	33.45	40	3.2	0.45	0.40
C3 Fluorenes	ND[d]	ND	ND	ND	ND	0.25
Phenanthrene	2.65	9.81	119	12.6	0.56	1.00
Anthracene	ND	0.49	65	4.4	0.38	1.00
C1 Phenanthrenes/anthracenes	32.96	42.31	155	14.8	1.03	0.40
C2 Phenanthrenes/anthracenes	134.72	147.36	180	23.0	3.31	0.16
C3 Phenanthrenes/anthracenes	148.31	99.93	72	10.6	1.95	0.15
C4 Phenanthrenes/anthracenes	99.40	46.92	13	ND	ND	0.14
Fluoranthene	0.07	0.01	75	9.9	1.07	1.00
Pyrene	0.27	0.24	77	10.1	1.00	1.00
C1 Fluoranthenes/pyrenes	4.38	2.07	130	16.8	1.46	0.45
Benz[*a*]anthracene	0.12	0.01	35	4.5	0.50	1.00
Chrysene	0.50	0.07	33	4.8	0.71	1.00
C1 Benz[*a*]anthracenes/chrysenes	4.25	4.89	65	13.4	1.27	0.47
C2 Benz[*a*]anthracenes/chrysenes	83.34	5.01	107	21.4	2.32	0.25
C3 Benz[*a*]anthracenes/chrysenes	82.58	ND	128	ND	ND	0.20
C4 Benz[*a*]anthracenes/chrysenes	32.71	ND	121	ND	ND	0.15
Benzo[*b*+*k*]fluoranthene[c]	0.11	0.01	23	4.0	0.69	1.00
Benzo[*e*]pyrene	0.04	0.02	11	2.4	0.36	1.00
Benzo[*a*]pyrene	0.06	ND	34	4.0	0.54	1.00
Perylene	0.85	ND	7	1.3	0.72	1.00
Indeno[1,2,3-*cd*]pyrene	0.03	ND	41	10.9	1.05	1.00
Dibenz[*a*,*h*]anthracene	0.03	ND	9	2.0	0.18	1.00
Benzo[*ghi*]perylene	0.04	ND	17	4.1	0.46	1.00

[a] Crude oil and diesel fuel PAH concentrations were normalized to a total sediment concentration of 1,000 μg/g to allow comparison with PAH concentrations on the MGP-contaminated sediments. A sediment organic carbon content of 1% was assumed to calculate the toxic units for sediment contaminated with the crude oil at a total PAH concentration of 1,000 μg/g.

[b] All relative response factors (RRFs) are based on the gas chromatography/mass spectrometry (GC/MS) peak area per ng of the alkyl PAH in a standard solution compared to that of its parent PAH as determined by GC/MS. The relative response factors of alkyl PAHs for which no standards were available were estimated on the basis of the closest analogous alkyl PAH. Alkyl PAHs used to determine the GC/MS relative response factors included alkyl naphthalenes (1-methyl-, 2-methyl-, 1, 2-dimethyl-, 1,3-dimethyl- 1, 8-dimethyl-, 2,7-dimethyl- and 1-ethyl, 2-ethyl, 1,4,5-trimethyl-, 2, 3, 5-trimethyl-, and 2-isopropyl-); 1-methylfluorene; 2-methyl- and 9-methylanthracene, 1-methyl-, 2-methyl-, and 3-methylphenanthrene, 9,10-dimethylanthracene; 2-ethylanthracene; 2-tertbutylanthracene; 1-methyl-7-isopropylphenanthrene; 1-methylpyrene; 7-methylbenz[*a*]anthracene; and 7,12-dimethylbenz[*a*]anthracene. Relative response factors for alkyl phenanthrenes/anthracenes, alkyl fluoranthenes/pyrenes, and alkyl benz[*a*]anthracenes/chrysenes are compared to phenanthrene.

[c] Benzo[*b*]fluoranthene and benzo[*k*]fluoranthene are reported as their sum because of insufficient chromatographic resolution.

[d] ND = not detected.

PAH from sediment to water and then from water to the organism increase in a similar manner with PAH size and thus tend to cancel each other out for determining the sediment concentration that represents one toxic unit [2].

Analysis of variance

The variance in the TU uncertainty factor was evaluated using an unconditional hierarchical random effects model [12]. Both sample location and site were treated as random variables, and the 95 and 99% confidence intervals were estimated using the unconditional mean model [13].

RESULTS AND DISCUSSION

Determining alkyl PAH and total PAH concentrations

The determination of total PAHs has largely been defined by the analysis method used to measure individual PAHs. Frequently, the total PAH concentration reported for a sample has been based on the sum of the 16 individual priority pollutant

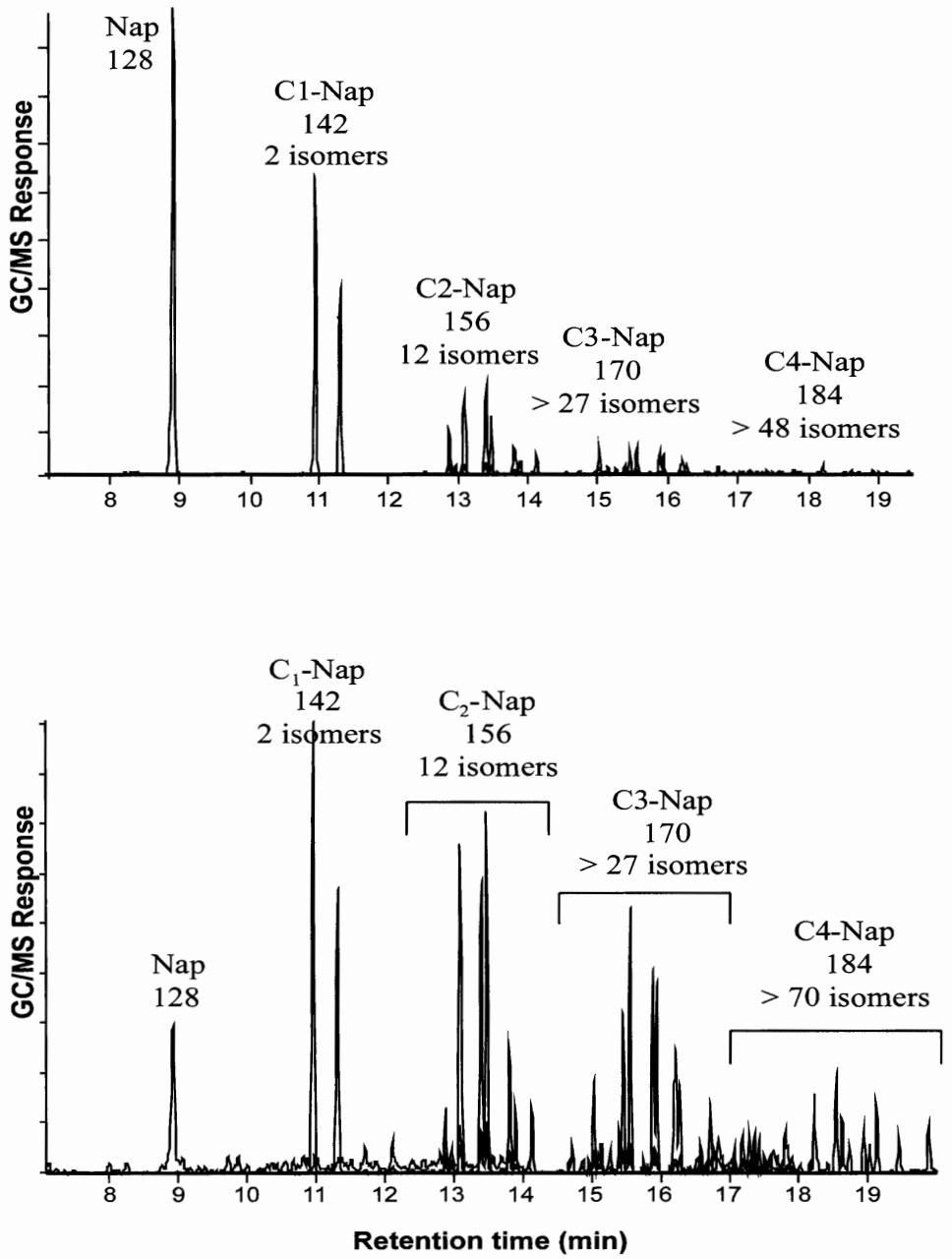


Fig. 1. Naphthalene and alkyl naphthalene distributions in pyrogenic polycyclic aromatic hydrocarbons (PAHs) from manufactured gas plant tar (top) and petrogenic PAHs from petroleum crude oil (bottom). GC/MS = gas chromatography/mass spectrometry.

PAHs determined by U.S. EPA Method 8310. For pyrogenic sources of PAHs (e.g., MGP processes), the parent PAHs are the predominant species, and the sum of the 16 U.S. EPA PAHs has been reported to represent about one-third to two-thirds of the actual total PAH concentrations [14]. In contrast, PAHs from petrogenic sources (e.g., petroleum) are dominated by alkyl derivatives [6–8], as shown by a comparison of MGP tar and petroleum crude oil (NIST Standard Reference Material 1582) in Figure 1 for the simplest case: naphthalene and its alkyl derivatives.

Two features are notable in Figure 1. First, the parent naphthalene dominates the pyrogenic MGP tar profile, while the alkyl derivatives of naphthalene dominate the petrogenic crude oil profile. In addition, the complicated nature of determining the concentration of one group of alkyl isomers is made apparent by the large number of individual compounds that constitute a single PAH group. For example, the petrogenic C4-naphthalene group contains more than 70 individual PAHs yet is reported only as a single compound in the total 34 PAH method. It should be noted that the alkyl naphthalenes represent the least number of possible isomers compared to the higher-molecular-weight PAHs. Therefore, the PAH concentrations reported for the 34 PAH method actually represent several hundred individual PAH compounds.

Additional care must also be taken to avoid including nontarget compounds in the integration of a group of alkyl PAHs. For example, dibenzothiophene elutes at the end of the C4-naphthalene cluster and frequently has a higher concentration than the total C4-naphthalene group. Since both dibenzothiophene and C4-naphthalenes have *m/z* 184 as their molecular ion, care must be taken not to include the peak area of dibenzothiophene in the C4-naphthalene peak area. Similarly, the

Table 2. Relative contributions of parent and alkyl polycyclic aromatic hydrocarbons (PAHs) in petroleum crude oil, diesel fuel, and manufactured gas plant (MGP)–contaminated sediments by PAH ring number

	Relative PAH concn. (% of total)					Relative PAH toxic units (% of total)				
			MGP-contaminated sediments					MGP-contaminated sediments		
	NIST crude oil	Diesel fuel	HD-5	HD-10	HD-22	NIST crude oil	Diesel fuel	HD-5	HD-10	HD-22
2-Ring										
Parent	0.4	0.6	6.1	3.5	1.1	0.7	1.0	10.5	6.3	2.1
C1 to C4 alkyl	33.5	57.5	15.9	12.4	7.0	43.4	64.0	21.3	17.3	9.9
3-Ring										
Parent	0.5	1.5	13.4	12.6	6.1	0.6	1.7	16.2	15.9	8.5
C1 to C4 alkyl	44.8	39.1	23.7	24.1	31.6	41.0	32.5	22.1	23.2	32.8
4-Ring										
Parent	0.1	0.0	9.8	12.6	14.4	0.1	0.0	8.9	11.8	14.8
C1 to C4 alkyl	20.7	1.2	24.7	22.3	22.2	14.1	0.8	16.8	17.2	18.8
5+6-Ring										
Parent	0.1	0.0	6.4	12.4	17.6	0.1	0.0	4.1	8.2	13.2

	PAH concn. (μg/g)					PAH toxic units				
			MGP-contaminated sediments					MGP-contaminated sediments		
	NIST crude oil[a]	Diesel fuel[a]	HD-5	HD-10	HD-22	NIST crude oil	Diesel fuel	HD-5	HD-10	HD-22
Total U.S. EPA 34 PAHs[b]	1,000	1,000	2,232	232	22.7	138	159	333	33.4	2.96
Total U.S. EPA 13 PAHs	9.4	21.5	711	75	6.1	1.9	4.3	125	12.2	0.88
Total U.S. EPA 16 PAHs	9.3	21.5	779	92	7.8	2	4.3	131	13.7	1.03
Total parent PAHs	10.2	21.5	797	95	8.9	2	4.3	133	14.1	1.14
% Parent vs total	1.0%	2.2%	35.7%	41.2%	39.2%	1.4%	2.7%	39.8%	42.3%	38.6%

[a] Crude oil and diesel fuel concentrations and toxic units were based on assuming a sediment concentration of 1,000 μg/g (total 34 PAHS) and a sediment organic concentration of 1 wt %. NIST = National Institute of Standards and Technology (Gaithersburg, MD, USA).

[b] Total 34 PAHs is the sum of the 18 parent PAHs and the 16 groups of alkyl PAHs listed in U.S. EPA [2], total 13 PAHs is the sum of the 13 parent PAHs used for sediment surveys as described in U.S. EPA [2], total 16 PAHs is the sum of the 16 U.S. EPA priority pollutant PAHs, and the total parent PAHs is the sum of the 18 parent PAHs determined in the total 34 PAH method.

deuterated internal standard, fluorene-d_{10}, elutes in the middle of the C3-alkylnaphthalene cluster and has an approximately 15% ion at *m/z* 170, which is the same mass as monitored to determine the C3-alkylnaphthalene compounds. For the majority of the samples in the present study, this interfering peak from fluorene-d_{10} was substantially larger than the total C3-alkylnaphthalene peaks, and therefore, care was needed to remove its peak area to avoid grossly overestimating the C3-alkyl naphthalene concentrations. Careful monitoring of secondary confirmatory ions can help avoid such misidentifications [9]. In addition, we routinely analyzed representative extracts in the full MS scan mode and confirmed individual peak identities on the basis of their full mass spectra.

Calibration for alkyl PAHs

The method for determining the 34 PAHs includes a calibration standard for only five out of the 16 groups of alkyl PAHs measured by the method. For the remaining alkyl PAHs, the GC/MS response factor for the parent PAH is used; that is, the response factor of the alkyl PAH compared to the parent is assumed to be 1.0 [2,9]. Unfortunately, this approach can yield substantial errors in the concentrations of the more highly alkylated PAHs. As shown in Table 1, the C3 and C4 alkyl PAHs have GC/MS responses of only approximately 15 to 25% of their related parent PAH. Thus, using the response factor of the parent PAH for an alkyl PAH will underestimate the concentration of C3 and C4 alkyl PAHs by a factor of four to seven. Since the pyrogenic PAHs have very low concentrations of highly alkylated PAHs, this inaccuracy does not greatly affect the total concentrations or TU reported. However, for petrogenic PAHs, the use of appropriate response factors for alkylated PAHs is necessary to obtain reasonable total PAH concentrations and TU values as discussed here. In the absence of appropriate alkyl PAH calibration compounds, the values in Table 1 could be used to improve the estimate of alkyl PAH concentrations.

Alkyl PAHs from petrogenic and pyrogenic sources

A quantitative comparison of petrogenic and pyrogenic PAH distributions is given in Tables 1 and 2 for the petroleum crude oil, diesel fuel, and three representative MGP sediments that are contaminated with low, medium, and high levels of pyrogenic PAHs. All samples were analyzed to determine the total PAHs based on the 34 alkyl and parent PAH list, the 18 parent PAHs in the 34 list, and the 16 U.S. EPA priority pollutant PAHs. The number of TU (as calculated by the U.S. EPA's narcosis model [2]) was also determined for each sample.

For the crude oil, only 1% of the total 34 PAH concentration and 1.4% of the total TU are contributed by the 18 parent PAHs on that list, while the 16 groups of alkyl isomers account for approximately 99% of the total 34 PAH concentrations and TU. (Note that the 34 PAH method does not include any alkyl derivatives of five- and six-ring PAHs.) Similarly, the parent PAHs accounted for only 2.2% of the total 34 PAH concentrations and 2.7% of the total TU in diesel fuel (Table 2). In contrast, the parent PAHs in the pyrogenic MGP samples account for 35 to 42% of the total PAH concentrations and TU.

Table 3. Underestimation of alkyl polycyclic aromatic hydrocarbons alkyl PAH concentrations and toxic units using parent PAH response factors for alkyl PAH calibration. MGP = manufactured gas plant; NIST = National Institute of Standards and Technology (Gaithersburg, MD, USA)

	NIST crude oil	Diesel fuel	MGP-contaminated sediments		
			HD-5	HD-10	HD-22
Total 34 PAH concn. (μg/g)					
Based on measured RRFs[a]	1,000	1,000	2,232	232	22.7
Based on assumed RRFs[b]	250	293	1,240	141	13
Ratio assumed vs measured RRFs	25%	29%	56%	61%	57%
Total 34 PAH toxic units					
Based on measured RRFs[a]	138	159	333	33.4	2.96
Based on assumed RRFs[b]	38	49	61	7.2	0.59
Ratio assumed vs measured RRFs	27%	31%	61%	63%	57%

[a] Alkyl PAH concentrations are based on relative response factors (RRFs) from Table 1.
[b] Alkyl PAH concentrations are based on parent PAH relative response factors as suggested in reference [9].

It should be noted that different crude oil sources and the degree of weathering will affect the fraction of alkyl PAHs, and the alkyl fraction of 99% for NIST crude oil is only a representative value. However, it remains true that petrogenic PAHs are still highly dominated by alkyl PAHs regardless of the petroleum source or degree of weathering [15,16]. Clearly, any error in measuring alkyl PAH concentrations more profoundly affects the calculation of TU for the petrogenic crude oil than for the sediments contaminated with the pyrogenic MGP PAHs.

As described previously, the alkyl PAH concentrations were based on determining the GC/MS response factors using 22 standard alkyl isomers. However, the National Oceanic and Atmospheric Administration method [9] that is the basis for the 34 PAH determinations states, "The response factor of the alkyl homologues was assumed to be equal to that of respective unsubstituted parent compounds." In order to determine the effect of this assumption on alkyl PAH concentrations and TU compared to the concentrations based on our experimentally determined response factors, we redetermined the total concentrations and TU for the same four samples by using the National Oceanic and Atmospheric Administration suggested assumption. As shown in Table 3, using the assumption that the alkyl PAH response factors are the same as the parent PAH underestimated the actual total PAH concentrations and TU by a factor of four for the crude oil and by a factor of three for the diesel fuel. For the MGP sediments, the error is not so great, but the concentrations and TU are underestimated by about 40% by using the parent response factors to calibrate for the alkyl PAHs. These discrepancies lead to substantial underestimation of the alkyl PAH concentrations and related TU and will certainly lead to incompatible data among different laboratories, particularly for petrogenic PAHs.

These results also demonstrate the need to carefully evaluate historical total PAH data in the context of the analytical method used and the source of PAHs (whether pyrogenic or petrogenic). For example, the determination of the so-called total PAHs in the United States has often been based on the sum of the 16 priority pollutant PAHs listed by the U.S. EPA. If two sediments were contaminated at an actual total PAH concentration of 1,000 mg/kg with a petrogenic source such as the petroleum crude oil and a pyrogenic source of PAHs such as MGP processes, the total PAH concentrations reported based on the 16 priority pollutant PAHs would be very different. Based on the results in Table 2, the total concentration reported based on the U.S. EPA's priority pollutant PAH method would be fairly reasonable for the MGP-contaminated sample, that is, approximately 350 mg/kg versus the true value of 1,000 mg/kg. However, the value reported for crude oil and diesel-contaminated samples would be only 10 and 20 mg/kg, respectively, rather than the true value of 1,000 mg/kg. These results support the recent report that the ecological risks of PAHs at petroleum-contaminated sites are underestimated because of the reliance on PAH data based only on the U.S. EPA's 16 priority pollutant PAHs [7].

Correlation of parent and alkyl PAH concentrations for MGP site sediments

The PAH concentrations of the 45 sediments are summarized in Table 4. The sediments used in this study ranged from coarse sand to soft organic muck having total organic carbon values from 0.6 to 11 wt %. Total 34 PAH concentrations

Table 4. Summary of 45 sediment characteristics; TOC = total organic carbon

Sample location (all USA)	No. of sediments	Total 34 PAH concn. (μg/g)		Total 34 PAH toxic units		Range in TOC, (wt %)
		Range	Median	Range	Median	
Hudson River	13	23–11,400	232	0.9–512	4.2	1.8–10.3
Lower Hudson River	5	197–1,010	307	10.–49	15	2.7–3.4
Oneonta, NY	4	107–1,040	530	3.5–24	9	3.1–11.0
Boston, MA	5	76–515	130	1.9–7.7	2.9	5.1–9.3
Plattsburgh, NY	8	16–3,430	235	0.5–137	22	0.7–6.5
Troy, NY	10	10–2,290	323	0.7–122	20	0.6–4.8

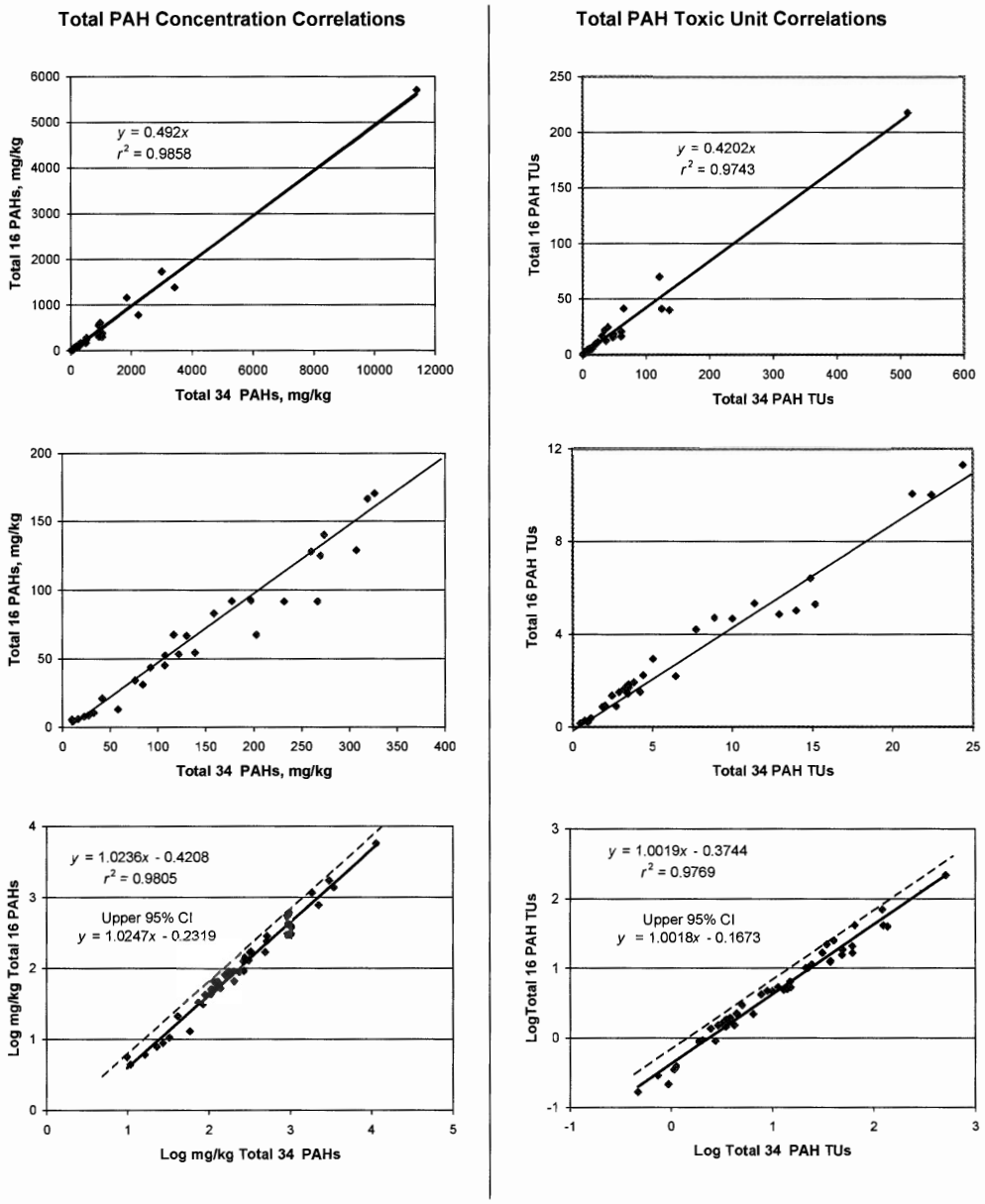


Fig. 2. Comparison of polycyclic aromatic hydrocarbon (PAH) concentrations (left side) and toxic units (right side) based on total 34 (alkyl and parent) PAHs and total 16 parent PAHs in 45 sediment samples from manufactured gas plant sites. The top two plots show linear correlations, with the center two plots showing an expanded scale of the top two plots. The bottom two plots show log-log correlations and the 95% upper confidence intervals (CI). TU = toxic units.

ranged from 10 to 11,400 μg/g, representing the range of near-ambient background concentrations to highly impacted. The individual PAH concentration reproducibility for replicate extractions and analyses of the sediments showed relative standard deviations typically 10 to 30% for quadruplicate samples. Several of the sediments had particles of pitch, bricks, and other residuals of the historic MGP industrial processes that contributed to the sediment heterogeneity.

As noted previously, the list of parent PAHs reported by various methods are not the same. The 34 PAH method includes the 18 parent PAHs listed in Table 1. The U.S. EPA's 16 priority pollutant PAHs include the same parent PAHs, with the exclusion of benzo[*e*]pyrene and perylene. Similarly, the 13 PAHs historically reported in sediment surveys [2] exclude those same two PAHs as well as indeno[1,2,3-*cd*]pyrene, dibenz[*a,h*]anthracene, and benzo[*ghi*]perylene. Fortunately, the concentrations of these excluded PAHs is typically low compared to the remaining parent PAHs, and their exclusion has little effect on either the total PAH concentrations or TU for either pore water or sediment as shown in Table 2. Therefore, the following discussions, unless otherwise noted, will be based on the 16 parent PAHs that are reported by the U.S. EPA's priority pollutant method. However, the same discussions apply whether the 18 or 13 parent PAHs are considered.

Even though the PAH concentrations found in the 45 sediments ranged over three orders of magnitude, the relative concentrations of alkyl versus parent PAHs remained quite constant for the sediment samples, as shown in the left side of Figure 2. The correlation coefficient (r^2) of the total 34 PAH concentrations with the sum of the 16 priority pollutant PAHs was 0.99 on a linear basis and 0.98 on a log basis. Similarly, the correlation of the TU calculated from the total 34 PAHs with the TU calculated only from the 16 parent PAHs is very high (r^2 = 0.97 on a linear basis and 0.98 on a log basis), as

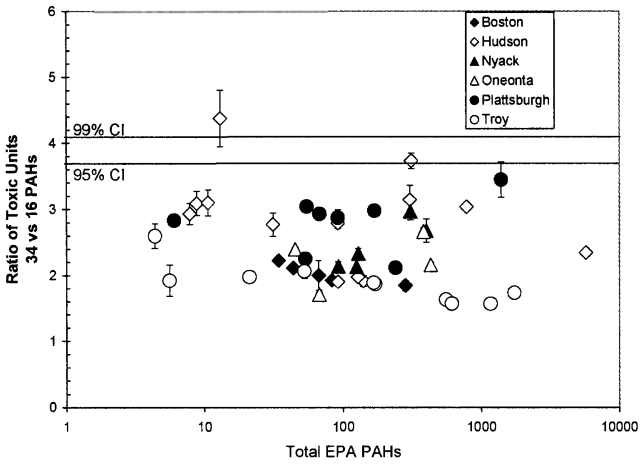


Fig. 3. Ratio of total toxic units based on the total 34 parent and alkyl polycyclic aromatic hydrocarbons (PAHs) compared to the total 16 parent PAHs for 45 sediments from manufactured gas plant sites, USA. CI = confidence intervals.

shown on the right side of Figure 2. (Note that the center plots on Fig. 2 use the same regression line as the top plots but only expand the axes so that the less contaminated samples can be observed.) Based on the strong correlations shown in Figure 2, a reasonable estimate of the total 34 PAH concentrations can be made for these 45 sediments by simply dividing the sum of the 16 parent PAH concentrations by the slope of the linear regression line (0.49).

Estimating toxic units from parent PAH concentrations

As shown in Figure 2, strong correlations exist between the sediment and pore-water TU calculated from the 34 alkyl and parent PAHs and those calculated from the 16 priority pollutant parent PAHs, indicating that predictions of total TU from only parent PAH data may be valid. In order to determine the accuracy of using parent PAHs to estimate the TU based on the 34 alkyl and parent PAHs, uncertainty factors were calculated for the total 34 PAH TU and for those based on the 16 parent PAHs for sediments from each sample site and sample location. As shown in Figure 3, the ratio of the TU from the 34 PAHs and the 16 PAHs were fairly similar for all 45 sediments from the six sites regardless of the level of contamination. A few samples exhibited higher ratios resulting from a greater contribution of alkylated PAHs associated with petroleum products used at the site. The presence of some petroleum contamination in these sediments was confirmed by GC/MS identification of petroleum alkanes in their extracts.

To further understand the sources of variability in the ratio of TU measured using 34 PAHs and 16 PAHs, an analysis of variance was conducted. This analysis demonstrated that both the sample location within a site and the location of the MGP site contributed significantly to the variability of the ratio. Approximately 36, 60, and 4%, respectively, of the variance was attributed to the MGP site location, sample location within the MGP site, and the replicate analysis of individual sediment samples.

As discussed earlier, data from 488 sediments was used by the U.S. EPA to determine that the number of total TU can be estimated within the 95% confidence interval by multiplying the number of TU calculated from the measurement of 13 parent PAHs (Table 1) by 11.5 [2]. However, the uncertainty factors calculated for the sites in the present study were considerably lower than that determined in the assessment of 488 samples by the U.S. EPA (Table 5), and the overall mean for the ratio of TU calculated from the measurement of 34 PAHs and 13 PAHs calculated in our study (2.9) compared favorably to that estimated in the study referenced by the U.S. EPA (2.8) [2]. The variability in the ratio was much smaller for MGP sites as evidenced by the lower values of upper 95 and 99% confidence intervals (4.2 and 4.6, respectively) compared to those reported by the U.S. EPA (11.5 and 16.9). The variability for total PAH concentrations is essentially the same as those shown in Table 5 for total TU; that is, the upper 95 and 99% confidence intervals for estimating the total 34 PAH concentrations were 3.5 and 3.9 for the 16 parent PAHs and 4.2 and 4.7 for the 13 parent PAHs. Based on these results and those shown in Figures 2 and 3, it seems reasonable to estimate total PAH concentrations and TU from the measurement of parent PAHs for sediments at sites known to be contaminated from MGP processes. However, it should be noted that any significant petroleum contamination could invalidate these estimates by increasing the proportion of alkyl PAHs relative to parent PAHs. As noted in the *Experimental* section, we routinely monitored petroleum alkanes in these samples, and no significant petroleum contamination was found at these MGP sites.

Table 5. Uncertainty factors for toxic units based on total 34 polycyclic aromatic hydrocarbons (PAHs) compared to 16 parent PAHs and 13 parent PAHs; CL = confidence limit

		34 PAHs vs 16 parent PAHs[a]			34 PAHs vs 13 parent PAHs[b]		
	n[c]	Mean	95% upper CL	99% upper CL	Mean	95% upper CL	99% upper CL
All sites (USA)	45	2.5	3.6	4.0	2.9	4.2	4.6
Hudson River	13	2.9	4.2	4.7	3.5	4.9	5.4
Lower Hudson River	5	2.5	3.2	3.4	2.9	3.4	3.6
Oneonta, NY	4	2.2	3.0	3.3	2.5	3.3	3.5
Boston, MA	5	2.0	2.3	2.4	2.5	2.9	3.0
Plattsburgh, NY	8	2.7	3.6	3.9	3.1	6.3	4.2
Troy, NY	10	1.9	2.5	2.6	2.2	3.0	3.2

[a] Toxic units based on the determination of 34 alkyl and parent PAHs versus the U.S. Environmental Protection Agency's 16 priority pollutants; PAHs determined by Method 8310 (http://www.epa.gov/SW-846/pdfs/8310.pdf).

[b] Toxic units based on the determination of 34 alkyl and parent PAHs versus the 13 parent PAHs used for correlation in U.S. EPA [2].

[c] Number of individual sediments from each site. Each sediment was analyzed in quadruplicate.

CONCLUSION

The accurate measurement of alkyl PAHs necessary to determine the total 34 PAH TU required for the U.S. EPA's narcosis risk model is hampered by the complex nature of the alkyl PAH distributions and by the lack of suitable standard alkyl PAH compounds. Assumptions made by different laboratories for alkyl PAH response factors will affect the reported concentrations (and associated TU), and the assumption that alkyl PAHs have the same GC/MS response factor as their parent PAH can result in an underestimation of total PAH concentrations and TU by as much as 75% for PAHs from petrogenic sources. The current approach of using the related parent PAH as a calibration standard results in artificially low values for the more highly alkylated PAHs, especially for petrogenic PAHs, since they are dominated by alkyl PAHs much more than pyrogenic PAHs. Since most historical sediment data includes only parent PAHs, predictive correlations are needed to estimate total TU from existing data. Although a high amount of uncertainty is associated with predicting the total PAH TU based on the measure of only parent PAHs for sediments contaminated from various PAH sources [2], the present study demonstrates that the uncertainty can be greatly reduced when the sediments are collected from areas containing primarily pyrogenic PAHs, such as those associated with MGP sites.

Acknowledgement—The financial support of the Gas Research Institute, New York State Electric and Gas, Niagara Mohawk Power, Northeast Gas Association, and the U.S. Department of Energy under Cooperative Agreement DE-FC26-98FT40321 is gratefully acknowledged. However, any opinions, findings, conclusions, or recommendations expressed herein are those of the authors and do not necessarily reflect the views of the sponsors.

REFERENCES

1. U.S. Environmental Protection Agency. 2004. Methods for the derivation of site-specific equilibrium partitioning sediment guidelines (ESGs) for the protection of benthic organisms: Nonionic organics. EPA/822/R/02/042. Office of Science and Technology, Washington, DC.
2. U.S. Environmental Protection Agency. 2003. Procedures for the derivation of ESBs for the protection of benthic organisms: PAH mixtures. EPA/600/R-02/013. Office of Research and Development, Washington, DC.
3. Di Toro DM, McGrath JA, Hansen DJ. 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. 1. Water and tissue. *Environ Toxicol Chem* 19:1951–1970.
4. Di Toro DM, McGrath JA. 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. II. Mixtures and sediments. *Environ Toxicol Chem* 19:1971–1982.
5. Kraaij R, Mayer P, Busser FJM, van Het Bolscher M, Seinen W, Tolls J, Belfroid AC. 2003. Measured pore-water concentrations make equilibrium partitioning work—A data analysis. *Environ Sci Technol* 37:268–274.
6. Electric Power Research Institute. 2000. Chemical source attribution at former MGP sites. 1000728. EPRI Technical Report. Palo Alto, CA, USA.
7. Barron MG, Holder E. 2003. Are exposure and ecological risks of PAHs underestimated at petroleum contaminated sites? *Human and Ecological Risk Assessment* 9:1533–1545.
8. Costa HJ, White KA, Ruspantini JJ. 2004. Distinguishing PAH background and MGP residues in sediments of a freshwater creek. *Environmental Forensics* 5:1–12.
9. U.S. Department of Commerce. 1998. Sampling and analytical methods of the national status and trends program. Mussel watch project: 1993–1996 update. NOAA Technical Memorandum NOS ORCA 130. National Oceanic and Atmospheric Administration, Silver Spring, MD.
10. U.S. Environmental Protection Agency. 2001. Methods for collection, storage and manipulation of sediments for chemical and toxicological analyses: Technical manual. EPA/823/B/01/002. Office of Science and Technology, Washington, DC.
11. Adams WJ, Burgess RM, Gold-Bouchot G, Leblanc L, Liber K, Williamson B. 2003. Porewater chemistry: Effects of sampling, storage, handling, and toxicity testing. In Carr RS, Nipper M, eds, *Porewater Toxicity Testing: Biological, Chemical, and Ecological Considerations.* SETAC, Pensacola, FL, USA, pp 95–124.
12. Littell R, Milliken GA, Stoup WW, Wolfinger RD. 1996. The variance in the TU uncertainty factor was evaluated using an unconditional hierarchical random effects model. In *SAS System for Mixed Models.* SAS Institute, Cary, NC, USA, pp 1–633.
13. Singer J. 1998. Using SAS PROC MIXED to fit multilevel models, hierarchical models, and individual growth models. *Journal of Educational and Behavioral Statistics* 24:323–355.
14. Hawthorne SB, Poppendieck DG, Grabanski CB, Loehr RC. 2002. Comparing PAH availability from manufactured gas plant soils and sediments with chemical and biological tests. 1. PAH release during water desorption and supercritical carbon dioxide extraction. *Environ Sci Technol* 36:4795–4803.
15. Douglas GS, Burns WA, Bence AE, Page DS, Boehm P. 2004. Optimizing detection limits for the analysis of petroleum hydrocarbons in complex environmental samples. *Environ Sci Technol* 38:3958–3964.
16. Page DS, Huggett RJ, Stegeman JJ, Parker KR, Woodin B, Brown JS, Bence AE. 2004. Polycyclic aromatic hydrocarbon sources related to biomarker levels in fish from Prince William Sound and the Gulf of Alaska. *Environ Sci Technol* 38:4928–4936.

Appendix. Polycyclic aromatic hydrocarbon (PAH) toxic units and detection limits

	Concn. for one toxic unit (μg/g OC)	Target detection limit[a] (μg/g sediment)	Method detection limit (μg/g sediment)
Naphthalene	385	0.11	0.001
2-Methylnaphthalene	444	0.13	0.001
1-Methylnaphthalene	444	0.13	0.001
C2 Naphthalenes	510	0.15	0.005
C3 Naphthalenes	581	0.17	0.01
C4 Naphthalenes	657	0.19	0.01
Acenaphthylene	452	0.13	0.001
Acenaphthene	491	0.14	0.001
Fluorene	538	0.16	0.001
C1 Fluorenes	611	0.18	0.005
C2 Fluorenes	686	0.20	0.01
C3 Fluorenes	769	0.23	0.03
Phenanthrene	596	0.18	0.001
Anthracene	594	0.17	0.001
C1 Phenanthrenes/anthracenes	670	0.20	0.005
C2 Phenanthrenes/anthracenes	746	0.22	0.01
C3 Phenanthrenes/anthracenes	829	0.24	0.02
C4 Phenanthrenes/anthracenes	913	0.27	0.03
Fluoranthene	707	0.21	0.001
Pyrene	697	0.21	0.001
C1 Fluoranthenes/pyrenes	770	0.23	0.005
Benz[*a*]anthracene	841	0.25	0.002
Chrysene	844	0.25	0.002
C1 Benz[*a*]anthracenes/chrysenes	929	0.27	0.01
C2 Benz[*a*]anthracenes/chrysenes	1,008	0.30	0.03
C3 Benz[*a*]anthracenes/chrysenes	1,112	0.33	0.05
C4 Benz[*a*]anthracenes/chrysenes	1,214	0.36	0.08
Benzo[*b*+*k*]fluoranthene[b]	980	0.29	0.002
Benzo[*e*]pyrene	967	0.28	0.002
Benzo[*a*]pyrene	965	0.28	0.002
Perylene	967	0.28	0.002
Indeno[1,2,3-*cd*]pyrene	1,115	0.33	0.002
Dibenz[*a*,*h*]anthracene	1,123	0.33	0.002
Benzo[*ghi*]perylene	1,094	0.32	0.002

[a] Target detection limits for both sediment and pore water are 1/34th of the concentration of each individual PAH that corresponds to one toxic unit as described in U.S. EPA [2]. Sediment values are based on 1% OC (organic carbon).

[b] Benzo[*b*]fluoranthene and benzo[*k*]fluoranthene are reported as their sum because of insufficient chromatographic resolution.

APPENDIX I

SOLID-PHASE MICROEXTRACTION MEASUREMENT OF PARENT AND ALKYL POLYCYCLIC AROMATIC BYDROCARBONS IN MILLILITER SEDIMENT PORE WATER SAMPLES AND DETERMINATION OF K_{DOC} VALUES

PUBLISHED IN *ENVIRONMENTAL SCIENCE TECHNOLOGY*

Environ. Sci. Technol. **2005**, *39,* 2795−2803

Solid-Phase Microextraction Measurement of Parent and Alkyl Polycyclic Aromatic Hydrocarbons in Milliliter Sediment Pore Water Samples and Determination of K_{DOC} Values

STEVEN B. HAWTHORNE,*,† CAROL B. GRABANSKI,† DAVID J. MILLER,† AND JOSEPH P. KREITINGER‡

Energy and Environmental Research Center, University of North Dakota, Box 9018, Grand Forks, North Dakota 58202, and The RETEC Group, Inc., Ithaca, New York 14850

The U.S. Environmental Protection Agency (EPA) narcosis model for benthic organisms in polycyclic aromatic hydrocarbon (PAH) contaminated sediments requires the measurement of 18 parent PAHs and 16 groups of alkyl PAHs ("34" PAHs) in pore water with desired detection limits as low as nanograms per liter. Solid-phase microextraction (SPME) with gas chromatographic/mass spectrometric (GC/MS) analysis can achieve such detection limits in small water samples, which greatly reduces the quantity of sediment pore water that has to be collected, shipped, stored, and prepared for analysis. Four sediments that ranged from urban background levels (50 mg/kg total "34" PAHs) to highly contaminated (10 000 mg/kg total PAHs) were used to develop SPME methodology for the "34" PAH determinations with only 1.5 mL of pore water per analysis. Pore water was obtained by centrifuging the wet sediment, and alum flocculation was used to remove colloids. Quantitative calibration was simplified by adding 15 two- to six-ring perdeuterated PAHs as internal standards to the water calibration standards and the pore water samples. Response factors for SPME followed by GC/MS were measured for 22 alkyl PAHs compared to their parent PAHs and used to calibrate for the 18 groups of alkyl PAHs. Dissolved organic carbon (DOC) ranging from 4 to 27 mg/L had no measurable effect on the freely dissolved concentrations of two- and three-ring PAHs. In contrast, 5−80% of the total dissolved four- to six-ring PAHs were associated with the DOC rather than being freely dissolved, corresponding to DOC/water partitioning coefficients (K_{DOC}) with log K_{DOC} values ranging from 4.1 (for fluoranthene) to 5.6 (for benzo[*ghi*]perylene). However, DOC-associated versus freely dissolved PAHs had no significant effect on the total "34" PAH concentrations or the sum of the "toxic units" (calculated by the EPA protocol), since virtually all (86−99%) of the dissolved PAH concentrations and toxic units were contributed by two- and three-ring PAHs.

Introduction

The U.S. Environmental Protection Agency (EPA) guidelines for protecting benthic organisms in polycyclic aromatic hydrocarbon (PAH) contaminated sediments are based on the concentrations of "freely dissolved" PAH concentrations in sediment interstitial water or "pore water" (*1*−*5*). The narcosis risk model uses the concentrations of "34" individual PAHs (18 two- to six-ring parent PAHs and 16 groups of prominent C_1 to C_4 alkyl derivatives) (*2*). The concentration of each PAH is then converted to a "toxic unit" on the basis of its partitioning behavior from water to an organism, and the risk to aquatic organisms is based on the sum of the individual PAH toxic units. Since higher molecular weight PAHs show stronger partitioning to biological lipids than lower molecular weight PAHs (which are more soluble in water), lower detection limits for higher molecular weight PAHs are required than for the lower molecular weight PAHs. The EPA provides a procedure for predicting PAH pore water concentrations based on an equilibrium partitioning model, but these predictions need to be validated and improved for the "34" PAHs by their measurement in field pore water samples (*1*, *2*).

The determination of polycyclic aromatic hydrocarbons (PAHs) in sediment pore water is complicated by several factors. Analytical sensitivities required to meet regulatory goals are in the low nanograms per liter range, which are difficult to achieve by conventional organic solvent extraction, even when large (liter) samples of pore water are obtained (*1*, *6*, *7*). Collecting, shipping, and storing such large samples under field conditions greatly increases the cost and complexity of site investigations. Filtering samples to obtain pore water and to remove colloidal material is not desirable because of potential losses of PAHs to the filtering material, and the use of centrifugation suggested by the EPA becomes increasingly difficult when liter samples of water must be generated from sediments, particularly when sandy material is collected (*2*, *6*, *7*). Routine use of in situ pore water samplers is also complicated by the problems of placement and recovery, long sampling times required, and lack of sufficient sensitivity for higher molecular weight PAHs (*1*, *2*, *6*, *7*). In addition, losses of hydrophobic compounds having log K_{OW} (octanol/water partitioning coefficient) values >4 can occur, which may make the use of such devices unsuitable for most PAHs since only the lowest molecular weight PAHs (naphthalene, alkyl naphthalenes, and acenaphthylene) have log K_{OW} values less than 4 (*3*, *4*, *6*−*8*).

Analytical methods for pore water PAH determinations must also be able to accommodate a very wide range in concentrations, both because the concentrations of interest for individual PAHs in pore water range from nanograms to milligrams per liter and because the saturation water solubilities of individual PAHs regulated by the EPA range from 0.2 μg/L for indeno[1,2,3-*cd*]pyrene to 31 000 μg/L (for naphthalene) (*9*). Thus, in a single water sample it may be necessary to determine milligram per liter concentrations of low molecular weight PAHs, and nanogram per liter concentrations of higher molecular weight PAHs.

Solid-phase microextraction (SPME) has been used for the determination of PAH concentrations in water samples (*5*, *8*, *10*, *11*), and has several attractive characteristics for determining pore water PAH concentrations. Since SPME is

* Corresponding author phone: (701)777-5256; fax: (701)777-5181; e-mail: shawthorne@undeerc.org.
† University of North Dakota.
‡ The RETEC Group, Inc.

10.1021/es0405171 CCC: $30.25 © 2005 American Chemical Society
Published on Web 03/18/2005

TABLE 1. PAH and Carbon Contents of Sediment Samples A−D and Associated Pore Water

	PAH concn, mg/kg dry wt[a]			
	sediment A	sediment B	sediment C	sediment D
2-ring	7.7	36.8	490	2903
3-ring	23.2	85.1	829	3761
4-ring	21.8	80.8	770	2299
5+6 ring	5.8	28.8	143	910
sum total "34" PAHs[b]	58	232	2232	9873
sum total "16" EPA PAHs[c]	13	92	779	4281
sediment TOC, dry wt %	8.5	2.9	3.3	4.6
sediment soot OC,[d] dry wt %	3.1	0.3	0.7	0.75
pore water DOC, mg/L				
after centrif	6.7	12.1	28	70
after centrif and flocc	3.7	5.1	17	27

[a] Individual concentrations of the "34" PAHs and standard deviations of quadruplicate analyses are given in Table S2, Supporting Information. [b] Total PAHs based on the sum of the18 parent and 16 groups of alkyl PAHs included in the total "34" PAH list (*2*). [c] Total PAHs based on the sum of the 16 parent PAHs as normally reported from EPA method 610. [d] Sediment soot carbon determined by 375 °C oxidation as described in ref 18.

an equilibrium (rather than an exhaustive) extraction method, the water sample size has little effect on the detection limit, regardless of the size of the PAH. For example, the mass of the five-ring PAH benzo[*a*]pyrene extracted by a 7 μm SPME fiber from a 1000 mL water sample is only about 1.5 times more than the mass extracted from a 1 mL sample, while there is essentially no change in the mass of naphthalene extracted from a 1 mL or 1000 mL water sample (*8*). However, SPME makes up for its inability to quantitatively extract large volumes of water by the fact that every molecule extracted by the SPME fiber is quantitatively transferred into the gas chromatograph, in contrast to solvent extracts where only a small fraction of the extracted analytes are introduced into the GC injection port. Since conventional quadrupole mass spectrometers can detect PAHs in the low picogram range, the potential detection limit of SPME for higher molecular weight PAHs from a 1 mL water sample is in the picograms per milliliter (nanograms per liter) range. In addition, the detection limits for individual PAHs are inversely related to their fiber/water partition coefficient, which is fortuitous since lower detection limits are required for higher molecular weight PAHs than for lower molecular weight PAHs (*2*).

The use of SPME for quantitative analyses is sometimes criticized because it is an equilibrium technique and because the actual time to come to equilibrium depends on the solute identity, the water and fiber sorbent volumes, and the mixing conditions (*8*, *10*). However, the use of suitable perdueterated PAHs (*d*-PAHs) as internal standards can compensate for these perceived disadvantages (*8*).

The present study developed a robust method for determining nanogram to milligram per liter pore water PAH concentrations that is also capable of determining the partitioning of PAHs between the freely dissolved and dissolved organic carbon (DOC) phases. Practical limitations on the method include the need for small sediment samples (to facilitate shipping and storage); the use of "certified clean" glassware for shipping, storage, and preparation steps; and sample preparation steps that are rapid enough to reduce sample storage time and to coincide with the analytical step.

Experimental Section

Sediment Samples. Sediment samples were collected with a Ponar grab sampler at 22 locations in a freshwater harbor near a former manufactured gas plant (MGP) site. Approximately 15 L each of sediment/water slurry was immediately transferred to a 20-L bucket, sieved through a 2-mm screen to remove debris, and briefly mixed before subsampling into new glass jars. Samples (ca. 200 g) were then cooled on ice in the dark and shipped to the laboratory by overnight air delivery. (The bulk of each sample was stored separately for subsequent biological testing.) Storage was at 4 °C in the dark. Four of the sediments representing the range of PAH concentrations found at the site were selected for developing and testing the SPME pore water method (Table 1). The sediment samples typically had ca. 50 wt % water as stored.

Pore Water Preparation. Pore water samples were prepared fresh daily as suggested by the EPA (*7*) by transferring ca. 40 mL of the sediment/water slurry to a "certified clean" 40 mL glass "VOA" vial and centrifuging for 30 min at 1000*g*. (Higher speed caused the glass vials to break.) This typically resulted in 10−15 mL of pore water that could be gently collected with a pipet. Flocculation of the water samples was performed twice with a 10 wt % solution of alum (aluminum potassium sulfate) added to the water at a 1:40 ratio. A few drops of 1 M NaOH was added and the vial was mixed to cause the flocculation. The vial was centrifuged again for 30 min and the supernatant water was collected with a pipet.

The pore water sample was then split into four 1.5 mL aliquots that were placed into new 2-mL silanized glass autosampler vials (Agilent, Wilmington, DE) containing a precleaned (sonicated overnight in acetone) 7-mm Teflon-coated stir bar, and the *d*-PAH internal standards were immediately added. The samples were then subjected to SPME analysis within a few minutes of preparation to ca. 4 h after preparation (for the fourth replicate sample). Daily blank and calibration water samples were prepared in the same manner with 1.5 mL of HPLC-grade water (Fisher Scientific, Pittsburgh, PA). Dissolved organic carbon (DOC) was determined by EPA method 5310C.

SPME and GC/MS Procedure. SPME analysis of the pore water samples was performed with commercially available PDMS [poly(dimethylsiloxane)] coated fibers from Supelco (Bellefonte, PA). SPME sorption was performed for 30 min. After the sorption period, the fiber was immediately desorbed into the GC/MS injection port in the splitless mode at 320 °C for 5 min. After an additional 10-min cleaning at 320 °C, sorption of the next water sample began. This sequence corresponded to the ca. 45 min GC/MS analysis time and allowed a new sample to be analyzed every 50 min.

Comparisons of commercially available fibers with 100 μm and 7 μm film thickness coatings of poly(dimethylsiloxane) (PDMS) showed little advantage in sensitivity for the higher molecular weight PAHs with the 100 μm (0.612 μL) fiber compared to the 7 μm (0.026 μL) fiber with the 30-min sorption time used. These results are consistent with early studies showing the 100 μm PDMS fiber requires longer

equilibration times and has fiber water distribution coefficients (K_D) that are lower than those of the 7 μm fibers for higher molecular weight PAHs (*8*, *10*, *11*). On the basis of reported apparent SPME K_D values determined with 5 h sorption times (*8*), the mass of three-ring and larger PAHs sorbed by the 100 μm fiber would only be ca. 1.5−2-fold more than that sorbed by the 7 μm fiber. In addition, PAH carryover and problems in obtaining low method blanks were substantially worse for the 100 μm fiber versus the 7 μm fiber. Thus, all subsequent studies were performed with the 7 μm fiber.

All analyses were performed with an Agilent model 5973 GC/MS equipped with a 60-m Agilent HP-5 MS column (0.25 μm film thickness, 250 μm i.d.) operated in the selected ion mode for the molecular ions of the target PAHs and *d*-PAHs. The oven temperature was held at 40 °C for 5 min during the SPME desorption, then programmed at 50 °C/min to 110 °C, followed by a temperature ramp of 12 °C/min to 320 °C (hold for 10 min).

Detection Limits and Calibration. The EPA's narcosis model predicts toxicity if the sum of the "toxic units" calculated for all "34" PAHs measured in a pore water sample meets or exceeds a value of 1. Therefore, the desired detection limits for individual PAH determinations were defined as the concentration of an individual PAH that would yield $^1/_{34}$ of a "toxic unit." (*2*). Under this definition, the required detection limit ranges from ca. 0.01 ng/mL for high molecular weight PAHs to a few nanograms per milliliter for low molecular weight PAHs (Table S1, Supporting Information). This distribution also mimics the relative concentrations of PAHs expected to be found in pore water, since lower molecular weight PAHs are more soluble and have lower K_{OC} values (*2*, *9*). Therefore, PAH calibration standards were prepared so that the distribution of individual PAH concentrations reflected the target detection limit. Because of the very wide range of concentrations needed, a separate calibration stock solution was prepared for low (naphthalene to pyrene) and for high (benz[*a*]anthracene and larger) molecular weight PAHs. Dilutions of these stock solutions were made in acetone for spiking into pure water for the SPME calibration standards so that no more than 20 μL of the diluted stock standards was needed for any of the calibration levels and so that no single PAH in the most concentrated water calibration standard exceeded its saturation solubility in water.

The individual PAH concentrations in the lowest calibration standard were typically at, or somewhat below, the target detection limit values (Table S1, Supporting Information), and ranged from ca. 0.001 ng/mL for the highest molecular weight PAHs to 2 ng/mL for naphthalene. Three-point standard curves (for benz[*a*]anthracene and larger PAHs) were generated from the concentrations of the lowest standard to 40-fold higher concentrations. Four-point standard curves (for pyrene and smaller PAHs) were generated starting with the lowest concentration standard and up to 800-fold higher. For phenanthrene and larger PAHs, the highest calibration standard contained approximately half the saturation solubility of the individual PAHs in water, while the concentration of lower molecular weight PAHs was substantially below their water saturation solubility.

Unless otherwise noted, all standard and sample peak areas were normalized to the 15 *d*-PAH internal standards ranging in size from naphthalene-d_8 to benzo[*ghi*]perylene-d_{12} (Table S1, Supporting Information), which were spiked into the water sample in 20 μL of acetone. When no deuterated analogue of a PAH was available, the *d*-PAH with the closest molecular structure was used (e.g., benzo[*a*]pyrene-d_{12} was used as the internal standard for benzo[*e*]pyrene, and the parent *d*-PAH was used for the related alkyl PAHs, except for 1-methylnaphthalene-d_{10}). Full calibration curves were determined periodically throughout the year-long study and were always found to be linear with essentially zero intercepts for each of the PAHs when the calibration peak area per nanogram of standard was normalized to the appropriate *d*-PAH internal standard. Therefore, routine analyses can be based on daily determinations of one of the middle concentration standards coupled with periodic verification of the full calibration curves.

While most parent PAHs (and many of their perdeuterated analogues) are available as pure standards, few alkyl PAHs are available, so their response must be estimated rather than measured in many cases. The calibration and quantitation of the more highly alkylated PAHs is increasingly complicated by the fact that a single group can have multiple alkylated isomers (even hundreds for C_3- and C_4-alkyl PAHs) that are listed as a single PAH in the total "34" PAH list. Finally, the MS response and the SPME sorption behavior of alkyl isomers will vary from isomer to isomer, and few SPME partitioning coefficients are available for alkyl PAHs.

In an effort to best determine and estimate the alkyl PAH response factors, we determined the SPME response (versus the appropriate parent *d*-PAH) of every alkyl PAH available as a pure standard from commercial sources. This attempt was complicated by the fact that stated purities of several of the alkyl PAHs were not accurate. Therefore, all of the alkyl PAHs we obtained from commercial sources were analyzed by GC/FID to determine their purity and by GC/MS to determine if their mass spectra were consistent with their reported identity. The chemical purity of the 22 alkyl PAHs tested ranged from ca. 70% to 98%, and these purities were used to correct their response factors. (Note that 1-methyl- and 2-methylnaphthalene were included in the parent PAH calibration standard discussed above.)

The 22 alkyl PAHs were prepared as a standard and were diluted in acetone to concentrations similar to those used for the preparation of the parent PAH calibration standards described above. This diluted alkyl PAH standard was then added to a normal water standard (containing the parent PAH calibration standard), and the combined water standard was analyzed by the SPME GC/MS procedure. The response factors for the alkyl PAHs were then normalized to those of their parent PAH [i.e., peak area per concentration (nanograms per milliliter) of the alkyl PAH divided by the peak area per concentration (nanograms per milliliter) of its parent PAH], to obtain the relative response factors, which include SPME partitioning and GC/MS response. The relative response factors determined range from 0.12 for C_4-phenanthrenes/anthracenes (i.e., the alkyl derivative is less sensitively detected by SPME-GC/MS than the parent phenanthrene) to 1.4 for C_2-naphthalenes (Table S1, Supporting Information). Since the alkyl standard stock solutions showed degradation of several individual species during the initial 1 month of calibration runs (compared to no changes in a year for the parent PAH solutions), their response factors were used in all future analyses to estimate the alkyl-PAH concentrations.

Results and Discussion

Four sediments were selected out of 22 sediments to represent the range of PAH concentrations found in the contaminated and urban background sediments. As shown in Table 1 (and Table S2, Supporting Information), the total "34" PAH concentrations varied from 58 to 10 000 mg/kg in the four samples.

Effect of Colloidal Material. Initial studies were performed to determine whether sufficient colloidal material remained in the pore water samples after centrifugation to cause losses of *d*-PAHs added as internal standards. All of the lower molecular weight *d*-PAHs (naphthalene-d_8, 1-methylnaphthalene-d_{10}, acenaphthene-d_{10}, fluorene-d_{10}, and phenan-

TABLE 2. Effect of Removing Colloids on Apparent Pore Water PAH Concentrations

	sediment C pore water concn (ng/mL)		
	before flocc (mean ± SD)	after flocc (mean ± SD)	% PAH on colloids
naphthalene	1589 ± 103	1561 ± 209	2
2-methylnaphthalene	499 ± 24	431 ± 84	14
1-methylnaphthalene	543 ± 55	438 ± 152	19
C2 naphthalenes	749 ± 82	425 ± 116	43
C3 naphthalenes	557 ± 54	210 ± 47	62
C4 naphthalenes	167 ± 14	34 ± 6	80
acenaphthylene	32 ± 13	16 ± 8	48
acenaphthene	351 ± 28	265 ± 94	24
fluorene	116 ± 9	80 ± 23	31
C1 fluorenes	78 ± 9	28 ± 7	64
C2 fluorenes	46 ± 2	10 ± 3	77
C3 fluorenes	31 ± 5	13 ± 5	57
phenanthrene	191 ± 3	122 ± 19	36
anthracene	57 ± 3	21 ± 4	63
C1 phenanthrenes/anthracenes	169 ± 12	41 ± 7	76
C2 phenanthrenes/anthracenes	110 ± 5	19 ± 3	83
C3 phenanthrenes/anthracenes	29.9 ± 2.2	2.2 ± 0.3	93
C4 phenanthrenes/anthracenes	14.4 ± 1.3	1.4 ± 0.8	90
fluoranthene	42.9 ± 2.8	9.5 ± 1.1	78
pyrene	45.1 ± 2.8	8.7 ± 1.0	81
C1 fluoranthenes/pyrenes	67.7 ± 3.5	3.5 ± 0.9	95
benz[*a*]anthracene	6.9 ± 0.3	0.5 ± 0.1	93
chrysene[a]	4.9 ± 0.2	0.5 ± 0.1	89
C1 chrysenes/benz[*a*]anthracenes	4.0 ± 0.3	0.3 ± 0.1	94
C2 chrysenes/benz[*a*]anthracenes	2.4 ± 0.4	ND	
C3 chrysenes/benz[*a*]anthracenes	ND	ND	
C4 chrysenes/benz[*a*]anthracenes	ND	ND	
benzo[*b*+*k*]fluoranthene	2.0 ± 0.2	0.12 ± 0.06	94
benzo[*e*]pyrene	1.2 ± 0.1	0.07 ± 0.02	94
benzo[*a*]pyrene	2.8 ± 0.3	0.11 ± 0.05	96
perylene[a]	0.45 ± 0.05	0.02 ± 0.01	95
indeno[1,2,3-*cd*]pyrene[a]	0.22 ± 0.05	0.01 ± 0.00	95
dibenz[*ah*]anthracene	0.18 ± 0.04	0.02 ± 0.01	91
benzo[*ghi*]perylene[a]	0.31 ± 0.06	0.02 ± 0.01	93
sum total "34" PAHs	5507	3742	32

[a] Concentrations determined before removal of the colloids exceeded their water solubilities for chrysene (2 μg/L), perylene (0.40 μg/L), indeno[1,2,3-*cd*]pyrene (0.19 μg/L), and benzo[*ghi*]perylene (0.26 μg/L) (*9*).

threne-d_{10}) showed the same concentration by SPME in pore water samples A, B, C, and D as was found in the standard water (within 90−110% based on a comparison of the raw peak areas). However, the loss of the higher molecular weight *d*-PAHs to colloidal material was substantial. Colloids sorbed 25−50% of the fluoranthene-d_{10} and pyrene-d_{10}, ca. 75−90% of the benz[*a*]anthracene-d_{12} and chrysene-d_{12}, and more than 90% of the 5- and 6-ring *d*-PAHs. Losses of the *d*-PAHs were the highest for pore water D, which had the highest DOC value (Table 1).

In contrast to the losses of added *d*-PAHs to colloidal material, the presence of colloids resulted in falsely high dissolved concentrations of higher molecular weight sample PAHs (based on external standard calibration). For example, the highly contaminated samples, pore waters C and D, showed several of the higher molecular weight PAHs to be present at concentrations higher than their saturation solubility concentrations (Table 2 and Table S3, Supporting Information). For these samples, particles could be seen adhered to the SPME fiber after analysis of their pore water, indicating that thermal desorption of the particles in the GC injection port could contribute to erroneously high pore water concentrations for the higher molecular weight PAHs. In addition, a second thermal desorption of these contaminated fibers (without exposure to a new sample) showed a similar distribution of the same higher molecular weight PAHs, also indicating that particles adhered to the SPME fiber were the source of erroneously high PAH values. Depletion of dissolved PAHs by the SPME fiber followed by desorption of colloid-associated PAHs has also been reported to cause falsely high values for higher molecular weight PAHs (*12*). In either case, for the proposed method described in the present work, removal of colloids is necessary to obtain accurate dissolved concentrations of higher molecular weight PAHs.

Removal of Colloids with Alum Flocculation. Hong et al. (*13*) determined that colloidal material could substantially contribute to erroneously high PAH concentrations in solvent-extracted pore water samples, and they investigated the use of alum flocculation to remove residual colloids after centrifugation. The flocculation method was validated by comparing the PAH concentrations in the water after flocculation to those obtained by using an air bridge and several months to obtain equilibrium. More recently, Ghosh further validated the use of flocculation by comparison to the air bridge technique for samples from the same site (pore water C) as were the subject of the present study [Upal Ghosh, personal communication].

Flocculation is simple to perform for the small samples used in our method, and its use has been validated to remove colloidal PAHs without changing the concentration of dissolved PAHs (*13*). Therefore, flocculation was evaluated by comparing the concentrations of PAHs in the four test samples before and after flocculation. Preliminary studies showed that two sequential flocculation steps removed a few percent more of the colloids from the water (based on DOC analysis of the produced water). Also, two flocculation steps yielded slightly lower remaining concentrations of the high molecular weight PAHs than one step, indicating some

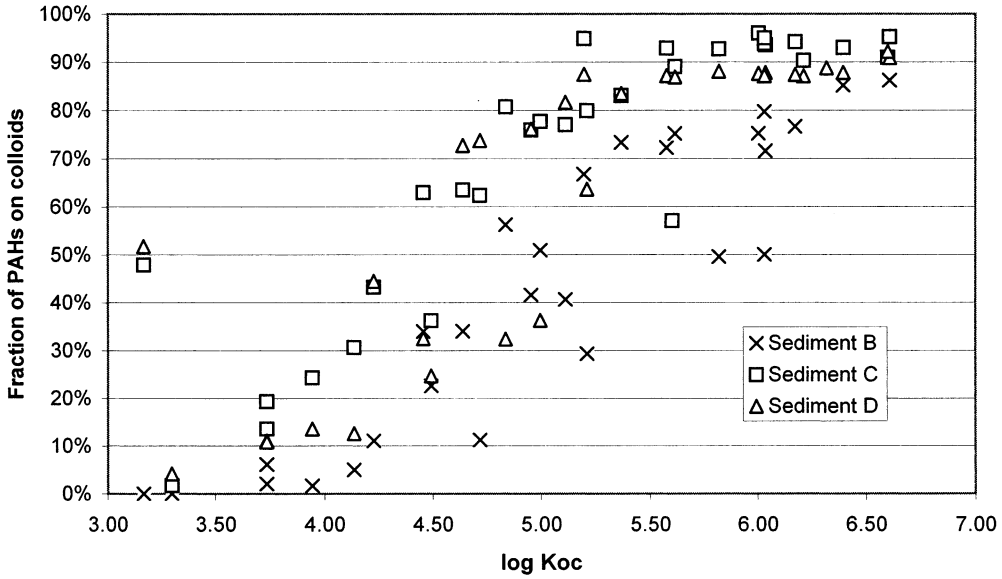


FIGURE 1. Fraction of individual PAHs associated with colloidal material removed by flocculation compared to their log K_{oc} values reported in ref 2.

remaining colloidal PAHs. A third flocculation did not change either the DOC or the concentrations of any of the PAHs (demonstrating that dissolved PAHs were not removed by the flocculation procedure), so it was concluded that two sequential flocculation steps were optimal for removing PAHs associated with colloids without affecting the dissolved PAH concentrations.

Generally speaking, the flocculation step removed ca. half of the measured DOC from the water sample (Table 1). (Note that, as discussed earlier, the samples were not filtered to remove colloids to prevent PAH losses, so the measured DOC before flocculation would include colloidal carbon.) The effect of flocculation on the concentrations of PAHs remaining in the pore water is shown in Table 2 and Table S3, Supporting Information. For pore water A, the sample with the lowest DOC after centrifugation, the flocculation had little or no detectable effect on the dissolved PAH concentrations (although the PAH concentrations were so low in this sample that small changes would be difficult to measure). However, the other three samples showed substantial changes in the concentrations of higher molecular weight PAHs after flocculation. For samples C and D, several of the higher molecular weight PAHs exceeded their water solubility limits before flocculation but were reduced to concentrations that did not exceed saturation after flocculation (Table 2 and Table S3, Supporting Information). As would be expected on the basis of their published K_{OC} values (*2*), the lower molecular weight PAHs (which have the highest water solubility and lowest K_{OC} values), are found in the water phase, while only a few percent are associated with the colloidal material (Figure 1). However, the fraction of PAHs associated with colloids (rather than dissolved in water) increases dramatically with molecular weight. Thus, ca. $^1/_4$ to $^1/_3$ of phenanthrene molecules in samples B, C, and D are found associated with colloids, and up to 96% of the higher molecular weight PAHs are found associated with colloidal material. Alkylated PAHs are also found to be more associated with the colloids than their parent PAHs (Table 2 and Table S3, Supporting Information), as would be expected since increasing alkylation increases their K_{OC} values (*2*).

These results clearly demonstrate that, after centrifugation, an additional step to remove colloids such as flocculation should be applied. Failure to remove the colloids before SPME (or solvent extraction) can have a significant effect on individual and total PAH concentrations.

Effect of Dissolved Organic Carbon on PAH Concentrations. According to the EPA protocol (*1*), PAHs in pore water samples are defined as existing in three separate phases, that is, associated with sediment particles and colloids, associated with the dissolved organic carbon (DOC), and dissolved in the water aside from the DOC (freely dissolved). After centrifugation and flocculation to remove the colloidal material, the PAHs are found partitioned between the water and the DOC. Poerschmann et al. (*14*, *15*) have demonstrated that *d*-PAHs added to a water sample become equilibrated with the DOC fraction in 1−2 min, and therefore, determining the concentration of an individual PAH based on its *d*-PAH analogue as an internal standard will represent the total PAH concentration in the water including the freely dissolved and DOC-associated PAHs. This sum will be referred to as the "total dissolved" concentration.

A comparison of the peak areas of the *d*-PAHs from the water calibration standards and pore waters A, B, C, and D shows that some loss of the higher molecular weight *d*-PAHs does occur to the DOC, but it is not significant for the lower molecular weight *d*-PAHs. On the basis of a comparison of the *d*-PAH peak areas compared to those found for the calibration standards by SPME analysis, partitioning of pyrene-d_{10}, fluoranthene-d_{10}, and all lower molecular weight *d*-PAH internal standards from the water to the DOC phases was insignificant, that is, the *d*-PAH peak areas in the four pore water samples ranged from 90% to 109% of those in the water calibration standards (typically within 1 standard deviation unit for quadruplicate analyses of each pore water sample). However, significant partitioning of the higher molecular weight *d*-PAHs from the freely dissolved phase to the DOC phase did occur, in agreement with earlier reports (*14*−*17*). For the four pore water samples, 25−40% of the benz[*a*]anthracene-d_{12} and chrysene-d_{12} partitioned to the DOC phase, while 40−60% of the five- and six-ring *d*-PAHs partitioned to the DOC phase for pore waters A, B, and C. However, pore water D (which has the highest DOC concentration) showed from 60% to 85% partitioning of the five- and six-ring *d*-PAHs to the DOC phase.

As previously discussed, quantitation of pore water PAHs by use of the full range of *d*-PAH internal standards gives the total dissolved concentrations in the combined freely dissolved and DOC phases (*14*, *15*). Since partitioning of lower molecular weight PAHs (naphthalene to fluoranthene and pyrene) to the DOC phase had no measurable effect on their

freely dissolved concentrations, the total dissolved and freely dissolved concentrations are effectively the same. Thus, the use of the analogous low molecular weight *d*-PAH internal standards gives the freely dissolved concentrations required by the EPA protocol. For the higher molecular weight PAHs, freely dissolved PAH concentrations can be calculated from the same analytical data set by basing the calibrations and sample concentration calculations on a lower molecular weight internal standard. Therefore, phenanthrene-d_{10} (which shows no measurable partitioning to the DOC phase) was used as the internal standard for all of the higher molecular weight PAHs to calculate their freely dissolved concentrations.

As shown in Table 3, the freely dissolved and total dissolved concentrations (freely dissolved plus DOC-associated) of the higher molecular weight PAHs are measurably different. Pore waters A, B, and C show as much as 3-fold higher concentrations of the five- and six-ring PAHs in the total dissolved versus freely dissolved fractions, and pore water D shows up to a 7-fold increase. On the basis of these data and the DOC concentrations (Table 1), the DOC/water partition coefficients (K_{DOC}) can be calculated for the higher molecular weight PAHs. As shown by representative four-, five-, and six-ring PAHs in Table 4, log K_{DOC} values range from 4.1 to 5.6 and are in good agreement with those reported by earlier investigators (*14*−*17*), thus validating the use of *d*-PAH internal standards with SPME to determine both the freely dissolved and total dissolved PAH concentrations from a single analysis.

While it is true that there are measurable differences in the freely dissolved and total dissolved (freely dissolved plus DOC-associated) concentrations of the higher molecular weight PAHs, the differences are insignificant when the sum total "34" PAH concentrations are compared (Table 3), since nearly all of the PAHs found in the pore water are two- or three-ring PAHs, which show no measurable partitioning into the DOC phase. For example, two- and three-ring PAHs account for 96% of the sum total "34" freely dissolved PAHs for the background sample A and 99% of the sum total "34" dissolved PAHs for the other three pore water samples (Table 3).

Effect of Dissolved Organic Carbon on Pore Water Toxic Units. According to the EPA protocol (*1*, *2*), risk from sediment PAHs to benthic organisms is based on the number of PAH "toxic units" freely dissolved in the sediment pore water. All PAHs are assumed to have the same toxicity (on a molar basis) in this model, and they differ only in their tendency to partition from pore water to the organism. In brief, the toxic units are calculated for each PAH from its measured freely dissolved concentration and its partitioning coefficient from water to an organism that is estimated from the PAH's octanol/water coefficient, K_{OW}, by the method of Di Toro et al. (*2*−*4*). These factors can be converted to a concentration value for each PAH. Because the partitioning coefficients increase dramatically with PAH molecular weight, freely dissolved concentrations of low molecular weight PAHs must be higher to represent 1 toxic unit than the concentrations required for high molecular weight PAHs. For example, the freely dissolved concentrations that represent 1 toxic unit range from 193 ng/mL for naphthalene to 10 ng/mL for pyrene and to 0.27 ng/mL for indeno[*1,2,3-cd*]pyrene (Table S1, Supporting Information). Thus, the toxic units contributed by each of the "34" PAHs is simply calculated by dividing its pore water concentration by the corresponding "concentration for one toxic unit" value from Table S1, Supporting Information.

Since much lower concentrations of high molecular weight PAHs contribute to the toxic units of a pore water than for the lower molecular weight PAHs, the distinction between total dissolved and freely dissolved PAH concentrations is potentially more important for the calculation of the sum of

TABLE 3. Freely Dissolved versus Total Dissolved Pore Water Concentrations and Toxic Units of Higher Molecular Weight PAHs[a]

	sediment A		sediment B		sediment C		sediment D	
	total	freely	total	freely	total	freely	total	freely
fluoranthene	0.14 ± 0.01	0.12 ± 0.01	0.99 ± 0.02	0.92 ± 0.02	9.5 ± 1.1	9.5 ± 1.1	26.9 ± 3.3	26.9 ± 3.3
pyrene	0.15 ± 0.04	0.12 ± 0.03	0.93 ± 0.01	0.85 ± 0.01	8.7 ± 1.0	8.7 ± 1.0	21.8 ± 0.5	21.7 ± 0.5
C1 fluoranthenes/ pyrenes	0.05 ± 0.01	0.04 ± 0.01	0.45 ± 0.06	0.42 ± 0.06	3.5 ± 0.9	3.5 ± 0.9	10.5 ± 1.9	10.4 ± 1.9
benz[*a*]anthracene	0.02 ± 0.00	0.01 ± 0.00	0.048 ± 0.002	0.032 ± 0.001	0.49 ± 0.11	0.36 ± 0.08	1.59 ± 0.09	0.95 ± 0.05
chrysene	0.02 ± 0.00	0.01 ± 0.00	0.049 ± 0.001	0.032 ± 0.001	0.53 ± 0.12	0.39 ± 0.09	1.17 ± 0.07	0.78 ± 0.05
C1 chrysenes/ benz[*a*]anthracenes	0.00 ± 0.00	0.00 ± 0.00	0.042 ± 0.025	0.029 ± 0.017	0.25 ± 0.10	0.19 ± 0.07	0.92 ± 0.06	0.55 ± 0.04
benzo[*b*+*k*]fluoranth ene	0.01 ± 0.00	0.01 ± 0.00	0.025 ± 0.006	0.012 ± 0.003	0.12 ± 0.06	0.07 ± 0.03	0.71 ± 0.05	0.29 ± 0.02
benzo[*e*]pyrene	0.01 ± 0.00	0.00 ± 0.00	0.014 ± 0.003	0.007 ± 0.002	0.07 ± 0.02	0.04 ± 0.01	0.48 ± 0.04	0.16 ± 0.01
benzo[*a*]pyrene	0.02 ± 0.01	0.01 ± 0.00	0.031 ± 0.004	0.015 ± 0.002	0.11 ± 0.05	0.07 ± 0.03	0.79 ± 0.07	0.26 ± 0.02
perylene	0.008 ± 0.000	0.004 ± 0.000	0.016 ± 0.012	0.009 ± 0.006	0.023 ± 0.011	0.014 ± 0.007	0.152 ± 0.016	0.051 ± 0.005
indeno[1,2,3-*cd*]pyrene	0.001 ± 0.000	0.001 ± 0.000	0.004 ± 0.001	0.002 ± 0.000	0.011 ± 0.005	0.005 ± 0.002	0.145 ± 0.019	0.022 ± 0.003
dibenz[*ah*]anthracene	0.008 ± 0.003	0.003 ± 0.001	ND	ND	0.016 ± 0.009	ND	0.131 ± 0.012	0.020 ± 0.002
benzo[*ghi*]perylene	0.006 ± 0.002	0.002 ± 0.001	0.009 ± 0.001	0.003 ± 0.000	0.022 ± 0.008	0.009 ± 0.003	0.287 ± 0.048	0.044 ± 0.007
sum total "34" PAHs	7.91	7.80	289.0	288.7	3742	3727	4196	4192
2+3-ring PAHs vs total concn, %	94.4	95.8	99.1	99.2	99.4	99.4	98.4	98.5
sum total toxic units (TU)	1.23	1.15	10.70	10.56	105	97	299	295
2+3-ring PAH TU vs total TU, %	86.4	92.1	94.8	96.0	96.0	96.1	94.8	96.2

[a] Total dissolved and freely dissolved pore water concentrations are given in nanograms per milliliter and are presented as mean ± SD. Total dissolved concentrations include the freely dissolved and the DOC-associated PAHs. There was no measurable difference in total dissolved and freely dissolved concentrations for lower molecular weight PAHs than those reported in the table.

TABLE 4. Experimental Dissolved Organic Carbon/Water Distribution Coefficients (log K_{DOC}) Values

	sediment A	sediment B	sediment C	sediment D	lit.	ref
fluoranthene	4.61	4.14			4.74	14
pyrene	4.79	4.26			4.83	14
benz[*a*]anthracene	5.37	4.96	4.31	4.40		
chrysene	5.23	5.01	4.31	4.27	5.04	16
benzo[*b*+*k*]fluoranthene	5.39	5.33	4.57	4.73		
benzo[*a*]pyrene	5.64	5.31	4.59	4.88	5.10	17
perylene	5.51	5.20	4.53	4.87		
benzo[*ghi*]perylene	5.62	5.58	4.88	5.31		

toxic units than the sum total PAH concentration for a pore water sample. However, as shown in Table 3, for these four samples, there is no significant effect on the sum of toxic units value regardless of whether the freely dissolved or total dissolved concentration is used.

Even though (on an equal concentration basis) lower molecular weight PAHs contribute much less to the toxic units than higher molecular weight PAHs, the pore water concentrations of two- and three-ring PAHs are so much higher than the larger PAHs that any differences caused by not accounting for DOC-associated PAHs is insignificant. This is equally true whether the sample is highly contaminated or has only background PAH concentrations. For example, determining the toxic units from the total dissolved concentration (i.e., ignoring the correction for DOC-associated PAHs) changes the sum of the toxic units for pore water C only from 295 to 299 (Table 3). Similarly, the sum of the toxic units calculated for the background sample A changes only from 1.15 (correctly calculated by use of the freely dissolved concentrations) to 1.23 when the DOC correction is ignored (Table 3).

Sediment/Pore Water Storage Stability. Ideally, a colloid-free pore water sample could be prepared in the field and shipped in a stable form to the lab for analysis. However, previous studies have demonstrated substantial losses (as much as 50−70%) of PAHs during transport and short-term storage of water samples, even when the samples are stored in the dark for as little as 48 h in silanized glass vials (*6*, *8*). In a detailed review of methods to determine sediment pore water PAH concentrations, Adams et al. (*6*) concluded, "that it is preferable to store pore water with its associated sediment, either in the form of a sediment core or a grab in a sealed container filled to the top with zero headspace, at 4 °C in the dark", and these recommendations were followed in our study. However, since changes in PAH concentrations could still occur (e.g., from vaporization of the lower molecular weight PAHs or from enhanced biodegradation as the sediment slurries are exposed to air during collection and homogenization), five samples collected during a 6-month sampling period were analyzed twice, that is, upon receipt at the laboratory and after a week or two of additional storage of the sediment/water slurry. In each case, fresh pore water was generated from the sediment/water slurry by the centrifugation and flocculation procedure on each day of pore water analyses.

Fortunately, no significant differences in PAH concentrations were found in the pore water samples before and after the additional storage time, i.e., the pore water concentrations of the individual PAHs in the fresh and stored samples were virtually the same within the analytical reproducibility of the SPME method as shown for sediment E in Table S4, Supporting Information. Although these results cannot guarantee that PAH concentrations are stable between sampling and transport to the laboratory, they do indicate that the pore water PAH concentrations remain stable for at least weeks when the sediment/water slurry is stored in accordance with the recommendations of Adams et al. (*6*).

Practical Considerations of the Method. As noted above, centrifugation at 1000*g* for 30 min was not sufficient to remove colloids and their associated PAHs. Although increasing the centrifugation speed may yield better colloid removal, the simple flocculation step allowed all storage and preparation steps to be performed with readily available disposable "certified clean" glassware that is commonly used in trace environmental studies. In addition, after implementation of the flocculation step, the robustness of the SPME fibers increased dramatically. Without flocculation, the usefulness of a fiber could be ruined by exposure to colloids in only one highly contaminated pore water sample. However, with flocculation only two fibers were used for the analysis of ca. 500 samples, standards, and blanks over a 1-year study without significant loss of method sensitivity or precision.

The use of *d*-PAH internal standards also enhanced the reproducibility of replicate analyses and greatly increased the stability of the quantitative calibration curves. With external standard calibration, changes in fiber capacity and MS response caused the calibration factors (e.g., peak area per nanogram of standard PAH in the calibration water) to vary by a factor of ca. 20−30 over the 1-year study. However, quantitative calibration curves based on the *d*-PAH internal standards were very stable over the 1-year study, as evidenced by the variation in the calibration PAH response factors (as normalized to the appropriate *d*-PAH internal standards) was only by 3−10% for the two-, three-, and four-ring PAHs and only by ca. 5−20% for the five- and six-ring PAHs over the 1-year study. With the proper precautions to maintain low SPME method blanks (discussed below), the detection limits also remained stable over the study. This stability in calibration utilizing the *d*-PAH internal standards significantly reduces the need for extensive daily calibration runs that are required when external standard calibration is used with SPME.

Since the GC/MS used in these analyses was capable of detecting low picogram levels of injected PAHs, obtaining good SPME blanks was critical to determining the overall method detection limits. Even with a new fiber, some low- and mid-molecular weight PAHs (primarily naphthalene, phenanthrene, fluoranthene, and pyrene) were present as background regardless of the amount of thermal cleaning used (as much as 6 h at 320 °C). It should also be noted that the SPME blanks should be prepared with pure water since the mass of background PAHs was higher than SPME blank analyses with no exposure to water. (The blank PAH background species were apparently not contributed by the water, since their amounts varied from fiber to fiber.) During pore water analyses, method blanks were routinely performed twice a day from pure water spiked with the same *d*-PAH internal standards as were used for the pore water samples. Any carryover of PAHs from the stir bars is also determined by method blanks and also found not to be measurable after the 14-h acetone sonication cleaning procedure. However, as an added precaution, stir bars were routinely discarded after exposure to the more highly contaminated samples.

TABLE 5. Effect of Ambient Air Contamination on Apparent PAH Concentrations in Pore Water from Sediment F

	mean ± SD,[b] ng/mL	
	high blank[a]	normal blank[a]
naphthalene	27.4 ± 1.1	29.4 ± 0.6
2-methylnaphthalene	4.4 ± 0.2	4.5 ± 0.1
1-methylnaphthalene	6.0 ± 0.3	5.8 ± 0.1
C2 naphthalenes	17.7 ± 2.0	13.3 ± 1.6
C3 naphthalenes	64.4 ± 19.0	5.3 ± 0.3
C4 naphthalenes	84.4 ± 18.5	ND
acenaphthylene	0.52 ± 0.17	0.62 ± 0.06
acenaphthene	6.96 ± 0.15	6.86 ± 0.03
fluorene	2.37 ± 0.13	1.94 ± 0.03
C1 fluorenes	5.06 ± 1.35	1.06 ± 0.11
C2 fluorenes	7.96 ± 1.49	0.90 ± 0.19
C3 fluorenes	22.08 ± 3.54	ND
phenanthrene	3.83 ± 0.36	2.43 ± 0.02
anthracene	0.47 ± 0.03	0.34 ± 0.01
C1 phenanthrenes/anthracenes	4.95 ± 1.17	1.15 ± 0.02
C2 phenanthrenes/anthracenes	5.81 ± 0.74	0.64 ± 0.11
C3 phenanthrenes/anthracenes	1.92 ± 0.50	0.60 ± 0.10
C4 phenanthrenes/anthracenes	0.79 ± 0.21	ND
fluoranthene	0.39 ± 0.03	0.36 ± 0.01
pyrene	0.35 ± 0.02	0.31 ± 0.00
C1 fluoranthenes/pyrenes	0.26 ± 0.04	0.17 ± 0.01

[a] Pore water from sediment F was analyzed on a day with high background ambient air levels of alkyl PAHs and on a day with normal background ambient air levels. [b] Concentrations and standard deviations are based on analyses of quadruplicate pore water samples.

Daily method blanks are also critical because of the potential for contamination of the fiber from ambient air. This problem was first identified when the concentrations of alkyl-substituted low molecular weight PAHs were substantially higher than expected in three different pore water samples and in the method blanks performed on the same days. Each of these days was associated with very hot days with south winds. On such days, an 1800 kW diesel generator is used to provide peak electrical power, and it is located ca. 100 m upwind of the laboratory. Since the distribution of alkyl PAHs is consistent with diesel exhaust, the contamination of the fiber with the alkyl PAHs was apparently from the generator exhaust. Table 5 shows the concentrations of the major parent and alkyl PAHs determined in a pore water sample from sediment F on a day when the generator was running and 9 days later when it was not used. The high concentrations of the alkyl PAHs found on the first day emphasize the need for daily method blanks. Since this observation was made, contamination from ambient air organics has been avoided by storing the fiber with the sheath needle inserted into an empty autosampler vial capped with a septum.

As shown in Table 2, the range in PAH concentrations in sediment pore waters can vary by more than 1000-fold. When the SPME fiber is exposed to high concentration samples, some carryover is possible even with the 5 min analytical desorption and the 10-min cleaning routinely performed. Therefore, it is also important to perform method blanks after pore water samples that have very high PAH concentrations are analyzed. With these precautions, the detection limits ranging from 0.002 to 0.5 ng/mL (Table S1, Supporting Information), have been routinely achieved with 1.5 mL water samples during 1 year of performing pore water analyses.

With proper care to remove colloids, to obtain low method blanks, and to base quantitative determinations on *d*-PAH internal standards, SPME provides a robust method to determine pore water PAH concentrations. The method is capable of determining nanogram per liter (part per trillion) concentrations of individual PAHs and can accurately measure individual PAH concentrations up to milligram per liter concentrations. Only 1.5 mL of pore water is needed per determination, which reduces the effort and cost in collecting, processing, shipping, and storing the large numbers of samples required for site surveys. A similar approach should also be applicable to other hydrophobic organics such as PCBs, and the recent introduction of commercial autosamplers makes the use of this method for routine analyses more favorable.

Regardless of whether PAHs were determined in the background pore water sample or in highly contaminated samples, the fraction of the two- and three-ring PAHs found in the DOC phase was insignificant (as compared to freely dissolved PAHs), but as much as 60−80% of the six-ring PAHs were found associated with the DOC. Therefore, differentiating freely dissolved versus total dissolved (freely dissolved plus DOC-associated) PAHs is important only for the individual four- to six-ring PAHs. However, since the lower molecular weight PAHs are present in pore water at much higher concentrations than the higher molecular weight PAHs, there is no significant difference in the sum total of the "34" PAH concentrations or the regulatory sum of the "toxic units" regardless of whether total dissolved or freely dissolved PAH concentrations are determined.

Acknowledgments

The financial support of the Gas Research Institute, Niagara Mohawk Power Corporation, and the U.S. Department of Energy under Cooperative Agreement DE-FC26-98FT40321 is gratefully acknowledged. However, any opinions, findings, conclusions, or recommendations expressed herein are those of the authors and do not necessarily reflect the views of the sponsors.

Supporting Information Available

Tables showing parent and alkyl PAH detection limits, toxic unit equivalents, and SPME-GC/MS relative response factors; sediment concentrations; effect of removing colloids from pore water on PAH concentrations, and pore water PAH concentrations (sediment E) before and after 14 days of storage. This information is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) U.S. Environmental Protection Agency. *Methods for the derivation of site-specific equilibrium partitioning sediment guidelines (ESGs) for the protection of benthic organisms: Nonionic organics*; EPA/822/R/02/042; Office of Science and Technology: Washington, DC, 2004.

(2) U.S. Environmental Protection Agency. *Procedures for the derivation of ESBs for the protection of benthic organisms: PAH mixtures*; EPA/600/R-02/013; Office of Research and Development: Washington, DC, 2003.

(3) Di Toro, D. M.; McGrath, J. A.; Hansen, D. J. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. 1. Water and tissue. *Environ. Toxicol. Chem.* **2000**, *19*, 1951−1970.

(4) Di Toro, D. M.; McGrath, J. A. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. II. Mixtures and sediments. *Environ. Toxicol. Chem.* **2000**, *19*, 1971−1982.

(5) Kraaij, R.; Mayer, P.; Busser, F. J. M.; van Het Bolscher, M.; Seinen, W.; Tolls, J.; Belfroid, A. C. Measured pore-water concentrations make equilibrium partitioning work—a data analysis. *Environ. Sci. Technol.* **2003**, *37*, 268−274.

(6) Adams, W. J.; Burgess, R. M.; Gold-Bouchot, G.; Leblanc, L.; Liber, K.; Williamson, B. Porewater chemistry: Effects of sampling, storage, handling, and toxicity testing. In *Porewater Toxicity Testing: Biological, Chemical, and Ecological Considerations*; Carr, R. S., Nipper, M., Eds.; Society for Environmental and Toxicological Chemistry: 2003; pp 95−124; ISBN 1-880611-65-1.

(7) U. S. Environmental Protection Agency. *Methods for collection, storage and manipulation of sediments for chemical and*

toxicological analyses: Technical manual; EPA-823-B-01-002; Office of Science & Technology: Washington, DC, 2001.
(8) Langenfeld, J. J.; Hawthorne, S. B.; Miller, D. J. Quantitative analysis of fuel-related hydrocarbons in surface water and wastewater samples by solid-phase microextraction. *Anal. Chem.* **1996**, *68*, 144−155.
(9) Mackay, D.; Shiu, W. Y. Aqueous solubility of polynuclear aromatic hydrocarbons. *J. Chem. Eng. Data* **1977**, *22* (4), 399−402.
(10) Paschke, A.; Popp, J. Solid-phase microextraction fibre−water distribution constants of more hydrophobic organic compounds and their correlations with octanol−water partition coefficients. *J. Chromatogr. A* **2003**, *999*, 35−42.
(11) Pörschmann, J. Sorption of hydrophobic organic compounds on nonpolar SPME fibers and dissolved humic organic matter − Part III: Application of the solubility parameter concept to interpret sorption on solid-phase microextraction (SPME) fiber coatings. *Microcolumn Sep.* **2000**, *12*, 603−612.
(12) Heringa, M.; Hermens, J. Measurement of free concentrations using negligible depletion-solid-phase microextraction. *Trends Anal. Chem.* **2003**, *22*, 575−587.
(13) Hong, L.; Ghosh, U.; Mahajan, T.; Zare, R. N.; Luthy, R. G. PAH sorption mechanism and partitioning behavior in Lampblack-impacted soils from former oil-gas plant sites. *Environ. Sci. Technol.* **2003**, *37*, 3625−3634.
(14) Poerschmann, J.; Zhang, Z.; Kopinke, F.-D.; Pawliszyn, J. Solid-phase microextraction for determining the distribution of chemicals in aqueous matrices. *Anal. Chem.* **1997**, *69*, 597−600.
(15) Poerschmann, J.; Kopinke, F.-D.; Pawliszyn, J. Solid-phase microextraction for determining the binding state of organic pollutants in contaminated water rich in humic organic matter. *J. Chromatogr. A* **1998**, *816*, 159−167.
(16) Pörschmann, J.; Kopinke, F.-D. Sorption of very hydrophobic organic compounds (VHOCs) on dissolved humic organic matter (DOM). 2. Measurement of sorption and application of a Flory−Huggins concept to interpret the data. *Environ. Sci. Technol.* **2001**, *35*, 1142−1148.
(17) Akkanen, J.; Kukkonen, J. V. K. Measuring the bioavailability of two hydrophobic organic compounds in the presence of dissolved organic matter. *Environ. Toxicol. Chem.* **2003**, *22*, 518−524.
(18) Accardi-Dey, A.; Gschwend, P. Assessing the combined roles of natural organic matter and black carbon as sorbents in sediments. *Environ. Sci. Technol.* **2002**, *36*, 21−29.

Received for review September 17, 2004. Revised manuscript received December 22, 2004. Accepted January 10, 2005.

ES0405171

APPENDIX J

GREATLY REDUCED BIOAVAILABILITY AND TOXICITY OF POLYCYCLIC AROMATIC HYDROCARBONS TO *HYALELLA AZTECA* IN SEDIMENTS FROM MANUFACTURED-GAS PLANT SITES

PUBLISHED IN *ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY*

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 26, No. 6, pp. 1146–1157, 2007
© 2007 SETAC
Printed in the USA
0730-7268/07 $12.00 + .00

GREATLY REDUCED BIOAVAILABILITY AND TOXICITY OF POLYCYCLIC AROMATIC HYDROCARBONS TO *HYALELLA AZTECA* IN SEDIMENTS FROM MANUFACTURED-GAS PLANT SITES

JOSEPH P. KREITINGER,*† EDWARD F. NEUHAUSER,‡ FRANCIS G. DOHERTY,§ and STEVEN B. HAWTHORNE‖
†Institute for Comparative and Environmental Toxicology and Department of Soil, Crop, and Atmospheric Sciences, Cornell University, Ithaca, New York 14851, USA
‡National Grid, 300 Erie Boulevard West, Syracuse, New York 13202, USA
§AquaTox Research, 1201 East Fayette Street, Syracuse, New York 13210, USA
‖Energy and Environmental Research Center, University of North Dakota, Box 9018, Grand Forks, North Dakota 58202, USA

(*Received* 24 *April* 2006; *Accepted* 29 *November* 2006)

Abstract—The toxicity of polycyclic aromatic hydrocarbons (PAHs) to *Hyalella azteca*, was measured in 34 sediment samples collected from four manufactured-gas plant (MGP) sites ranging in total PAH_{16} (sum of 16 U.S. Environmental Protection Agency priority pollutant PAHs) concentrations from 4 to 5,700 mg/kg, total organic carbon content from 0.6 to 11%, and soot carbon from 0.2 to 5.1%. The survival and growth of *H. azteca* in 28-d bioassays were unrelated to total PAH concentration, with 100% survival in one sediment having 1,730 mg/kg total PAH_{16}, whereas no survival was observed in sediment samples with concentrations as low as 54 mg/kg total PAH_{16}. Twenty-five of the 34 sediment samples exceeded the probable effects concentration screening value of 22.8 mg/kg total PAH_{13} (sum of 13 PAHs) and equilibrium partitioning sediment benchmarks for PAH mixtures (on the basis of the measurement of 18 parent PAHs and 16 groups of alkylated PAHs, [PAH_{34}]); yet, 19 (76%) of the 25 samples predicted to be toxic were not toxic to *H. azteca*. However, the toxicity of PAHs to *H. azteca* was accurately predicted when either the rapidly released concentrations as determined by mild supercritical fluid extraction (SFE) or the pore-water concentrations were used to establish the bioavailability of PAHs. These results demonstrate that the PAHs present in many sediments collected from MGP sites have low bioavailability and that both the measurement of the rapidly released PAH concentrations with mild SFE and the dissolved pore-water concentrations of PAHs are useful tools for estimating chronic toxicity to *H. azteca*.

Keywords—Polycyclic aromatic hydrocarbon Manufactured-gas plant *Hyalella azteca* Pore water Supercritical fluid extraction

INTRODUCTION

Remediation and management of contaminated sediments is often technically difficult and can be very expensive when large volumes of contaminated material require treatment. The National Research Council has recently reviewed the implications and science regarding the bioavailability of contaminants in sediments and has determined that there is a need to improve risk-based assessments by including more explicit consideration of bioavailability processes [1]. Inadequate scientific understanding has hampered the widespread consideration of bioavailability processes in remedial decision making to date. In their report, the National Research Council recommends specific steps that can be taken to improve the understanding of bioavailability processes at individual sites for use in regulatory decision making.

The technical approach for assessing risk to aquatic life from complex mixtures of polycyclic aromatic hydrocarbons (PAHs) is not well defined and is being debated at a national level. To address this debate, the U.S. Environmental Protection Agency (U.S. EPA) has developed equilibrium partitioning sediment benchmarks (ESBs) for PAH mixtures that are designed to protect benthic aquatic invertebrate organisms [2]. These benchmarks incorporate equilibrium partitioning theory (EqP) and the hydrocarbon narcosis model of toxicity [3,4]. The U.S. EPA's approach for deriving ESBs is based on the observation that the toxicity of organic contaminants in sediment is more closely related to the measurement of 34 PAHs (18 parent PAHs and 16 groups of alkylated PAHs designated as PAH_{34}) in sediment (expressed either on a sediment organic carbon basis or as that measured as freely dissolved in pore water) than to the concentration in bulk sediment. However, to support the use of U.S. EPA's ESB approach and to demonstrate why they are more appropriate than conservative empirical methods, a database is needed that incorporates measures of PAH bioavailability.

Characterization of the biological and chemical availability of PAHs in sediments has indicated that in certain environments the aqueous concentrations and toxicity of PAHs are much lower than previously assumed. Numerous reports have been published showing that some sediments have high concentrations of PAHs but lack observable toxicity to aquatic organisms [5–10]. For example, no correlation could be shown between the concentration of total PAHs in sediments and toxicity to the marine amphipod *Rhepoxynius abronius* at an aluminum smelter in British Columbia, Canada, despite PAH concentrations up to 10,000 mg total PAH/kg sediment [9]. Similar results on PAH bioavailability have recently been published comparing the predicted and observed uptake of PAHs by the freshwater aquatic invertebrate *Lumbriculus variegatus* in contaminated sediments collected near Superior, Wisconsin (USA) [10]. In this study, standard default assumptions for

* To whom correspondence may be addressed (jkreitinger@retec.com).

Any opinions, findings, conclusions, or recommendations expressed herein are those of the authors and do not necessarily reflect the views of the sponsors.

estimating PAH exposure to this aquatic invertebrate resulted in an overestimate of chemical uptake by 50- to well over 1,000-fold. However, predictions of PAH uptake were greatly improved when the pore-water concentration of chemicals was used to estimate contaminant bioavailability.

The observed lack of toxicity at high sediment PAH concentrations is believed to be associated with reduced exposure resulting from the stronger sorption of PAHs to sediment organic carbon than is normally assumed. It is believed that the PAHs sorbed to anthropogenic sources of ''black'' or ''soot'' carbon (e.g., charcoal, soot, coal or coke fines, coal tar pitch) are more strongly sorbed and less bioavailable than PAHs associated with natural sources of ''soft'' organic carbon (e.g., natural organic matter) [11–20]. A number of methods have been proposed for evaluating the bioavailability of pollutants in soils and sediments, including the analysis of pore water [21,22] and mild supercritical carbon dioxide extraction (SFE) [23]. The rapidly released concentrations of PAHs by SFE have been correlated previously with aqueous desorption, field biodegradation, uptake of PAHs by earthworms, and the potential for dermal and gut uptake by humans [23–26].

The objective of this study was to evaluate the feasibility of estimating PAH bioavailability in aquatic sediments as a means to improve predictions of toxicity to benthic aquatic organisms. Two different methods for determining bioavailable PAHs were evaluated for their ability to improve predictions of PAH toxicity to *Hyalella azteca* exposed to 34 sediment samples collected from four MGP sites. These methods included the analyses of dissolved PAHs in interstitial sediment pore water and analyses of the rapidly released concentration of PAHs in sediment by mild SFE. *Hyalella azteca* was selected as the test organism because it is sensitive to narcotic contaminants such as petroleum hydrocarbons and PAHs, ecologically important, typically in direct contact with sediment, and tolerant of a wide range of physical/chemical sediment properties [2,27]. The pore water and SFE rapidly released concentrations of PAHs were used as input data for the equilibrium partitioning and hydrocarbon narcosis models to develop predictions of sediment toxicity. These predictions were then compared with chronic toxicity to *H. azteca* in 28-d bioassays.

MATERIALS AND METHODS

Sediment samples

Thirty-four sediment samples were collected from four former MGP sites; two sites were located adjacent to the Hudson River (Hudson and Troy, NY, USA), a third site was located adjacent to Lake Champlain and Saranac River (Plattsburgh, NY, USA), and a fourth site was located adjacent to a small creek in central New York (Oneonta, NY, USA). Gas manufacturing from coal occurred at these sites from approximately 50 to 140 years ago, and all sites had ceased MGP operations approximately 50 to 70 years ago. Sediment samples were collected with a Ponar grab sampler or with a shovel. Approximately 15 L of sediment was transferred to a 20-L bucket, sieved through a 2-mm screen to remove debris, and briefly mixed. After homogenization, sediment samples were placed into new 64-oz (~175-ml) glass jars with Teflon® lined lids and stored in the dark at 4°C for less than 28 d before testing. Each sediment sample was initially screened for its concentration of total PAH_{34} and total organic carbon (TOC) content to select a set of 34 sediment samples having a broad range in PAH concentration and carbon content.

A laboratory control sediment sample, JAM, was collected from a rural area (Jamesville Reservoir) near Syracuse, New York (USA) with a measured 2.8 weight % (wt %) TOC and 6.4 mg/kg total PAH_{34} (PAH_{13} = 2.1 mg/kg). Several (2–4) potential field reference sediments were collected near each MGP site from locations having low concentrations of PAHs and considered not affected by MGP contamination. Samples selected as the field reference sediment for each site had the lowest PAH concentration with physical and chemical characteristics (i.e., field characterization of grain size and TOC content) similar to the test samples. Two field reference samples were collected from the Troy MGP site to evaluate the effect of different TOC content on amphipod survival and growth. Samples TR22 and TR11 were selected for their low and intermediate TOC contents (0.6 and 2.1 wt %, respectively).

Sediment characterization

Total organic carbon and soot carbon were determined on air-dried sediment samples according to the method proposed by Gschwend and others [15,17] with a Teledyne model CE440 elemental analyzer (Leeman Labs, Hudson, NH, USA). Total solids and grain size were determined according to U.S. EPA Method 160.3 and American Society for Testing and Materials Method D422-63/D421-85, respectively [28,29].

Determination of total and rapidly released PAHs in sediment

Sediment samples were extracted for analysis of PAHs with a combination of SFE followed by Soxhlet extraction, as previously described [24]. Sediments were briefly centrifuged to remove interstitial pore water, and then 2-g samples were mixed with 4 g of sodium sulfate before SFE. After the extraction of rapidly released PAHs by SFE (200 bar, 50°C for 40 min), each residue was quantitatively transferred to a Soxhlet thimble and then extracted overnight in a Soxhlet apparatus with 1:1 (v/v) methylene chloride/acetone. Polycyclic aromatic hydrocarbon concentrations in all extracts were determined by gas chromatography/mass spectrometry (GC/MS) with an Agilent Model 5973 instrument (Agilent Technologies, Santa Clara, CA, USA) operated in the selected ion mode by monitoring the molecular ion of each PAH. Separation of the PAHs was performed with a 60-m HP-5 (0.25 mm i.d., 0.25-μm film thickness) column supplied by Agilent. Quantitation was achieved with the aid of perdeuterated PAHs (d-PAHs), which were added to each extract as internal standards [23].

Determination of PAHs in pore water

Methods used to collect and analyze pore water have been described previously [30]. In brief, pore-water samples were obtained by centrifuging the wet sediment, and alum flocculation was used to remove colloids. The pore-water samples were prepared fresh daily from sediment samples within 28 d of sample collection. Polycyclic aromatic hydrocarbon concentrations in pore-water samples were determined after solid-phase microextraction (SPME) and GC/MS analysis according to the procedures described above for the molecular ions of the target PAHs and d-PAHs, which were added to the pore-water samples as internal standards.

Analysis of 34 parent and alkylated PAHs

Analysis of PAHs in sediment and pore water included the analysis of 18 parent and 16 groups of alkylated compounds

(PAH_{34}) [2]. All standard and sample peak areas were normalized to the d-PAH internal standards, ranging in size from naphthalene-d_8 to benzo[*ghi*]perylene-d_{12}. When no deuterated analog of a PAH was available, the d-PAH with the closest molecular structure was used (e.g., benzo[*a*]pyrene-d_{12} was used as the internal standard for benzo[*e*]pyrene). The parent d-PAH was used for the related alkyl PAHs (except for 1-methylnaphthalene-d_{10}). Quantitations were based on the peak areas of the molecular ions (compared with those of the relevant d-PAH internal standards) and the relative response factors as previously reported [30,31]. Peak identities were routinely verified by analyzing representative extracts in the full scan GC/MS mode.

Pore-water dissolved organic carbon

The dissolved organic carbon (DOC) content for each pore-water sample was determined after alum flocculation by U.S. EPA Method 415.1 [32].

Toxicity testing

Chronic toxicity to the freshwater amphipod *H. azteca* was conducted in accordance with the procedures outlined in U.S. EPA Test Method 100.4 for a 28-d sediment exposure period to assess the bioavailability of PAHs in sediments [27]. Amphipods were obtained as 4-d-old juveniles (Environmental Consulting and Testing, Superior, WI, USA) and acclimated to a temperature of approximately 23°C for 3 d before the start of a toxicity test. Amphipods were fed a mixture of yeast–cerophyl–trout chow (Purina Mills, St. Louis, MO, USA) and cultures of green algae (*Selenastrum capricornutum*) during acclimatization. During testing, replicate beakers received daily 1.2-ml yeast–cerophyl–trout chow only, with a solids concentration of 1.6 g/L [27]. Approximately 100 ml of overlying water was poured off and renewed once daily for the duration of the 28-d sediment exposure period. Chemistry of aged overlying water was measured either daily (dissolved oxygen) or intermittently (total ammonia, conductivity, pH, alkalinity, and hardness). The test design entailed the exposure of four replicates of 10 amphipods to control, field reference, and test samples. Following the 28-d sediment exposure period, the surviving amphipods were harvested, counted, and weighed. Comparisons of survival and dry weights among treatments were performed with the use of TOXSTAT Version 3.5 statistical software (Western EcoSystems Technology, Cheyenne, WY, USA). Comparisons of survival among treatments were conducted with arc sine (square root)–transformed values subjected to either the parametric Dunnett's test or nonparametric Steel's many-one rank test procedures. The dry weight among treatments was analyzed by the Tukey method of multiple comparisons. All statistical comparisons were made at the 95% confidence level ($p < 0.05$).

Sediment spiking experiment

The toxicity of PAHs associated with sediment TR15 (a highly contaminated, but nontoxic, sediment) were evaluated by extracting them and then spiking this extract onto the field reference sediment HD22. Approximately 650 grams (wet wt) of sediment TR15 was centrifuged to decant as much water as possible, then air dried overnight. Polycyclic aromatic hydrocarbons were extracted by adding 350 ml of pesticide residue–grade acetone and sonicating for approximately 20 h. The acetone was decanted from the sediment, followed by a fresh addition of approximately 225 ml of acetone (enough to fully cover the sediment) and an additional 20 h of sonication. This procedure was repeated four times to yield a total extract volume of approximately 1 L. The 1-L extract bottle was then placed in a 25°C water bath, and the acetone was evaporated under a gentle stream of pure nitrogen to approximately 25 ml. At this point, the bottle was rotated while the remaining acetone was evaporated to deposit the PAHs in a shell around the bottle's interior. All procedures were performed in brown glass bottles in a darkened laboratory hood to avoid photochemical decomposition of the PAHs.

After evaporation of the acetone was completed, 650 g (wet wt) of the uncontaminated field reference sediment HD22 was added to the spiking bottle, and the wet sediment/PAH slurry was rotated for 20 h at approximately six rotations per minute. A replicate sample of HD22 was prepared in an identical manner by evaporating 1 L of acetone and mixing the residue with fresh HD22 sediment. Quadruplicate 2-g subsamples were removed from the sediment amended with PAHs and analyzed by GC/MS in a manner identical to that used for the sediment samples as described above.

Predicting toxicity with EqP and hydrocarbon narcosis models

Predictions of sediment toxicity were made by determining narcotic potential of the PAHs present in each sample [2,4]. The aqueous PAH-specific final chronic values or the sediment critical PAH concentration expressed on an organic carbon basis were used to calculate the number of toxic units associated with each individual parent PAH or group of alkylated PAHs in pore water or sediments, respectively [2]. A toxic unit (TU) is a hazard quotient that is defined as the measured concentration of PAHs in sediment or pore water compared with the concentration of PAH expected to result in toxic effects. For a mixture of PAHs, the TUs for the individual parent PAH and groups of alkylated PAHs are summed. The combined models predict that PAHs in sediment or pore water will be toxic when the sum of TUs for 18 parent PAHs and 16 groups of alkylated PAHs exceeds one. The total PAH concentration determined by Soxhlet extraction, the bioavailable PAH concentration determined by mild SFE extraction, and the dissolved PAH concentration in pore water were used to estimate the narcotic potential of the PAHs in each sediment.

RESULTS

To investigate the bioavailability of PAHs in sediments at MGP sites, 34 sediment samples were selected from four sites representing a broad range of properties. The sediments ranged in physical composition from highly organic muck to nearly pure sand with TOC content ranging from 0.6 to 11 wt %, soot carbon content ranging from 0.2 to 5.1 wt %, and the concentration of DOC in pore water ranging from 2.5 to 40 mg/L (Table 1). Table 2 shows a comparison of the total PAH concentrations from the measurement of the 34 parent PAHs and groups of alkyl PAHs (PAH_{34}) specified for determining equilibrium partitioning sediment benchmarks by the U.S. EPA [2] and the total PAH concentrations that have traditionally been reported on the basis of the sum of the 16 parent PAHs (PAH_{16}) measured by U.S. EPA Method 8310. As shown in Table 2, the sum of 16 priority pollutant PAHs ranged by three orders of magnitude in concentration from 4 to 5,700 mg/kg, whereas the concentration of the sum of 34 PAHs, representing both the predominant parent and alkylated PAHs, ranged from 10 to 11,400 mg/kg. A third definition of total PAH from the

Table 1. Range in sediment physical and chemical characteristics. DOC = dissolved organic carbon

Site (NY, USA)	No. of samples (*n*)	Sand min–max (wt %)	Total organic carbon min–max (wt %)	Soot carbon min–max (wt %)	Pore-water DOC min–max (mg/L)
Hudson	13	3–70	0.4–10	0.3–5.1	3.7–27
Oneonta	4	40–64	3.1–11	0.4–5.0	4.1–15
Plattsburgh	7	20–93	0.7–6.5	0.3–0.7	3.1–24
Troy	10	47–94	0.6–4.8	0.2–4.1	3.9–40

sum of 13 parent PAH (PAH_{13}) concentrations has also been used for predicting the toxicity of PAH mixtures in sediment samples. For example, the probable effects concentration (PEC) is the PAH_{13} concentration above which toxic effects are considered likely [33], and the threshold effects concentration is the PAH_{13} concentration expressed on a sediment organic carbon basis below which toxicity is considered unlikely [34]. However, total PAH concentrations from PAH_{13} and PAH_{16} are very similar, as shown in Appendix A.

The sediments had a wide range in the fraction of SFE rapidly released PAH_{34} (0.7–94%) and a very wide range in the PAH_{34} concentration measured in pore water by SPME (0.06–10,700 μg/L). In addition to a wide range in PAH concentrations, the samples ranged significantly in the apparent degree of weathering, as estimated by the relative concentration of low–molecular weight PAHs (2-ring) compared with the total concentration of PAHs (Table 3).

The field reference sediments HD22, ON5, PL1, TR11, and TR22 were determined to have total PAH_{34} concentrations of 23, 107, 16, 11, and 10 mg/kg dry weight, respectively (Table 3). The concentration of total PAHs expressed as the sum of 13 parent compounds, PAH_{13}, was determined to be 6.1, 40.2, 5.0, 3.6, and 4.8 mg/kg dry weight, respectively, and when normalized for the organic carbon content, the PAH_{13} concentration measured in sediment samples was 215, 878, 103, 174, and 872 mg PAH_{13}/kg organic carbon, respectively (Appendix A). All of the field reference samples, with the exception of ON5 and TR22, were below the proposed threshold effects concentration of 290 mg PAH_{13}/kg organic carbon, below which toxic effects are considered to be unlikely [34].

SFE rapidly released PAHs

The fraction of PAHs that were rapidly released by SFE ranged dramatically among sediment samples (Table 2). For example, 0.7% (20 mg/kg) of the total Soxhlet-extracted PAH_{34} (2,990 mg/kg) in sample TR15 was rapidly released by mild SFE (Tables 3 and 4). In contrast, 95% of the total Soxhlet-extracted PAHs (3,430 mg/kg) in sample PL8 was rapidly released by SFE. Of the 34 sediment samples evaluated, 28 of the samples (82%) were observed to have low concentrations of rapidly released PAHs (<10% of the total PAH_{34} mass was extracted by SFE). It is also interesting to note the wide range in the total PAH_{34} concentrations among the sediment samples that had 1% or less of their PAH molecules characterized as rapidly released. Only 1.0, 0.9, and 0.7% of the PAH_{34} were rapidly released in samples HD19, TR1, and TR15 respectively; yet, the total PAH_{34} concentration in these samples ranged from 58 to 2,990 mg/kg. The rapidly released fraction of PAH_{34} was generally low for sediment samples collected from the Oneonta and Troy sites, ranging from 2.4 to 22% and 0.7 to 17%, respectively (Table 4). In contrast, a wide range in the rapidly released fraction was observed for sediment samples collected at the Hudson and Plattsburgh sites. For these sites, the rapidly released fraction was observed to range from 1 to 79% and 2.9 to 94%, respectively.

SPME pore-water concentrations

The concentrations of PAHs measured in pore water were not related to the total extractable PAH concentrations in sediment, as in the SFE rapidly released concentrations discussed above. For example, the concentrations of total PAH_{34} measured in pore water were 694 and 3,390 μg/L for sediment samples TR15 and PL11, respectively (Table 5). However, the concentrations of total Soxhlet-extractable PAH_{34} for sediment samples TR15 and PL11 were 2,990 and 139 mg/kg, respectively (Table 3). As expected, on the basis of their higher water solubility, 2-ring PAHs are the primary PAHs detected in sediment pore water (Table 5).

Table 2. Range of total sediment polycyclic aromatic hydrocarbon (PAH) concentrations, supercritical fluid extraction (SFE) rapidly released PAH fractions, and pore-water dissolved PAH concentrations

		Sediment			
		Total PAH concentration (mg/kg)			
Site (NY, USA)	No. of samples (*n*)	PAH_{16} min–max[a]	PAH_{34} min–max[b]	SFE rapidly released fraction min–max (%)[c]	Pore-water PAH_{34} concn. min–max (μg/L)[b]
Hudson	13	8–5,700	23–11,400	1.0–79	0.7–5,330
Oneonta	4	45–429	107–1,040	2.4–22	2.5–46
Plattsburgh	7	6–1,380	16–3,430	2.9–94	0.06–10,700
Troy	10	4–1,730	10–2,990	0.7–17	1.0–694
All locations	34	4–5,700	10–11,400	0.7–94	0.06–10,700

[a] Sum of 16 U.S. Environmental Protection Agency priority pollutant PAHs noted below with an asterisk.

[b] Sum of 34 PAHs includes 18 parent PAHs and 16 groups of alkylated PAHs: naphthalene*, C1 naphthalenes, C2 naphthalenes, C3 naphthalenes, C4 naphthalenes, acenaphthylene*, acenaphthene*, fluorene*, C1 fluorenes, C2 fluorenes, C3 fluorenes, phenanthrene*, anthracene*, C1 phenanthrenes/anthracenes, C2 phenanthrenes/anthracenes, C3 phenanthrenes/anthracenes, C4 phenanthrenes/anthracenes, fluoranthene*, pyrene*, C1 fluoranthenes/pyrenes, benz[*a*]anthracene*, chrysene*, C1 chrysenes, C2 chrysenes, C3 chrysenes, C4 chrysenes, benzo[*b*+*k*]fluoranthene*, benzo[*e*]pyrene, benzo[*a*]pyrene*, perylene, indeno[1,2,3-*cd*]pyrene*, dibenz[*a,h*]anthracene*, benzo[*ghi*]perylene*.

[c] SFE rapidly released fraction is PAH_{34} concn. determined by SFE (Table 4)/PAH_{34} concn. determined by Soxhlet extraction (Table 3).

Table 3. Concentration of polycyclic aromatic hydrocarbons (PAHs) in sediment, PAH distribution by ring number, and number of toxic units

Site (NY, USA)	Sample name	Sediment PAH concentration (mg/kg)						Toxic units[b]
		PAH_{34}[a]	2-ring	3-ring	4-ring	5-ring	6-ring	
Hudson	HD3	977	318	388	219	32	21	50 ± 4
	HD4	33	4	14	11	2	1	2.5 ± 0.8
	HD5	2,230	569	750	770	84	59	100 ± 10
	HD6	1,030	74	320	527	61	47	47 ± 17
	HD8	11,400	3,830	3,960	2,650	602	353	510 ± 80
	HD9	84	15	34	25	7	4	3.8 ± 0.5
	HD10	232	45	77	81	14	15	12 ± 2
	HD12	27	2	11	9	2	2	1.0 ± 0.0
	HD13	273	10	68	135	31	29	3.5 ± 0.8
	HD14	260	11	58	126	27	38	4.4 ± 0.6
	HD15	177	7	39	85	22	22	3.4 ± 0.6
	HD19	58	8	22	22	4	2	0.9 ± 0.2
	HD22	23	2	8	8	2	2	1.1 ± 0.0
Oneonta	ON3	117	4	23	59	20	11	5.0 ± 0.7
	ON5	107	13	37	41	12	4	3.5 ± 0.3
	ON6	1,040	58	383	472	93	35	13 ± 1
	ON7	944	37	294	442	120	50	24 ± 1
Plattsburgh	PL1	16	2	4	6	3	1	0.5 ± 0.0
	PL2	203	15	63	91	24	10	6.5 ± 0.5
	PL4	267	24	90	112	29	12	15 ± 3
	PL8	3,430	1,650	1,010	628	101	41	140 ± 20
	PL11	139	44	53	32	8	2	37 ± 6
	PL13	493	98	201	156	27	11	62 ± 8
	PL14	122	6	39	55	17	5	22 ± 15
Troy	TR1	326	19	96	148	42	21	31 ± 7
	TR3	962	44	266	444	155	52	34 ± 9
	TR7	319	14	83	153	43	26	8.8 ± 2.3
	TR9	41	4	9	19	6	3	3.7 ± 1.6
	TR11	11	1	4	3	2	1	0.7 ± 0.1
	TR12	1,840	100	524	811	303	104	64 ± 16
	TR13	911	44	226	442	153	47	40 ± 4
	TR15	2,990	219	1,040	1,140	410	181	120 ± 20
	TR17	108	9	26	50	16	7	3.4 ± 0.5
	TR22	10	1	2	4	2	1	2.4 ± 1.4

[a] PAH_{34} is the sum of 18 parent PAHs and 16 groups of alkylated PAHs (see Table 2).
[b] Toxic units estimated from Soxhlet-extracted sediment PAH concentrations according to equilibrium partitioning and hydrocarbon narcosis models.

Toxicity of MGP sediments to amphipods

Survival and growth of amphipods exposed to test sediments were compared with laboratory control (HD22 and JAM) and field reference (HD22, ON5, PL1, and TR11) sediments. Sample HD22 was used as either a laboratory control sample or as a combined field reference/laboratory control sample, depending on the toxicity test performed. The percent survival of amphipods in the laboratory control samples was 98% or greater for both HD22 and JAM (Tables 6 and 7). The growth of amphipods exposed to HD22 and JAM was not significantly different ($p < 0.05$) when both laboratory control sediments were tested simultaneously.

The survival and growth of amphipods exposed to field reference sediments were not significantly different from the survival and growth of amphipods exposed to the JAM or HD22 laboratory control samples, with an exception for the field reference sediment PL1 (Table 6). All of the amphipods exposed to PL1 survived (100%); however, growth was 71% (0.25 mg dry wt/organism) of the growth observed in amphipods exposed to the laboratory control JAM (0.35 mg dry wt/organism). Neither survival nor growth of amphipods exposed to the low TOC field reference sediment TR22 was significantly different from the lab control sediment JAM. Even though the mean survival of amphipods exposed to TR22 was 83 ± 12% and 78 ± 21% in two separate tests, the large variances are likely a result of the coarse grain size and low TOC content of TR22. These characteristics have previously been observed to reduce amphipod survival in uncontaminated sediments (F. Doherty, unpublished data). Because of the large variance, test samples from the Troy site were not compared with TR22 but were instead compared with the field reference sample TR11.

The chronic toxicity measured with *H. azteca* in 28-d bioassays was not correlated to the total concentration of PAH_{34} despite the wide range in the concentration of PAH_{34} (10–11,400 mg/kg) (Fig. 1). No amphipods survived in three sediment samples collected from the Hudson MGP site and in three sediment samples collected from the Plattsburgh MGP site (Table 6). The dry weights of amphipods collected from nonlethal sediments after 28 d of exposure were not significantly lower than those obtained from amphipods exposed to field reference samples.

Quite unexpectedly, no mortality or reduction in growth was observed in several sediments with very high concentrations of PAHs, whereas other samples with much lower concentrations of PAHs were highly toxic. For example, PL11 (with a total PAH_{34} concentration of 139 mg/kg) had no surviving amphipods at the end of the 28-d test, whereas 100% of the amphipods exposed to sediment TR15 (having a total PAH_{34} concentration of 2,990 mg/kg) survived and showed no

Table 4. Supercritical fluid extraction (SFE) rapidly released sediment polycyclic aromatic hydrocarbon (PAH) concentrations and PAH distribution by ring number

Site (NY, USA)	Sample name	Rapidly released PAH concentration (mg/kg)						Toxic units[b]
		PAH_{34}[a]	2-Ring	3-Ring	4-Ring	5-Ring	6-Ring	
Hudson	HD3	297	135	116	42	3	1	16 ± 3
	HD4	1.9	0.7	0.7	0.4	0.1	<0.1	0.2 ± 0.1
	HD5	977	408	369	185	11	4	52 ± 6
	HD6	24.3	5.5	11.2	6.6	0.9	0.1	1.4 ± 1.1
	HD8	9,040	3,490	3,440	1,840	200	80	430 ± 90
	HD9	3.8	1.5	1.4	0.8	0.1	<0.1	0.2 ± 0.0
	HD10	25.7	10.4	9.2	5.3	0.7	0.2	1.5 ± 0.5
	HD12	0.7	0.1	0.4	0.2	<0.1	<0.1	<0.1
	HD13	35.4	2.0	14.6	16.7	1.5	0.6	0.5 ± 0.1
	HD14	58.5	2.7	20.1	30.5	3.2	2.0	1.0 ± 0.2
	HD15	27.4	1.8	10.1	13.3	1.5	0.7	0.5 ± 0.3
	HD19	0.6	0.2	0.1	0.2	<0.1	<0.1	<0.1
	HD22	0.7	0.2	0.3	0.3	<0.1	<0.1	<0.1
Oneonta	ON3	24.5	1.6	7.2	12.8	2.3	0.7	1.1 ± 0.2
	ON5	23.7	2.6	10.5	9.2	1.3	0.2	0.7 ± 0.2
	ON6	37.5	3.2	19.2	13.4	1.5	0.2	<0.1
	ON7	22.6	1.3	9.1	10.6	1.4	0.2	0.6 ± 0.2
Plattsburgh	PL1	0.5	0.1	0.2	0.1	0.1	<0.1	<0.1
	PL2	10.0	0.9	4.0	4.3	0.6	0.1	0.3 ± 0.1
	PL4	69.6	7.6	30.0	27.4	3.7	0.8	4.1 ± 1.6
	PL8	3,240	1,550	970	600	90	30	130 ± 21
	PL11	102	36	40	21	4	1	29 ± 6
	PL13	223	59	104	54	5	1	30 ± 11
	PL14	7.1	0.6	3.1	2.9	0.4	0.1	1.4 ± 0.4
Troy	TR1	2.9	0.5	1.2	1.0	0.2	<0.1	0.3 ± 0.1
	TR3	15.1	1.3	7.0	5.9	0.7	0.1	0.6 ± 0.0
	TR7	32.9	1.8	13.0	15.7	2.0	0.4	0.9 ± 0.3
	TR9	5.4	0.7	1.9	2.4	0.3	0.1	0.5 ± 0.2
	TR11	0.4	0.1	0.2	0.1	<0.1	<0.1	<0.1
	TR12	129	8	59	56	5	1	4.9 ± 1.5
	TR13	77.3	4.6	33.2	35.6	3.5	0.4	3.7 ± 1.5
	TR15	20.0	4.4	11.6	6.9	0.8	0.1	1.1 ± 0.2
	TR17	1.5	0.1	0.5	0.7	0.1	<0.1	0.1 ± 0.0
	TR22	1.6	0.1	0.6	0.7	0.2	<0.1	0.4 ± 0.2

[a] PAH_{34} is the sum of 18 parent PAHs and 16 groups of alkylated PAHs (see Table 2).
[b] Toxic units estimated from SFE rapidly released sediment PAH concentrations according to equilibrium partitioning and hydrocarbon narcosis models.

significant reduction in growth. To better understand the surprising lack of toxicity observed in sample TR15, PAHs in this sample were extracted and then added to the field reference sediment HD22. The field reference sediment spiked with PAHs extracted from sample TR15 was highly toxic and resulted in 100% mortality of *H. azteca* (Table 7). The survival and growth of amphipods exposed to the control treatment (consisting of the field reference sediment spiked with only the carrier solvent) was not different than for amphipods exposed to the field reference sediment.

The ability to predict which sediment samples were nontoxic was not substantially improved by calculation of TUs with the measurement of Soxhlet-extractable PAHs (Fig. 2). The concentration of Soxhlet-extractable PAHs in sediment TR15 was determined to be equivalent to 118 TU, and as identified above, no toxicity was observed in this sample. Twenty-three of the 29 sediment samples that were measured to have PAH concentrations exceeding one toxic unit were observed to be nontoxic to *H. azteca*.

Although a relationship between the total concentration of PAHs in sediment and toxicity (Figs. 1 and 2) was not apparent, estimates of PAH bioavailability with the use of either the rapidly released PAH concentrations measured by SFE (Fig. 3) or the concentrations of PAHs measured in pore water (Fig. 4) were clearly able to differentiate between toxic and nontoxic sediment samples. The highest PAH concentration determined by SFE that did not result in toxicity to *H. azteca* was 5.0 TU (sediment TR12). However, this same sediment sample was determined to have 65 TU when total Soxhlet-extractable PAHs were used to predict toxicity. Interestingly, sediment TR15, which was nontoxic to *H. azteca*, was estimated to have a PAH concentration of 118 TU on the basis of Soxhlet extraction, whereas the number of TUs calculated on the basis of the SFE rapidly released PAH concentrations was 1.1 TU. The maximum pore-water PAH concentration determined by SPME that did not result in toxicity to *H. azteca* was 25 toxic units (sediment TR15).

DISCUSSION

Sediment screening guidelines have been developed for PAHs that are used widely by regulatory agencies for estimating the toxicity of PAH-affected freshwater sediments [33,34]. The PEC and median effects concentration (MEC), 22.8 mg total PAH_{13}/kg sediment and 1,800 mg total PAH_{13}/kg organic carbon, respectively, are widely believed to represent the maximum concentration of PAHs that can be present in sediment, above which harmful effects are likely to be observed. In our study, 19 out of 25 sediment samples (76%) that exceeded the PEC and 12 out of 18 sediment samples (67%) that exceeded the MEC were not toxic to *H. azteca* in

Table 5. Pore-water polycyclic aromatic hydrocarbon (PAH) concentrations and PAH distribution by ring number

Site (NY, USA)	Sample name	Pore-water PAH concentration (μg/L)						Toxic units[b]
		PAH_{34}[a]	2-Ring	3-Ring	4-Ring	5-Ring	6-Ring	
Hudson	HD3	5,330	4,860	449	24.1	0.24	0.022	310 ± 60
	HD4	71.8	64.2	6.61	0.89	0.12	0.011	2.3 ± 0.2
	HD5	3,850	3,450	372	25.2	0.35	0.038	110 ± 10
	HD6	299	237	55.4	5.97	0.15	0.010	18 ± 2
	HD8	3,710	3,210	439	52.4	3.47	0.427	130 ± 10
	HD9	42.4	39.4	2.38	0.62	<0.01[c]	0.006	1.4 ± 0.2
	HD10	280	254	23.2	2.39	0.09	0.014	9.5 ± 0.9
	HD12	5.0	2.0	2.57	0.40	0.04	0.004	0.4 ± 0.0
	HD13	2.7	1.66	0.51	0.54	<0.01	0.010	0.2 ± 0.0
	HD14	9.5	5.7	1.80	1.74	0.17	0.024	3.1 ± 0.7
	HD15	15.7	3.3	8.84	2.70	0.74	0.126	4.3 ± 0.3
	HD19	0.7	0.2	0.23	0.25	0.03	0.007	0.1 ± 0.0
	HD22	2.3	1.8	0.28	0.22	0.04	0.006	0.3 ± 0.1
Oneonta	ON3	2.5	0.8	1.15	0.49	0.04	0.005	0.3 ± 0.1
	ON5	28.2	16.6	9.61	1.92	0.05	<0.004	3.4 ± 0.3
	ON6	46.2	20.4	20.3	5.41	0.09	0.010	5.7 ± 0.3
	ON7	28.6	11.0	12.0	5.37	0.14	0.015	3.2 ± 0.1
Plattsburgh	PL1	<0.5	<0.5	<0.1	0.06	<0.01	<0.004	<0.1
	PL2	16.9	8.4	5.80	2.57	0.12	0.013	2.9 ± 0.2
	PL4	83.1	56.1	21.8	5.08	0.13	0.016	7.8 ± 0.6
	PL8	10,700	10,300	374	30.9	1.08	0.156	140 ± 10
	PL11	3,390	2,970	387	38.7	0.45	0.055	120 ± 10
	PL13	1,130	830	255	39.2	1.42	0.262	81 ± 8
	PL14	8.3	5.2	2.49	0.59	<0.01	<0.004	0.7 ± 0.0
Troy	TR1	104	79.0	21.9	2.58	0.02	<0.004	3.0 ± 0.1
	TR3	46.0	23.5	19.8	2.71	0.02	<0.004	2.6 ± 0.1
	TR7	6.9	2.8	2.9	1.21	0.03	<0.004	0.7 ± 0.1
	TR9	6.5	4.4	1.8	0.30	0.01	<0.004	0.3 ± 0.0
	TR11	2.7	2.1	0.5	0.10	<0.01	<0.004	0.1 ± 0.0
	TR12	49.9	29.7	17.6	2.53	0.04	<0.004	2.4 ± 0.1
	TR13	49.7	23.7	19.6	6.33	0.11	0.010	4.0 ± 0.7
	TR15	694	486	188	19.5	0.34	0.019	25 ± 2
	TR17	7.0	4.1	0.9	1.89	0.12	0.016	0.6 ± 0.1
	TR22	1.0	0.6	0.3	0.19	<0.01	<0.004	<0.1

[a] PAH_{34} is the sum of 18 parent PAHs and 16 groups of alkylated PAHs (see Table 2).
[b] Toxic units estimated from pore-water PAH concentrations according to equilibrium partitioning and hydrocarbon narcosis models.
[c] Detection limits range from 0.5 mg/L for naphthalene to 0.002 mg/L for individual 6-ring PAHs. Detection limits for each PAH have been previously reported in MacDonald et al. [33].

28-d bioassays. The lack of toxicity observed in these sediments indicate that the PEC and MEC screening values do not provide a reliable basis for predicting the toxicity of PAHs in sediments at some MGP sites.

Evidence for reduced bioavailability of PAHs

Multiple lines of evidence indicate that PAHs present in sediments at some MGP sites often have low availability. This evidence includes low rapidly released PAH fractions determined by SFE; dissolved PAH concentrations in sediment pore water that are much lower than expected from assumed aqueous partitioning to natural organic carbon; low toxicity observed in sediments predicted to be toxic by the combined EqP and hydrocarbon narcosis models; and finally, the lack of PAH toxicity in a sediment sample that, when the PAHs were extracted and added to a nontoxic field reference sediment sample, were highly toxic. It has been suggested that bioavailability of PAHs in sediments might be overestimated by the analysis of bulk sediments when they contain soot, and under these conditions, the consensus screening values will overestimate toxicity and ecological effects [9,34]. The sediment samples used in our study that exceeded the PEC were observed to contain 0.2 to 5.1 wt % soot carbon that represented 9 to 84% of the TOC present in these samples. In addition to the multiple lines of evidence described above, our results are consistent with the suggestion by others that soot carbon could modify the bioavailability of PAHs in sediments [14,15]. On the basis of the multiple lines of evidence indicting the low availability of PAHs in the samples we investigated, it is not surprising that PAH screening values and ESBs are not reliable predictors of toxicity to aquatic invertebrates in MGP site sediments.

Spiking the extracted PAHs from sediment TR15 onto a nontoxic field reference sediment (HD22) clearly demonstrated the large difference in the bioavailability and toxicity of freshly added and field-aged compounds in our study. Previous research has compared the bioavailability of PAHs with aquatic amphipods in field-contaminated sediment and in sediment spiked with added PAHs [35]. In these experiments, the biota–sediment accumulation factors for the spiked PAHs were only 1.4 to 3.3 times higher than the field-aged compounds. The toxicity observed in our sediment with spiked PAHs suggests that the difference in bioavailability of freshly added and field-aged PAHs could be substantively greater than that reported by others and that PAHs in some sediments at MGP sites are in a highly advanced state of sequestration.

Improving predictions of sediment toxicity

The concentration of rapidly released PAHs determined by SFE greatly improved predictions of toxicity to *H. azteca* com-

Table 6. Survival and growth (mean ± 1 SD) of *Hyalella azteca* in 28-d bioassays

Sample type	Sample name	Survival (%)	Growth (mg dry wt/organism)	Sample type	Sample ID	Survival (%)	Growth (mg dry wt/organism)
Hudson, NY, USA				Troy, NY, USA			
Lab control/field reference	HD22	100 ± 0	0.31 ± 0.04	Lab control	JAM	100 ± 0	0.39 ± 0.06
	HD22	100 ± 0	0.27 ± 0.02		JAM	100 ± 0	0.45 ± 0.08
Test	HD4	100 ± 0	0.25 ± 0.03		HD22	98 ± 5	0.43 ± 0.05
	HD6	100 ± 0	0.30 ± 0.02		HD22	100 ± 0	0.39 ± 0.03
	HD9	100 ± 0	0.33 ± 0.04	Field reference	TR11	98 ± 5	0.40 ± 0.04
	HD12	100 ± 0	0.28 ± 0.03		TR11	98± 5	0.36 ± 0.03
	HD13	100 ± 0	0.33 ± 0.03		TR22	78 ± 21	0.36 ± 0.05
	HD14	100 ± 0	0.28 ± 0.02		TR22	83 ± 13	0.37 ± 0.02
	HD10	98 ± 5	0.28 ± 0.02	Test	TR9	100 ± 0	0.40 ± 0.04
	HD19	98 ± 5	0.35 ± 0.04		TR13	100 ± 0	0.38 ± 0.04
	HD15	95 ± 6	0.22 ± 0.03		TR15	100 ± 0	0.30 ± 0.03[b]
	HD3	0	ND[a]		TR17	100 ± 0	0.35 ± 0.05[b]
	HD5	0	ND		TR1	98 ± 5	0.44 ± 0.03
	HD8	0	ND		TR3	98 ± 5	0.39 ± 0.08
					TR12	98 ± 5	0.38 ± 0.03
					TR7	80 ± 16	0.34 ± 0.08
						—	
Oneonta, NY, USA				Plattsburgh, NY, USA			
Lab control	JAM	100 ± 0	0.35 ± 0.03	Lab control	JAM	100 ± 0	0.35 ± 0.03
Field reference	ON5	88 ± 13	0.32 ± 0.03	Field reference	PL1	100 ± 0	0.25 ± 0.02[b]
Test	ON3	100 ± 0	0.38 ± 0.03	Test	PL4	100 ± 0	0.34 ± 0.03
	ON7	100 ± 0	0.27 ± 0.03[b]		PL2	100 ± 0	0.28 ± 0.01[b]
	ON6	95 ± 10	0.29 ± 0.05		PL14	100 ± 0	0.28 ± 0.02[b]
		—			PL8	0	ND
		—			PL11	0	ND
		—			PL13	0	ND

[a] ND = no growth data collected in test sample because of 100% mortality.
[b] Significant reduction in survival or growth compared with corresponding laboratory control sediment. No significant reductions in survival or growth were observed for comparisons of test sediments to field reference sediments. All tests conducted at $p < 0.05$.

pared with predictions determined by the concentration of total extractable PAHs. Other researchers have used various methods to estimate the uptake and bioavailability of PAHs by aquatic invertebrates [36,37]. Much of the variability in the biota–sediment accumulation factor of field-contaminated and freshly added PAHs observed for an aquatic deposit-feeding amphipod (*Corophia volutator*) could be explained by the rapidly desorbing fraction measured with Tenax TA resin over a 25-d period [35]. The concentration of rapidly released PAHs determined during a 40-min extraction with supercritical carbon dioxide has been shown to be directly correlated to the rapidly desorbing fraction measured by a 14-d aqueous extraction with XAD_2 resin [23]. In this study, the 40-min extraction with mild supercritical carbon dioxide was observed to correlate with the toxicity observed in sediments having a wide range of characteristics (Fig. 3).

The PAH concentrations measured in pore water were also able to clearly discriminate between toxic and nontoxic sediments (Fig. 4). Others have also shown that the concentrations of PAHs measured in sediment pore water are a more accurate predictor of sediment toxicity than the concentrations of PAHs in the bulk sediment [8,10]. This has lead to the proposed use of pore-water PAH concentrations and the combined EqP and hydrocarbon narcosis models as means to estimate sediment toxicity and to develop sediment remediation goals (Electrical Power Research Institute, Palo Alto, CA, USA, Technical Update 1010371, http://www.epri.com). It appears surprising that in our study, no toxicity was observed in pore-water samples having up to 25 TU (sample TR12). On the basis of the hydrocarbon narcosis model, one would expect to observe toxicity when the concentration of PAHs in pore-water samples exceeds 1.0 TU. However, the basis for calculating a toxic unit is the estimated critical body burden (final chronic value, 2.24 μmol/g lipid) believed to protect 95% of all aquatic genera from chronic toxic effects, and this criteria is not specific to *H. azteca* [2]. The mean critical body residue resulting in 50%

Table 7. Toxicity (mean ± 1 SD) of polycyclic aromatic hydrocarbons (PAHs) to *Hyalella azteca* when added to field reference sediment compared with field-contaminated sediments

Sediment				*H. azteca* toxicity			
Source	Name	Treatment	Total PAH_{34} concn. (mg/kg)	Survival (%)	Growth (mg dry wt/organism)	Total organic carbon (wt %)	Soot carbon (wt %)
Test	TR15	None	2,990	98 ± 5	0.30 ± 0.05	3.5	0.7
Field reference	HD22	PAH-spiked	2,730	0	ND[a]	2.9	0.5
Field reference	HD22	Solvent-spiked	23	100 ± 0	0.38 ± 0.05	2.9	0.5
Field reference	HD22	None	23	98 ± 5	0.38 ± 0.01	2.9	0.5
Lab control	JAM	None	6.4	98 ± 5	0.43 ± 0.04	2.8	—[a]

[a] ND = no data collected in spiked field reference treatment because of 100% mortality; — = not determined.

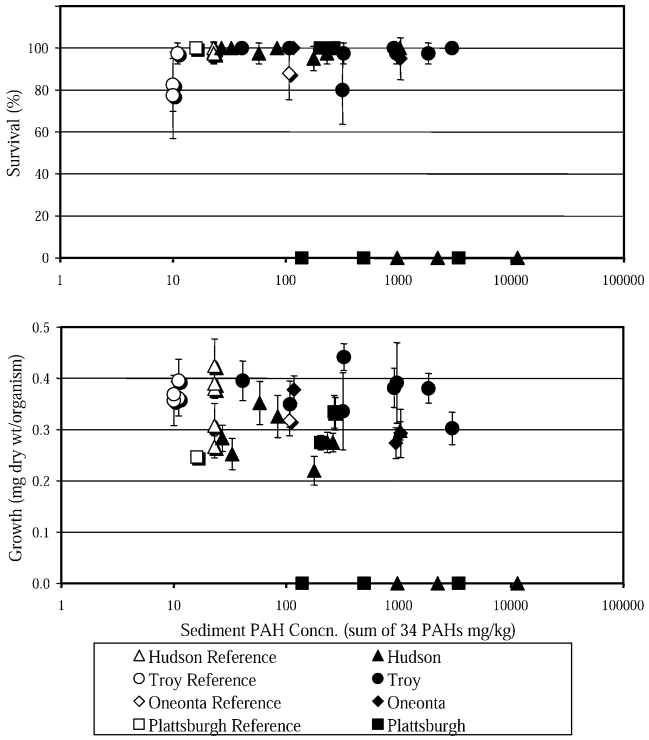


Fig. 1. Total Soxhlet-extractable polycyclic aromatic hydrocarbon (PAH) concentrations in sediment as a predictor of *Hyalella azteca* survival and growth after 28-d bioassays. Error bars represent ±1 SD. Sites located in New York, USA.

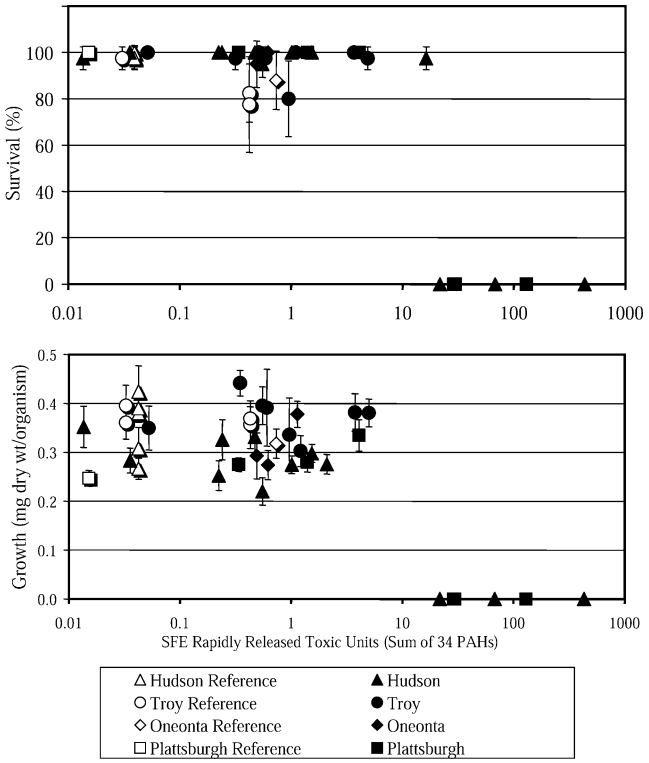


Fig 3. Toxic units in sediment determined by measuring supercritical fluid extraction (SFE) rapidly released polycyclic aromatic hydrocarbons (PAHs) as a predictor of *Hyalella azteca* survival and growth after 28-d bioassays. Error bars represent ±1 SD. Sites located in New York, USA.

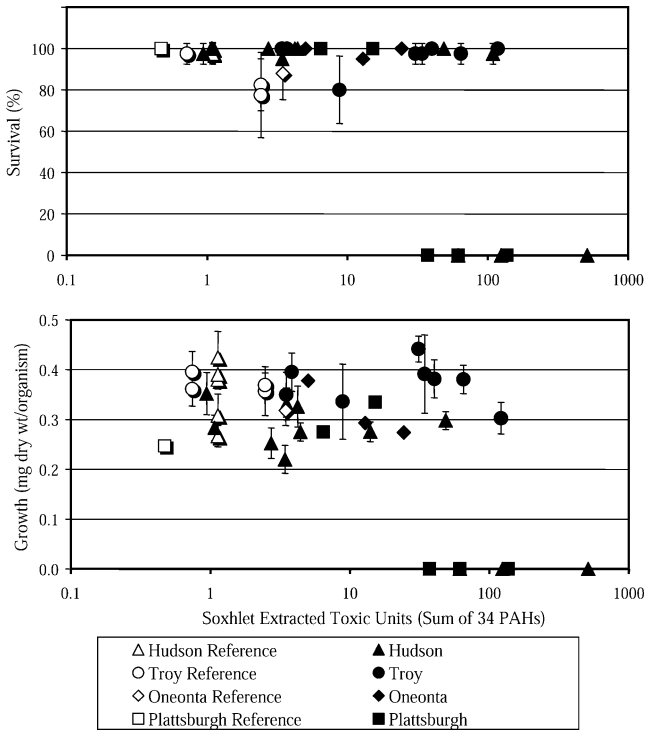


Fig. 2. Toxic units in sediment determined by measuring Soxhlet-extractable polycyclic aromatic hydrocarbons (PAHs) as a predictor of *Hyalella azteca* survival and growth after 28-d bioassays. Error bars represent ±1 SD. Sites located in New York, USA.

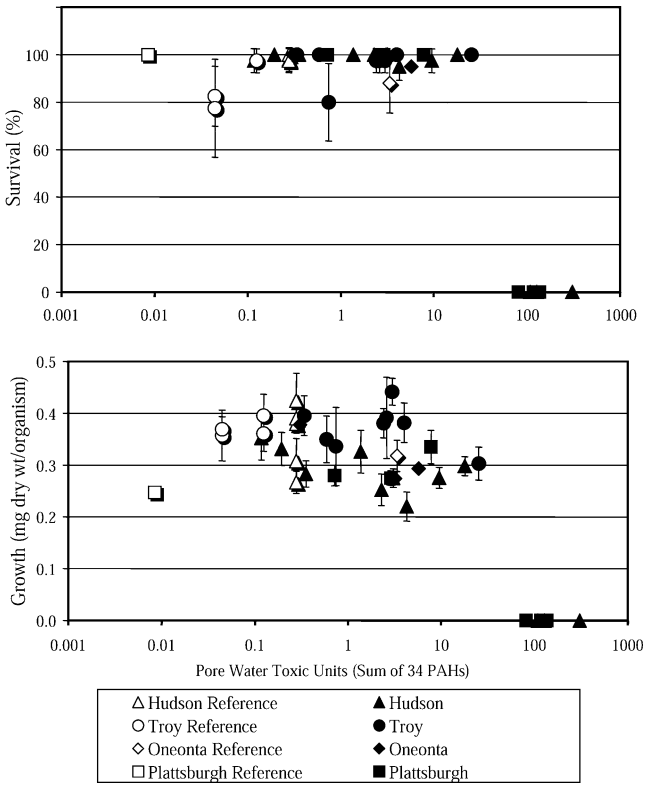


Fig. 4. Toxic units determined by measuring freely dissolved polycyclic aromatic hydrocarbons (PAHs) in pore water as a predictor of *Hyalella azteca* survival and growth after 28-d bioassays. Error bars represent ±1 SD. Sites located in New York, USA.

mortality (LR50) has been estimated by others to be closer to 56 μmol/g lipid, with a 95% confidence interval ranging from 40 to 114 μmol/g lipid (i.e., 3,550 μmol/g dry wt for organisms having 6.3% lipid) [38]. On the basis of this reported critical body burden, we would expect acute toxic effects when the PAH concentration in pore water is between 18 and 51 TU (as calculated by the use of the final chronic value, 2.24 μmol/g lipid, in the U.S. EPA method for deriving ESBs) [2]. Regardless of the criteria used for predicting toxicity by the hydrocarbon narcosis model, it is clear that the analysis of porewater PAHs provided a means to differentiate between samples that were toxic and those that were nontoxic to *H. azteca*.

Some have suggested that DOC reduces the concentration of freely dissolved PAHs in pore water, thus reducing its toxicity. As a result of this, it is recommended that estimates of PAH toxicity in pore water should be adjusted for the PAHs that are associated with the DOC phase [39]. Our analysis of PAHs in pore water with the use of SPME is designed to avoid this problem by allowing the measurement of freely dissolved and total dissolved (i.e., freely dissolved plus PAHs associated with the DOC) PAH concentrations [30]. For all of the samples in our study, the effect of DOC on our determination of toxic units was minimal because the lower molecular mass PAHs (that show no significant partitioning to DOC) contribute nearly all of the toxicity to pore water. For example, when the pore-water PAH concentrations from TR12 were adjusted for the DOC present in the sample, the number of TUs was only reduced from 25.4 to 24.1 TU.

CONCLUSIONS

It is surprising to observe a lack of toxicity in the majority of sediments that exceed the PEC and MEC screening values. Several sediment samples had no apparent toxic effects to *H. azteca* even when the total Soxhlet-extractable PAH_{34} concentrations were as high as 2,990 mg/kg PAHs (PAH_{16} = 1,730 mg/kg). Clearly, these screening values for PAHs do not apply to the MGP sediments investigated in this study that have a significant percentage of the TOC characterized as heat-stable soot. Future investigations should focus on evaluating the predictive ability of these screening values for additional species, various toxicity endpoints, and other industrial sources of PAHs. The analysis of rapidly released PAHs demonstrated a wide range in the availability of PAHs in sediments at MGP sites, and all of the four MGP sites investigated had some sediment samples with very low rapidly released fractions, as determined by SFE. A comparison of the rapidly released PAH concentrations to the observed toxic effects on *H. azteca* indicated that SFE might be an efficient tool for predicting the toxicity in sediment samples collected from MGP sites. Application of the EqP and hydrocarbon narcosis models, adjusted for the bioavailability of PAHs by measuring either the SFE rapidly released PAH concentrations or the pore-water PAH concentrations, was effective at predicting toxicity to *H. azteca*.

Acknowledgement—Financial support for this research was provided by the Gas Research Institute, National Grid, Northeast Gas Association, and U.S. Department of Energy under Cooperative agreement DE-F26-98FT40321. The authors thank James Edwards, Carol Grabanski, and David Miller for sample collection and analytical support and Susan Kane Driscoll and David Nakles for helpful discussions and encouragement.

REFERENCES

1. National Research Council. 2003. *Bioavailability of Contaminants in Soils and Sediments: Processes, Tools and Applications.* The National Academies Press, Washington, DC.
2. U.S. Environmental Protection Agency. 2003. Equilibrium partitioning sediment benchmarks (ESBs) for the protection of benthic organisms: PAH mixtures. EPA/600/R-02/013. Final/Technical Report. Washington, DC.
3. Di Toro DM, McGrath JA, Hansen DJ. 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. I. Water and tissue. *Environ Toxicol Chem* 18:1951–1970.
4. Di Toro DM, McGrath JA. 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. II. Mixtures and sediments. *Environ Toxicol Chem* 18:1971–1982.
5. Bender ME, Roberts MH, deFur PO. 1987. Unavailability of polynuclear aromatic hydrocarbons from coal particles to the eastern oyster. *Environ Pollut* 44:243–260.
6. Chapman PM, Downie J, Maynard A, Taylor LA. 1996. Coal and deodorizer residues in marine sediments—Contaminants or pollutants? *Environ Toxicol Chem* 15:638–642.
7. Maruya KA, Risebrough RW, Horne AJ. 1997. The bioaccumulation of polynuclear aromatic hydrocarbons by benthic invertebrates in an intertidal marsh. *Environ Toxicol Chem* 16:1087–1097.
8. Ozretich RJ, Ferraro SP, Lamberson JO, Cole FA. 2000. Test of (Σ) polycyclic aromatic hydrocarbon model at a creosote-contaminated site, Elliott Bay, Washington, USA. *Environ Toxicol Chem* 19:2378–2389.
9. Paine MD, Chapman PM, Allard PJ, Murdoch MH, Minifie D. 1996. Limited bioavailability of sediment PAH near an aluminum smelter: Contamination does not equal effects. *Environ Toxicol Chem* 15:2003–2018.
10. West CW, Kosian PA, Mount DR, Makynen EA, Pasha MS. 2001. Amendment of sediments with a carbonaceous resin reduces bioavailability of polycyclic aromatic hydrocarbons. *Environ Toxicol Chem* 20:1104–1111.
11. Socha SB, Carpenter R. 1987. Factors affecting pore water hydrocarbon concentrations in Puget Sound sediments. *Geochem Cosmochim Acta* 51:1273–1284.
12. McGroddy SE, Farrington JW. 1995. Sediment pore water partitioning of polycyclic aromatic hydrocarbons in three cores from Boston Harbor, Massachusetts. *Environ Sci Technol* 29:1542–1550.
13. McGoddy SE, Farrington JW, Gschwend PM. 1996. Comparison of the in situ and desorption sediment–water partitioning of polycyclic aromatic hydrocarbons. *SAR QSAR Environ Res* 2:237–247.
14. Maruya KA, Risenbrough RW, Horne AJ. 1996. Partitioning of polynuclear aromatic hydrocarbons between sediments from San Francisco Bay and their pore waters. *Environ Sci Technol* 30: 2942–2947.
15. Gustafsson O, Haghseta F, Chan C, Macfarlane J, Gschwend PM. 1997. Quantification of the dilute sedimentary soot phase: Implications for PAH speciation and bioavailability. *Environ Sci Technol* 31:203–209.
16. Ghosh U, Talley JW, Luthy RG. 2001. Particle-scale investigation of PAH desorption kinetics and thermodynamics from sediment. *Environ Sci Technol* 35:3468–3475.
17. Accardi-Dey D, Gschwend PM. 2002. Assessing the combined roles of natural organic matter and black carbon as sorbents in sediments. *Environ Sci Technol* 36:21–29.
18. Ingersoll CG, Brunson EL, Wang N, Dwyer J, Ankley GT, Mount DR, Huckins J, Petty J, Landrum PF. 2003. Uptake and depuration of nonionic organic contaminants from sediment by the oligochaete, *Lumbriculus variegatus*. *Environ Toxicol Chem* 22:872–885.
19. Jonker MTO, Koelmans AA. 2002. Sorption of polycyclic aromatic hydrocarbons and polychlorinated biphenyls to soot and soot-like materials in the aqueous environment: Mechanistic considerations. *Environ Sci Technol* 36:3725–3734.
20. Talley JW, Ghosh U, Tucker SG, Furey JS, Luthy RG. 2002. Particle-scale understanding of the bioavailability of PAHs in sediment. *Environ Sci Technol* 36:477–483.
21. Ronday R. 1997. Centrifugation method for soil pore water assessment of the bioavailability of organic chemicals in soil. *Commun Soil Sci Plant Anal* 28:777–785.
22. Kraaij R, Mayer P, Busser FJM, Bolscher MV, Seinen W, Tolls

J. 2003. Measured pore-water concentrations make equilibrium partitioning work—A data analysis. *Environ Sci Technol* 37:268–274.
23. Hawthorne SB, Poppendieck DG, Grabanski CB, Loehr RC. 2002. Comparing PAH availability from manufactured gas plant soils and sediments with chemical and biological tests. 1. PAH release during water desorption and supercritical carbon dioxide extraction. *Environ Sci Technol* 36:4795–4803.
24. Hawthorne SB, Poppendieck DG, Grabanski CB, Loehr RC. 2001. PAH release during water desorption, supercritical carbon dioxide extraction, and field bioremediation. *Environ Sci Technol* 35:4577–4583.
25. Stroo HF, Roy TA, Liban CB, Kreitinger JP. 2005. Dermal bioavailability of benzo[*a*]pyrene on lampblack: Implications for risk assessment. *Environ Toxicol Chem* 24:1568–1572.
26. Stroo HF, Nakles DV, Kreitinger JP, Loehr RC, Hawthorne SB, Luthy RG, Holman HY, LaPierre A. 2005. Improving risk assessments for manufactured gas plant soils by measuring PAH availability. *Integr Environ Assess Manag* 1:259–266.
27. U.S. Environmental Protection Agency. 2000. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates, 2nd ed. EPA-600-R-99-064. Final/Technical Report. Washington, DC.
28. U.S. Environmental Protection Agency. 1983. Methods for chemical analysis of water and wastes. EPA 600/4-79-020. Final/Technical Report. Washington, DC.
29. American Society for Testing Materials. 1990. Standard methods for dry preparation of soil samples for particle size analysis and determination of soil constants, particle-size analysis of soils. D422-63, D421-85D. In *Annual Book of ASTM Standards* Vol 4.08. ASTM, Philadelphia, PA, USA, pp 89–97.
30. Hawthorne SB, Grabanski CB, Miller DJ, Kreitinger JP. 2005. SPME measurement of parent and alkyl PAHs in milliliter sediment pore water samples and determination of K_{DOC} values. *Environ Sci Technol* 39:2795–2803.
31. Hawthorne SB, Miller DJ, Kreitinger JP. 2006. Measurement of ''total'' PAH concentrations and toxic units used for sediment risk assessment at manufactured gas plant sites. *Environ Toxicol Chem* 25:287–296.
32. U.S. Environmental Protection Agency. 1971. Methods for the chemical analysis of water and wastes. EPA-600-4-79-020. Cincinnati, OH.
33. MacDonald DD, Ingersoll CG, Berger TA. 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. *Arch Environ Contam Toxicol* 39:20–31.
34. Swartz RC. 1999. Consensus sediment quality guidelines for polycyclic aromatic hydrocarbon mixtures. *Environ Toxicol Chem* 18:780–787.
35. Kraaij RH, Ciarelli S, Tolls J, Kater BJ, Belfroid A. 2001. Bioavailability of lab-contaminated and native polycyclic aromatic hydrocarbons to the amphipod *Corophium volutator* relates to chemical desorption. *Environ Toxicol Chem* 20:1716–1724.
36. Parkerton TF, Stone MA, Letinski DJ. 2000. Assessing the aquatic toxicity of complex hydrocarbon mixtures using solid phase microextraction. *Toxicol Lett* 112:273–282.
37. Verbruggen EMJ, Vaes WHJ, Parkerton TF, Hermens LM. 2000. Polyacrylate coated SPME fibers as a tool to simulate body residues and target concentrations of complex organic mixtures for estimation of baseline toxicity. *Environ Sci Technol* 34:324–331.
38. Driscoll SK, Landrum PF. 1997. A comparison of equilibrium partitioning and critical body residue approaches for predicting toxicity of sediment-associated fluoranthene to freshwater amphipods. *Environ Toxicol Chem* 16:2179–2186.
39. U.S. Environmental Protection Agency. 2000. Method for the derivation of site-specific equilibrium partitioning sediment guidelines (ESGs) for the protection of benthic invertebrates: Nonionic organics. EPA-822-R-00-007. Office of Science and Technology, Office of Research and Development, Washington, DC.

APPENDIX. Sediment physical and chemical characteristics[a]

Site (NY, USA)	Sample name	PAH concentration (mg/kg dry wt)			Grain size (wt %)[a]			Total organic carbon (wt %)	Soot carbon (wt %)	Pore-water DOC (mg/L)[e]
		PAH_{13}[b]	PAH_{16}[c]	PAH_{34}[d]	Sand	Silt	Clay			
Hudson	HD3	286	310	971	25	38	37	3.2	0.5	11.1
	HD4	9.3	10.6	32.5	62	15	23	1.8	0.7	6.7
	HD5	711	779	2,230	9	49	43	3.3	0.6	17.3
	HD6	246	302	1,030	19	40	41	2.8	0.4	6.5
	HD8	5,260	5,700	11,400		ND		4.6	0.7	27.0
	HD9	26.8	31.2	84.2	3	57	40	3.2	0.4	7.9
	HD10	74.6	91.7	232	3	56	41	0.4	0.3	5.1
	HD12	6.3	8.8	27.1	18	47	35	3.5	0.4	8.1
	HD13	104	140	273	67	14	19	10.3	3.9	7.9
	HD14	84.4	128	260	70	13	18	7.7	1.7	9.9
	HD15	65.0	92.0	177	69	14	17	6.8	5.1	9.7
	HD19	10.6	13.0	58.1	31	41	28	8.5	3.1	3.7
	HD22	6.1	7.8	22.7	8	49	43	2.9	0.5	3.9
Oneonta	ON3	54.8	67.4	117	57	33	11	3.1	0.4	15.0
	ON5	40.2	45.1	107	58	27	16	4.6	1.9	4.7
	ON6	339	380	1,040	64	25	11	11.0	5.0	4.1
	ON7	372	429	944	40	42	18	5.1	2.5	5.4
Plattsburgh	PL1	5.0	6.0	16.2	20	61	19	4.8	0.7	2.5
	PL2	56.2	67.6	203	61	31	8	4.2	0.7	3.1
	PL4	77.6	91.7	267	87	7	6	2.4	0.5	9.1
	PL8	1,340	1,380	3,430	76	15	9	6.5	0.3	24.0
	PL11	52.3	54.5	139	93	3	4	0.7	0.5	23.1
	PL13	156	168	493	79	10	11	1.2	0.4	10.9
	PL14	48.3	53.5	122	90	5	5	0.7	0.2	7.1
Troy	TR1	145	171	326	65	26	9.2	1.5	0.4	4.3
	TR3	547	609	962	70	18	12	3.9	1.8	3.9
	TR7	135	167	319	94	4	2	4.8	4.1	39.7
	TR9	17.6	21.1	41.3	76	15	10	1.5	0.7	8.8
	TR11	3.6	4.4	10.8	85	7	8	2.1	1.2	24.0
	TR12	1,030	1,160	1,840	62	26	12	3.9	1.2	4.4
	TR13	498	553	911	47	37	17	3.1	1.1	9.0
	TR15	1,510	1,730	2,990	71	18	11	3.6	0.7	6.7
	TR17	44.4	52.5	108	76	14	10	4.3	3.0	9.4
	TR22	4.8	5.6	9.8	85	10	6	0.6	0.2	17.1

[a] ND = not determined.
[b] Sum of 13 polycyclic aromatic hydrocarbons (PAHs) noted below with an asterisk.
[c] Sum of 16 PAHs noted below with an dagger.
[d] Sum of 34 PAHs: naphthalene*†, C1 naphthalenes, C2 naphthalenes, C3 naphthalenes, C4 naphthalenes, acenaphthylene*†, acenaphthene*†, fluorene*†, C1 fluorenes, C2 fluorenes, C3 fluorenes, phenanthrene*†, anthracene*†, C1 phenanthrenes/anthracenes, C2 phenanthrenes/anthracenes, C3 phenanthrenes/anthracenes, C4 phenanthrenes/anthracenes, fluoranthene*†, pyrene*†, C1 fluoranthenes/pyrenes, benz[*a*]anthracene*†, chrysene*†, C1 chrysenes, C2 chrysenes, C3 chrysenes, C4 chrysenes, benzo[*b*+*k*]fluoranthene*†, benzo[*e*]pyrene, benzo[*a*]pyrene*†, perylene, indeno[1,2,3-*cd*]pyrene†, dibenz[*a,h*]anthracene†, benzo[*ghi*]perylene†.
[e] Dissolved organic carbon after flocculation to remove colloids as described in MacDonald et al. [33].

APPENDIX K

PREDICTING BIOAVAILABILITY OF SEDIMENT POLYCYCLIC AROMATIC HYDROCARBONS TO *HYALELLA AZTECA* USING EQUILIBRIUM PARTITIONING, SUPERCRITICAL FLUID EXTRACTION, AND PORE WATER CONCENTRATIONS

PUBLISHED IN *ENVIRONMENTAL SCIENCE TECHNOLOGY*

Environ. Sci. Technol. **2007**, *41,* 6297−6304

Predicting Bioavailability of Sediment Polycyclic Aromatic Hydrocarbons to *Hyalella azteca* using Equilibrium Partitioning, Supercritical Fluid Extraction, and Pore Water Concentrations

STEVEN B. HAWTHORNE,*,† NICHOLAS A. AZZOLINA,‡ EDWARD F. NEUHAUSER,§ AND JOSEPH P. KREITINGER‡

Energy and Environmental Research Center, University of North Dakota, Grand Forks, North Dakota 58201, The RETEC Group, Incorporated, 1001 West Seneca Street, Suite 204, Ithaca, New York 14850, and National Grid, 300 Erie Boulevard West, Syracuse, New York 13202

Polycyclic aromatic hydrocarbon (PAH) bioavailability to *Hyalella azteca* was determined in 97 sediments from six former manufactured-gas plants and two aluminum smelter sites. Measurements of Soxhlet extractable, rapidly released based on mild supercritical fluid extraction, and pore water dissolved concentrations of 18 parent and 16 groups of alkyl PAHs (PAH_{34}) were used to predict 28 day survival based on equilibrium partitioning and hydrocarbon narcosis models. Total PAH concentrations had little relationship to toxicity. Amphipods survived in sediments with PAH_{34} concentrations as high as 2990 μg/g, while sediments as low as 2.4 μg/g of PAH_{34} resulted in significant mortality. Equilibrium partitioning using either total extractable or rapidly released concentrations significantly improved predictions. However, pore water PAH_{34} concentrations were best for predicting amphipod survival and correctly classified toxic and nontoxic sediment samples with an overall model efficiency of 90%. Alkyl PAHs accounted for 80% of the toxicity, demonstrating that careful measurement of the 16 alkyl clusters in pore water is required. Regression analysis of the pore water PAH_{34} data from 97 field sediments against amphipod survival resulted in a mean 50% lethal residue value of 33 μmol/g of lipid, consistent with 32 μmol/g of lipid for fluoranthene determined by others in controlled laboratory conditions, thus demonstrating the applicability of EPA's hydrocarbon narcosis model when using pore water PAH_{34} concentrations.

Introduction

Industries that historically produced or utilized coal tars have been a major source of polycyclic aromatic hydrocarbons (PAHs) to the environment. Many such industries were (or are) located on waterways, with the result that PAH-contaminated sediments are often associated with these sites. In addition, sediments that have not been impacted by these industries contain background levels of PAHs from other sources such as atmospheric deposition of combustion particulates and background levels of PAHs that typically exceed baseline regulatory concentrations. For example, the regulatory based probable effects concentration (PEC, the total concentration of 13 parent PAHs above which toxicity is expected to be likely) and the threshold effects concentration (TEC, below which toxicity is considered unlikely) are 22.8 and 1.6 μg/g, respectively (*1*), but few urban sediments have PAH concentrations below either criteria. In a recent study of 114 field-collected sediments (both background and impacted), only 27 had PAH_{13} concentrations below the PEC, and only four sediments were below the TEC (*2*).

Efforts to improve predictive methods for the risks posed by sediments contaminated with PAHs have used equilibrium partitioning models to predict the partitioning of PAHs from sediments to water based on natural organic carbon−water partitioning coefficients (K_{OC}) (*3*−*6*). However, historically contaminated sediments often have much lower pore water concentrations of PAHs than predicted using literature K_{OC} values (*2*, *7*), supposedly because of a greatly reduced availability of PAHs as sequestration processes occur. Measured K_{OC} values for historically contaminated sediments from manufactured-gas plant (MGP) sites have been reported to be as much as 3 orders of magnitude higher than literature K_{OC} values used in equilibrium partitioning models (*2*, *7*). There are also an increasing number of reports demonstrating that many different carbon types (such as coal, combustion soots, charcoal, and coal tar pitch) are present in many sediments that bind PAHs much more tightly (and result in much less partitioning to water) than predicted by equilibrium partitioning models using K_{OC} values based on natural organic carbon (*2*, *7*−*12*).

Recently, there has been an increasing amount of evidence that the bioavailability of PAHs can also be overestimated using equilibrium partitioning models based on K_{OC} values that are normally used to describe partitioning with natural organic carbon. Several investigators have reported the lack of observable toxicity to aquatic organisms, despite high sediment concentrations of PAHs (*13*−*16*), and PAH uptake in *Lumbriculus variegatus* was found to be as much as 1000-fold less than predicted by sediment PAH concentrations and equilibrium partitioning models (*17*). In a recent report, the toxicity of PAHs in sediments from MGP sites to *Hyalella azteca* was found to be much lower than predicted by the equilibrium partitioning model but was more accurately predicted when either pore water PAH concentrations or the rapidly released PAH concentrations measured by mild supercritical fluid extraction (SFE) were used rather than sediment concentrations (*18*). The use of mild SFE to obtain rapidly released PAH concentrations has also previously been reported to correlate with water desorption of PAHs from soils (*19*, *20*) and to improve the prediction of earthworm uptake of PAHs from soil (*21*).

In the present study, we compare three approaches to predict the availability of PAHs to *H. azteca* based on a 28 day chronic toxicity test. Ninety-seven background and industrially impacted sediments were studied from six different MGP sites and from two aluminum smelting operations. Both 18 parents and 16 groups of alkyl PAHs (PAH_{34}) suggested by the U.S. EPA (*5*) were measured in the sediment, the pore water, and the rapidly released or available fraction based on mild SFE (*19*−*21*). Predictions of the internal

* Corresponding author phone: (701)777-5256; fax: (701)777 5181; e-mail: shawthorne@undeerc.org.
† University of North Dakota.
‡ The RETEC Group, Inc.
§ National Grid.

10.1021/es0702162 CCC: $37.00 © 2007 American Chemical Society
Published on Web 08/04/2007

PAH concentrations expressed on a lipid basis (and thus, toxicity to the organism) were based on the U.S. EPA's PAH equilibrium partitioning/hydrocarbon narcosis model (*3*−*6*), which uses generally accepted K_{OC} values to predict pore water PAH concentrations from sediment concentrations, followed by the use of octanol−water coefficients (K_{OW}) to predict the organism lipid PAH concentrations. Biota lipid PAH concentrations were also predicted using the rapidly released sediment PAH_{34} concentrations and the measured pore water PAH_{34} concentrations as input data to the equilibrium partitioning model proposed by the U.S. EPA (*3*−*6*).

Experimental Procedures

Sediment Collection and Characterization. Sediment collection procedures and analytical methods have been described in detail in earlier reports (*2*, *22*, *23*). In brief, approximately 150 sediments were collected using a Ponar grab sampler or, in a few cases, with a shovel. Approximately 15 L of the sediment−water slurry was transferred to a clean bucket, sieved through a 2 mm screen, briefly mixed, transferred to new glass jars with Teflon-lined lids, and immediately placed on ice. This procedure resulted in sediment−water slurries with approximately 40−70% water content. Samples were shipped by overnight air to the analytical and toxicology laboratories and stored at ca. 4 °C until use. All analytical tests were completed, and all biological tests were begun within 28 days of sample collection. All sediments were initially screened for PAH concentrations and total organic carbon (TOC) to select 97 sediments that best represented the range of PAH concentrations, organic carbon contents, and sediment textures existing at each site, as well as to spatially represent the site. Field reference sediments were also selected from each site that were not contaminated by the MGP or aluminum smelter activities (based on their PAH concentrations).

TOC and BC (black or soot carbon) were determined by elemental analysis (C,H,N) after acidification with HCl to remove inorganic carbonates. Samples for BC were prepared by oxidation under air at 375 °C for 24 h in a gas chromatographic oven (*24*). Pore water dissolved organic carbon (DOC) was determined after alum flocculation using the U.S. EPA method 415.1.

The 34 PAHs (18 parent and 16 groups of alkyl PAHs) listed for the sediment PAH narcosis model by the U.S. EPA (*5*) were determined in all sediment Soxhlet extracts, SFE extracts, and pore water samples. Each method used several 2- to 6-ring perdeuterated PAHs as analytical internal standards to aid in quantitation (*22*, *23*). Pore water PAH concentrations were obtained in quadruplicate by briefly centrifuging the sediment−water slurry, flocculating with alum twice to remove colloids, adding the perdeuterated internal standards, and quantitative analysis of the dissolved pore water PAH concentrations with solid-phase microextraction (SPME) and GC-MS with selected ion monitoring (*22*). SFE available and total extractable sediment PAH concentrations were based on extractions of quadruplicate 2 g samples of the sediment, after the pore water fraction was removed by centrifugation, and the sediments were mixed with 4 g of sodium sulfate (*23*). SFE was performed for 40 min with pure carbon dioxide at 200 bar, 50 °C, and a flow rate of 1.0 mL/min (*19*, *20*). Soxhlet extraction was performed for 18 h with 150 mL of acetone−methylene chloride (1:1).

Toxicity Testing. Toxicity to *H. azteca* was determined using EPA method 100.4 for a 28 day exposure period as previously described (*18*).

Data Analysis. Statistical analysis of amphipod survival data was performed to estimate the dose response for each of the three chemical measurements of PAHs. A Probit regression model was run in SAS (SAS Institute, Cary, NC) using the Probit procedure to estimate the mean amphipod survival. The statistical fits (χ^2 test of significance) of the Probit regression models were evaluated for significance ($\alpha = 0.05$), and the mean dose and probability were presented as the Probit regression line. The 85% survival dose was determined by the lower 95% confidence interval for a probability of 85% survival, and the 15% survival dose was represented by the upper 95% confidence interval for a probability of 15% survival. The modeled dose response for each of the three chemical measurements of PAHs was evaluated for statistical fit using a binary logistic regression model and the Goodman−Kruskal γ (*25*). The Goodman−Kruskal γ is a rank-order correlation statistic used as a measure of association for the ability of a predictor variable (e.g., pore water concentrations) to explain the response variable (the binary variable toxic or nontoxic). The γ value ranges from −1.0 (no predictive ability) to 1.0 (perfect predictor).

TABLE 1. Summary of Sediment and Pore Water Characteristics

	units	minimum	maximum	median
Bulk Sediment[a]				
total PAH_{34}	μg/g	1.31	17 600	277
total PAH_{16}	μg/g	0.22	8580	128
TOC	wt %	0.3	42.4	3.3
BC	wt %	0.1	39.7	0.8
Fraction (BC/TOC)	%	5.3	100	33.4
% alkylated PAHs[b]	%	25	94	53
2- and 3-ring PAHs/ total PAH_{34}[c]	%	6	96	38
Sediment Pore Water				
total PAH_{34}	ng/mL	0.02	10 900	17.4
total PAH_{16}	ng/mL	0.02	9250	5.84
DOC	mg/L	1.4	114	4.35

[a] Sediment PAH concentrations are on a dry weight basis. [b] Total concentration of alkyl PAHs divided by total PAH_{34} concentration. [c] Sum concentration of all 2- and 3-ring PAHs divided by total PAH_{34} concentration.

Results and Discussion

Sediment Characteristics and PAH Concentrations. Sediment textures ranged from coarse sand to fine-grained silts and clays. The TOC ranged from 0.3 to 42 wt %, and BC ranged from 0.1 to 40 wt %, indicating both low and high impact from industrial carbon residues. The fraction of BC as compared to TOC ranged from 5% (indicating no significant contribution of BC) to 100% (indicating that all organic carbon in that sediment was present as BC).

PAH concentrations for the 97 test sediments are summarized in Table 1 and Table S1. PAH concentrations ranged from very low background concentrations (<μg/g) to sediments contaminated as high as 1.8 wt % (17 600 μg/g) total PAH_{34}. The fraction of low molecular weight PAHs (sum of 2- and 3-ring PAHs) as compared to the total EPA_{34} PAHs (2−6-ring) ranged from 6% (indicating highly weathered PAHs from coal tar or coal tar pitch) to 96% (indicating unweathered coal tar PAHs) (Table 1). Pore water concentrations also ranged over several orders of magnitude, from a lowest detected concentration of 0.02 ng/mL to a highest detected concentration of 10 900 ng/mL (PAH_{34}). A more complete description of the individual 34 PAH concentrations for sediment, the SFE rapidly released fractions, and the pore water concentrations is given in Table S1.

Approximately 10 impacted sediments showed significant heterogeneity in the quadruplicate Soxhlet extracts and in the SFE extracts. For these sediments, the relative standard deviations (RSDs) for individual and total PAH_{34} concentrations in the quadruplicate extracts sometimes exceeded 50

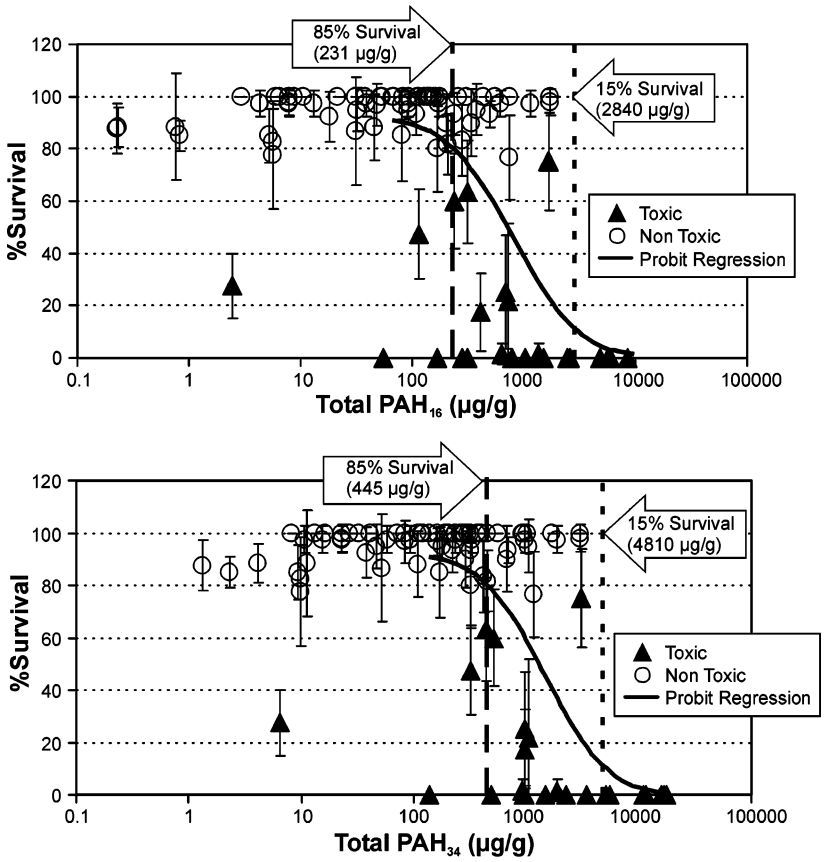


FIGURE 1. ***H. azteca*** **survival as compared to total extractable PAH_{16} and PAH_{34} sediment concentrations.**

or 100%, and for such sediments, the replicate extracts usually ranged from light yellow to dark brown. When this occurred, a second set of quadruplicate samples was extracted and analyzed, and in all cases, the sediment PAH heterogeneity was confirmed, demonstrating that the sediments contained blebs of nonaqueous-phase coal tars, pieces of coal tar pitch, or pieces of other material that were highly contaminated with PAHs. In many cases, visual observation under a low-power microscope confirmed the presence of pitch particles and/or tar droplets. In contrast, the quadruplicate pore water samples from each sediment showed good reproducibility, with % RSDs typically less than 10% for low- and mid-molecular weight PAHs and typically less than 15% for higher molecular weight PAHs.

Survival Predictions Based on Sediment Total Extractable PAH Concentrations. Out of the 97 sediments used to compare toxicity predictions, 25 were found to result in reduced survival to *H. azteca* following 28 day exposures. Both MGP and aluminum smelter sites had toxic sediments, with 17 out of the 73 MGP sediments causing reduced survival and eight of the 24 aluminum smelter sediments causing reduced survival.

The poor relationship between total PAH concentrations expressed as micrograms per gram (whether EPA_{16} or EPA_{34}) and *H. azteca* survival is shown in Figure 1. When compared to the probable effects concentration (PEC) of 22.8 μg/g (PAH_{13}) (*1*), 73 of the sediments have total PAH_{13} concentrations that exceed the PEC, yet 67% of those that exceed the PEC value are nontoxic. Similarly, the total extractable PAH_{34} concentration shows little relationship to *H. azteca* survival (Figure 1). Except for sediments with very low or very high PAH concentrations, total PAH concentrations have little or no ability to predict toxicity. (Note that the plot for total PAH_{13} versus toxicity is essentially identical to that shown in Figure 1 for total PAH_{16} since PAH_{13} concentrations are only a few percent lower than the PAH_{16} values for all of these sediments.)

The use of equilibrium partitioning models as developed by DiToro and others and proposed as a regulatory sediment guidance approach by the U.S. EPA (*3*−*6*) to improve toxicity predictions is explored in Figure 2. This model first predicts pore water concentrations from sediment concentrations using literature and modeled K_{OC} values. The resultant pore water PAH concentrations are then used to predict biota lipid concentrations using K_{OW} values for each of the 34 parent and groups of alkyl PAHs (*3*−*6*). Thus, proper prediction of the final lipid total molar PAH concentration depends heavily on the assumption that K_{OC} values used for each individual PAH (and each group of alkyl clusters) are correct and that each PAH has a single K_{OC} value that applies to all sediments.

As shown in Figure 2 (top), the use of total extractable PAH_{34} sediment concentrations and the equilibrium partitioning model (*5*, *6*) significantly improves the ability to predict survival to *H. azteca* over the simple use of total extractable (PAH_{34} or PAH_{16}) concentrations shown in Figure 1. The overall model efficiency (% of correct predictions) improves from 48 to 80% (Table 2). However, substantial overlap between toxic and nontoxic sediments still exists, and 26 of the 97 sediment samples lie within the region of 85−15% survival where it is difficult to make statistically strong predictions of toxicity (Table 2). The scatter in predicted versus actual survival shown in the top of Figure 2 might be expected based on a recent report where the K_{OC} values for each individual PAH from a similar set of sediments (including most of the sediments reported here) varied by nearly 3 orders of magnitude for the same PAH from different sediments. For example, while a log K_{OC} value for pyrene of 4.84 is used for all sediments in the model (*5*), measured values of log K_{OC} in 114 sediments ranged from 4.17 to 7.40, and the median log K_{OC} was 5.81, nearly one log unit higher

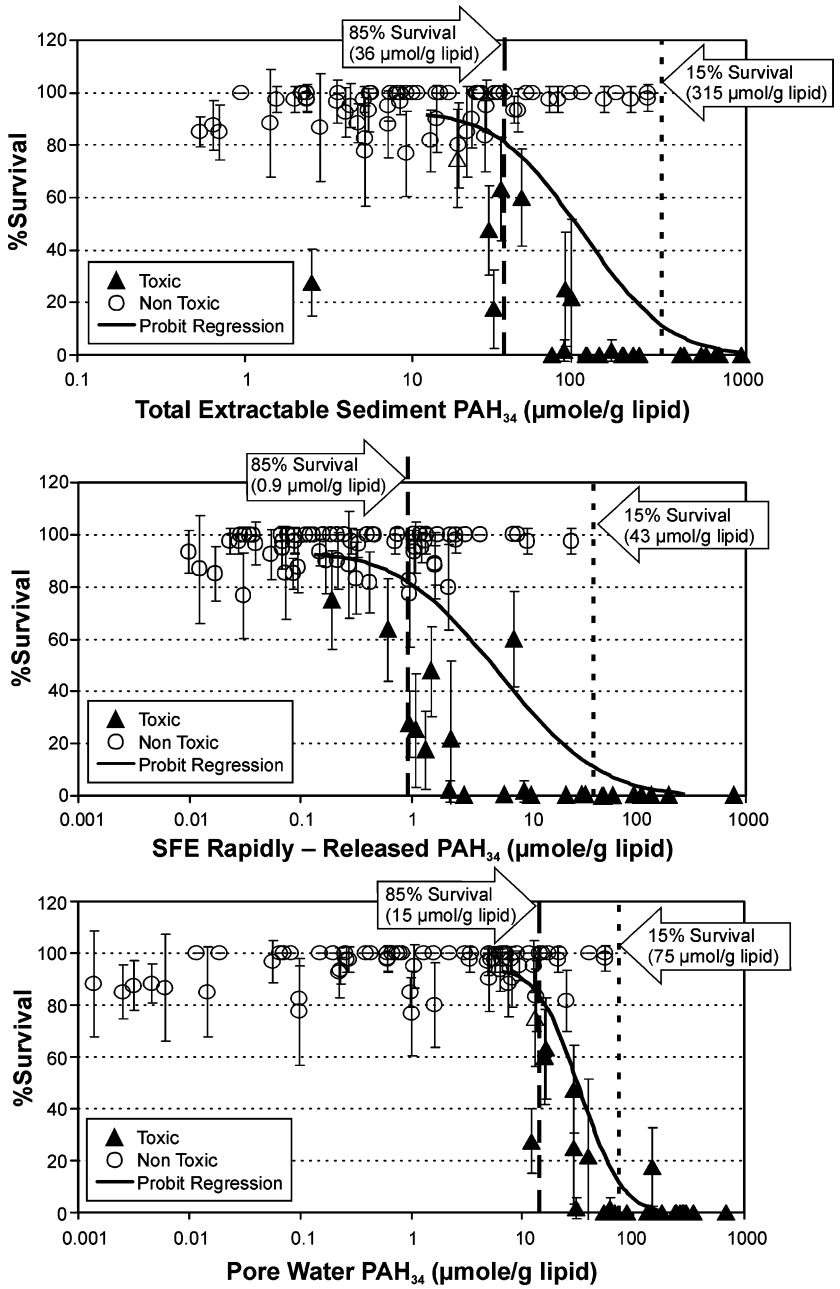


FIGURE 2. ***H. azteca*** **survival (28 day) as compared to predicted micromol per gram of lipid for 97 sediments based on total extractable sediment, SFE rapidly released, and pore water PAH_{34} concentrations.**

than the value used in the model (*2*, *5*). Thus, the essential assumption in the equilibrium partitioning model that K_{OC} values for a single PAH are constant for all sediments is not supported by experimental data on field sediments (*2*, *7*).

Survival Predictions Based on SFE Rapidly Released PAH Concentrations. The next approach we investigated was to replace the total Soxhlet extractable concentrations for each PAH by the concentration that was measured to be rapidly released by mild SFE, then predicting the lipid PAH concentrations in the same manner as that used for the total extractable concentrations using equilibrium partitioning modeling based on literature K_{OC} and K_{OW} values (*3*−*6*). As shown in Figure 2 (middle), replacing the total extractable PAH concentrations with rapidly available PAH concentrations does not appear to significantly differentiate toxic and nontoxic sediment samples. The Goodman−Kruskal γ increases modestly from 0.78 to 0.80, and the model sensitivity (correctly identifying toxic samples) increases from 80 to 92%. However, in gaining sensitivity, the model specificity (correctly identifying nontoxic samples) decreased significantly to 64%, with an even greater number of samples (forty) within the uncertain region of 85−15% survival, forcing the overall model efficiency to 71% (Table 2).

Survival Predictions Based on Pore Water PAH Concentrations. Finally, the predicted biota lipid PAH concentrations were determined based on measured pore water concentrations for PAH_{34}. The pore water approach has the obvious advantage that only K_{OW} values for each PAH need to be accurate (and apply to all water samples) (*3*−*6*), which is certainly more valid than the assumption that K_{OC} values are correct and apply to all sediment samples (*2*, *7*−*12*). As shown in Figure 2 (bottom), the use of pore water concentrations and lipid−water partitioning coefficients based on K_{OW} values significantly improves the prediction of toxic and nontoxic sediments over the other two approaches discussed previously. The Goodman−Kruskal γ improves dramatically, to 0.95. In addition, the model sensitivity (correctly predicted toxicity) reaches 92%, and the specificity (correctly predicted

TABLE 2. Survival Predictions for *H. azteca* using Total Extractable, SFE Rapidly Released, and Pore Water PAH_{34} Concentrations from 97 Field Sediments

method	15−85% survival range (μmol/g of lipid)[a]	no. of sediments in 15−85% range	prediction efficiencies			Goodman−Kruskal γ
			sensitivity[b] (%)	specificity[c] (%)	overall[d] (%)	
PAH_{13} concn > 1.6 mg/kg (TEC)[e]			100	6	30	0.73
PAH_{13} concn > 22.8 mg/kg (PEC)[f]			96	32	48	0.75
PAH_{34} concn	36−315	26	80	81	80	0.78
SFE rapidly released PAH_{34}	0.9−43	40	92	64	71	0.80
pore water PAH_{34}	15−75	17	92	89	90	0.95

[a] Lower 95% confidence interval for 85% survival and upper 95% confidence interval for 15% survival. [b] Sensitivity is the extent to which a test correctly classifies a toxic sample as toxic and is therefore protective of the environment. [c] Specificity is defined as the rate at which a test correctly classifies a nontoxic sample as nontoxic. [d] Overall efficiency is the fraction of correct predictions for all samples. [e] TEC is the sum of 13 parent PAH concentrations below which toxicity is considered unlikely (*1*). [f] PEC is the sum of 13 parent PAH concentrations above which toxicity is considered likely (*1*).

nontoxic) reaches 89%, yielding an overall model efficiency of 90% (Table 2).

Since the data presented in Figure 2 come from sediments from eight different MGP and aluminum smelter sites, and since they encompass such a large range of carbon concentrations and types (as well as PAH concentrations), it would seem likely that specific sites or sediment characteristics may strongly relate to the toxicity and/or ability of the models to predict toxicity. However, there is no apparent relationship between toxicity and model behavior among the different sites or between MGP and aluminum sites (Supporting Information Figure S1). In addition, there appears to be no relationship between total TOC (Supporting Information Figure S2), BC (Supporting Information Figure S3), the fraction of BC as compared to TOC (Supporting Information Figure S4), or sediment texture (not shown) on the predictive abilities of the models based on total extractable PAH, SFE available PAH, or porewater PAH concentrations.

As shown in Table 2 and Figure 2, Probit regression analysis shows that the number of sediment samples within the 95% confidence interval for 15−85% survival was significantly fewer for the pore water predictions than for either SFE rapidly released or total extractable concentrations. With the pore water data, only two toxic sediments were incorrectly predicted to have less than 85% survival (i.e., had a predicted μmol/g of lipid PAH concentration below the 85% survivability line). However, it should be noted that the toxicity for the sediment with ca. 30% survival (Figure 2, bottom) is unlikely to be caused by PAHs since the total sediment concentrations were only 2.4 μg/g (PAH_{16}) and 6.4 μg/g (PAH_{34}). In fact, only three sediments out of the 97 shown in Figure 2 had lower total extractable PAH concentrations. This sediment also had only 0.2 wt % natural organic carbon (0.8 wt % TOC minus 0.6 wt % SOC) and was composed primarily of sand. Recent studies with uncontaminated sandy sediments show that the lack of essential nutrients may cause mortality, even with the addition of food as per EPA method 100.4 (Francis Doherty, personal communication). Unidentified toxic agents may have also been present. However, none could be found by full-scan GC-MS analysis of the pore water and sediment extracts. The other sample defined as toxic (by failing the 85% survival criteria) but predicted as nontoxic had a survival of 75 ± 19% (Figure 2, bottom), which is very close to the conservative 85% survival value used in our study as the cutoff for nontoxic sediments.

Ideally, any method used to predict biological effects of pollutants would be conservative (i.e., tend to overpredict rather than underpredict toxicity). The pore water predictions of toxicity had a sensitivity of 92%, classifying only two sediments (just discussed previously) out of 25 toxic sediment samples as nontoxic (Table 2). The pore water predictions also had fewer sediments in the uncertain region between 85 and 15% survival as compared to the rapidly released and total extractable PAH_{34} approaches (Table 2). These results from Probit regressions show that (to a 95% confidence level) sediments falling outside the 85−15% survival range would not need to be tested during field surveys but could be directly classified as toxic or nontoxic based on pore water PAH concentrations. The sediments falling between the predicted 85 and 15% survival range would then require toxicity testing. Thus, for the 97 sediments, 26 sediments would require biological testing based on total extractable sediment PAH_{34} concentrations, 40 sediments for rapidly available sediment concentrations, while only 17 sediments would need biological testing for the predictions based on pore water concentrations (Table 2).

The relatively poor ability of rapidly released PAH concentrations to predict toxicity was a surprise since our initial report comparing pore water and SFE predictions showed both methods to improve toxicity predictions over using total extractable concentrations and the equilibrium partitioning model (*18*). All of the sediments studied in the initial report were from MGP sites, while the present study includes sites near aluminum smelters that had higher relative proportions of high molecular weight PAHs (likely associated with coal tar pitch used for anode production) than the MGP sites (likely contaminated with MGP tars having a lower molecular weight distribution). There are no apparent differences in toxicity (or the ability to predict toxicity) between MGP and aluminum sites as shown in Figure S1.

The poorer predictive ability of mild SFE is likely a result of the types of available PAH molecules measured by the two techniques. Pore water PAH measurements determine the equilibrium (or near equilibrium) pore water PAH concentrations found in the sediment−water slurry. In contrast, SFE measures the capacity of the sediment to rapidly release PAHs to water (*19*). Since *H. azteca* presumably absorbs solvated PAHs from the water phase, it would seem logical that pore water concentrations may more closely reflect exposure in the toxicity tests. It should also be noted that sediment heterogeneity (discussed previously) also likely reduces the predictive abilities of both total extractable and rapidly released PAH concentrations, as compared to the good homogeneity shown by pore water PAH concentrations. In any case, the results in Figure 2 and Table 2 clearly demonstrate that pore water concentrations are superior to either total extractable PAH_{34} or SFE rapidly available PAH_{34} concentrations in predicting *H. azteca* mortality.

Since the two methods measure different phenomena (capacity to rapidly release PAHs vs equilibrium pore water concentrations), it was hoped that a combination of the two approaches would further increase the ability to predict

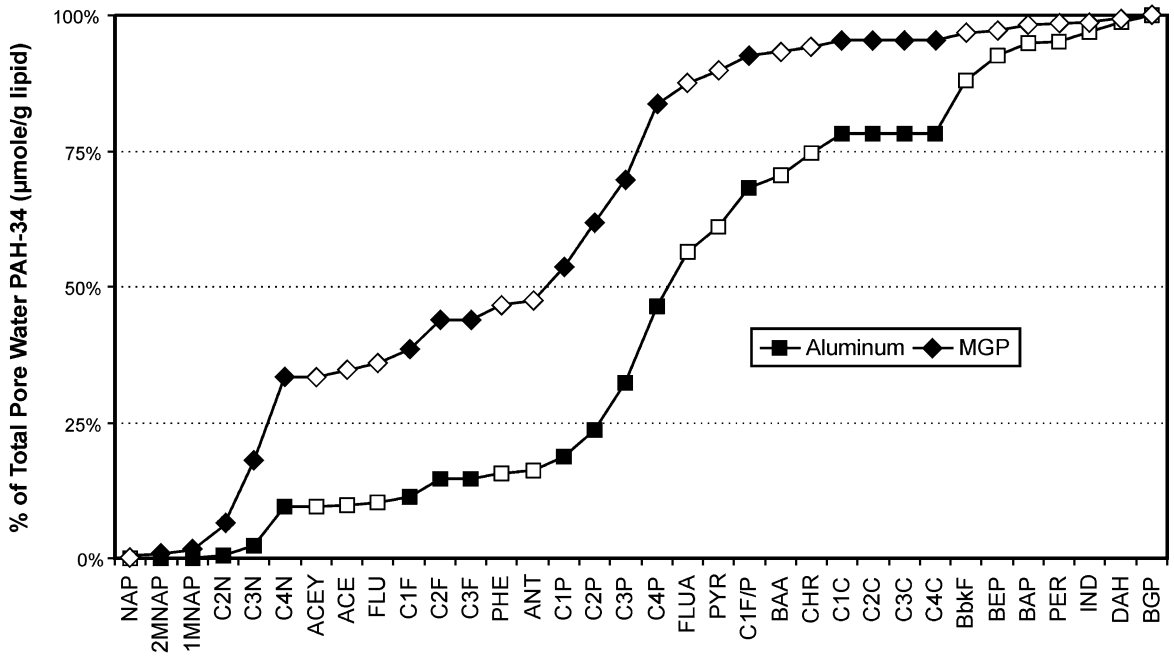


FIGURE 3. Cumulative average relative contribution to lipid PAH burden of each of the 34 PAHs in MGP and aluminum smelter sediment pore water samples. Alkyl PAHs are designated with a solid symbol and parent PAHs with an open symbol.

toxicity. As would be expected, the molar PAH lipid concentrations predicted by SFE and pore water measurement concentrations are correlated, but not strongly, and a linear regression of the log SFE μmol/g of lipid versus log pore water μmol/g of lipid only has an r^2 value of 0.55. A binary logistic regression model using the molar PAH lipid concentrations predicted by pore water as the predictor and toxic/nontoxic as the response was statistically significant ($p < 0.0001$) and explained approximately 95% of the variance in toxicity (Goodman−Kruskal $\gamma = 0.95$). Unfortunately, adding the molar PAH lipid concentrations predicted by SFE as an additional predictor was not statistically significant ($p = 0.657$), nor did it change the Goodman−Kruskal γ, indicating that adding SFE to the pore water measurements did not improve the predictions of toxicity. Thus, based on the results from the 97 sediments, there was no added value in obtaining the SFE rapidly released PAH_{34} concentrations for the prediction of *H. azteca* toxicity in freshwater sediments.

Effect of Nonaqueous-Phase Hydrocarbon Liquids (NAPL). As might be expected for sediments collected in industrial waterways, approximately one-third of the samples had a sheen or NAPL phase observed during sample collection, which was later confirmed by independent observation at the analytical laboratory. Any significant amount of a NAPL hydrocarbon phase (whether PAHs or other hydrocarbons such as petroleum alkanes) could change the mechanism from sediment−water partitioning to liquid−liquid partitioning. Since the equilibrium partitioning model assumes that PAH partitioning occurs between natural organic carbon on the sediment and pore water, the presence of the NAPL phase in one-third of our test sediments may contribute to the model's inability to predict pore water concentrations and thus reduce its ability to predict toxicity.

Surprisingly, the presence or absence of NAPL did not have a significant relationship to toxicity or on the predicted μmol/g of lipid PAH concentrations, as shown in Supporting Information Figure S5. Out of the 97 sediments tested, 27 had NAPL phases, of which 17 were toxic and 10 were nontoxic. Note also that removing the NAPL containing sediments from the sample set did not improve the prediction of toxicity based on the total Soxhlet extractable PAH_{34} concentrations or the rapidly available PAH_{34} concentrations.

Field versus Laboratory Determination of Threshold Toxicity Concentration. As shown in Figure 2, the sediment samples used in this study had a fairly broad distribution of nontoxic, moderately toxic, and toxic samples that allows a lethal residue (LR_{50}) to be calculated using Probit regression modeling. For the field sediments used in this study, the LR_{50} lipid concentration (total PAH_{34} molar concentration) was 33.0 μmol/g of lipid (31−35 μmol/g of lipid, 95% CI). This value is in good agreement with a laboratory-determined value recently reported by Schuler et al. (*26*) for fluoranthene with 28 day exposures to *H. azteca* of 32 μmol/g of lipid (26−40 μmol/g of lipid, 95% CI). This good agreement from controlled laboratory exposure and from 97 field sediments validates the EPA's narcosis model with pore water concentrations for describing PAH toxicity to sensitive benthic organisms such as *H. azteca*.

Relative Contributions of Alkyl and Parent PAHs to Toxicity Predictions. Historically, only 16 (or 13) parent PAHs determined by EPA method 8270 are considered in regulatory processes, as well as in the majority of scientific studies reported in the literature. This is potentially misleading since the majority of PAHs found in the environment are likely to be alkylated rather than parent PAHs. For example, a recent report showed that ca. 99% of the PAHs in a petroleum crude oil was alkylated and that ca. 60−70% of the PAHs in MGP coal tars was alkylated (*23*). In recognition of the potential importance of alkylated PAHs, the EPA's PAH hydrocarbon narcosis model suggests that a total of 18 prominent parent PAHs and 16 groups of alkyl PAHs be measured as was done in the present study (*5*). (Note that, with the exception of the two methylnaphthalene isomers, each group of alkyl PAHs can contain a few to nearly 100 isomers, so that this list of 16 groups of alkyl PAHs represents several hundreds of PAHs (*23*).) The potential impact of the alkyl PAHs on the predicted toxicity is potentially quite large since their modeled K_{OW} values are significantly larger than the related PAH (*3*−*5*), and therefore, the concentration of a particular group of alkyl PAHs can be much lower to account for one predicted toxic unit than the concentration required for the related

parent PAH. For example, the pore water concentrations required for one toxic unit in the EPA model (equivalent to a concentration of 2.24 μmol/g of lipid) for naphthalene, and its C1, C2, C3, and C4 isomers, were 194, 82, 30, 11, and 4 ng/mL, respectively (*5*, *22*). Therefore, C4 naphthalenes contribute nearly 50-fold higher toxic units than the same concentration of naphthalene in pore water. In contrast, the concentrations of sediment PAHs that account for one toxic unit only vary by a factor of 2, essentially since the partitioning coefficients (K_{OC} and K_{OW}) used in the model tend to cancel each other's effect on the predicted lipid concentration (*5*).

Figure 3 shows the average relative contributions of the different PAHs and groups of alkyl PAHs in pore water to predicted bioaccumulation and related PAH narcosis for all non-background sediments (i.e., sediments that had the majority of PAHs as nondetected are not included). Two features are important to note in these data. First, an average of 96% (MGP) and 78% (aluminum) of the predicted toxicity is contributed by 2−4 four-ring PAHs (e.g., naphthalene through chrysene), and the measurement of 5- and 6-ring PAHs in pore water does little to change the predicted lipid molar PAH concentration. This is fortunate since the measurement of 5- and 6-ring PAHs at the required picogram per milliliter detection limits is difficult (*22*). The results in Figure 3 also show that, since only the higher molecular weight PAHs have significant partitioning into dissolved organic matter (DOM) (*22*), there is no significant difference in the predicted lipid PAH concentrations (or the EPA toxic units (*5*)), whether freely dissolved (PAHs associated only with the pore water phase) or total dissolved (freely dissolved PAHs and PAHs associated with DOM). For the 97 sediments used in the present study, predicting the lipid PAH concentrations using freely dissolved or total dissolved PAH concentrations did not yield significantly different results in model interpretations. Therefore, the distinction of freely dissolved and total dissolved PAH concentrations for predicting PAH toxicity does not appear to be important as long as PAHs associated with colloids are removed by flocculation prior to analysis of the pore water (*22*).

The second observation from Figure 3 is that alkyl PAHs contribute an average of 81% (MGP) and 55% (aluminum) of the total predicted lipid molar PAH concentrations (and therefore 81 and 55% of the predicted toxicity, respectively). For the combined sites, 69% of the total toxicity was caused by the C1 to C4 alkyl naphthalene and phenanthrene/anthracene isomers. The importance of the alkyl PAHs to toxicity clearly demonstrates the need to apply methods capable of measuring both parent and alkyl PAHs (*22*, *23*) rather than relying on methods that only determine parent PAHs.

The results of this study involving 97 field sediments clearly demonstrate that the measurement of pore water PAHs is superior to the use of total extractable or SFE rapidly available PAH_{34} concentrations to accurately predict the bioavailability and toxicity of PAHs from background and impacted sediments using the EPA's hydrocarbon narcosis model. Considering sediment matrix characteristics such as TOC, BC, texture, and presence of NAPL did not appear to improve predictions based on total sediment PAH concentrations. In addition, it is clear that the common practice of measuring only the parent PAHs is not sufficient to describe environmental effects and that the high contribution of alkyl PAHs to the predicted sediment toxicity emphasizes the need for consistent and accurate methods to calibrate for, and quantitate, the complex clusters of isomers that make up most alkylated groups on the PAH_{34} list.

Acknowledgments

Financial support was provided by National Grid, New York State Electric and Gas, Alcoa, and U.S. Department of Energy (Cooperative Agreement DE-FC26-98FT40321). However, any opinions, findings, conclusions, or recommendations expressed herein are those of the authors and do not necessarily reflect the views of the sponsors. David Nakles, Todd Bridges, Daniel Farrar, and Francis Doherty are thanked for helpful discussions. Dave Miller, Carol Grabanski, Cory McNemar, Jessica Coleman, and William Blackburn are thanked for technical support.

Supporting Information Available

Five additional figures and a Table. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) MacDonald, D. D.; Ingersoll, C. G.; Berger, T. A. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. *Arch. Environ. Contam. Toxicol.* 2000, *39*, 20−31.

(2) Hawthorne, S. B.; Grabanski, C. B.; Miller, D. J. Measured partitioning coefficients for parent and alkyl polycyclic aromatic hydrocarbons in 114 historically contaminated sediments. Part 1. K_{OC} values. *Environ. Toxicol. Chem.* **2006**, *25*, 2901−2911.

(3) Di Toro, D. M.; McGrath, J. A.; Hansen, D. J. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. 1. Water and tissue. *Environ. Sci. Technol.* **2000**, *19*, 1951−1970.

(4) Di Toro, D. M.; McGrath, J. A. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. II. Mixtures and sediments. *Environ. Sci. Technol.* **2000**, *19*, 1971−1982.

(5) U.S. Environmental Protection Agency. *Procedures for the Derivation of ESBs for the Protection of Benthic Organisms: PAH Mixtures*; EPA/600/R-02/013; Office of Research and Development: Washington, DC, 2003.

(6) U.S. Environmental Protection Agency. *Methods for the Derivation of Site-Specific Equilibrium Partitioning Sediment Guidelines (ESGs) for the Protection of Benthic Organisms: Nonionic Organics*; EPA/822/R/02/042; Office of Science and Technology: Washington, DC, 2004.

(7) Dondelle, M. M.; Loehr, R. C. Comparison of estimated and experimentally obtained soil water distribution coefficients. *Pract. Period. Hazard., Toxic, Radioact. Waste Manage.* **2002**, 218−226.

(8) Khalil, M. F.; Ghosh, U.; Kreitinger, J. P. Role of weathered coal tar pitch in the partitioning of polycyclic aromatic hydrocarbons in manufactured gas plant site sediments. *Environ. Sci. Technol.* **2006**, *40*, 5681−5687.

(9) Cornelissen, G.; Gustafsson, O.; Bucheli, T. D.; Jonker, M. T. O.; Koelmans, A. A.; van Noort, P. C. M. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: Mechanisms and consequences for distribution, bioaccumulation, and biodegradation. *Environ. Sci. Technol.* **2005**, *39*, 6881−6895.

(10) Lohmann, R.; MacFarlane, J. K.; Gschwend, P. M. Importance of black carbon to sorption of native PAHs, PCBs, and PCDDs in Boston and New York harbor sediments. *Environ. Sci. Technol.* **2005**, *39*, 141−148.

(11) Jonker, M. T. O.; Koelmans, A. A. Sorption of polycyclic aromatic hydrocarbons and polychlorinated biphenyls to soot and soot-like materials in the aqueous environment: Mechanistic considerations. *Environ. Sci. Technol.* **2002**, *36*, 3725−3734.

(12) Cornelissen, G.; Breedveld, G. D.; Kalaitzidis, S.; Christanis, K.; Kibsgaard, A.; Oen, A. M. P. Strong sorption of native PAHs to pyrogenic and unburned carbonaceous geosorbents in sediments. *Environ. Sci. Technol.* **2006**, *40*, 1197−1203.

(13) Bender, M. E.; Roberts, M. H.; deFur, P. O. Unavailability of polynuclear aromatic hydrocarbons from coal particles to the eastern oyster. *Environ. Pollut.* **1987**, *44*, 243−260.

(14) Chapman, P. M.; Downie, J.; Maynard, A.; Taylor, L. A. Coal and deodorizer residues in marine sediments: Contaminants or pollutants? *Environ. Toxicol. Chem.* **1996**, *15*, 638−642.

(15) Maruya, K. A.; Risebrough, R. W.; Horne, A. J. The bioaccumulation of polynuclear aromatic hydrocarbons by benthic invertebrates in an intertidal marsh. *Environ. Toxicol. Chem.* **1997**, *16*, 1087−1097.

(16) Ozretich, R. J.; Ferraro, S. P.; Lamberson, J. O.; Cole, F. A. Test of (Σ) polycyclic aromatic hydrocarbon model at a creosote-contaminated site, Elliott Bay, WA. *Environ. Toxicol. Chem.* **2000**, *19*, 2378−2389.

(17) Paine, M. D.; Chapman, P. M.; Allard, P. J.; Murdoch, M. H.; Minifie, D. Limited bioavailability of sediment PAH near an

aluminum smelter: Contamination does not equal effects. *Environ. Toxicol. Chem.* **1996**, *15*, 2003−2018.
(18) Kreitinger, J. P. Greatly reduced bioavailability and toxicity of PAHs to *Hyalella azteca* in sediments from manufactured-gas plant sites. *Environ. Toxicol. Chem*. **2007**, *26*, 1146−1157.
(19) Hawthorne, S. B.; Poppendieck, D. G.; Grabanski, C. B.; Loehr, R. C. Comparing PAH availability from manufactured gas plant soils and sediments with chemical and biological tests. 1. PAH release during water desorption and supercritical carbon dioxide extraction. *Environ. Sci. Technol.* **2002**, *36*, 4795−4803.
(20) Hawthorne, S. B.; Poppendieck, D. G.; Grabanski, C. B.; Loehr, R. C. PAH release during water desorption, supercritical carbon dioxide extraction, and field bioremediation. *Environ. Sci. Technol.* **2001**, *35*, 4577−4583.
(21) Kreitinger, J. K.; Quiñones-Rivera, A.; Neuhauser, E. F.; Alexander, M.; Hawthorne, S. B.; Supercritical carbon dioxide extraction as a predictor of polycyclic aromatic hydrocarbon bioaccumulation and toxicity by earthworms in manufactured-gas plant site soils. *Environ. Toxicol. Chem.* **2007**, *26*, 1809−1817.
(22) Hawthorne, S. B.; Grabanski, C. B.; Miller, D. J.; Kreitinger, J. P. Solid-phase microextraction measurement of parent and alkyl polycyclic aromatic hydrocarbons in milliliter sediment pore water samples and determination of K_{DOC} values. *Environ. Sci. Technol.* **2005**, *39*, 2795−2803.
(23) Hawthorne, S. B.; Miller, D. J.; Kreitinger, J. P. Measurement of total PAH concentrations and toxic units used for estimating risk to benthic invertebrates at manufactured-gas plant sites. *Environ. Toxicol. Chem*. **2006**, *25*, 287−296.
(24) Gustafsson, O.; Haghseta, F.; Chan, C.; MacFarlane, J.; Gschwend, P. M. Quantification of the dilute sedimentary soot phase: Implications for PAH speciation and bioavailability. *Environ. Sci. Technol.* **1997**, *31*, 203−209.
(25) Goodman, L. A.; Kruskal, W. H. *Measures of Association for Cross Classification*; Springer-Verlag: Berlin, 1979.
(26) Schuler, L. J.; Landrum, P. F.; Lydy, M. Comparative toxicity of fluoranthene and penatachlorobenzene to three freshwater invertebrates. *Environ. Toxicol. Chem*. **2006**, *25*, 985−994.

Received for review January 27, 2007. Revised manuscript received June 20, 2007. Accepted July 5, 2007.

ES0702162

APPENDIX L

STANDARD TEST METHOD FOR DETERMINATION OF PARENT AND ALKYL POLYCYCLIC AROMATICS IN SEDIMENT PORE WATER USING SOLID-PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY/MASS SPECTROMETRY IN SELECTED ION MONITORING MODE

PUBLISHED IN *ASTM INTERNATIONAL*

Designation: D 7363 – 07

Standard Test Method for Determination of Parent and Alkyl Polycyclic Aromatics in Sediment Pore Water Using Solid-Phase Microextraction and Gas Chromatography/Mass Spectrometry in Selected Ion Monitoring Mode[1, 2]

This standard is issued under the fixed designation D 7363; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 The U.S. Environmental Protection Agency (USEPA) narcosis model for benthic organisms in sediments contaminated with polycyclic aromatic hydrocarbons (PAHs) is based on the concentrations of dissolved PAHs in the interstitial water or "pore water" in sediment. This test method covers the separation of pore water from PAH-impacted sediment samples, the removal of colloids, and the subsequent measurement of dissolved concentrations of the required 10 parent PAHs and 14 groups of alkylated daughter PAHs in the pore water samples. The "24 PAHs" are determined using solid-phase microextraction (SPME) followed by Gas Chromatography/Mass Spectrometry (GC/MS) analysis in selected ion monitoring (SIM) mode. Isotopically labeled analogs of the target compounds are introduced prior to the extraction, and are used as quantification references.

1.2 Lower molecular weight PAHs are more water soluble than higher molecular weight PAHs. Therefore, USEPA-regulated PAH concentrations in pore water samples vary widely due to differing saturation water solubilities that range from 0.2 µg/L for indeno[1,2,3-cd]pyrene to 31 000 µg/L for naphthalene. This method can accommodate the measurement of milligram per litre concentrations for low molecular weight PAHs and nanogram per litre concentrations for high molecular weight PAHs.

1.3 The USEPA narcosis model predicts toxicity to benthic organisms if the sum of the toxic units (ΣTU_c) calculated for all "34 PAHs" measured in a pore water sample is greater than or equal to 1. For this reason, the performance limit required for the individual PAH measurements were defined as the concentration of an individual PAH that would yield 1/34 of a toxic unit (TU). However, the focus of this method is the 10 parent PAHs and 14 groups of alkylated PAHs (Table 1) that contribute 95 % of the toxic units based on the analysis of 120 background and impacted sediment pore water samples.[3] The primary reasons for eliminating the rest of the 5-6 ring parent PAHs are: *(1)* these PAHs contribute insignificantly to the pore water TU, and *(2)* these PAHs exhibit extremely low saturation solubilities that will make the detection of these compounds difficult in pore water. This method can achieve the required detection limits, which range from approximately 0.01 µg/L, for high molecular weight PAHs, to approximately 3 µg/L for high molecular weight PAHs.

1.4 The test method may also be applied to the determination of additional PAH compounds (for example, 5- and 6-ring PAHs as described in Hawthorne et al).[4] However, it is the responsibility of the user of this standard to establish the validity of the test method for the determination of PAHs other than those referenced in 1.1 and Table 1.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* For specific hazard statements, refer to Section 9.

2. Referenced Documents

2.1 *ASTM Standards:* [5]

D 1192 Guide for Equipment for Sampling Water and

[1] This test method is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

Current edition approved Aug. 1, 2007. Published August 2007.

[2] Standard methods under the jurisdiction of ASTM Committee D19 may be published for a limited time preliminary to the completion of full collaborative study validation. Such standards are deemed to have met all other D19 qualifying requirements but have not completed the required validation studies to fully characterize the performance of the test method across multiple laboratories and matrices. Preliminary publication is done to make current technology accessible to users of Standards, and to solicit additional input from the user community.

[3] Hawthorne, S. B., Grabanski, C. B., and Miller, D. J., "Measured Partitioning Coefficients for Parent and Akyl Polycyclic Aromatic Hydrocarbons in 114 Historically Contaminated Sediments: Part I, Koc Values," *Environmental Toxicology and Chemistry*, 25, 2006, pp. 2901-2911.

[4] Hawthorne, S. B., Grabanski, C.B., Miller, D .J., and Kreitinger, J. P., "Solid Phase Microextraction Measurement of Parent and Alkyl Polycyclic Aromatic Hydrocarbons in Milliliter Sediment Pore Water Samples and Determination of K_{DOC} Values," *Environmental Science Technology*, 39, 2005, pp. 2795-2803.

[5] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

Copyright © ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, United States.

TABLE 1 Relative Response Factors[A]

Analyte	SPME-GC/MS RRF[B] versus Parent	Basis for Performance Limit[C]
Naphthalene	1.00	B
2-Methylnaphthalene[D]	1.00	B
1-Methylnaphthalene	1.00	B
C2-Naphthalenes	1.44	B
C3-Naphthalenes	0.88	B
C4-Naphthalenes	0.71	C
Acenaphthylene	1.00	B
Acenaphthene	1.00	B
Fluorene	1.00	B
C1-Fluorenes	0.73	B
C2-Fluorenes	0.59	B
C3-Fluorenes	0.35	S
Phenanthrene	1.00	B
Anthracene	1.00	B
C1-Phenanthrenes/Anthracenes	0.57	B
C2-Phenanthrenes/Anthracenes	0.32	B
C3-Phenanthrenes/Anthracenes	0.29	B
C4-Phenanthrenes/Anthracenes	0.12	S
Fluoranthene	1.00	B
Pyrene	1.00	B
C1-Fluoranthenes/Pyrenes	0.51	C
Benz[a]anthracene	1.00	B
Chrysene	1.00	B
C1-Chrysenes/Benz[a]anthracenes	0.62	C

[A] From Hawthorne, S. B., Grabanski, C.B., Miller, D .J., and Kreitinger, J. P., "Solid Phase Microextraction Measurement of Parent and Alkyl Polycyclic Aromatic Hydrocarbons in Milliliter Sediment Pore Water Samples and Determination of K_{DOC} Values," *Environmental Science Technology*, 39, 2005, pp. 2795-2803.

[B] All relative response factors are based on the SPME-GC/MS peak area per ng of the alkyl PAH in a water standard compared to that of its parent PAH as determined by SPME followed by GC/MS.When several isomers were available, (for example, C2-naphthalenes), the mean relative response factor is reported. The relative response factors of alkyl PAHs for which no standards were available were estimated based on the closest analogous alkyl PAH as described in reference 2.1.

[C] Performance limits were determined as 3 times the background concentrations from the SPME fiber based on the analysis of water blanks ("B"), the lowest calibration standard which consistently yielded a signal to noise ratio of at least 3:1 ("C"), or (for when no calibration standard was available) for the lowest concentrations consistently found in pore water samples with a signal to noise ratio of at least 3:1 ("S"). Detection limits for alkyl PAHs are based on a single isomer.

[D] Alkyl PAHs used to determine the SPME-GC/MS relative response factors including alkyl naphthalenes (1-methyl-, 2-methyl-, 1,2-dimethyl-, 1,3-dimethyl-1,8-dimethyl-, 2,7-dimethyl-, 1-ethyl, 2-ethyl, 1,4,5-trimethyl-, 2,3,5-trimethyl-, and 2-isopropyl-), 1-methylfluorene, 2-methyl- and 9-methylanthracene, 1-methyl-, 2-methyl-, and 3-methylphenanthrene, 9,10-dimethylanthracene, 2-ethylanthracene, 2-tertbutylanthracene, 1-methyl-7-isopropylphenanthrene, 1-methylpyrene, 7-methylbenz[a]anthracene, and 7,12-dimethylbenz[a]anthracene.

Steam in Closed Conduits[6]
D 1193 Specification for Reagent Water
D 2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water
D 3370 Practices for Sampling Water from Closed Conduits
D 5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis
E 178 Practice for Dealing With Outlying Observations

3. Terminology

3.1 *Definitions:*

3.1.1 *calibration standard*—a solution prepared from a secondary standard, stock solution, or both, and used to calibrate the response of the instrument with respect to analyte concentration.

[6] Withdrawn.

3.1.2 *calibration verification standard (VER)*—the mid-point calibration standard (CS3) that is analyzed daily to verify the initial calibration.

3.1.3 *CS1, CS2, CS3, CS4*—shorthand notation for calibration standards.

3.1.4 *data acquisition parameters*—parameters affecting the scanning operation and conversion of the analytical signal to digitized data files. These include the configuration of the ADC circuitry, the ion dwell time, the MID cycle time, and acquisition modes set up for the method. Examples of acquisition modes for the HP5973 include SIM mode, and Low Mass Resolution Mode.

3.1.5 *performance limit*—performance limit for individual PAH is defined as the concentration of an individual PAH that would yield 1/34 of a toxic unit. For performance limit of individual PAH, refer to Table 2 (see 4.6).

3.1.6 *deuterated PAH (d-PAH)*—polycyclic aromatic hydrocarbons in which deuterium atoms are substituted for all hydrogens (that is, perdeuterated). In this method, d-PAHs are used as internal standards.

3.1.7 *GC*—gas chromatograph or gas chromatography.

3.1.8 *HRGC*—high resolution GC.

3.1.9 *LRMS*—low resolution MS.

3.1.10 *internal standards*—isotopically labeled analogs (d-PAHs) of the target analytes that are added to every sample, blank, quality control spike sample, and calibration solution. They are added to the water samples immediately after completing the flocculation step and transferring the water aliquot to the autosampler vial, and immediately after adding the calibration PAH solution to water calibration standards, but before SPME extraction. The internal standards are used to calculate the concentration of the target analytes or estimated detection limits.

3.1.11 *laboratory blank*—see *method blank*.

3.1.12 *method blank*—an aliquot of reagent water that is extracted and analyzed along with the samples to monitor for laboratory contamination. Blanks should consistently meet concentrations at or less than one-third of the performance limits for individual PAHs stated in Table 2. Alternatively, if the PAH concentrations calculated from the water blank immediately preceding the test samples are <20 % of the test sample concentrations, the blank is acceptable.

3.1.13 *low calibration level (LCL)*—the level at which the entire analytical system must give a recognizable signal and acceptable calibration point for the analyte. It is equivalent to the concentration of the lowest calibration standard assuming that all method-specified sample weights, volumes, and cleanup procedures have been employed.

3.1.14 *high or upper calibration level (UCL)*—the concentration or mass of analyte in the sample that corresponds to the highest calibration level in the initial calibration. It is equivalent to the concentration of the highest calibration standard, assuming that all method-specified sample weights, volumes, and cleanup procedures have been employed.

3.1.15 *MS*—mass spectrometer or mass spectrometry.

3.1.16 *PAH*—polycyclic aromatic hydrocarbon, or alternately, polynuclear aromatic hydrocarbon.

TABLE 2 Toxic Unit Factors and Performance Limits[A]

Analyte	Added d-PAH Internal Standard	d-PAH Internal Std. for Calculation	SPME-GC/MS RRF versus Parent	Conc. for One Toxic Unit, C_{tu}, (ng/mL)	Performance Limit (ng/mL)
Naphthalene	A	A	1.00	193.47	5.69
2-Methylnaphthalene		B	1.00	81.69	2.40
1-Methylnaphthalene	B	B	1.00	81.69	2.40
C2-Naphthalenes		A	1.44	30.24	0.89
C3-Naphthalenes		A	0.88	11.10	0.33
C4-Naphthalenes		A	0.71	4.05	0.12
Acenaphthylene		C	1.00	306.85	9.03
Acenaphthene	C	C	1.00	55.85	1.64
Fluorene	D	D	1.00	39.30	1.16
C1-Fluorenes		D	0.73	13.99	0.41
C2-Fluorenes		D	0.59	5.30	0.16
C3-Fluorenes		D	0.35	1.92	0.06
Phenanthrene	E	E	1.00	19.13	0.56
Anthracene		E	1.00	20.72	0.61
C1-Phenanthrenes/Anthracenes		E	0.57	7.44	0.22
C2-Phenanthrenes/Anthracenes		E	0.32	3.20	0.09
C3-Phenanthrenes/Anthracenes		E	0.29	1.26	0.04
C4-Phenanthrenes/Anthracenes		E	0.12	0.56	0.02
Fluoranthene	F	F	1.00	7.11	0.21
Pyrene	G	G	1.00	10.11	0.30
C1-Fluoranthenes/Pyrenes		G	0.51	4.89	0.14
Benz[a]anthracene		H	1.00	2.23	0.066
Chrysene	H	H	1.00	2.04	0.060
C1-Chrysenes/Benz[a]anthracenes		H	0.62	0.86	0.025

[A] From Hawthorne, S. B., Grabanski, C.B., Miller, D .J., and Kreitinger, J. P., "Solid Phase Microextraction Measurement of Parent and Alkyl Polycyclic Aromatic Hydrocarbons in Milliliter Sediment Pore Water Samples and Determination of K_{DOC} Values," *Environmental Science Technology*, 39, 2005, pp. 2795-2803.

3.1.17 *percent difference (%D)*—the difference between the analyzed concentration and expected concentration, expressed as a percentage of the expected concentration.

3.1.18 *relative response factor (RRF)*—the empirically determined ratio between the area ratio (analyte to internal standard) and the unit mass of analyte in the calibration standard (area ratio/ng) for available alkyl PAHs in a given homolog and their parent PAH.

3.1.19 *selected ion monitoring (SIM)*—a mode of operation for the mass spectrometer in which specific ions are monitored. This mode of operation differs from the full scan mode, in which the MS acquires all ions within a range. Because the spectrometer is monitoring fewer ions in the SIM mode, more acquisition (dwell) time is possible for each ion. This results in greater instrument sensitivity for the selected ions. Spectral scanning and library searching, used for tentatively identified compounds, are not supported in this mode.

3.1.20 *signal-to-noise ratio*—the ratio of the mass spectrometer response of a GC peak to the background noise signal.

4. Summary of Test Method

4.1 Either the use of an autosampler, or a manual approach can be used to perform the SPME extraction and the subsequent injection of collected analytes into the GC/MS. An autosampler (Leap Technologies Compi-Pal or equivalent) is much preferred over the manual method because: *(1)* the autosampler yields lower and more reproducible blanks, *(2)* the manual method requires the use of a stir bar that can cause sample cross-contamination, *(3)* the manual method is highly labor-intensive and requires multiple timed manipulations per analysis leading to operator fatigue and resultant errors, and *(4)* the autosampler reduces the technician time required to prepare samples for a 24-h run sequence to approximately 3 h, while the manual method requires 24-h operator attendance. Therefore, the method procedures are written assuming the use of an autosampler, with modifications to the autosampler procedures listed for the manual method.

AUTOSAMPLER METHOD

4.2 *Pore Water Separation and Preparation*—The pore water is separated from wet sediment samples by centrifugation and supernatant collection. Colloids are removed from the separated pore water samples by flocculation with aluminum potassium sulfate (alum) and sodium hydroxide as described in Hawthorne et al.[4] A second flocculation and centrifugation, followed by supernatant collection completes the colloid removal. The prepared pore water samples are then split into the required number of replicate aliquots (1.5 mL each) and placed into silanized glass autosampler vials. The 8 perdeuterated PAH internal standards (d-PAHs) are then added immediately. All of the water preparation steps beginning with the centrifugation and ending with the addition of d-PAH internal standards should be conducted continuously and in the minimum amount of time possible.

4.2.1 The SPME fiber should be cleaned at the beginning of each sampling set (and after very contaminated samples) for 1 h by placing in the cleaning chamber under helium flow at 320°C. This can conveniently be performed while the pore waters are being prepared.

4.3 *Solid-Phase Microextraction*—The SPME extraction of the pore water samples is performed using a commercially available (available from Sigma-Aldrich, formerly Supleco, or equivalent) 7 µm film thickness polydimethylsiloxane (PDMS)-coated fused silica fiber for 30 min while the water sample is mixed by the precession of the autosampler mixing chamber at a rate of 250 revolutions per minute. The target

PAHs and d-PAH internal standards adsorb to the nonpolar PDMS phase at equivalent rates. The use of the d-PAHs (that is, isotopic dilution) to quantitate the target PAHs compensates for variations in equilibrium partitioning and kinetics.

4.4 *GC/MS SIM Analysis*—Following the sorption period, the SPME fiber is immediately desorbed to a GC/MS injection port in the splitless mode at 320°C for 5 min. The GC/MS system specified uses a 60 m narrow-bore (250 µm ID) HP5-MS or equivalent capillary column to achieve high resolution for PAHs. Following the 5 min desorption period, the SPME fiber is inserted into the cleaning port and additionally cleaned for 15 min under helium flow at 320°C. At the end of the cleaning period, sorption of the next water sample is begun.

MANUAL METHOD

4.5 *Alternate Procedures for Manual Method*—Samples are prepared as for the autosampler method, except that a small Teflon-coated stir bar is placed in the silanized autosampler vial prior to adding the water and d-PAH internal standard solution. A new stir bar should be used for each sample, calibration standard, and blank to avoid cross-contamination caused by carryover on the stir bar. To perform the SPME step, the vial is set on a stir plate and the stirring rate adjusted so that no large vortex is formed. The SPME fiber should be inserted into the water so that the entire 1-cm active length is exposed to the water sample, but not so low that the fiber comes into contact with the stir bar or that the metal needle sheath contacts the water. All time sequences should be the same as specified for the autosampler method. A spare GC split/splitless injection port at 320°C and under helium flow can be used for the 15-min cleaning step between samples as well as for the initial 1-h cleaning step at the beginning of each experimental day.

4.6 The mass spectrometer is operated in the SIM mode for the molecular ions of the target PAHs and d-PAHs to achieve low limits of detection. Analyte concentrations are quantified by three methods:

4.6.1 PAHs for which an exact deuterated analog is included in the internal standard mix are quantified by isotope dilution.

4.6.2 Parent PAHs (that is, unsubstituted PAHs) for which an exact deuterated analog is not included in the internal standard mix are quantified by reference to a deuterated analog of a PAH with the same number of rings as the analyte.

4.6.3 Alkyl PAHs are quantified using the experimentally and empirically-determined relative response factors from Hawthorne et al.[4] and as shown in Table 1. The laboratory may use updated response factors, if additional alkyl PAH standards become commercially available. However, the laboratory must correct for purities of less than 98 %.

4.7 *Conversion of Quantified Concentration to Toxic Units*—The USEPA narcosis model predicts toxicity to benthic organisms if the sum of the toxic units calculated for all "34 PAHs" measured in a pore water sample is greater than or equal to 1. For this reason, the performance limits required for the individual PAH measurements were defined as the concentration of an individual PAH that would yield 1/34 of a toxic unit. See Table 2. This distribution reflects the relative concentrations of PAHs expected to be found in pore water because the lower molecular weight PAHs are more soluble and have lower organic carbon partition coefficients (Koc), and reflects the lower partitioning of lower molecular weight PAHs to the receptor organism since they have smaller octanol/water coefficients (Kow). The performance limits are essentially benchmarks to ensure that the adequate sensitivity is achieved to predict toxicity.

5. Significance and Use

5.1 This method directly determines the concentrations of dissolved PAH concentrations in environmental sediment pore water samples. The method is important from an environmental regulatory perspective because it can achieve the analytical sensitivities to meet the goals of the USEPA narcosis model for protecting benthic organisms in PAH contaminated sediments. Regulatory methods using solvent extraction have not achieved the wide calibration ranges from nanograms to milligrams per litre and the required levels of detection in the nanogram-per-litre range. In addition, conventional solvent extraction methods require large aliquot volumes (litre or larger), use of large volumes of organic solvents, and filtration to generate the pore water. This approach entails the storage and processing of large volumes of sediment samples and loss of low molecular weight PAHs in the filtration and solvent evaporation steps.

5.2 This method can be used to determine nanogram to milligram per litre PAH concentrations in pore water. Small volumes of pore water are required for SPME extraction, only 1.5 mL per determination and virtually no solvent extraction waste is generated.

6. Interferences

6.1 Non-target hydrocarbons can cause peaks on selected ion current profiles (SICPs) intended for other PAHs. Pattern recognition must be employed for identifying interfering peaks, and peak series that should not be considered for the homolog or target PAH under consideration. Analysts should be intimately familiar with both parent and alkyl PAH analyses in complex environmental samples. Representative samples having higher PAH concentrations should periodically be analyzed by full scan GC/MS so that pattern recognition of alkyl PAHs (and interfering species) can be verified by their full mass spectra. This procedure is particularly important for newer operators.

6.2 Solvents, reagents, glassware and other sample processing hardware may yield discrete artifacts or elevated baselines that may cause misinterpretation of the chromatographic data. All of these materials must be demonstrated to be free from interferences under the conditions of analysis by performing laboratory method blanks. Analysts should avoid using PVC gloves, powdered gloves, or gloves with measurable levels of phthalates.

NOTE 1—The use of high purity reagents and solvents helps minimize interference problems.

7. Apparatus

7.1 *Centrifuge*, capable of sustaining 1000 g with cups for securing 40 mL and 20 mL vials.

7.2 *SPME Fiber Holder*, compatible with 7-µm SPME fiber and compatible with either the autosampler or the manual method.

TABLE 3 Primary Material Hazards

Material	Hazards	Exposure Limit[A]	Signs and Symptoms of Exposure
Alum (Aluminum Potassium Sulfate)	Irritant	2 mg/M^3 TWA	May cause skin irritation, especially under repeated or prolonged contact, or when moisture is present. May irritate or burn the eyes. Dust or mist inhalation at levels above the TLV may cause irritation to the respiratory tract. May irritate the gastrointestional tract.
Acetone	Flammable	1000 ppm-TWA	Inhalation of vapors irritates the respiratory tract. May cause coughing, dizziness, dullness, and headache.
Dichloromethane (DCM)	Carcinogen, Irritant	25 ppm-TWA, 125 ppm-STEL	Causes irritation to respiratory tract. Has a strong narcotic effect with symptoms of mental confusion, light-headedness, fatigue, nausea, vomiting and headache. Causes irritation, redness and pain to the skin and eyes. Prolonged contact can cause burns. Liquid degreases the skin. May be absorbed through skin.
Sodium Hydroxide	Corrosive	2 mg/M^3 TWA	Causes skin irritation, chemical burns, permanent injury or scarring, and blindness. Vinegar is a mild acid that will neutralize lye if it were to make contact with the skin. Harmful if inhaled or ingested. Causes Sore throat, cough labored breathing, shortness of breath, and abdominal pain. Symptoms may be delayed.

[A] Exposure limit refers to the OSHA regulatory exposure limit.

7.3 *SPME Fibers*, 7-µm diameter, coated with polydimethylsiloxane (PDMS).

7.4 *PTFE Coated Stir Bars (Stir Fleas)*, of a size effective for stirring 1.5 mL water without vortexing (for manual method only).

7.5 *Magnetic Stir Plate (for manual method only)*.

7.6 *SPME Holder Stand (for manual method only) or GC/MS Autosampler*, capable of SPME extraction and injection.

7.7 *Cleaning Port*, capable of purging SPME fibers in a helium-swept atmosphere at 320°C.

7.8 *GC/MS Analysis*:

7.8.1 *Gas Chromatograph* shall have split/splitless injection port for capillary column, temperature program with isothermal hold.

7.8.2 *GC Column*, 60 mm × 0.25 mm ID × 25 µm film thickness HP5-MS or equivalent.

7.8.3 *Inlet Liner*, 2 mm ID silanized glass.

7.8.4 *GC Inlet*, 320°C, splitless mode.

7.8.5 *Oven Program*—Isothermal 5 min hold at 40°C. Ramp at 50°C/min to 110°C, followed by a temperature ramp of 12°C/min to 320°C (hold for 10 min).

7.8.6 *Mass Spectrometer*—Electron impact ionization with the ionization energy optimized for best instrument sensitivity (typically 70 eV), stability and signal to noise ratio. Shall be capable of repetitively selectively monitoring at least 12 m/z during a period of approximately 1 s and shall meet all manufacturers' specifications.

7.8.7 *GC/MS Interface*—The mass spectrometer (MS) shall be interfaced to the GC such that the end of the capillary column terminates within 1 cm of the ion source but does not intercept the electron or ion beam.

7.8.8 *Data System*, capable of collecting, recording, and storing MS data.

8. Reagents and Materials

8.1 *Purity of Reagents*—Reagent grade chemicals must be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.[7]

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water that meets the purity specifications of Type I or Type II water, presented in Specification D 1193.

8.3 *40 mL Vials*, with Teflon-lined caps.

8.4 *20 mL Vials*, with Teflon-lined caps.

8.5 *Silanized 2.0 mL Autosampler Vials*.

8.6 *Internal Standard Stock Solution*—A dichloromethane solution of d-PAH internal standards used for preparing spiking solutions by dilution into acetone (see 12.2).

8.7 *Internal Standard Spiking Solution*—A dilution of the internal standard stock solution in acetone used to spike d-PAH internal standards into all sample, calibration, and blank water vials.

8.8 *Calibration Stock Solution*—A dichloromethane solution of PAHs used for preparing calibration standards (see 12.2).

8.9 *Calibration Spiking Solutions*—A series of solutions prepared by diluting the calibration stock solution with acetone (see 12.2).

8.10 *Calibration Standards*—Prepared by adding internal standard and calibration spiking solutions in reagent water (see 12.2).

8.11 *Acetone*.

8.12 *Dichloromethane* (DCM).

8.13 *Sodium Hydroxide* (NaOH).

8.14 *Aluminum Potassium Sulfate Dodecahydrate* ($AlK(SO_4)_2 \cdot 12H_2O$).

8.15 *Alum Solution*—Add 20 g ($AlK(SO_4)_2 \cdot 12H_2O$) to 80 mL reagent water.

9. Hazards

9.1 The effluents of sample splitters for the gas chromatograph and roughing pumps on the mass spectrometer must be

[7] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals,* BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary,* U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

vented to the laboratory hood exhaust system or must pass through an activated charcoal filter.

9.2 *Primary Materials Used*—The table contains a summary of the primary hazards listed in the MSDS. A complete list of materials used in the method can be found in the reagents and materials section. Practitioners must review the information in the MSDS for each material before using it for the first time or when there are major changes to the MSDS.

10. Sampling and Sample Preservation

10.1 Collect the sediment sample in accordance with Practices D 3370 and Specification D 1192, as applicable.

10.2 Prior to shipment, the samples should be mixed well. Sieve the slurry of sediment and site water through a 2-mm screen to remove debris. If the sieved slurry is to be stored or shipped before use, store in 250 mL to 1 L jars with PTFE-lined lids. Great care must be taken to clean the lid of the jar before capping with the lid to avoid leakage of the water during shipment.

10.3 Ship in an ice chest with adequate ice to maintain 0 to 6°C. Store at the laboratory in the dark at 0 to 6°C.

11. Preparation of Apparatus

11.1 Set up the GC system using the following parameters.

11.1.1 GC Column Agilent HP-5MS column (0.25 µm film thickness, 0.25 mm ID) or equivalent.

11.1.2 Inlet liner 2-mm ID silanized glass.

11.1.3 GC Inlet 320°C, splitless mode.

11.1.4 *Oven Program*—Isothermal 5 min hold at 40°C. Ramp at 50°C/min to 110°C, followed by a temperature ramp of 12°C/min to 320°C. (Hold for 10 min.)

MS Quad Temperature	150°C, maximum 200°C
MS Source Temperature	230°C, maximum 250°C

11.1.5 Set up SIM Groups to monitor the quantitation and internal standard ions shown in Table 4. Each ion dwell time should be set at 25 ms. Twelve ions are monitored in each group.

NOTE 2—Some ions (for example, m/z 184.1 for C4 naphthalenes) are included in two ion groups to ensure that the target peaks are adequately monitored. Table 4 should be used with the chromatograms in Appendix X1 to aid the analyst in setting proper retention time windows and recognition of target and contaminant peaks, especially for the alkyl clusters.

12. Calibration

12.1 Determine the absolute and relative retention times of the first and last characteristic peak in each homolog with the aid of the examples in Appendix X1.

12.1.1 Set up a SIM program with the necessary ions to acquire all the alkyl-PAH homologs using the ion groups shown in Table 4 and 25 ms dwell time per ion.

12.1.2 Update the expected retention times in the method section of the quantitation software using the d-PAH internal standards of previous runs as relative retention time markers and the representative chromatograms in Appendix X1. Assure that the SIM windows for the homologs are set to at least 8 s before the first, and 30 s after the last characteristic peaks to assure coverage of the elution range.

12.2 *Analyze Initial Calibration*:

TABLE 4 SIM Ion Groups and Retention Time Windows

NOTE—Retention times must be verified by the user.

Analyte	SIM Ion Group	Target m/z	Retention Time (min)	
			Start	Stop
Naphthalene	1	128.1	7	17
2-Methylnaphthalene	1	142.1	7	17
1-Methylnaphthalene	1	142.1	7	17
C2-Naphthalenes	1	156.1	7	17
C3-Naphthalenes	1	170.1	7	17
C4-Naphthalenes	1,2	184.1	7	21
Acenaphthylene	1	152.1	7	17
Acenaphthene	1	154.1	7	17
Fluorene	1	166.1	7	17
C1-Fluorenes	2	180.1	17	21
C2-Fluorenes	2	194.1	17	21
C3-Fluorenes	2,3	208.1	17	25
Phenanthrene	2	178.1	17	21
Anthracene	2	178.1	17	21
C1-Phenanthrenes/Anthracenes	2	192.1	17	21
C2-Phenanthrenes/Anthracenes	2,3	206.1	17	30
C3-Phenanthrenes/Anthracenes	2,3	220.1	17	30
C4-Phenanthrenes/Anthracenes	3	234.1	21	30
Fluoranthene	2,3	202.1	17	30
Pyrene	2,3	202.1	17	30
C1-Fluoranthenes/pyrenes	3	216.1	21	30
Benz[a]anthracene	3	228.1	21	30
Chrysene	3	228.1	21	30
C1-Chrysenes	3	242.1	21	30
d-PAH Internal Standards				
Naphthalene-d8	1	136.1	7	17
1-Methylnaphthalene-d10	1	152.1	7	17
Acenaphthene-d10	1	164.1	7	17
Fluorene-d10	1	176.1	7	17
Phenanthrene-d10	2	188.1	17	21
Fluoranthene-d10	2,3	212.1	17	30
Pyrene-d10	2,3	212.1	17	30
Chrysene-d12	3	240.2	21	30

12.2.1 Prepare stock solutions of PAHs and internal standard stock solutions of d-PAHs at approximately the concentrations shown in Table 5. These concentrations were based on the PAH distributions previously determined in 120 sediment pore water samples. Stocks are prepared in DCM. Spiking solutions are prepared by dilution of intermediate stocks in acetone. For calibration solutions, spiking solutions are added to reagent water.

12.2.1.1 Prepare calibration standard spiking solutions. These are prepared by adding acetone to the stock to give the calibration solution concentrations (CS1–CS4), as described below:

(1) For CS1, take 5 µL stock to 100 mL in acetone.

(2) For CS2 take 50 µL to 100 mL in acetone.

(3) For CS3, take 25 µL to 10 mL in acetone.

(4) For CS4, take 100 µL to 10 mL in acetone.

12.2.1.2 Spike 4 µL of each calibration solution into 1.5 mL of reagent water to give a calibration series with the low calibration limits (LCLs) and upper calibration limits (UCLs) shown in Table 5. Spike 10 µL of internal standard spiking solution at the concentrations shown in Table 5 into each vial.

12.2.1.3 Extract and analyze the calibration series.

(1) Extract and analyze two water blank solutions.

(2) Extract and analyze the water calibration solutions, as described in 13.4 and 13.5. Begin with the CS1-spiked sample,

TABLE 5 Initial Calibration Standard Series

Analyte	DCM Stock Conc. mg/mL	LCL			UCL
		CS1	CS2	CS3	CS4
		ng/1.5 mL	ng/1.5 mL	ng/1.5 mL	ng/1.5 mL
Naphthalene	41.5	8.3	83	415	1660
1-Methylnaphthalene	23.9	4.78	47.8	239	956
2-Methylnaphthalene	20.42	4.084	40.84	204.2	816.8
Acenaphthylene	9.02	1.804	18.04	90.2	360.8
Acenaphthene	11	2.2	22	110	440
Fluorene	7.55	1.51	15.1	75.5	302
Anthracene	0.6	0.12	1.2	6	24
Phenanthrene	5.5	1.1	11	55	220
Fluoranthene	2.11	0.422	4.22	21.1	84.4
Pyrene	1.8	0.36	3.6	18	72
Benz[a]anthracene	0.08	0.016	0.16	0.8	3.2
Chrysene	0.03	0.006	0.06	0.3	1.2
Deuterated Analogs of Mix A Compounds	Stock Solution	CS1	CS2	CS3	CS4
Naphthalene-d8	5	50.0	50.0	50.0	50.0
1-Methylnaphthalene-d10	6	60.0	60.0	60.0	60.0
Acenaphthene-d10	1.23	12.3	12.3	12.3	12.3
Fluorene-d10	1.2	12.0	12.0	12.0	12.0
Phenanthrene-d10	0.96	9.6	9.6	9.6	9.6
Fluoranthene-d10	0.93	9.3	9.3	9.3	9.3
Pyrene-d10	0.84	8.4	8.4	8.4	8.4
Chrysene-d12	0.033	0.33	0.33	0.33	0.33

followed by sequentially more concentrated calibration standards. Follow by two water blanks.

12.2.1.4 Calculate the performance parameters for the calibration.

(1) Generate ion chromatograms for the masses listed in Table 4 that encompass the expected retention windows of the target analytes. Integrate the selected ion current profiles of the quantitation ions shown in the table. Integration of alkyl clusters should be as the total area of the cluster integrated from the baseline before the first peak in the cluster to the baseline after the last peak in the cluster peaks. Cluster peaks should never be integrated using the valley-to-valley method. The peak areas of non-target peaks (see Appendix X1) must be removed from the alkyl cluster peak area before any calculation.

(2) Calculate the area ratio (analyte peak area divided by internal standard peak area) per unit mass of analyte, using the area of the appropriate internal standard listed in Table 1. Quantitative calculations are based on a comparison of the area ratio per ng from the calibration and sample waters. The area ratio per ng is calculated for calibration runs by dividing the calibration peak area by the peak area of its most closely associate d-PAH internal standard (the deuterated parent PAH, in most cases), and dividing this result by the ng of the calibration PAH present in the vial (that is, its mass in the vial, not its concentration). Calibration standards are given in Table 5.

$$area\ ratio\ per\ ng\ (ar\ rat/ng) = \frac{[(peak\ area\ cal.\ std)/(peak\ area\ d{-}PAH)]}{(mass\ of\ std\ in\ cal\ vial)} \quad (1)$$

(3) Calculate the mean ar rat/ng. The mean relative response factor for these duplicate daily calibration standards should agree with those from the 4-point (or 3-point) standard curve within 20 % for the two and three-ring PAHs, and within 25 % for the four-ring PAHs. No sample data will be reported if these calibration criteria are not met. Calculate the mean area ratio/ng and the standard deviation of the relative response factors for each calibration standard solution using the following equations:

$$\overline{ar\ rat/ng} = \frac{1}{n}\sum_{i=1}^{n}(ar\ rat/ng)_i \quad (2)$$

where:

$(ar\ rat/ng)_i$ = ar rat/ng calculated for calibration solution "i" using the equation in 12.2.1.4*(2)*, and

n = number of calibration points in the curve.

(4) Calculate the percent relative standard deviation:

$$\%RSD = \frac{SD}{\overline{ar\ rat/ng}} \times 100 \quad (3)$$

where:

$\overline{ar\ rat/ng}$ = mean ar rat/ng calculated above, and

SD = sample standard deviation of the replicate area rat/ng values used to calculate the mean ar rat/ng.

12.3 *Criteria for Acceptable Initial Calibration*—Prior to analyzing any samples, the standard curves are prepared using the identical analysis procedures as used for sample waters. To be acceptable, the linearity of each PAH standard curve should be $r^2 > 0.99$, and the relative response factor per ng for each concentration should show a relative standard deviation of <25 % for two- to three-ring PAHs, and <30 % for four-ring PAHs. See Section 16. If acceptable initial calibration is not achieved, identify the root cause, perform corrective action, and repeat the initial calibration. If the root cause can be traced to an abnormal disruption of an individual acquisition (for example, injector malfunction) repeat the individual analysis and recalculate the percent relative standard deviation. If the

calibration is acceptable, document the problem and proceed; otherwise repeat the initial calibration.

12.3.1 Because of the large range of calibration concentrations required, the wide range of water solubilities of the individual PAHs, and the desire to require only one stock calibration solution, some PAHs may only have a three point linear calibration curve that meets the above criteria. This is most likely to occur for the higher molecular weight PAHs, because the dilution of lowest calibration standard is likely to be below detection limits for many labs (and is also below the required detection limits needed for the method, so it does not negatively impact the analyses). In such cases, the lowest calibration standard is ignored, and the "J" level adjusted appropriately. Less frequently, the highest concentrations of the lowest molecular weight PAHs may exceed the linear dynamic range of the GC/MS response. In such cases the laboratory should investigate lowering the MS multiplier voltage to autotune voltage or slightly below and rerun the calibration curve. If the highest calibration standard still exceeds the detector linearity, it is acceptable to reject the highest concentration for those specific PAHs (and adjust the "E" value accordingly), as long as a minimum of a three-point standard curve is generated for each PAH.

12.3.1.1 It is recommended that a 4-point (or 3-point) initial calibration be established every two weeks, when continuing calibration criteria are not met, or when service is performed on the GC/MS instrument system.

12.3.2 The signal to noise ratio (S/N) for the GC signals present in every selected ion current profile (SICP) must be ≥10:1 for the labeled internal standards and unlabeled calibration compounds.

12.4 *Calibration Verification*—Continuing calibration is performed daily at the beginning of a 24-h period. The injection of the first continuing calibration begins the 24-h window, within which all pore water samples must be injected. Duplicate daily standards are analyzed.

12.4.1 Into 1.5 mL of reagent water, add 4 µL of the CS3 spiking solution and 10 µL of the d-PAH internal standards.

12.4.2 Analyze duplicate vials of the Calibration Standard Solution CS3. Use the same data acquisition parameters as those used during the initial calibration. Check for GC resolution and peak shape. If peak shape or retention times are unacceptable, perform column and injector maintenance. If this fails to correct the problem, the column must be replaced and the calibration repeated.

12.4.3 *Criteria for Acceptable Daily Calibration Check*—The criteria listed below for acceptable calibration must be met at the beginning of each 24-h period that samples are analyzed. The mean relative response factor for these duplicate daily calibration standards should agree with those from the 4-point (or 3-point) standard curve within 20 % for the two- and three-ring PAHs, and within 25 % for the four-ring PAHs. No sample data will be reported if these calibration criteria are not met. If the continuing calibration criteria are not met, identify the root cause, perform corrective action and repeat the continuing calibration. If the second consecutive continuing calibration does not meet acceptance criteria, additional corrective action must be performed.

12.4.4 The signal to noise ratio (S/N) for the GC signals present in every selected ion current profile (SICP) must be ≥10:1 for the labeled internal standards and unlabeled calibration compounds.

12.5 *Method Blanks*—Method blanks are prepared and analyzed daily in duplicate following the continuing calibration and between analysis of replicate sets of the same pore water sample. See 12.5.2.2.

12.5.1 For each method blank, add 10 µL of the d-PAH internal standards solution into 1.5 mL of reagent water.

12.5.2 Two types of sources of background PAHs must be considered. For the higher molecular weight PAHs, typical GC/MS criteria for signal to noise are appropriate, since their detection limits are normally controlled by GC/MS sensitivity. However, for lower molecular weight PAHs, atmospheric contaminants can cause significant background peaks, especially for low MW alkyl PAHs. This problem is most likely to be significant in urban areas impacted by atmospheric PAHs (for example, from diesel exhaust), and with laboratories using manual techniques, rather than the SPME autosampler.

12.5.2.1 *Background PAHs from Ambient Air*—Concentrations of each PAH in the water blanks should be calculated in the same manner as a sample. Should the blank prior to the subsequent pore water sample have detectable background concentrations more than ⅓ of the target detection limit given in Table 3, the analyses should not continue until the fiber is sufficiently cleaned as demonstrated by a clean water blank. The mean of the calculated concentrations of the PAHs in the blanks analyzed immediately before and immediately after sample pore waters should be subtracted from the sample pore water concentrations.

12.5.2.2 *Carryover from Highly Contaminated Samples*—Carryover blanks are analyzed between each new pore water sample (not including replicates). Significant carryover can occur if the previous sample was highly contaminated. Should the blank prior to the subsequent pore water sample have detectable background concentrations more than ⅓ of the target detection limit, the analyses should not continue until the fiber is sufficiently cleaned as demonstrated by a clean water blank. Alternatively, if the concentrations determined in the blanks are less than 20 % of those found in the related sample, the data can be accepted.

13. Procedure

13.1 At the laboratory, store samples and extracts in the dark at 0 to 6°C.

13.2 *Holding Times*:

13.2.1 Pore waters must be generated within 28 days of sediment sample collection.

13.2.2 Pore waters must be generated and flocculated as quickly as possible, and then immediately spiked with 10 µL of d-PAH solution.

13.2.3 Solid phase micro-extraction must be completed within 24 h of flocculation.

13.3 *Generation of Pore Water*:

13.3.1 Stir the slurry and transfer approximately 40 mL (containing a solids and liquids in proportion to the slurry provided) to a clean 40 mL vial. Cap the vial with a PTFE-lined cap. Place the vials in a centrifuge. Spin for 30 min at 1000 g.

TABLE 6 Example of a 24-h Analytical Sequence[A]

Example Analytical Sequence					
Run Type	Minutes	Cumulative Minutes to Start	Cumulative Minutes to End	Cumulative Hours to Start[A]	Cumulative Hours to End
Standard	50	0	50	0.0	0.8
Standard	50	50	100	0.8	1.7
Blank	50	100	150	1.7	2.5
Blank	50	150	200	2.5	3.3
Sample	50	200	250	3.3	4.2
Sample	50	250	300	4.2	5.0
Blank	50	300	350	5.0	5.8
Blank	50	350	400	5.8	6.7
Sample	50	400	450	6.7	7.5
Sample	50	450	500	7.5	8.3
Blank	50	500	550	8.3	9.2
Blank	50	550	600	9.2	10.0
Sample	50	600	650	10.0	10.8
Sample	50	650	700	10.8	11.7
Blank	50	700	750	11.7	12.5
Blank	50	750	800	12.5	13.3
Sample	50	800	850	13.3	14.2
Sample	50	850	900	14.2	15.0
Blank	50	900	950	15.0	15.8
Blank	50	950	1000	15.8	16.7
Sample	50	1000	1050	16.7	17.5
Sample	50	1050	1100	17.5	18.3
Blank	50	1100	1150	18.3	19.2

[A] The last pore water sample must be injected within 24 h of the flocculation step (that is, the value for cumulative hours to start must be ≤24).

Using a new, graduated serological pipette, transfer 10 mL of the supernatant to a new 20 mL vial.

13.3.2 *Flocculation of Pore Water*—Flocculation must be performed no more than 24 h prior to extraction.

13.3.2.1 If a flocculation blank is to be analyzed, create the blank by placing 10 mL of reagent water in clean a 40 mL vial. Process this blank along with pore water samples.

13.3.2.2 Add the working alum solution (see Section 9) to each vial of pore water (and QC samples). The volume of the alum solution should be 1/40th of the sample volume. After the addition, swirl the vial for several rotations to incorporate the solution.

13.3.2.3 Add 3 to 5 drops of NaOH working solution (see Section 9) to each vial. Swirl to incorporate the NaOH.

13.3.2.4 Shake the vial for 15 s.

13.3.2.5 Centrifuge for 30 min at 1000 g.

13.3.2.6 Collect the supernatant into a clean 20 mL vial.

13.3.2.7 Repeat 13.3.2.2 through 13.3.2.6 once.

13.3.2.8 Immediately transfer 1.5 mL aliquots to new silanized autosampler vials and immediately add the internal standard solution as described below. Vials are weighed before and after adding the water sample to determine the exact sample water mass.

13.4 *Extraction and Analysis of Flocculated Pore Water*:

13.4.1 Split the prepared pore water samples into the required number of replicate samples, placing 1.5 mL aliquots of each into a new silanized glass autosampler vial. For QC samples, add 1.5 mL of reagent water.

NOTE 3—The SPME fiber should be cleaned at the beginning of each sampling set (and after very contaminated samples) for 1 h by placing in the cleaning chamber under helium flow at 320°C. This can conveniently be performed while the pore waters are being prepared.

13.4.2 Immediately add 10 µL of the d-PAH solution to each sample and QC sample.

NOTE 4—All of the water preparation steps beginning with the centrifugation and ending with the addition of d-PAH internal standards should be conducted continuously and in the minimum amount of time possible.

13.4.3 Load the autosampler following the recommended analytical sequence in Table 6. Verify the sequence against documented sequence following the loading process.

13.5 The recommended analytical sequence described in Table 6 is based on a 24-h "clock."

13.5.1 Two calibration verification standards are analyzed (122 min). The sequence begins with analysis of the first continuing calibration standard.

13.5.2 Analyze two method blanks (61 min each).

13.5.3 Analyze pore water samples (in duplicate at a minimum) (61 min each).

14. Data Analysis and Calculations

14.1 Generate ion chromatograms for the masses listed in Table 4 that encompass the expected retention windows of the target analytes (see Appendix X1). Integrate the selected ion current profiles of the quantitation ions shown in the table.

14.1.1 *Qualitative Identification Criteria for Individual Analytes*—For a gas chromatographic peak to be identified as a target analyte, it must meet all of the following criteria:

14.1.1.1 The quantitation ion must be present, with a signal-to-noise ratio of at least 3:1 for environmental samples.

14.1.1.2 The relative retention time (RRT) of the parent PAHs (and the 2 and 1-methylnaphthalene compounds) compared to the RRT for the labeled-standards must be within ±3 s of the relative retention times obtained from the continuing calibration (or initial calibration if this applies). Alkyl clusters must be identified based on their relative retention times to the parent PAHs and related d-PAHs, and also by observation of their characteristic fingerprints by an experienced analyst.

14.1.2 *Qualitative Identification Criteria for Total Homolog Groups* (for example, total C2 or C3 alkylnaphthalenes)—Integration of the alkyl PAHs requires hands-on labor from a highly experienced analyst. Retention time windows, like those used for the parent PAHs are inadequate for identifying alkyl clusters (that can be minutes wide). Proper identification of alkyl clusters is critical, as is the proper identification of non-target species that occur at the same nominal mass. Mental pattern recognition must be used to avoid including non-target species that may occur at the same mass and retention time window as the target alkyl PAHs. All alkyl clusters should be integrated baseline to baseline to sum the total area of the cluster (adjusting the baseline for detector drift), but not valley to valley. Manual control of the integration is required for alkyl clusters.

14.1.2.1 Representative selected ion chromatograms from coal tar contaminated sediment pore water for all target species are shown in Appendix X1. The top chromatogram on each page is the d-PAH internal standard used for the parent and alkyl PAHs associated with that parent. For example, the first page shows d8-naphthalene (m/z 136) followed by naphthalene (m/z 128), the two methylnaphthalene isomers (m/z 142), the C2 naphthalene cluster (m/z 156), the C3-naphthalene cluster (m/z 170), and the C4 naphthalene cluster (m/z 184). The chromatogram also shows a typical interference that occurs in sediments for the C4-naphthalene cluster, that is, the dibenzothiophene isomers that occur in the same selected ion chromatogram as the C4-naphthalene cluster. These interfering dibenzothiopenes are crossed out, and the correct cluster for integration (based on full scan analyses of several different contaminated sediment pore waters) are indicated by brackets. Similar designations are used to indicate common interfering peaks and the correct target species in the subsequent chromatograms.

14.1.3 The retention time (RT) of the analyte must be no more than 5 s before the expected RT of the first isomer in the homolog, based on the continuing windowing solution analysis.

14.1.4 The retention time (RT) of the analyte must be no more than 5 s after the expected RT of the last isomer in the homolog, based on the continuing windowing solution analysis.

14.2 *Quantitation for Target Analytes*:

14.2.1 Sample water concentrations are calculated by dividing the peak area of the sample peak by the peak area of its d-PAH internal standard, and then dividing the result by the calibration area ratio per ng, and dividing that result by the sample water weight.

$$Concentration\ (ng/mL) = \frac{(area\ sample\ peak)/(area\ d-PAH\ peak)}{(area\ ratio\ per\ ng\ cal.\ std) \times (sample\ weight)} \quad (4)$$

14.2.2 The mean calibration area ratio per ng values from the daily calibration runs is used for sample concentration calculations (assuming QA/QC checks with the full calibration curve meet criteria).

14.2.3 The concentrations of alkyl PAH clusters are based on the calibration response of their parent PAH as adjusted for the relative response factor (rrf) for that cluster of species (including SPME and GC/MS responses) taken from Table 1. Thus, the concentrations of alkyl clusters are calculated by:

$$Concentration\ (ng/mL) = \frac{(area\ sample\ cluster)/(area\ d-PAH\ peak)}{(area\ ratio\ per\ ng\ parent\ cal\ std) \times (sample\ weight)} \quad (5)$$

NOTE 5—The two methylnaphthalene isomers are individual alkyl peaks (not clusters as in all other alkyl cases) and are treated as parent PAHs in the calculations.

14.2.4 If no peaks are present at a signal to noise value ≥3 to 1 in the region of the ion chromatogram where the compounds of interest are expected to elute, report the result as "Not Detected" (that is, ND) at the reporting limit.

14.2.5 Depending on project objectives, the results may be reported to TDLs or estimated detection limits (EDLs).

14.2.5.1 If project-specific guidance requires analysis-specific EDLs, calculate the detection limit for that compound according to the following equation:

$$Estimated\ Detection\ Limit = \frac{N \times 2.5}{His \times (ar\ rat/ng)} \quad (6)$$

where:

N = height of peak to peak noise of quantitation ion signal in the region of the ion chromatogram where the compound of interest is expected to elute,

His = peak height of quantitation ion for appropriate internal standard, and

$ar\ rat/ng$ = mean ar rat/ng of compound obtained during daily calibration.

14.2.5.2 If project-specific guidance requires total toxic units (TTU) to be reported, calculate the detection limit for that compound according to the following equations:

$$TU_c = Ctu \times result(ng/mL)^{-1} \quad (7)$$

$$Total\ Toxic\ Units\ (TTU) = \sum_{1}^{34} TU_c \quad (8)$$

where:

TU_c = toxic unit concentration for each individual compound or homolog (ng/mL),

Ctu = concentration for one toxic unit (ng/mL), see Table 2,

$result$ = individual pore water result for a compound or homolog (ng/mL), and

TTU = total toxic units for all 34 compounds and homologs.

14.2.6 Flag all compound results in the sample which were estimated below the lowest calibration level with a "J" qualifier.

14.2.7 Flag all compound results in the sample which were estimated above the upper calibration level with an "E" qualifier.

15. Precision and Bias

15.1 *Single Analyst Precision Statement*:

15.1.1 The recommendations of the ASTM task group members were followed in performing the single-laboratory study. Three environmental sediment samples were selected from archived sediments to represent low, medium, and high

TABLE 7 Precision Statement for SPME Pore Water PAHs

Target Analyte	Statistic/Parameter	Study Pore Water Samples		
		HP-24	HP-3	HP-4
		Low	Medium	High
Naphthalene	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	ND[A]	130.9	975.3
	Single Operator Std. Deviation (So)		4.2	42.6
	Relative Standard Deviation (%)		3.2	4.4
2-Methylnaphthalene	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	ND	20.2	245.4
	Single Operator Std. Deviation (So)		0.64	9.89
	Relative Standard Deviation (%)		3.2	4.0
1-Methylnaphthalene	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	ND	81.7	209.6
	Single Operator Std. Deviation (So)		2.4	7.1
	Relative Standard Deviation (%)		3.0	3.4
C2-Naphthalenes	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	0.33	125.4	324.2
	Single Operator Std. Deviation (So)	0.0259	8.61	23.7
	Relative Standard Deviation (%)	7.8	6.9	7.3
C3-Naphthalenes	Number of Retained Values	7	7	6
	Mean Recovery (ng/mL)	0.41	124.9	212.5
	Single Operator Std. Deviation (So)	0.029	12.7	5.99
	Relative Standard Deviation (%)	7.1	10.2	2.8
C4-Naphthalenes	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	0.14	44.6	53.0
	Single Operator Std. Deviation (So)	0.025	6.05	5.3
	Relative Standard Deviation (%)	17.7	13.6	10.0
Acenaphthylene	Number of Retained Values	7	7	6
	Mean Recovery (ng/mL)	ND	0.16	7.52
	Single Operator Std. Deviation (So)		0.020	0.09
	Relative Standard Deviation (%)		12.5	1.3
Acenaphthene	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	0.21	44.1	84.8
	Single Operator Std. Deviation (So)	0.0125	1.28	2.79
	Relative Standard Deviation (%)	6.1	2.9	3.3
Fluorene	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	0.11	23.2	31.6
	Single Operator Std. Deviation (So)	0.0071	0.75	1.48
	Relative Standard Deviation (%)	6.7	3.2	4.7
C1-Fluorenes	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	0.11	22.4	25.8
	Single Operator Std. Deviation (So)	0.011	0.86	1.50
	Relative Standard Deviation (%)	10	3.8	5.8
C2-Fluorenes	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	ND	12.7	16.1
	Single Operator Std. Deviation (So)		0.88	1.85
	Relative Standard Deviation (%)		6.9	11.5
C3-Fluorenes	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	ND	ND	ND
	Single Operator Std. Deviation (So)			
	Relative Standard Deviation (%)			
Phenanthrene	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	0.1	31.3	39.2
	Single Operator Std. Deviation (So)	0.0069	1.84	3.16
	Relative Standard Deviation (%)	6.8	5.9	8.1
Anthracene	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	0.03	6.2	8.2
	Single Operator Std. Deviation (So)	0.0007	0.37	0.72
	Relative Standard Deviation (%)	2.6	5.9	8.9
C1-phenanthrenes/anthracenes	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	0.13	31.9	45.2
	Single Operator Std. Deviation (So)	0.0088	1.97	5.76
	Relative Standard Deviation (%)	6.9	6.2	12.7
C2-Phenanthrenes/Anthracenes	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	0.01	10.3	16.1
	Single Operator Std. Deviation (So)	0.0014	0.98	3.43
	Relative Standard Deviation (%)	11	9.5	21.3
C3-Phenanthrenes/Anthracenes	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	ND	4.4	4.4
	Single Operator Std. Deviation (So)		0.62	1.55
	Relative Standard Deviation (%)		14.1	35.5
C4-Phenanthrenes/Anthracenes	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	ND	1.2	ND
	Single Operator Std. Deviation (So)		0.24	

TABLE 7 *Continued*

Target Analyte	Statistic/Parameter	Study Pore Water Samples		
		HP-24	HP-3	HP-4
		Low	Medium	High
	Relative Standard Deviation (%)		20.6	
Fluoranthene	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	0.04	5.6	5.8
	Single Operator Std. Deviation (So)	0.0028	0.61	0.87
	Relative Standard Deviation (%)	6.7	10.9	15.1
Pyrene	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	0.06	6.2	7.7
	Single Operator Std. Deviation (So)	0.0038	0.75	1.28
	Relative Standard Deviation (%)	6.2	12.1	16.8
C1-Fluoranthenes/Pyrenes	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	0.04	5.0	6.1
	Single Operator Std. Deviation (So)	0.0033	0.78	1.79
	Relative Standard Deviation (%)	7.3	15.8	29.2
Benz[a]anthracene	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	ND	0.76	0.75
	Single Operator Std. Deviation (So)		0.16	0.33
	Relative Standard Deviation (%)		20.8	44.5
Chrysene	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	0.01	0.77	0.79
	Single Operator Std. Deviation (So)	0.0009	0.16	0.35
	Relative Standard Deviation (%)	10.7	20.5	44.7
C1-Chrysenes	Number of Retained Values	7	7	7
	Mean Recovery (ng/mL)	ND	0.54	0.50
	Single Operator Std. Deviation (So)		0.11	0.33
	Relative Standard Deviation (%)		21.2	64.9
Total Toxic Units	Number of Retained Values	7	7	7
	Mean Recovery (units)	0.15	50.4	81.4
	Single Operator Std. Deviation (So)	0.01	3.52	5.23
	Relative Standard Deviation (%)	4.8	7.0	6.4

[A] ND: Analyte not detected in the associated sample.

concentrations of pore water PAHs. Efforts were made to ensure that sediments were chosen that had a full distribution of target PAH ring sizes, a range of PAH concentrations found in environmental sediment samples, and a representative range in total organic carbon concentration and texture.

15.1.2 The quantitations were based on three- or four-point calibration curves as verified by daily analysis of duplicate calibration verification standards at the medium-high concentration level. Prior to sample analysis, the initial calibration curves must have a coefficient of determination greater than 0.990, and the relative response factors must have a relative standard deviation of less than 25 % for two to three-ring PAHs, and less than 30 % for four-ring PAHs. The calibration verification mean relative response factor must agree with those of the initial calibration curve within 20 % for two to three-ring PAHs, and less than 25 % for four-ring PAHs. No sample data were reported if these criteria were not met. All method blanks met the requirement that the concentrations be at or less than 20 % of the Performance Limits for individual PAHs.

15.1.3 As directed in section 10.3 of Practice D 2777, the data were evaluated for outliers. The data were evaluated using the one-sided t-test at the upper 5 % significance level as described in Practice E 178, Section 6. Two outlying observations were found for high-level sample HP-4. One C3-naphthalenes result and one acenaphthylene result for sample HP-4 were outliers. The mean and single operator standard deviation were recalculated for sample HP-4 C3-naphthalenes and acenaphthylene without the outlying observations (that is, n = 6).

15.1.4 The precision statements for each analyte are shown on Table 7. For this single-laboratory study, it was assumed that the calculated standard deviation is equivalent to the single operator standard deviation (S_o). Replicate determinations of sample PAH concentrations typically had relative standard deviations (RSDs) less than 10 %, with somewhat higher RSDs for higher molecular weight compounds. The only unusually high RSDs occurred for the highest molecular weight PAHs from high-level sample HP 4. The reason for this is that the saturation limits may have been reached for the high molecular weight PAHs (that is, C1-phenanthrenes/anthracenes through C1-chrysenes).

15.1.5 Finally, the variation of individual PAH determinations had no significant effect on the repeatability of the total toxic unit determinations. See Table 7. This was demonstrated even though the statistical outliers found in sample HP-4 were not omitted in the calculation of total toxic units. The RSDs for the total toxic unit results ranged from 5 to 7 %.

15.2 *Single Analyst Bias Statement*:

15.2.1 A single laboratory study was performed using the perdeuterated PAHs d12-benz(a)anthracene and d10-2-methylnaphthalene spiked at low, medium, and high levels into environmental sediment samples. The quality control statements for each analyte level sample, obtained from the perdeuterated spike study, are shown in Tables 8-10. The quality control statements can also be considered precision and bias statements because the true spiking levels of the perdeuterated PAHs were known. The graphs and regression equations show the relationship between single-operator standard deviation and concentration, and mean measured value and

TABLE 8 HP-24 Low Concentration Quality Control

Analyte	True Spiked Value (ng/mL)	Number of Retained Values	Mean Recovery (ng/mL)	Mean Recovery (%)	Single Standard Deviation (So)	Relative Standard Deviation (%)
2-Methylnaphthalene-d10	4.68	7	4.33	92.6	0.3161	7.3
Benz[a]anthracene-d12	0.0429	7	0.0352	81.9	0.0031	8.8

TABLE 9 HP-3 Medium Concentration Quality Control

Analyte	True Spiked Value (ng/mL)	Number of Retained Values	Mean Recovery (ng/mL)	Mean Recovery (%)	Single Standard Deviation (So)	Relative Standard Deviation (%)
2-Methylnaphthalene-d10	26.7	7	26.7	100.1	0.859	3.2
Benz[a]anthracene-d12	0.25	7	0.199	81.0	0.015	7.5

TABLE 10 HP-4 High Concentration Quality Control

Analyte	True Spiked Value (ng/mL)	Number of Retained Values	Mean Recovery (ng/mL)	Mean Recovery (%)	Single Standard Deviation (So)	Relative Standard Deviation (%)
2-Methylnaphthalene-d10	283.9	7	230.7	81.3	11.0	4.8
Benz[a]anthracene-d12	2.61	7	2.13	81.7	0.13	5.9

concentration for both perdeuterated PAHs (see Figs. 1-4). The figures show the linearity of precision and accuracy with increasing concentration. The d12-benz(a)anthracene recoveries were consistently around 80 %. This may possibly indicate the consistent suppression of the mass spectral signal by a near-eluting compound. The recoveries for d10-2-methylnaphthalene ranged from 81 to 112 %. The repeatability for the known spike recoveries was consistent; the known spike RSDs ranged from 3 to 9 %. PAH concentration had no significant effect on the repeatability of the technique.

16. Quality Control Criteria

16.1 *Initial Calibration*:

16.1.1 The following acceptance criteria will be used for initial calibration: *(1)* The signal to noise (S/N) ratio for the GC signals present in every selected ion current profile (SICP) must be ≥10:1 for the labeled internal standards and calibration compounds; *(2)* The percent relative standard deviation (RSD) for the mean area ratio/ng for labeled internal standards and the calibration compounds must be less than 30 % for high molecular weight PAHs and less than 25 % for low molecular weight PAHs, and the $r^2 > 0.99$. The calibration curve must not be forced through the origin; *(3)* The number of calibration standards may be reduced from four to three based on the criteria in 12.3 of this test method.

16.1.2 The following corrective action will be adopted for initial calibration: *(1)* Initial calibration must be re-established if the RSD(s) exceed the limit(s); *(2)* The calibration will not be re-established in response to a nonconforming RSD if the sample results are less than the PQL.

16.2 *Daily Duplicate Calibration Verifications*:

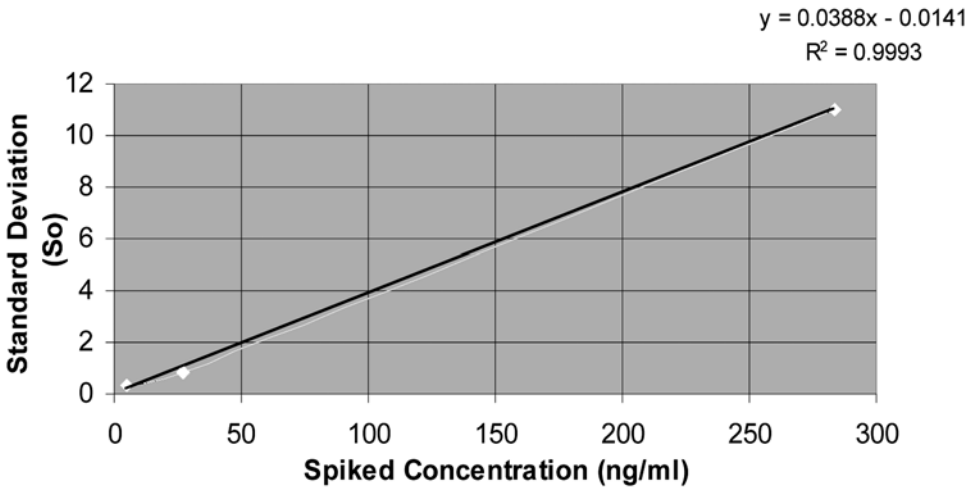


FIG. 1 2-Methylnaphthalene-d10 Single Standard Deviation versus Spiked Concentration

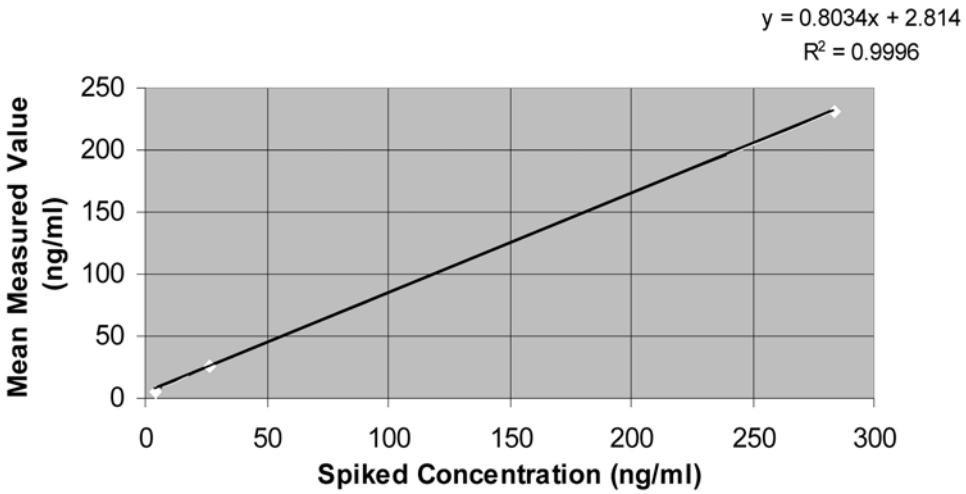


FIG. 2 Methylnaphthalene-d10 Mean Measured Value versus Spiked Concentration

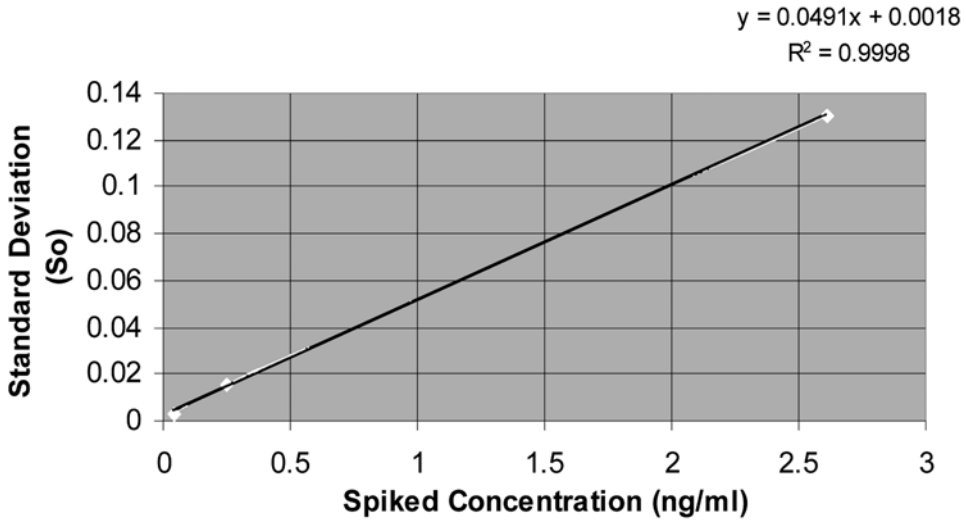


FIG. 3 Benz[a]anthracene-d12 Single Standard Deviation versus Spiked Concentration

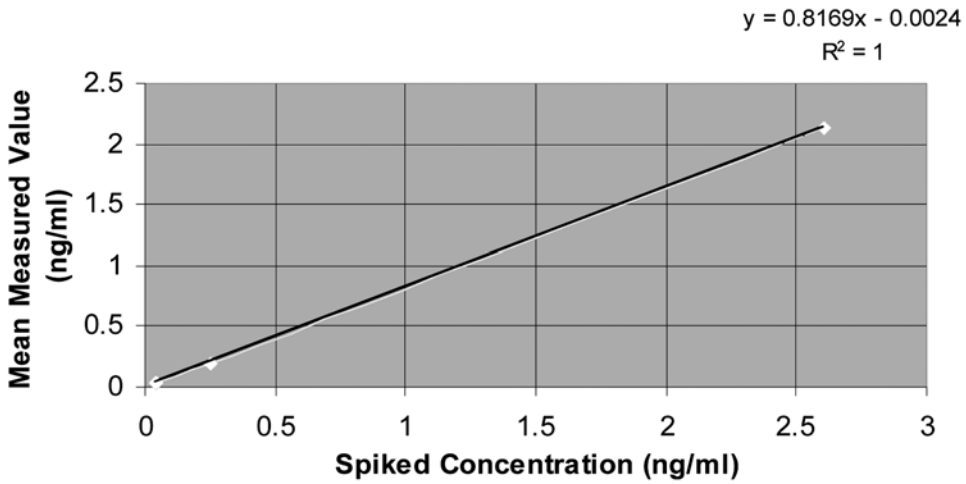


FIG. 4 Benz[a]anthracene-d12 Mean Measured Value versus Spiked Concentration

16.2.1 The following acceptance criteria will be used for daily duplicate calibration verifications: *(1)* The S/N ratio for the GC signals present in every SICP must be ≥10:1 for the labeled internal standards and the calibration compounds; *(2)*

The percent differences for the measured area ratio/ng of all analytes must be within ±25 % for high molecular weight PAHs and less than ±20 % for low molecular weight PAHs of the mean values established during the initial calibration.

16.2.2 The following corrective action will be adopted for daily duplicate calibration verifications if the first acceptance criterion is not satisfied: a new initial calibration curve must be established before sample extracts can be analyzed.

16.3 *Flocculation Blanks*:

16.3.1 The following acceptance criterion will be used for flocculation blanks: Prepared as needed to assess contamination from flocculation reagents and handling. Target analytes must not be detected above ⅓ of the target detection limits or >20 % of the associated sample result(s).

16.3.2 The following corrective action will be adopted for flocculation blanks: Locate the source of the contamination; correct the problem. Re-extract and reanalyze associated samples that are less than ten times the level of the contaminants present in the method blank.

16.4 *Extraction and Analytical Blanks*:

16.4.1 The following acceptance criterion will be used for extraction and analytical blanks: Analyzed between every sample to monitor the baseline. Target analytes must not be detected above ⅓ of the target detection limits or >20 % of the associated sample result(s).

16.4.2 The following corrective action will be adopted for extraction and analytical blanks: Locate the source of the contamination; correct the problem. Re-extract and reanalyze associated samples that are less than ten times the level of the contaminants present in the method blank.

16.5 *Signal to Noise Ration*:

16.5.1 The following acceptance criterion will be used for signal to noise ratio: The signal to noise (S/N) ratio for the GC signals present in every selected ion current profile (SICP) must be ≥3:1 for target compounds in environmental samples and ≥10:1 for the labeled internal standards.

16.5.2 The following corrective action will be adopted for signal to noise ratio: Reanalyze the sample unless obvious matrix interference is present.

APPENDIX

(Nonmandatory Information)

X1. ION PLOTS

X1.1 Selected ion chromatograms from a typical coal tar impacted pore water of d-PAH internal standards (top chromatogram of each page), and the related target parent and alkyl PAHs. Target species are indicated with brackets, and interfering species are marked with an “X.”

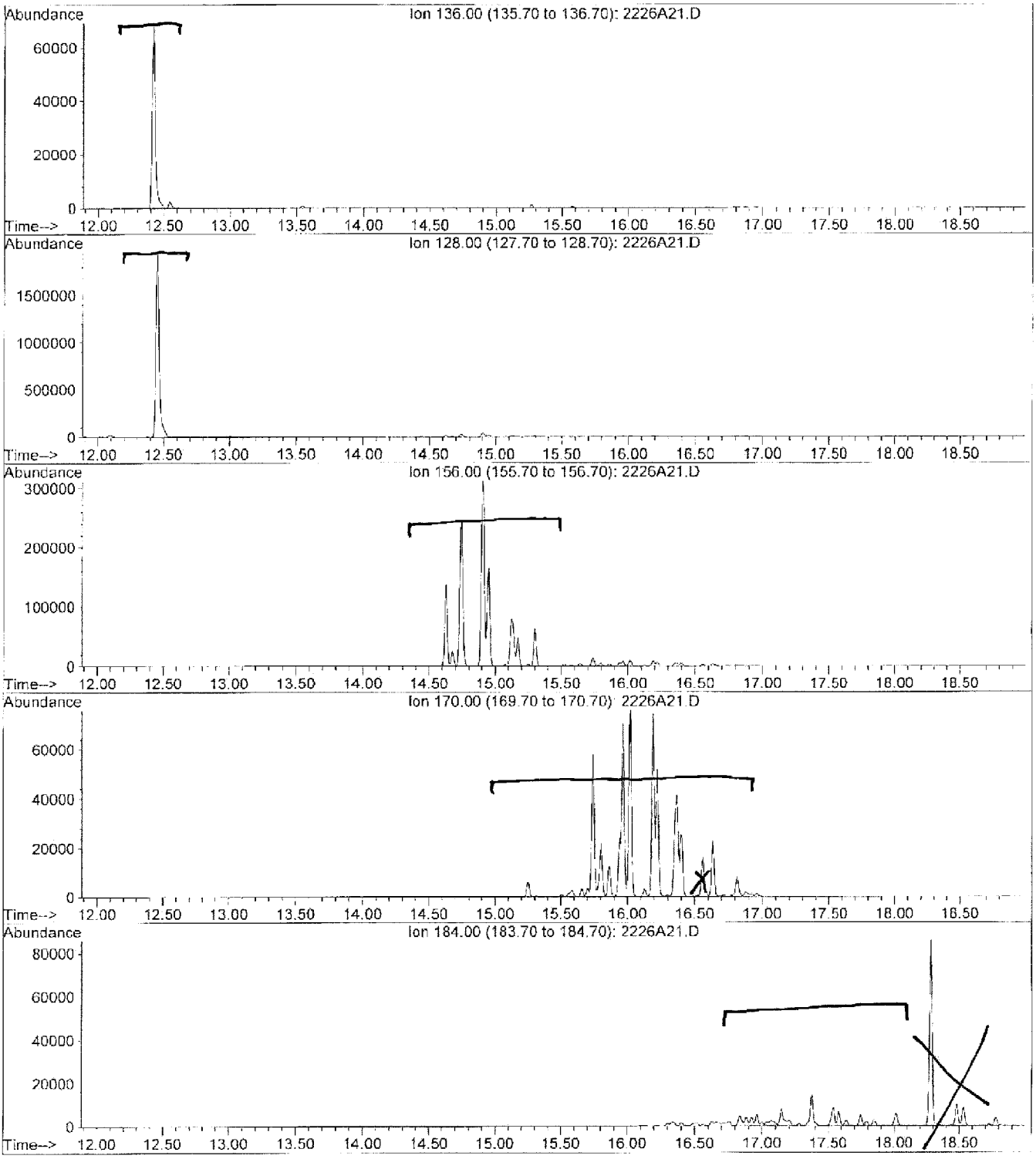


FIG. X1.1 Naphthalenes

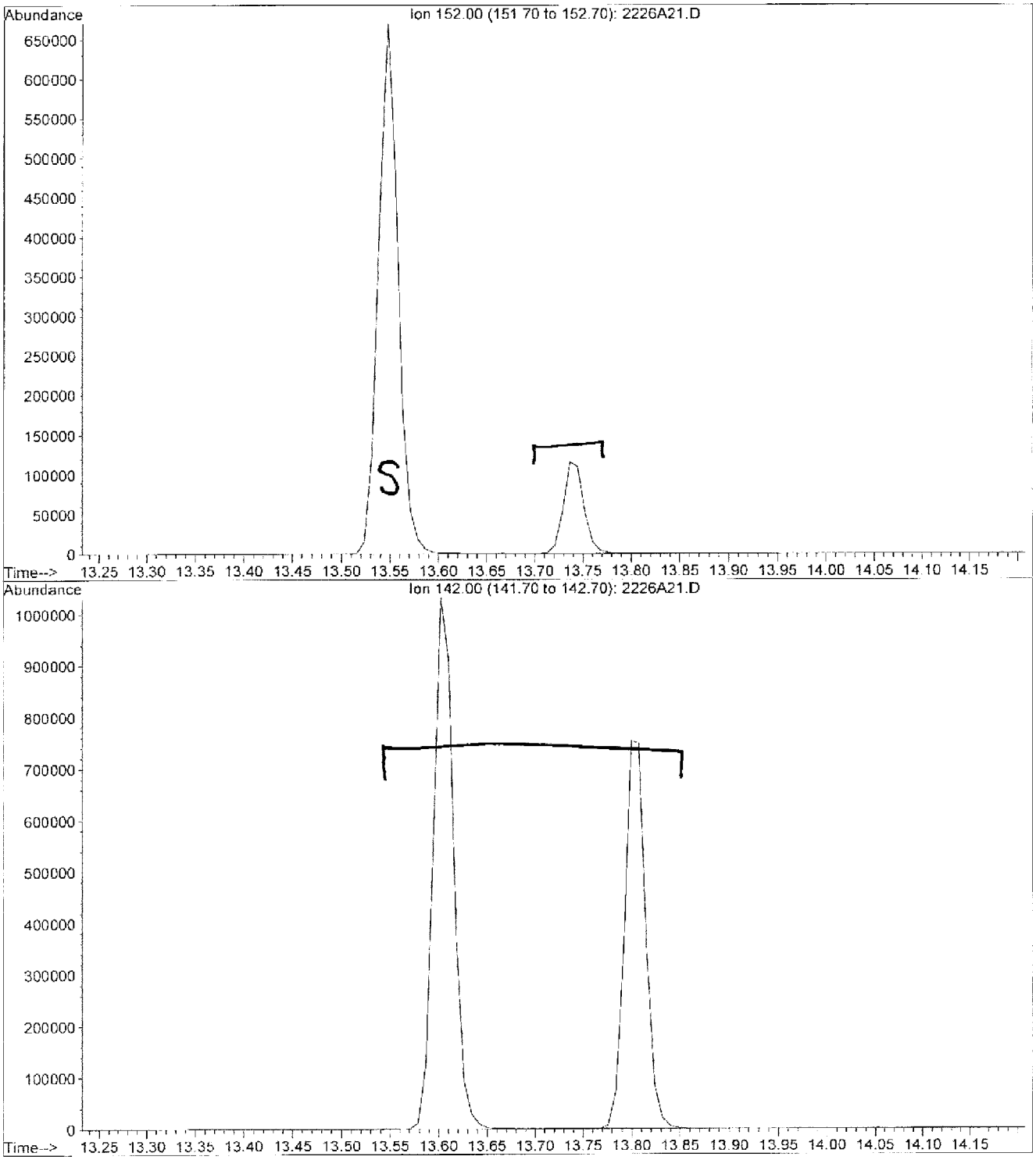


FIG. X1.2 Methylnaphthalenes
("s" is a spiked d_{10}-methylnaphthalene surrogate)

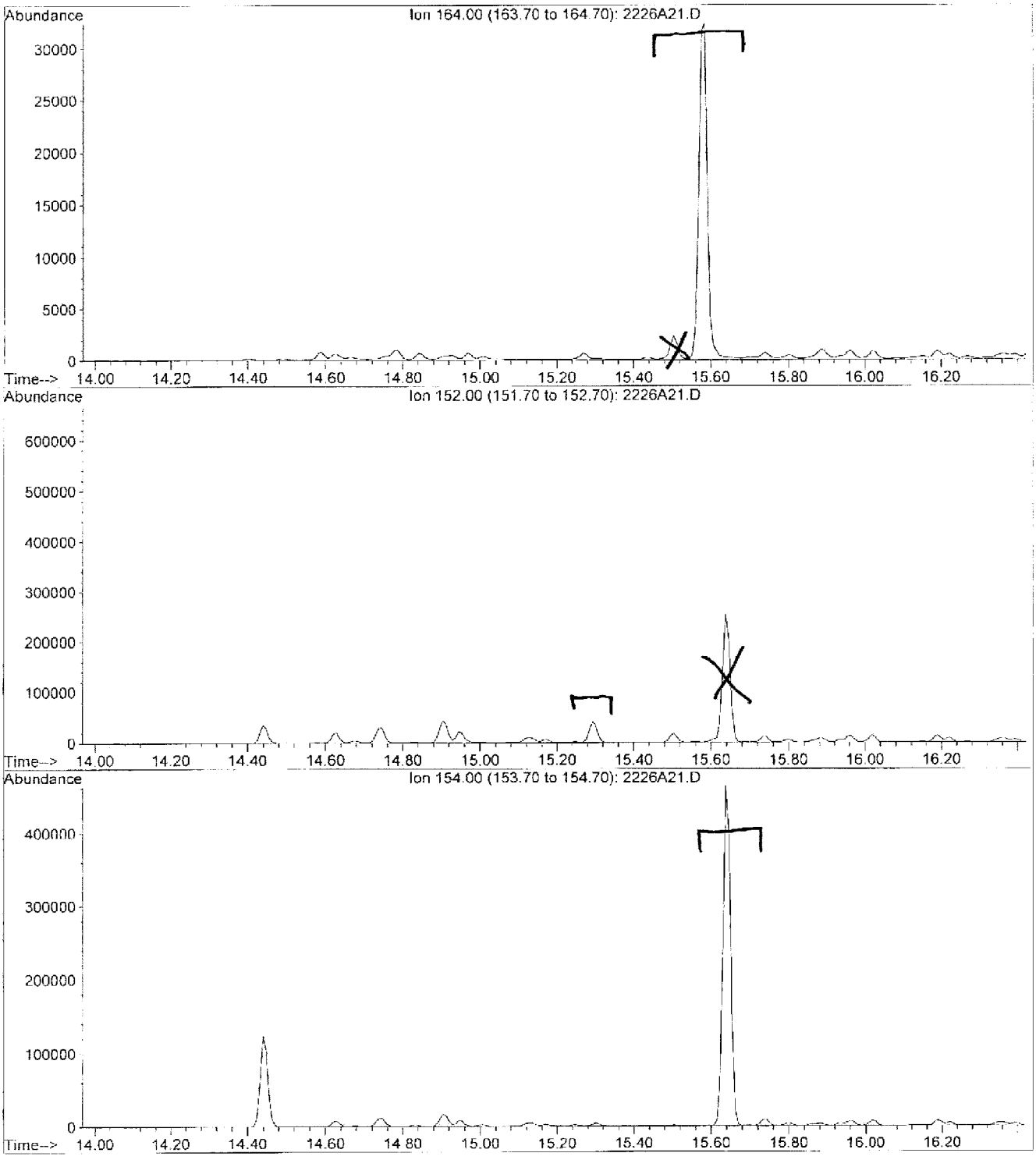


FIG. X1.3 Acenaphthylene/Acenaphthene

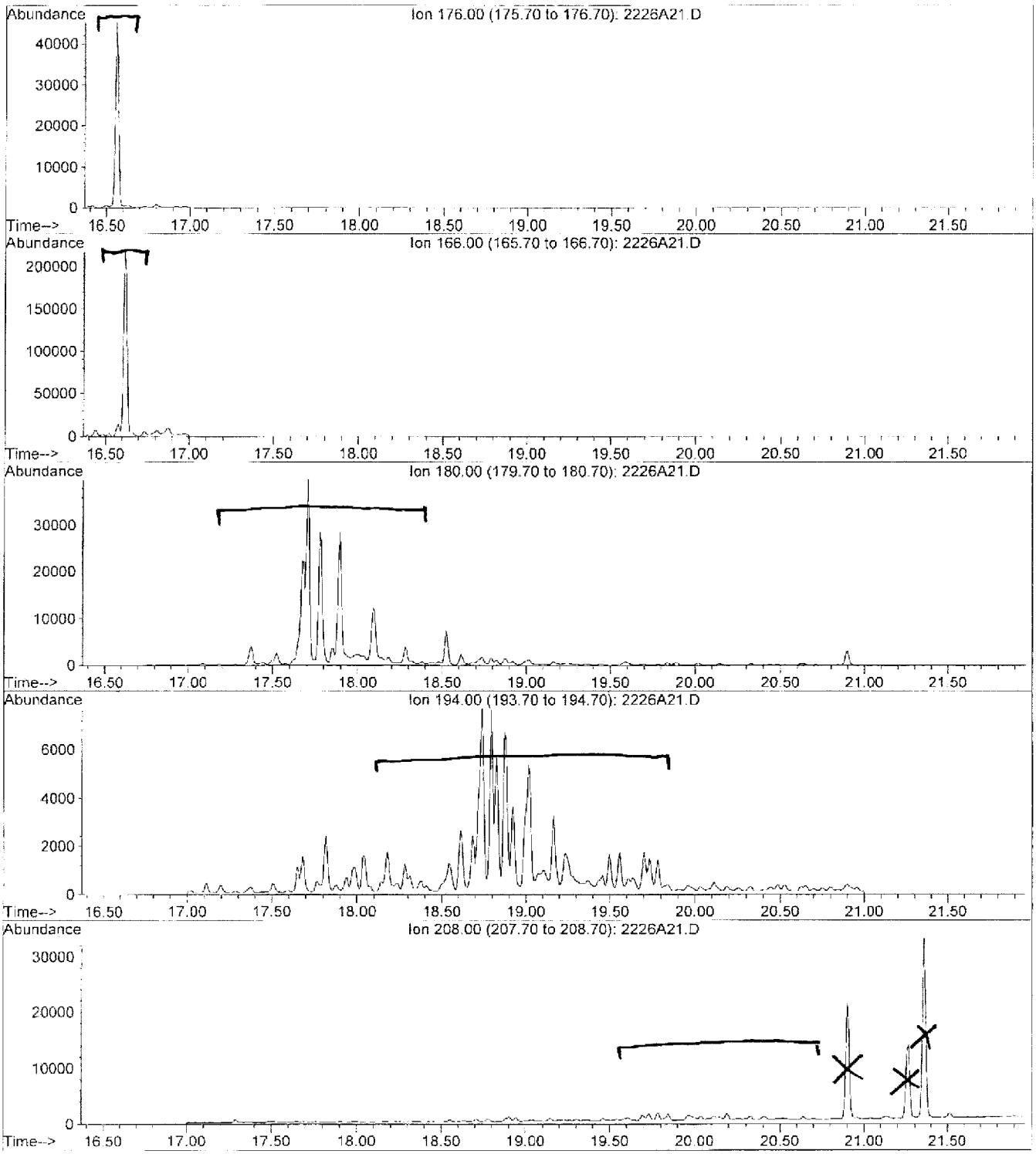


FIG. X1.4 Fluorenes

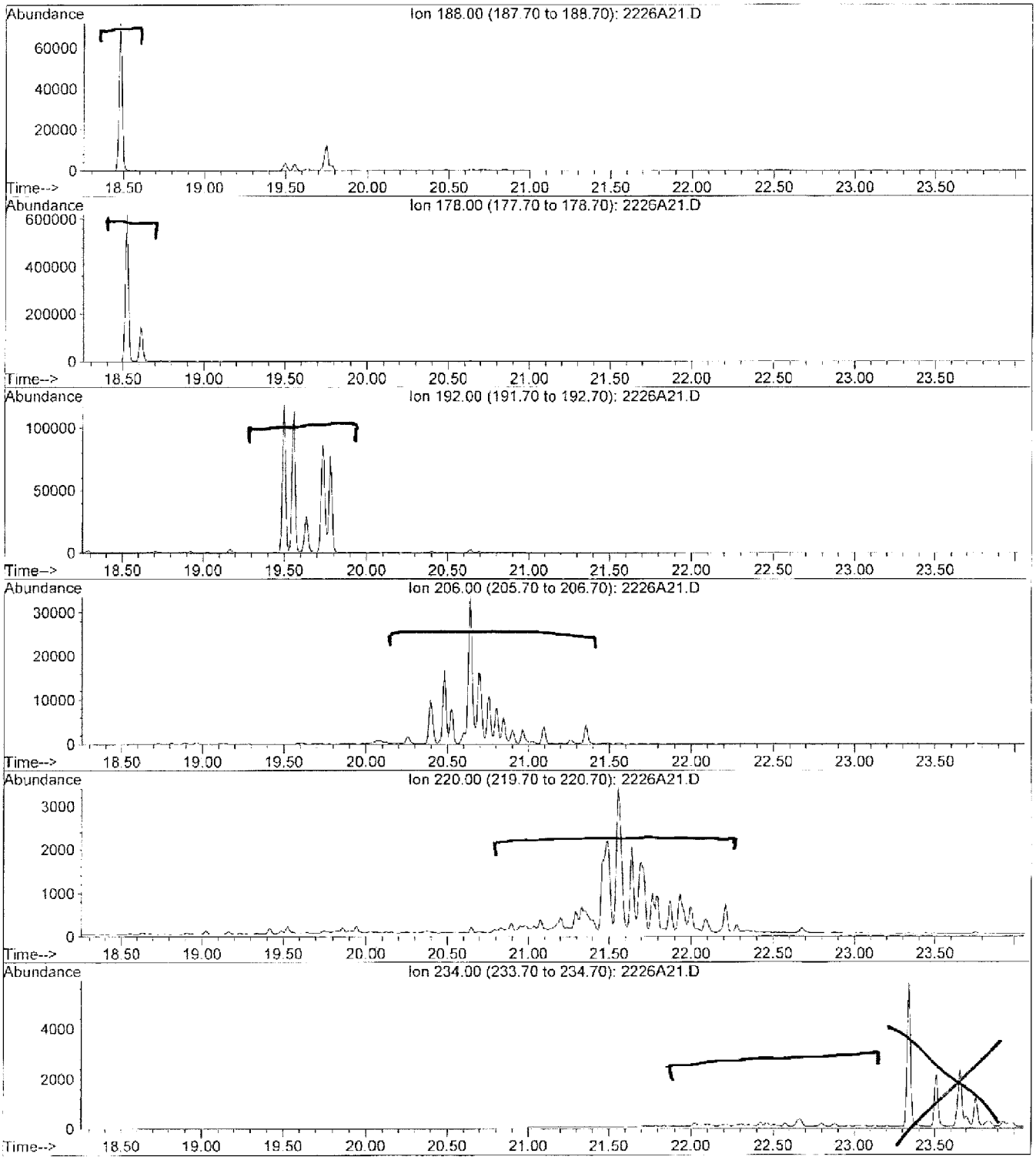


FIG. X1.5 Phenanthrenes/Anthracenes

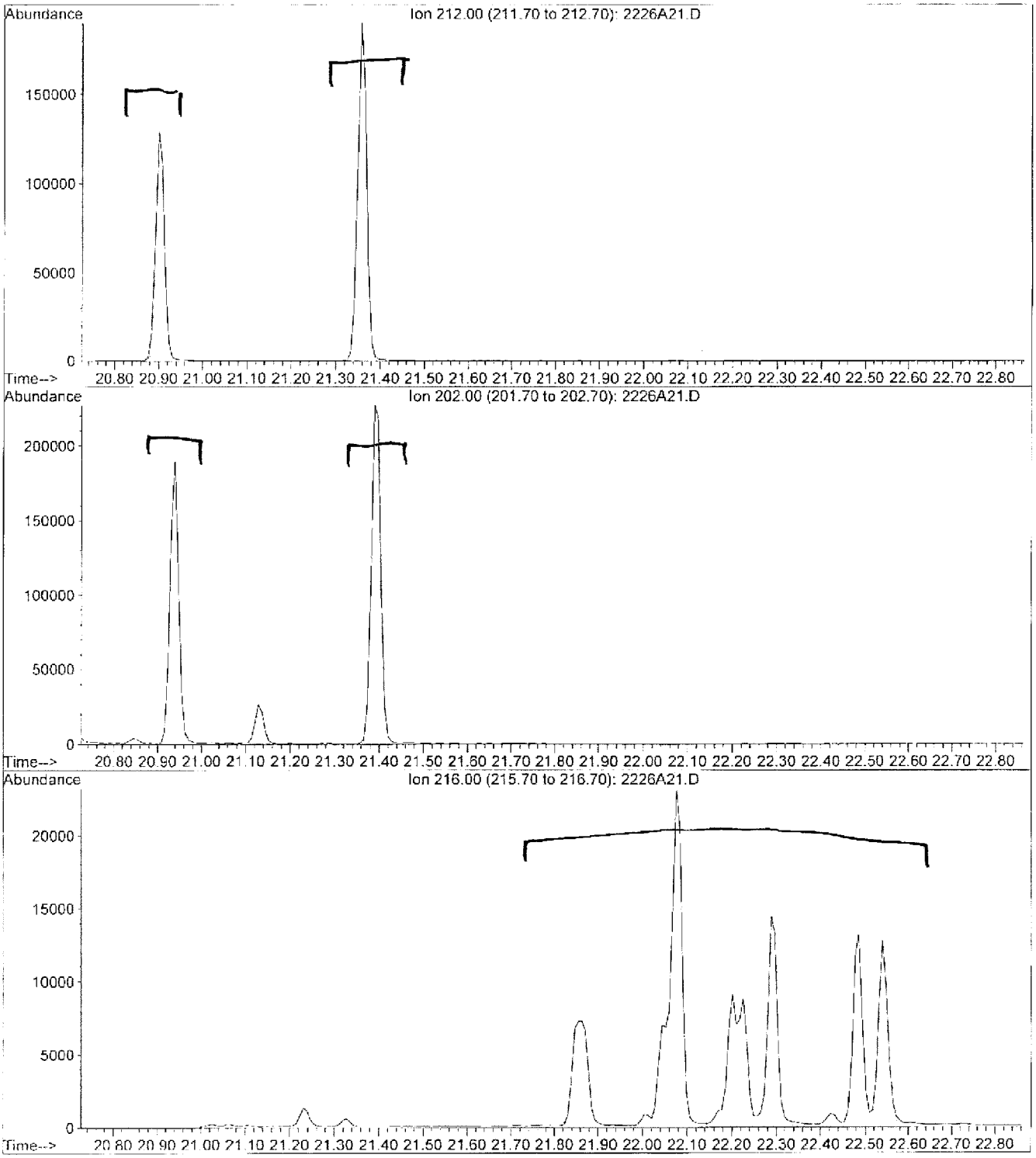


FIG. X1.6 Fluoranthenes/Pyrenes

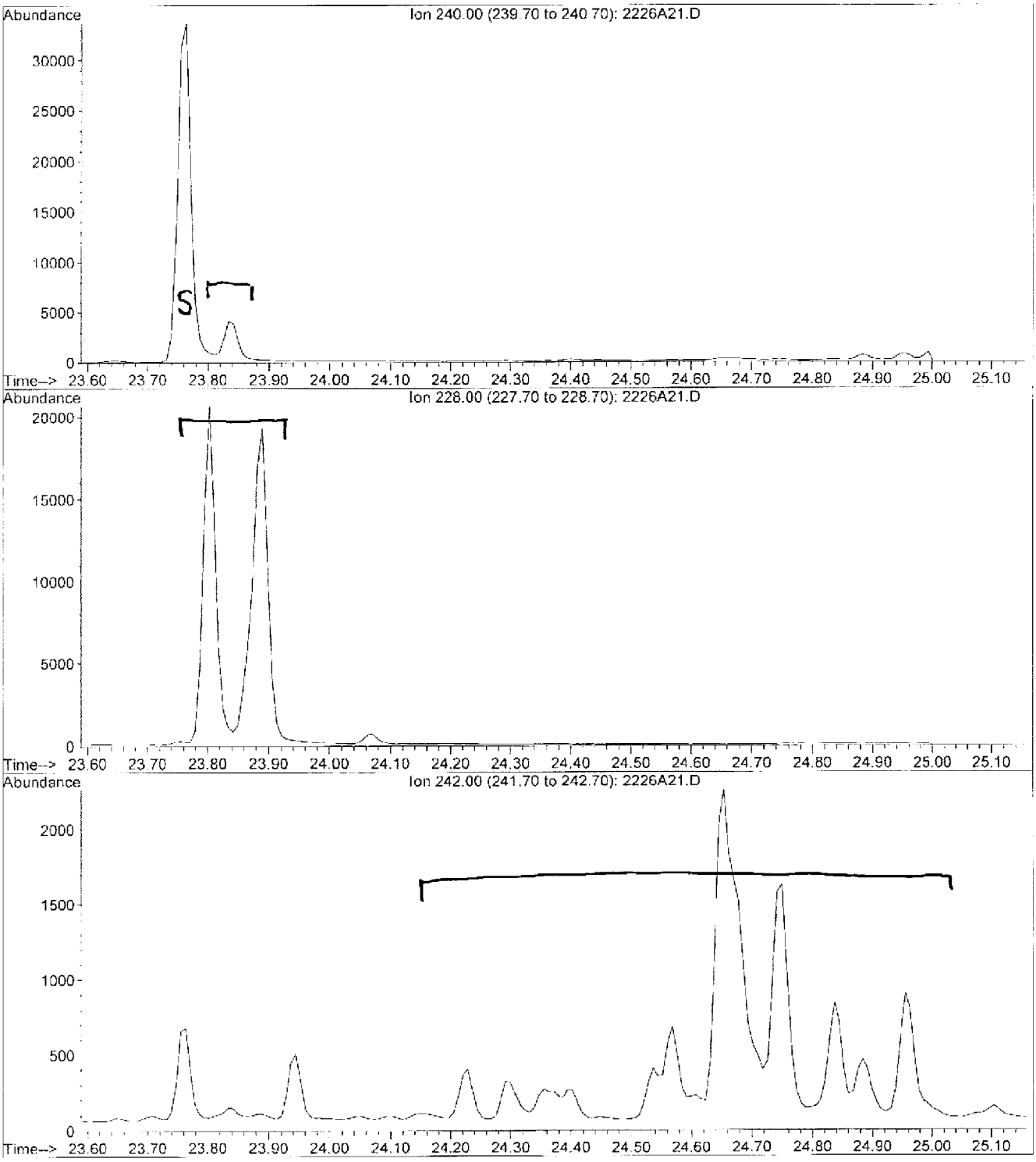


FIG. X1.7 Benz[a]anthracenes/Chrysenes
("s" is a spiked d_{12}-benz[a]anthracene surrogate)

ASTM International takes no position respecting the validity of any patent rights asserted in connection with any item mentioned in this standard. Users of this standard are expressly advised that determination of the validity of any such patent rights, and the risk of infringement of such rights, are entirely their own responsibility.

This standard is subject to revision at any time by the responsible technical committee and must be reviewed every five years and if not revised, either reapproved or withdrawn. Your comments are invited either for revision of this standard or for additional standards and should be addressed to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend. If you feel that your comments have not received a fair hearing you should make your views known to the ASTM Committee on Standards, at the address shown below.

This standard is copyrighted by ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, United States. Individual reprints (single or multiple copies) of this standard may be obtained by contacting ASTM at the above address or at 610-832-9585 (phone), 610-832-9555 (fax), or service@astm.org (e-mail); or through the ASTM website (www.astm.org).

APPENDIX M

MEASURED PARTITIONING COEFFICIENTS FOR PARENT AND ALKYL POLYCYCLIC AROMATIC HYDROCARBONS IN 114 HISTORICALLY CONTAMINATED SEDIMENTS: PART 1 K_{OC} VALUES

PUBLISHED IN *ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY*

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 25, No. 11, pp. 2901–2911, 2006
© 2006 SETAC
Printed in the USA
0730-7268/06 $12.00 + .00

MEASURED PARTITIONING COEFFICIENTS FOR PARENT AND ALKYL POLYCYCLIC AROMATIC HYDROCARBONS IN 114 HISTORICALLY CONTAMINATED SEDIMENTS: PART 1. K_{OC} VALUES

STEVEN B. HAWTHORNE,* CAROL B. GRABANSKI, and DAVID J. MILLER
Energy and Environmental Research Center, University of North Dakota, Box 9018, Grand Forks, North Dakota 58202, USA

(*Received* 6 *March* 2006; *Accepted* 30 *May* 2006)

Abstract—Polycyclic aromatic hydrocarbon (PAH) partitioning coefficients between sediment organic carbon and water (K_{OC}) values were determined using 114 historically contaminated and background sediments collected from eight different rural and urban waterways in the northeastern United States. More than 2,100 individual K_{OC} values were measured in quadruplicate for PAHs ranging from two to six rings, along with the first reported K_{OC} values for alkyl PAHs included in the U.S. Environmental Protection Agency's (U.S. EPA) sediment narcosis model for the prediction of PAH toxicity to benthic organisms. Sediment PAH concentrations ranged from 0.2 to 8,600 μg/g (U.S. EPA 16 parent PAHs), but no observable trends in K_{OC} values with concentration were observed for any of the individual PAHs. Literature K_{OC} values that are commonly used for environmental modeling are similar to the lowest measured values for a particular PAH, with actual measured values typically ranging up to two orders of magnitude higher for both background and contaminated sediments. For example, the median log K_{OC} values we determined for naphthalene, pyrene, and benzo[*a*]pyrene were 4.3, 5.8, and 6.7, respectively, compared to typical literature K_{OC} values for the same PAHs of 2.9, 4.8, and 5.8, respectively. Our results clearly demonstrate that the common practice of using PAH K_{OC} values derived from spiked sediments and modeled values based on *n*-octanol–water coefficients can greatly overestimate the actual partitioning of PAHs into water from field sediments.

Keywords—Manufactured gas plant Polycyclic aromatic hydrocarbons Sediment K_{OC} values Partitioning

INTRODUCTION

Polycyclic aromatic hydrocarbon (PAH) partitioning coefficients between sediment organic carbon and water (K_{OC} values) are used to predict the bioavailability and general environmental behavior of individual PAH species. In many applications, the K_{OC} value for a particular PAH is assumed to be a constant that can be applied to all sediments. For example, the U.S. Environmental Protection Agency's (U.S. EPA) narcosis model requires the measurement of 18 parent and 16 groups of alkyl polycyclic aromatic hydrocarbons (PAH_{34}) in sediments to calculate the number of PAH toxic units (TU_{34}) available to benthic organisms. Sediment concentrations for each PAH (or group of alkyl PAHs) are used along with sediment organic carbon–water partitioning coefficients (K_{OC}) and octanol–water partitioning coefficients (K_{OW}) to predict toxicity [1–4]. However, few experimentally determined values exist for higher-molecular-weight and alkyl PAHs, especially for historically contaminated sediments.

An increasing number of reports have showed that measured K_{OC} values for PAHs can be substantially higher than those typically used for modeling and predictive investigations [5–14], and therefore the water concentrations of PAHs are often lower than those predicted using standard K_{OC} values. Given the increasing evidence in the literature that there are multiple carbon types present in sediments and that these carbon types can have different sorption affinities for PAHs (and thus result in different partitioning behavior into water), it seems unlikely that commonly accepted K_{OC} values are adequate to predict environmental behavior of PAHs in field sediments [5]. Several recent reports have shown that PAH K_{OC} values from sediments having more sorptive carbon forms such as coke, soot (or black carbon), and pitch can be one to three orders of magnitude higher than commonly accepted K_{OC} values for the same PAH [5–14]. In a recent review of measured K_{OC} values for PAHs [5], Cornelissen et al. concluded that ''the use of generic organic carbon-water distribution coefficients in the risk assessment of organic compounds is not warranted and that bioremediation endpoints could be evaluated on the basis of freely dissolved concentrations instead of total concentrations in sediment/soil.'' This statement emphasizes the need to determine water PAH concentrations and K_{OC} values for field sediments in order to accurately assess their environmental partitioning behavior.

Previous reports normally deal with a limited number of individual PAHs and a small number of sediment samples, making any generalization of the potential range of PAH K_{OC} values that actually exist in real-world sediment samples difficult. None of the previous studies has determined K_{OC} values for the two- to six-ring PAHs (and their alkyl derivatives) that are of scientific and regulatory concern from a single sediment, and no studies have reported values from a large enough number of sediment samples to allow the range of K_{OC} values that actually exist in the environment to be explored. Limits in analytical sensitivity and the resultant need for large sediment pore-water samples have made such studies prohibitively difficult, especially for the less soluble five- and six-ring PAHs. However, the recent application of solid-phase microextraction for trace determination of PAHs using only 1- to 2-ml samples of pore water makes determining K_{OC} values for two- to six-ring PAHs in a large number of sediments more realistic [15].

The purpose of the present study is to present measured

* To whom correspondence may be addressed (shawthorne@undeerc.org).

K_{OC} values from 114 historically contaminated and background sediments collected from eight different sites. More than 2,100 K_{OC} values are reported, including the first experimentally determined K_{OC} values for several higher-molecular-weight PAHs and alkyl PAHs for multiple sediment samples. The K_{OC} values from field sediments are compared to those typically reported and used in partitioning studies, and the relationships among PAH identities, PAH concentrations, sediment organic carbon, and K_{OC} values are discussed. Subsequent reports will include partitioning coefficients to black carbon (K_{BC} values) and to dissolved organic carbon (K_{DOC}) for the same suite of samples. It is the intention of the authors to provide the resultant data to the scientific community as an electronic database so that these results can be used to better understand and predict the partitioning of PAHs that occurs under the broad range of characteristics that exist in contaminated and background sediments.

EXPERIMENTAL

Sediment samples

More than 200 sediment samples were collected using a Ponar grab sampler (Forestry Suppliers, Jackson, MS, USA) at eight different sites in 2003, 2004, and 2005 (Table 1). Six of the sites were thought to be impacted by former manufactured gas plant (MGP) activities and two by aluminum smelters. All the MGP sites had ceased production prior to 1960. Former MGP activities were the most likely source of PAHs for three of the sites (''rural, light industrial'' in Table 1), but the remaining three MGP sites were in heavily industrial or highly urbanized areas that included other potential PAH sources (e.g., petroleum storage, transport, and use).

Approximately 15 L each of sediment–water slurry was immediately transferred to a 20-L bucket, sieved through a 2-mm screen to remove debris, and briefly mixed before subsampling into new glass jars with Teflon®-lined lids. Samples (~200 g) were then cooled on ice in the dark and shipped to the laboratory by overnight air delivery. (The bulk of each sample was stored separately for subsequent biological testing.) Storage was at 4°C in the dark. The sediment samples typically had approximately 50 weight % water as stored. Sediment collection and storage procedures were based on previous recommendations [1,2,16], and all sediments and associated pore waters were analyzed within 28 d of sample collection [15,17].

A preliminary estimate of PAH concentrations on each sediment was performed by mixing 2 g of the wet sediment with 2 g of sodium sulfate and extracting with 20 ml of 1:1 acetone/methylene chloride for 18 h in a bath sonicator and analyzing the extracts for the so-called 34 PAHs as previously described [17]. On the basis of these initial estimates of PAH concentrations, 114 sediments were selected for additional study to represent the range of textures, total organic carbon (TOC), and PAH concentrations (from background to highly contaminated) found at the eight sites.

Sediment preparation and analysis

All sediment and pore-water analyses were performed within 28 d of sample collection. Pore-water and sediment samples were prepared fresh daily as suggested by the U.S. EPA [16] by transferring approximately 40 ml of the sediment–water slurry to a certified clean 40-ml glass VOA vial and centrifuging for 30 min at 1,000 *g*. (Higher speed caused the glass vials to break.) This typically resulted in 10 to 20 ml of pore water that could be removed with a pipette. Colloidal material in the pore water was removed by alum flocculation [15]. Perdeuterated PAHs ranging from two to six rings were added as internal standards, and the pore-water concentrations of the 34 parent and alkyl PAHs were determined using solid-phase microextraction as previously described [15].

After removal of the pore water, the remaining wet sediment was recovered, quadruplicate 2-g samples of the sediment were mixed with an equal weight of sodium sulfate to get a dry and pourable sample, and each replicate was extracted for 18 h in a Soxhlet apparatus with 150 ml of 1:1 acetone/methylene chloride as previously described [17]. Each extract was then spiked with 5 μl of a mixture of two- to six-ring perdeuterated PAHs (*d*-PAHs) as internal standards and analyzed using gas chromatography/mass spectrometry (GC/MS) with selected ion monitoring [17]. More dilute extracts were concentrated under a gentle stream of clean nitrogen prior to GC/MS analysis. No sediment samples were air dried prior to extraction in order to avoid any losses of the more volatile PAHs. Replicate portions of each sediment were dried overnight at 80°C to allow their moisture content to be determined and to allow presentation of the concentration data on a dry-weight basis.

All pore-water analyses and sediment extractions and analyses were performed in quadruplicate. Detection limits for the individual PAHs in sediment are given in detail in Hawthorne et al. [17] and were approximately 1 to 2 ng/g for parent PAHs and approximately 2 to 30 times higher for the alkyl PAHs. Detection limits for pore water are reported in detail elsewhere [15] and vary from approximately 0.5 ng/ml for naphthalene to approximately 0.05 ng/ml for three- and four-ring PAHs to approximately 0.002 ng/ml for six-ring PAHs. All analyses were performed with an Agilent model 5973 GC/MS (Agilent Technologies, Wilmington, DE, USA) operated in the selected ion mode for the molecular ions of the target PAHs and *d*-PAHs and equipped with a 60-m Agilent HP-5 MS column (0.25-μm film thickness, 250-μm inner diameter). The possible presence of petroleum contaminants was routinely evaluated by monitoring an ion (m/z = 85) that is characteristic for petroleum alkanes.

TOC

Total organic carbon was determined on air-dried sediment (80°C for 2 h) after grinding and acidification with 1 M HCl to remove inorganic carbonates. Elemental analysis (C, H, N) was performed with a Leeman Labs Model CE440 elemental analyzer (Hudson, NH, USA). The TOC values were determined in triplicate for each sediment.

Data acceptance or rejection

All pore-water and sediment PAH analyses were performed in quadruplicate for all 114 sediment samples. No K_{OC} values from any of the sediments were rejected. In a few cases, a replicate pore-water or replicate Soxhlet extract was lost during laboratory handling. However, a minimum of triplicate pore-water and sediment analyses were completed for all sediment samples. For samples near the method detection limits, a minimum of three detected values were also required, or K_{OC} data were not reported. Typical relative standard deviations (RSDs) for replicate pore water analyses were <15% for lower-molecular-weight PAHs and <20% for the higher-molecular-weight PAHs. Only 5% of the pore-water determinations showed RSDs >30%, and these were normally very low concentrations of the higher-molecular-weight PAHs. For sedi-

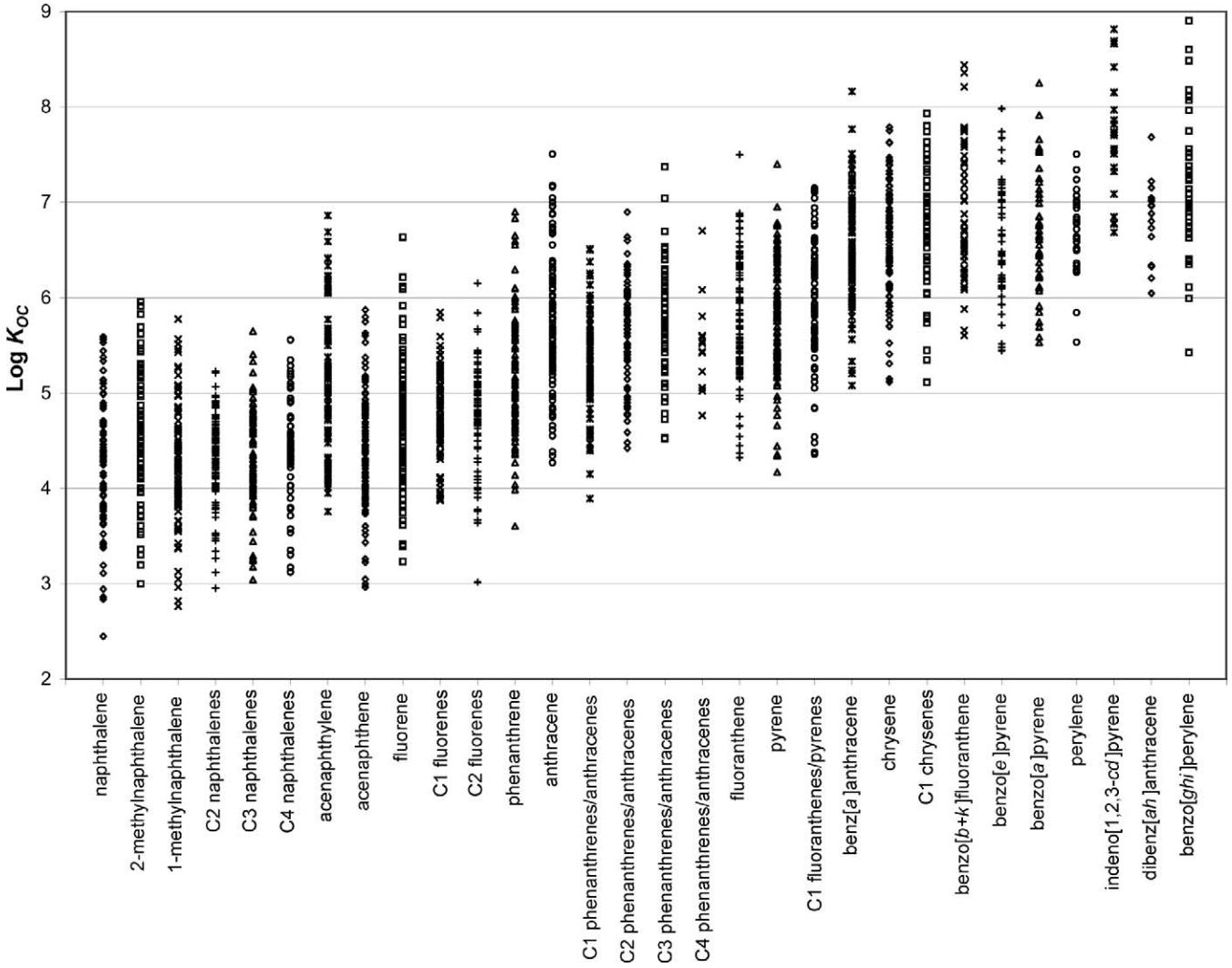


Fig. 1. Log K_{OC} values for parent and alkyl polycyclic aromatic hydrocarbons from 114 sediments.

ment concentrations, the RSDs for the replicate determinations were typically <20%. However, some sediments were quite inhomogeneous and displayed higher RSDs. Approximately 19% of the quadruplicate sediment measurements of individual PAHs had RSDs >40%. For such sediments, an additional set of quadruplicate samples were extracted and analyzed, and the mean of the eight analyses was used for the sediment PAH concentrations.

Table 1. Sediment sample description

Site	Location	Surroundings	Likely PAH[a] sources(s)	Sampling date	Sediment texture, min-max wt%			Total organic carbon, wt%		
					Sand	Silt	Clay	Low	High	Median
A	Freshwater river, eastern New York ([NY], USA)	Rural, light industrial	MGP[b]	2003, 2005	3-93	5-57	2-43	0.4	31.0	4.91
B	Freshwater river and bay, eastern NY	Rural, light industrial	MGP	2004	20-93	3-61	4-19	0.7	6.5	2.8
C	Freshwater creek, central NY	Rural, light industrial	MGP	2004	40-64	25-43	11-18	3.0	11.0	4.9
D	Oligohaline river, eastern NY	Urban, commercial	MGP	2003, 2005	12-77	15-55	8-33	2.5	5.4	3.1
E	Freshwater river, eastern NY	Urban, industrial	MGP	2003	47-94	4-37	2-17	0.6	4.8	3.3
F	Freshwater river, central NY	Urban, industrial	MGP, general industry	2005	5-97	1-63	2-46	0.5	13.8	2.4
G	Freshwater lake, south central North Carolina, USA	Rural, industrial	Aluminum smelter	2005	1-88	10-40	2-64	0.3	42.4	5.5
H	Freshwater river, northeastern NY	Rural, industrial	Aluminum smelter	2005	10-56	20-62	10-29	0.7	3.9	3.2

[a] Polycyclic aromatic hydrocarbons.
[b] Manufactured gas plant.

Table 2. Summary of sediment polycyclic aromatic hydrocarbon concentrations (μg/g)

Sample site		All sites			A		B		C		D		E		F		G		H	
no. of samples		114			33		8		4		14		10		21		12		12	
	n	min	max	median	min	max	min	max	min	max	min	max	min	max	min	max	min	max	min	max
naphthalene	114	0.02	1197	0.8	0.10	1197	0.07	381	0.79	5	0.24	5	0.08	37	0.07	702	0.02	3	0.05	0.8
2-methylnaphthalene	114	0.01	559	0.4	0.07	559	0.06	338	0.18	3	0.14	3	0.04	16	0.03	521	0.01	2	0.03	0.4
1-methylnaphthalene	114	0.003	383	0.3	0.07	348	0.03	196	0.12	2	0.10	16	0.02	9	0.03	383	0.00	1	0.01	0.4
C2 naphthalenes	114	0.08	1105	2.1	0.38	1016	0.72	378	0.53	12	0.67	57	0.28	43	0.19	1105	0.08	10	0.19	3.0
C3 naphthalenes	114	0.05	651	1.9	0.41	411	0.29	148	0.51	13	0.85	33	0.12	24	0.15	651	0.05	9	0.16	5.4
C4 naphthalenes	105	ND	360	1.6	0.35	219	0.22	37	0.38	9	0.93	11	ND	7	0.00	360	0.05	4	ND	8.7
acenaphthylene	113	ND	132	1.1	0.04	106	0.30	25	0.68	10	0.55	12	0.18	79	0.04	132	0.01	0	ND	0.2
acenaphthene	114	0.01	675	0.7	0.07	675	0.03	140	0.27	3	0.32	21	0.03	4	0.05	371	0.01	10	0.03	7.0
fluorene	114	0.01	547	0.7	0.06	547	0.03	19	0.42	7	0.29	8	0.04	64	0.04	255	0.01	8	0.01	8.4
C1 fluorenes	114	0.09	391	2.0	0.20	391	0.21	99	0.75	17	0.72	24	0.15	45	0.13	389	0.09	9	0.14	9.3
C2 fluorenes	105	ND	383	2.2	0.32	226	0.19	30	0.52	13	0.97	14	ND	20	ND	383	0.21	7	ND	9.4
C3 fluorenes	3	ND	28	ND	ND	28	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
phenanthrene	114	0.05	1443	6.5	0.54	1443	0.23	278	5.09	49	3.10	58	0.33	196	0.09	793	0.05	85	0.05	44.9
anthracene	113	ND	1202	4.0	0.20	1202	0.18	98	1.40	13	1.71	32	0.17	223	0.08	532	0.01	34	0.00	83.7
C1 phenanthrenes/anthracenes	114	0.12	1481	10.8	0.70	1481	0.58	283	4.71	98	3.47	100	0.61	240	0.22	1424	0.12	63	0.24	63.9
C2 phenanthrenes/anthracenes	114	0.25	2422	17.6	0.79	1387	1.19	168	6.13	123	4.18	141	0.72	189	0.29	2422	0.42	56	0.25	72.7
C3 phenanthrenes/anthracenes	113	ND	1606	8.3	0.54	755	0.97	35	3.11	53	3.74	32	0.22	47	0.12	1606	0.23	61	ND	39.9
C4 phenanthrenes/anthracenes	95	ND	328	2.4	ND	160	0.75	4	0.96	16	1.15	7	ND	13	ND	328	0.08	67	ND	9.3
fluoranthene	114	0.02	741	13.0	0.43	741	0.67	85	5.74	73	5.95	47	0.20	178	0.10	394	0.03	187	0.02	181.3
pyrene	114	0.02	737	13.0	0.52	737	0.78	153	6.10	69	5.80	68	0.61	170	0.12	513	0.02	169	0.02	140.1
C1 fluoranthenes/pyrenes	114	0.03	1163	17.5	0.29	1012	1.33	187	8.97	92	4.22	135	0.82	331	0.10	1163	0.03	214	0.04	205.0
benz[*a*]anthracene	113	ND	373	7.5	0.15	373	0.44	46	3.11	34	2.54	23	0.35	131	0.05	235	0.00	133	ND	142.6
chrysene	113	ND	396	8.5	0.18	396	0.45	38	3.09	39	3.00	33	0.45	114	0.06	348	0.01	161	ND	307.9
C1 chrysenes	113	ND	788	15.2	0.17	502	1.34	85	7.52	108	3.91	89	0.65	134	0.08	788	0.02	319	ND	158.8
C2 chrysenes	96	ND	555	8.2	ND	260	0.87	35	5.05	85	2.77	30	ND	68	ND	555	ND	212	ND	39.3
C3 chrysenes	53	ND	189	ND	ND	128	ND	0.4	ND	0	ND	5	ND	16	ND	189	ND	178	ND	11.5
C4 chrysenes	3	ND	121	ND	ND	121	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
benzo[*b*+*k*]fluoranthene	114	0.02	418	10.6	0.25	418	1.22	33	5.65	53	5.00	32	0.75	185	0.07	330	0.02	253	0.02	238.7
benzo[*e*]pyrene	114	0.005	151	4.2	0.09	116	0.39	14	1.72	19	1.93	15	0.31	39	0.04	151	0.01	104	0.005	95.5
benzo[*a*]pyrene	114	0.01	339	8.5	0.16	339	0.56	42	3.02	35	4.16	25	0.35	130	0.07	302	0.01	158	0.02	103.5
perylene	114	0.004	72	2.3	0.21	72	0.36	5	1.28	7	1.48	4	0.14	18	0.07	52	0.00	52	0.05	24.1
indeno[1,2,3-*cd*]pyrene	112	ND	314	9.4	0.11	314	0.57	24	2.62	31	2.61	26	0.42	133	ND	267	0.01	289	ND	123.2
dibenz[*a,h*]anthracene	105	ND	89	1.8	0.06	88	0.11	5	0.59	7	0.51	6	0.08	37	ND	89	0.00	65	ND	38.3
benzo[*ghi*]perylene	112	ND	165	4.6	0.09	165	0.39	17	1.70	19	1.41	15	0.21	48	ND	143	0.01	135	ND	63.7
Total "34" PAHs[a]		1.3	17583	232	8	17583	16	3427	107	1041	70	1012	10	2990	2.3	16728	4.1	3060	1.3	2225
Total U.S. EPA "16" PAHs[b]		0.2	8577	92	3.0	8577	6.0	1384	45	429	37	398	4.4	1732	0.8	4821	0.2	1693	0.2	1482
% alkyl vs total PAHs		17	94	49	44	75	49	65	38	61	42	65	34	54	36	70	29	94	17	78
% 2+3-ring vs total PAHs		6	96	42	26	72	29	78	23	47	27	52	30	46	38	80	9	96	6	87

ND=not detected. Detection limits for each PAH and alkyl PAH are given in Hawthorne et al. [17].

[a]Sum of all parent and alkyl PAHs in the table using the PAH species defined by the U.S. Environmental Protection Agency (U.S. EPA) hydrocarbon narcosis model [2].

[b]The sum of the 16 parent PAHs as defined in U.S. EPA method 8270 (http://www.epa.gov/).

Table 3. Summary of pore water polycyclic aromatic hydrocarbon (PAH) concentrations (ng/g)

Sample site	All Sites				A		B		C		D		E		F		G		H	
no. of samples	114				33		8		4		14		10		21		12		12	
	n	min	max	median	min	max	min	max	min	max	min	max	min	max	min	max	min	max	min	max
naphthalene	75	ND	8405	0.32	ND	8405	ND	6712	ND	ND	ND	55	0.21	309	ND	2093	ND	0.10	ND	1.7
2-methylnaphthalene	75	ND	1288	0.04	ND	551	ND	1288	ND	ND	ND	9.1	ND	36	ND	306	ND	0.04	ND	0.2
1-methylnaphthalene	88	ND	1141	0.16	ND	472	ND	1141	ND	0.5	ND	131	0.1045	38	ND	316	ND	0.41	ND	2.2
C2 naphthalenes	89	ND	952	1.41	ND	952	ND	635	ND	4.0	ND	164	ND	60	ND	351	ND	20.5	ND	6.7
C3 naphthalenes	88	ND	851	1.13	ND	851	ND	259	0.24	8.3	ND	79	ND	30	ND	120	ND	28.1	ND	13.2
C4 naphthalenes	62	ND	400	0.52	ND	400	ND	53	ND	4.6	ND	12	ND	5.8	ND	37	ND	12.2	ND	9.0
acenaphthylene	92	ND	41	0.11	ND	41	ND	35	ND	0.3	ND	7.4	0.07	2.0	0.007	16	ND	0.15	ND	0.4
acenaphthene	108	ND	462	0.49	0.06	462	ND	394	0.20	3.6	ND	96	0.06	5.6	0.032	184	ND	0.34	ND	20.2
fluorene	101	ND	143	0.23	ND	143	ND	121	0.19	4.1	ND	18	0.03	58	0.011	49	ND	0.14	ND	14.9
C1 fluorenes	83	ND	69	0.50	ND	69	ND	60	0.09	2.9	ND	11	ND	13	ND	30	ND	1.5	ND	7.4
C2 fluorenes	70	ND	51	0.60	ND	51	ND	20	0.10	2.1	ND	3.2	ND	4.7	ND	30	ND	3.6	ND	3.8
C3 fluorenes	2	ND	40	ND	ND	40	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
phenanthrene	93	ND	155	0.48	ND	155	ND	140	0.48	4.4	ND	54	ND	73	ND	69	ND	0.53	ND	35.1
anthracene	101	ND	35	0.10	ND	35	ND	25	0.05	0.3	ND	6.0	ND	15	0.004	13	ND	0.13	ND	6.9
C1 phenanthrenes/anthracenes	82	ND	72	0.48	ND	72	ND	64	0.16	4.6	ND	23	ND	18	ND	62	ND	2.0	ND	16.3
C2 phenanthrenes/anthracenes	70	ND	45	0.54	ND	37	ND	19	ND	2.4	ND	4.9	ND	6.9	ND	45	ND	3.4	ND	6.7
C3 phenanthrenes/anthracenes	57	ND	32	ND	ND	6.7	ND	2.7	0.03	0.5	ND	0.6	ND	0.9	ND	32	ND	1.8	ND	3.2
C4 phenanthrenes/anthracenes	15	ND	11	ND	ND	11	ND	0.9	ND	0.1	ND	ND	ND	ND	ND	ND	ND	0.64	ND	ND
fluoranthene	107	ND	33	0.38	ND	21	0.0118	12	0.21	2.5	ND	2.6	ND	10.2	ND	13	ND	0.51	ND	32.6
pyrene	107	ND	21	0.36	ND	20	0.0210	17	0.17	2.2	ND	4.0	ND	6.3	ND	19	ND	0.46	ND	20.9
C1 fluoranthenes/pyrenes	86	ND	25	0.25	ND	7.4	0.0233	8.9	0.08	1.1	ND	1.5	ND	2.3	ND	25	ND	0.78	ND	16.8
benz[*a*]anthracene	87	ND	3.1	0.02	ND	2.0	ND	1.1	ND	0.07	ND	0.14	0.004	0.35	ND	3.1	ND	0.04	ND	1.7
chrysene	91	ND	2.9	0.03	ND	1.4	ND	1.1	0.020	0.12	ND	0.15	0.005	0.31	ND	2.9	ND	0.07	ND	2.1
C1 chrysenes	51	ND	5.1	ND	ND	0.73	ND	0.65	ND	0.04	ND	0.04	ND	0.14	ND	5.1	ND	0.03	ND	0.9
C2 chrysenes	1	ND	0.6	ND	ND	0.63	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C3 chrysenes	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C4 chrysenes	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
benzo[*b+k*]fluoranthene	51	ND	2.1	ND	ND	0.96	ND	0.44	0.014	0.04	ND	0.02	ND	0.09	ND	1.6	ND	0.02	ND	2.1
benzo[*e*]pyrene	49	ND	2.0	ND	ND	0.44	ND	0.28	0.005	0.03	ND	0.02	ND	0.06	ND	2.0	ND	0.02	ND	1.0
benzo[*a*]pyrene	47	ND	2.8	ND	ND	1.41	ND	0.58	0.016	0.06	ND	0.03	ND	0.11	ND	2.8	ND	0.01	ND	0.58
perylene	30	ND	0.47	ND	ND	0.47	ND	0.07	ND	0.01	ND	ND	ND	0.03	ND	0.29	ND	ND	ND	0.15
indeno[1,2,3-*cd*]pyrene	34	ND	0.16	ND	ND	0.16	ND	0.11	0.002	0.004	ND	ND	ND	0.01	ND	ND	ND	ND	ND	0.15
dibenz[*a,h*]anthracene	16	ND	0.19	ND	ND	0.19	ND	0.05	ND	ND	ND	ND	ND	0.06	ND	ND	ND	ND	ND	0.11
benzo[*ghi*]perylene	42	ND	0.26	ND	ND	0.26	ND	0.16	0.0036	0.01	ND	0.00	ND	0.01	ND	ND	ND	ND	ND	0.22
Total "34" PAHs		ND	10867	16.0	0.21	10867	0.06	10707	2.1	46	ND	682	0.97	694	0.22	3357	ND	77	0.11	208
Total EPA "16" PAHs		ND	9246	5.1	0.21	9246	0.03	7452	1.4	15	ND	243	0.50	480	0.15	2292	ND	2.3	0.10	127

ND=not detected. Detection limits for each PAH and alkyl PAH are given in Hawthorne et al. [15].

[a]Sum of all parent and alkyl PAHs in the table using the PAH species defined by the U.S. Environmental Protection Agency (U.S. EPA) hydrocarbon narcosis model [2].

[b]The sum of the 16 parent PAHs as defined in the U.S. EPA method 8270 (http://www.epa.gov/).

Table 4. Summary of log sediment organic carbon–water partitioning coefficients (K_{OC}) values. K_{OC} units are (μg/kg carbon)/(μg/L)

	n[a]	Log K_{OC} min	Log K_{OC} max	Log of mean K_{OC} values[b]	Mean of log K_{OC} values[c]	Median of log K_{OC} values	Calculated log K_{OC}[d]
Naphthalene	76	2.45	5.59	4.75	4.26	4.31	3.08
2-Methylnaphthalene	75	3.00	5.96	5.01	4.56	4.55	3.62
1-Methylnaphthalene	88	2.76	5.78	4.71	4.26	4.23	3.63
C2 naphthalenes	89	2.95	5.23	4.53	4.32	4.39	
C3 naphthalenes	89	3.04	5.65	4.61	4.33	4.31	
C4 naphthalenes	61	3.12	5.56	4.69	4.41	4.43	
Acenaphthylene	91	3.76	6.86	5.71	5.11	5.04	3.70
Acenaphthene	109	2.97	5.87	4.82	4.40	4.39	3.68
Fluorene	101	3.23	6.63	5.24	4.71	4.66	3.93
C1 fluorenes	84	3.87	5.85	5.01	4.78	4.82	
C2 fluorenes	69	3.02	6.15	5.07	4.76	4.86	
Phenanthrene	93	3.60	6.90	5.74	5.20	5.10	4.21
Anthracene	100	4.27	7.51	6.29	5.75	5.69	4.20
C1 phenanthrenes/anthracenes	83	3.89	6.51	5.65	5.34	5.30	
C2 phenanthrenes/anthracenes	70	4.42	6.90	5.93	5.60	5.65	
C3 phenanthrenes/anthracenes	58	4.52	7.37	6.20	5.79	5.82	
C4 phenanthrenes/anthracenes	13	4.77	6.70	5.86	5.52	5.52	
Fluoranthene	108	4.32	7.50	6.20	5.79	5.76	4.68
Pyrene	111	4.17	7.40	6.20	5.82	5.81	4.61
C1 fluoranthenes/pyrenes	87	4.36	7.15	6.36	5.96	5.93	
Benz[*a*]anthracene	87	5.08	8.16	6.95	6.55	6.57	5.37
Chrysene	91	5.12	7.79	6.93	6.60	6.64	5.37
C1 chrysenes	51	5.12	7.93	7.11	6.73	6.76	
Benzo[*b*+*k*]fluoranthene	52	5.61	8.44	7.42	6.88	6.70	5.82
Benzo[*e*]pyrene	50	5.45	7.98	7.00	6.59	6.47	
Benzo[*a*]pyrene	49	5.53	8.25	7.13	6.68	6.65	5.67
Perylene	30	5.53	7.50	6.84	6.66	6.63	
Indeno[1,2,3-*cd*]pyrene	34	5.78	8.82	7.96	7.57	7.59	6.26
Dibenz[*a,h*]anthracene	16	6.05	7.68	7.00	6.82	6.92	6.19
Benzo[*ghi*]perylene	43	5.43	8.91	7.76	7.13	7.03	6.26

[a] The number of sediments that had sufficient pore-water concentrations to allow K_{OC} values to be determined.
[b] Log of the average K_{OC} values.
[c] Mean calculated from the log K_{OC} values.
[d] Log K_{OC} values based on the correlation of Xia [19] and suggested literature K_{OW} values from the Syracuse Research Corporation, Syracuse, New York, USA. CHEMFATE data base (http://www.syrres.com/esc/chemfate.htm) accessed March 1, 2006.

RESULTS AND DISCUSSION

Sediment characteristics

The 114 sediments used to determine PAH K_{OC} values represented a large range of pore-water and sediment PAH concentrations as well as TOC and sediment texture (Tables 1 to 3). Sediment textures ranged from gel-like muck to very coarse sand and gravel. Approximately one half of the sediments had numbers of mollusks and shell fragments that were largely removed by sieving with the 2-mm screen. Texture analyses (sand/silt/clay) showed that the sediments ranged from nearly pure sand to nearly pure silt and clay (Table 1). In addition to natural sediment materials such as gravel and mollusk shells, several showed high amounts of brick fragments and other industrial residue.

The TOC values range from 0.3 to 42 weight % carbon (dry basis), although the median TOC values are similar among the eight sites (Table 1). Sediment PAH concentrations vary over a very large range, with total sediment PAH concentrations as low as 0.2 μg/g as the total U.S. EPA 16 parent PAHs (U.S. EPA_{16}, the sum of the 16 parent PAHs normally reported from U.S. EPA method 8270; http://www.epa.gov/) to as high as 8,600 μg/g as total U.S. EPA_{16} (17,600 μg/g as total U.S. EPA_{34}; Table 2). Similarly, total pore-water concentrations ranged from 0.02 ng/ml as total U.S. EPA_{16} to 9,200 ng/ml as total U.S. EPA_{16} (corresponding to 10,900 ng/ml as total U.S. EPA_{34}). Pore-water concentrations were not sufficient for a few of the alkyl PAHs (C3-fluorenes and the C2- to C4-chrysenes) to allow quantitation in any of the pore-water samples. It is important to note that none of the individual PAH concentrations determined in pore-water samples exceeded their saturation water solubility since a flocculation step was used prior to their determination to remove colloidal material from the water samples as previously described [15].

PAH partitioning coefficients

Sediment–water partitioning coefficients (K_D) determined for the parent and alkyl PAHs showed nearly four orders of magnitude variation for each individual PAH, although a large range in K_D values might be expected on the basis of the range in sediment characteristics, especially TOC (Table 1). However, the common practice of describing partitioning behavior using K_{OC} rather than K_D does little to reduce the range in partitioning coefficients. As shown in Figure 1 for the 2,158 values measured in this study, the K_{OC} values showed a high degree of variation, up to three orders of magnitude, for nearly all the individual PAHs. Also note that, although there are only a few extreme K_{OC} values (e.g., the lowest value for naphthalene in Fig. 1), the K_{OC} values for each PAH are fairly evenly

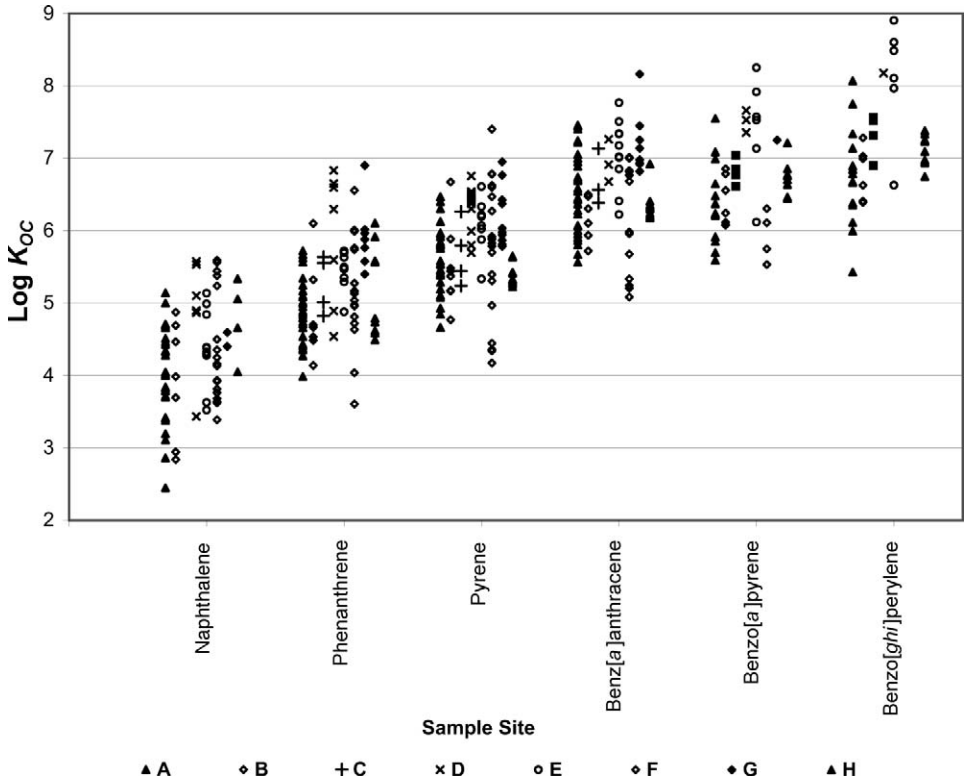


Fig. 2. Log sediment organic carbon–water partitioning coefficients (K_{OC}) values for representative polycyclic aromatic hydrocarbons from each site. The characteristics of sample sites A to H are listed in Table 1.

distributed over two to three orders of magnitude. These results clearly show that the use of a single K_{OC} value to predict the behavior of a particular PAH is not sufficiently accurate for the range of sediments and PAH partitioning behaviors that actually exist in field sediment sites, likely as a result of a range of carbon types having different sorption characteristics that exist at such sites [5,18].

The range, mean, and median K_{OC} values are summarized in Table 4, along with those from modeled values based on available literature K_{OC} values and K_{OW} values [19]. Note that few measured K_{OC} values from historically contaminated sediments are actually available for use in these models (especially for higher-molecular-weight and alkyl PAHs), and the majority of the K_{OC} values used for the model correlations have been determined in spiked (rather than historically contaminated) sediments. In general, the median K_{OC} value determined in our sediments was an order of magnitude higher than the literature K_{OC} values. Although the lowest K_{OC} values we measured were somewhat lower than the literature K_{OC} values, the majority of our measured K_{OC} values were significantly higher than the measured literature values or the predicted K_{OC} values (Table 4). It should also be noted that other common models used to estimate log K_{OC} values, including those proposed by Karickhoff [20] and the U.S. EPA's SPARC model [2], give log K_{OC} values similar to those listed in Table 4 based on the model of Xia [19] and thus are generally much lower than the measured sediment K_{OC} values.

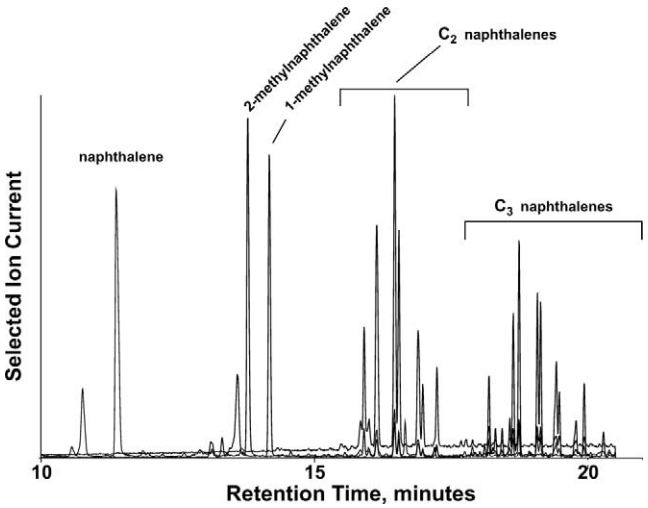


Fig. 3. Selected ion current chromatograms of naphthalene and alkyl naphthalenes from sediment pore water.

The high values of K_{OC} we obtained for many sediments is supported by an increasing number of reports that have been recently reviewed [5], although only a limited number of PAHs and sediments have been studied. In one of the more extensive studies, Jonker and Smedes [21] reported K_{OC} values for three- to six-ring PAHs that are nearly identical to our highest K_{OC} values shown in Table 4. For example, the highest log K_{OC} values they reported for phenanthrene, benz[*a*]anthracene, and benzo[*ghi*]perylene were 6.7, 8.0, and 9.3, respectively, which compare closely to the highest log K_{OC} values we measured for the same PAHs of 6.9, 8.2, and 8.9.

The dependence of K_{OC} values on the site location appeared to be significant for some sites; however, it did not appear to be related to the likely source of PAHs as shown for representative PAHs in Figure 2. For example, the aluminum smelter site G consistently had high K_{OC} values, while the other aluminum smelter site (H) had among the lowest K_{OC} values.

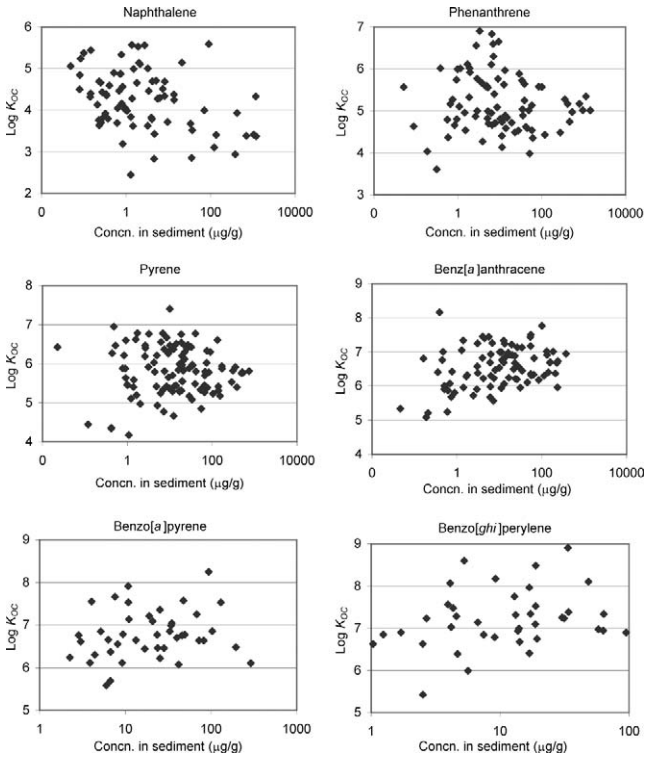


Fig. 4. Effect of polycyclic aromatic hydrocarbon (PAH) sediment concentrations on sediment organic carbon–water partitioning coefficients (K_{OC}) values for representative two- to six-ring PAHs.

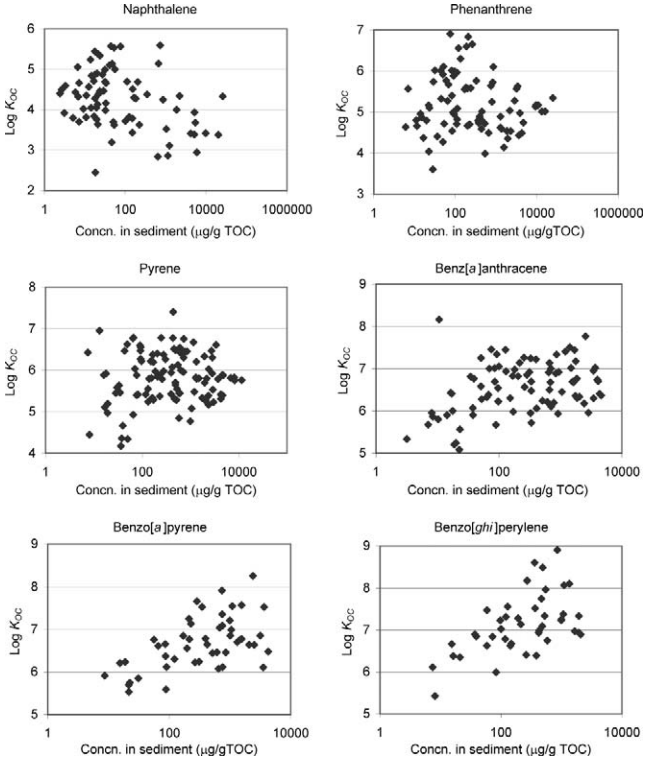


Fig. 5. Effect of polycyclic aromatic hydrocarbon (PAH) sediment concentration per gram of organic carbon on sediment organic carbon–water partitioning coefficients (K_{OC}) values for representative two- to six-ring PAHs. TOC = total organic carbon.

Table 5. Log sediment organic carbon–water partitioning coefficients (K_{OC}) values for sediments with and without nonaqueous-phase liquids (NAPL) phases

	No NAPL or sheen present				NAPL or sheen present			
	Mean log K_{OC}	SD[a]	Median log K_{OC}	*n*	Mean log K_{OC}	SD	Median log K_{OC}	*n*
Naphthalene	4.40	0.62	4.41	50	4.03	0.85	3.93	27
2-Methylnaphthalene	4.66	0.57	4.63	45	4.45	0.75	4.41	31
1-Methylnaphthalene	4.36	0.58	4.30	57	4.13	0.73	4.10	32
C2 naphthalenes	4.41	0.41	4.50	60	4.17	0.56	4.17	30
C3 naphthalenes	4.41	0.48	4.36	59	4.17	0.57	4.12	31
C4 naphthalenes	4.46	0.43	4.43	35	4.35	0.65	4.43	26
Acenaphthylene	5.27	0.72	5.18	60	4.83	0.67	4.73	32
Acenaphthene	4.47	0.57	4.41	77	4.27	0.64	4.38	33
Fluorene	4.77	0.54	4.72	69	4.60	0.79	4.60	33
C1 fluorenes	4.79	0.44	4.84	55	4.79	0.51	4.78	30
C2 fluorenes	4.77	0.55	4.84	42	4.75	0.61	4.86	27
Phenanthrene	5.28	0.58	5.16	61	5.07	0.77	5.02	33
Anthracene	5.87	0.65	5.79	68	5.49	0.69	5.57	33
C1 phenanthrenes/anthracenes	5.44	0.51	5.39	55	5.16	0.54	5.19	29
C2 phenanthrenes/anthracenes	5.61	0.54	5.61	44	5.58	0.61	5.73	26
C3 phenanthrenes/anthracenes	5.79	0.60	5.86	32	5.80	0.61	5.77	26
C4 phenanthrenes/anthracenes	5.63	0.83	5.52	4	5.48	0.31	5.52	9
Fluoranthene	5.87	0.61	5.76	75	5.62	0.59	5.76	34
Pyrene	5.91	0.60	5.88	78	5.61	0.59	5.77	34
C1 fluoranthenes/pyrenes	6.03	0.64	5.93	57	5.83	0.63	6.01	31
Benz[*a*]anthracene	6.60	0.59	6.57	58	6.47	0.62	6.59	30
Chrysene	6.67	0.57	6.63	61	6.47	0.59	6.66	31
C1 chrysenes	6.69	0.62	6.64	31	6.80	0.69	6.91	20
Benzo[*b*+*k*]fluoranthene	6.89	0.72	6.70	33	6.85	0.62	6.70	19
Benzo[*e*]pyrene	6.59	0.68	6.44	31	6.59	0.55	6.59	19
Benzo[*a*]pyrene	6.76	0.65	6.68	34	6.49	0.49	6.48	15
Perylene	6.74	0.43	6.75	18	6.55	0.41	6.44	12
Indeno[1,2,3-*cd*]pyrene	7.57	0.65	7.62	21	7.57	0.59	7.54	13
Dibenz[*a*,*h*]anthracene	6.82	0.48	6.80	9	6.81	0.38	6.97	7
Benzo[*ghi*]perylene	7.19	0.77	7.06	30	7.01	0.51	6.99	13

[a] Standard deviation.

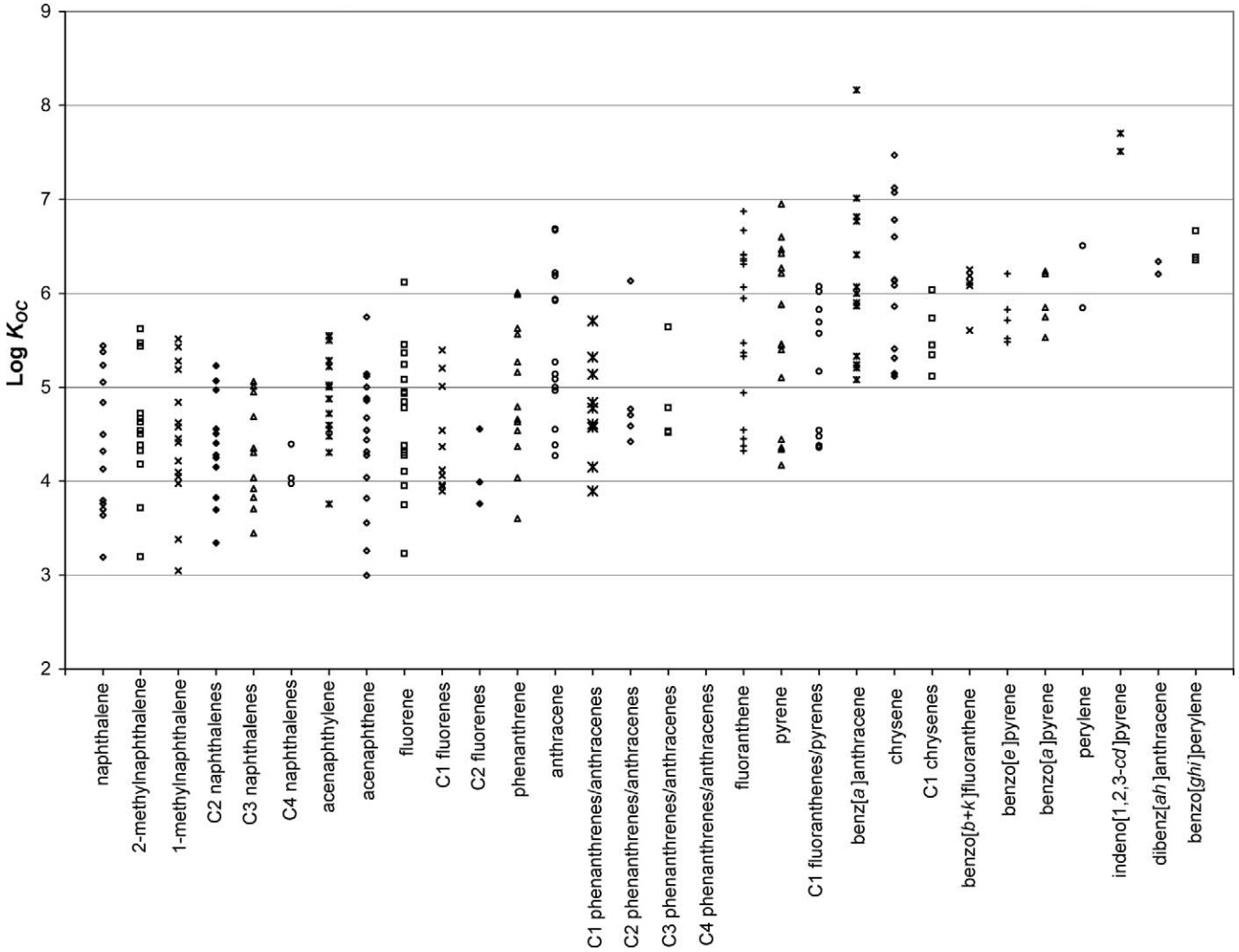


Fig. 6. Log sediment organic carbon–water partitioning coefficients (K_{OC}) values for background sediments (U.S. $EPA_{16} < 10$ mg/kg). U.S. EPA_{16} refers to the sum of the 16 parent polycyclic aromatic hydrocarbons as defined in the U.S. Environmental Protection Agency method 8270 (http://www.epa.gov/).

Similarly, the MGP site D had consistently high K_{OC} values, while the remaining MGP sites had K_{OC} values fairly evenly distributed across the range shown by the other sites.

K_{OC} *values for alkyl PAHs*

With the exception of 1- and 2-methylnaphthalene, each alkyl PAH included in the EPA_{34} list actually consists of several isomers that are reported as one compound. For example, the selected ion chromatograms in Figure 3 show the naphthalene and alkyl naphthalene isomers from a pore-water sample. Note that the so-called C2-naphthalene PAH actually consists of seven major (and several minor) different dimethyl- or ethyl-naphthalene compounds. Similarly, the C3-naphthalene PAH includes 12 major isomers and several minor isomers. All the other alkyl PAHs on the U.S. EPA_{34} list includes a similarly complex (or more complex) mixture of isomers. Thus, the alkyl PAHs on the U.S. EPA_{34} list actually summarize the concentrations of several hundred individual alkyl PAH isomers into only 16 groups of alkyl isomers.

Unfortunately, there are very few standard compounds available for alkyl PAHs that allow quantitative calibration of individual alkyl PAH isomers. Therefore, the concentrations of the alkyl PAHs are reported for each isomeric group based on calibration with the few alkyl PAH standards available [2, 15,17]. As a consequence, the log K_{OC} values for alkyl PAHs shown in Figure 1 and Table 4 represent the average partitioning behavior of all the isomers present in each particular isomeric group. For example, log K_{OC} values reported for the C2-alkylnaphthalenes shown in Figure 3 represent the average partitioning behavior for the seven major isomers that make up the C2-alkylnaphthalene group.

Effect of NAPL phase on K_{OC} *values*

Sediment samples collected from urban and industrial sites frequently have hydrocarbon phases (sheen or nonaqueous phase liquids [NAPLs]) separate from the bulk sediment that could affect PAH partitioning. Any realistic approach to the regulation and mitigation of real-world sites must be able to incorporate such sediments into predictive modeling efforts. In the present study, 34 out of 114 sediments had observable NAPL and/or sheen (based on field observations during sample collection and homogenation). Since the presence of a separate hydrocarbon phase could influence the observed K_{OC} values, the K_{OC} values from sediments containing NAPL were compared to those that contained no observable NAPL phase. Surprisingly, removal of the NAPL-containing sediments did not

reduce the variation in K_{OC} values seen in the other sediments, as shown in Table 5. Regardless of the presence or absence of a NAPL phase, there were no significant differences in the range and median log K_{OC} values for all the PAHs (Table 5), and all the 34 PAH K_{OC} values agree with those in Table 4 that represent all 114 sediments.

Effect of PAH sediment concentrations on measured K_{OC} *values*

Nonlinearity in PAH sorption is normally assumed as PAH concentrations increase and has led to the use of Freundlich parameters to adjust K_{OC} values as sorption sites become increasingly occupied [10,22,23]. When single sediments are spiked at increasing concentrations, the K_{OC} values tend to drop as much as an order of magnitude [10]. However, PAH concentrations do not appear to have any significant influence on K_{OC} values when measured for multiple sediment samples, as shown for representative PAHs in Figure 4. For example, the sediment concentration of pyrene varies from 0.02 to 740 μg/g, yet no trend in K_{OC} values with concentration exists (Fig. 4). Similarly, the sediment concentrations of the other PAHs shown in Figure 4 also have ranges in concentration of four to five orders of magnitude, but no apparent effect of concentration on log K_{OC} values can be observed. When the concentrations are plotted per gram TOC (rather than per gram dry sediment), once again no significant relationship of K_{OC} to PAH concentration is observed (Fig. 5).

A comparison of the K_{OC} values determined for the background sediments and the impacted sediments also showed no observable differences. Figure 6 shows the log K_{OC} values of representative two- to six-ring PAHs determined for sediments having total (U.S. EPA_{16}) PAH concentrations of <10 μg/g (corresponding to ~ 30 μg/g of the total U.S. EPA_{34} PAHs). Although many fewer K_{OC} values could be determined in the background sediments (especially for the higher-molecular-weight PAHs), a comparison of Figure 6 with the K_{OC} data for the entire sample set (Fig. 1) shows no major shift in the K_{OC} values for the background versus impacted sediments.

CONCLUSION

The K_{OC} values determined for parent and alkyl PAHs from 114 background and contaminated sediments demonstrate that a better understanding of PAH partitioning behavior with different types of sediment carbon is needed to support the use of equilibrium partitioning models for their application to real-world sediments. It is important to note that most prior partitioning studies utilized a much narrower range of PAH concentrations and of sediment types than those reported here. However, all the sediments used in the present study are from sites that are under regulatory scrutiny and therefore are representative of the types of sites that are of highest concern to regulatory agencies and industry. Therefore, despite the difficulty in working with such a wide range of sediment characteristics and PAH concentrations, it is important that partitioning data that are used for regulatory models be relevant to sites where those models are applied to determine mitigation responses.

Acknowledgement—The financial support of the Gas Research Institute, National Grid, New York State Electric and Gas, Niagara Mohawk Power, Northeast Gas Association, and the U.S. Department of Energy under Cooperative Agreement DE-FC26-98FT40321 is gratefully acknowledged. However, any opinions, findings, conclusions, or recommendations expressed herein are those of the authors and do not necessarily reflect the views of the sponsors. The authors also appreciate helpful discussions with Joe Kreitinger and Ed Neuhauser and the assistance of Nick Azzolina and Thea Rielkoff in reducing the data.

REFERENCES

1. U.S. Environmental Protection Agency. 2004. Methods for the derivation of site-specific equilibrium partitioning sediment guidelines (ESGs) for the protection of benthic organisms: Nonionic organics. EPA/822/R/02/042. Office of Science and Technology, Washington, DC.
2. U.S. Environmental Protection Agency. 2003. Procedures for the derivation of ESBs for the protection of benthic organisms: PAH mixtures. EPA/600/R-02/013. Office of Research and Development, Washington, DC.
3. Di Toro DM, McGrath JA, Hansen DJ. 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. 1. Water and tissue. *Environ Toxicol Chem* 19:1951–1970.
4. Di Toro DM, McGrath JA. 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. II. Mixtures and sediments. *Environ Toxicol Chem* 19:1971–1982.
5. Cornelissen G, Gustafsson O, Bucheli TD, Jonker MTO, Van Noort PCM. 2005. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: Mechanisms and consequences for distribution, bioaccumulation, and biodegradation. *Environ Sci Technol* 39:6881–6895.
6. Jonker MTO, Sinke AJC, Brils JM, Koelmans AA. 2003. Sorption of polycyclic aromatic hydrocarbons to oil contaminated sediment: Unresolved complex? *Environ Sci Technol* 37:5197–5203.
7. Nguyen TH, Sabbah I, Ball WP. 2004. Sorption nonlinearity for organic contaminants with diesel soot: Method development and isotherm interpretation. *Environ Sci Technol* 38:3595–3603.
8. Cornelissen G, Kukulska Z, Kalaitzidis S, Christanis K, Gustafsson O. 2004. Relations between environmental black carbon sorption and geochemical sorbent characteristics. *Environ Sci Technol* 38:3632–3640.
9. Cornelissen G, Haftka J, Parsons J, Gustafsson O. 2005. Sorption to black carbon of organic compounds with varying polarity and planarity. *Environ Sci Technol* 39:3688–3694.
10. Nguyen TH, Sabbah I, Ball WP. 2004. Sorption nonlinearity for organic contaminants with diesel soot: Method development and isotherm interpretation. *Environ Sci Technol* 38:3595–3603.
11. Reeves WR, McDonald TJ, Cizmas L, Donnelly KC. 2004. Partitioning and desorption behavior of polycyclic aromatic hydrocarbons from disparate sources. *Sci Total Environ* 332:183–192.
12. McLeod PB, Van Den Heuvel-Greve MJ, Allen-King RM, Luoma SN, Luthy RG. 2004. Effects of particulate carbonaceous matter on the bioavailability of benzo[*a*]pyrene and 2,2′,5,5′-tetrachlorobiphenyl to the clam, *Macoma balthica*. *Environ Sci Technol* 38:4549–4556.
13. Hong L, Ghosh U, Mahajan T, Zare RN, Luthy RG. 2003. PAH sorption mechanism and partitioning behavior in lampblack-impacted soils from former oil-gas plant sites. *Environ Sci Technol* 37:3625–3634.
14. Ahn S, Werner D, Karapanagioti HK, McGlothlin DR, Zare RN, Luthy RG. 2005. Phenanthrene and pyrene sorption and intraparticle diffusion in polyoxymethylene, coke, and activated carbon. *Environ Sci Technol* 39:6516–6526.
15. Hawthorne SB, Grabanski CB, Miller DJ, Kreitinger JP. 2005. Solid-phase microextraction measurement of parent and alkyl polycyclic aromatic hydrocarbons in milliliter sediment pore water samples and determination of K_{DOC} values. *Environ Sci Technol* 39:2795–2803.
16. U.S. Environmental Protection Agency. 2001. Methods for collection, storage and manipulation of sediments for chemical and toxicological analyses: Technical manual. EPA/823/B/01/002. Office of Science and Technology, Washington, DC.
17. Hawthorne SB, Miller DJ, Kreitinger JP. 2006. Measurement of total polycyclic aromatic hydrocarbon concentrations in sediments and toxic units used for estimating risk to benthic invertebrates at manufactured gas plant sites. *Environ Toxicol Chem* 25:287–295.
18. Nguyen TH, Goss K-U, Ball WP. 2005. Polyparameter linear free

energy relationships for estimating the equilibrium partition of organic compounds between water and the natural organic matter in soils and sediments. *Environ Sci Technol* 39:913–924.
19. Xia G. 1998. Sorption behavior of nonpolar organic chemicals on natural sorbents. PhD thesis. Department of Geography and Environmental Engineering, Johns Hopkins University, Baltimore, MD, USA.
20. Karickhoff SW, Brown DS, Scott TA. 1979. Sorption of hydrophobic pollutants on natural sediments. *Water Res* 13:241–248.
21. Jonker MTO, Smedes F. 2000. Preferential sorption of planar contaminants in sediments from Lake Ketelmeer, The Netherlands. *Environ Sci Technol* 34:1620–1626.
22. Wang X, Sato T, Xing B. 2005. Sorption and displacement of pyrene in soils and sediments. *Environ Sci Technol* 39:8712–8718.
23. Kim HS, Pfaender FK. 2005. Effects of microbially mediated redox conditions on PAH-soil interactions. *Environ Sci Technol* 39:9189–9196.

APPENDIX N

MEASURED PARTITION COEFFICIENTS FOR PARENT AND ALKYL POLYCYCLIC AROMATIC HYDROCARBONS IN HISTORICALLY CONTAMINATED SEDIMENTS: PART 2. TESTING THE $K_{OC}K_{BC}$ TWO CARBON–TYPE MODEL

PUBLISHED IN *ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY*

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 26, No. 12, pp. 2505–2516, 2007
© 2007 SETAC
Printed in the USA
0730-7268/07 $12.00 + .00

Environmental Chemistry

MEASURED PARTITION COEFFICIENTS FOR PARENT AND ALKYL POLYCYCLIC AROMATIC HYDROCARBONS IN 114 HISTORICALLY CONTAMINATED SEDIMENTS: PART 2. TESTING THE $K_{OC}K_{BC}$ TWO CARBON–TYPE MODEL

STEVEN B. HAWTHORNE,* CAROL B. GRABANSKI, and DAVID J. MILLER
Energy & Environmental Research Center, University of North Dakota, Box 9018, Grand Forks, North Dakota 58202, USA

(*Received* 3 *February* 2007; *Accepted* 14 *June* 2007)

Abstract—Polycyclic aromatic hydrocarbon (PAH) desorption partition coefficients between black carbon and water (K_{BC}) were determined using 114 historically contaminated and background sediments from eight different rural and urban waterways. Black carbon was measured after oxidation at 375°C for 24 h. Organic carbon–water partition coefficients (K_{OC}) required for the calculation of K_{BC} values were determined for two- to six-ring parent and C1- to C4-alkyl PAHs based on the lower range of measured K_{OC} values from the same sediments and comparisons to literature K_{OC} values. Approximately 2,050 log K_{BC} values were determined on sediments having a range of total organic carbon from 0.3 to 42% by weight, black carbon from 0.1 to 40% by weight, and total PAH concentrations (U.S. Environmental Protection Agency 16 parent PAHs) from 0.2 to 8,600 μg/g. Contrary to expectations, PAH partitioning was not better explained using the combined K_{OC} and K_{BC} models rather than the simple K_{OC} model (i.e., K_{BC} values for each individual PAH ranged nearly three orders of magnitude). No effect of PAH concentration on measured K_{BC} values was apparent. Values of K_{BC} also showed no trends with total organic carbon, black carbon, or the presence or absence of a non–aqueous phase liquid. Multiple linear regression analysis with K_{OC} and K_{BC} as fitted values also failed to explain the variance of the experimental data (r^2 values typically less than 0.20, and standard errors greater than two orders of magnitude). These results demonstrate that models of PAH partitioning that account for different carbon types, although useful for understanding partitioning mechanisms, cannot yet be used to accurately predict PAH partitioning from historically contaminated sediments.

Keywords—Manufactured gas plant Polycyclic aromatic hydrocarbons Sediment Black carbon–water partition coefficients Organic carbon–water partition coefficients

INTRODUCTION

It has become increasingly obvious that conventional organic carbon–water partition coefficients (K_{OC}) are not sufficient for predicting polycyclic aromatic hydrocarbon (PAH) partitioning behavior in sediments and that the use of conventional literature K_{OC} values often overpredicts water PAH concentrations [1–7]. In an effort to increase the ability of equilibrium partitioning models to predict PAH water concentrations based on measured sediment concentrations, recent studies have included a second carbon phase for soot or black carbon (BC) in addition to the conventional natural organic carbon (OC) phase [7–18]. Thus, the measured partition coefficient (K_D) is explained by the combined effects of natural and soot carbon with the following equation: $K_D = (f_{OC}K_{OC}) + (f_{BC}K_{BC})$, where f_{OC} and f_{BC} are the fraction of natural OC and BC, respectively, and K_{OC} and K_{BC} are the water partition coefficients for natural OC and BC, respectively [14].

Improved descriptions of PAH partitioning have been reported using this approach. Only a small number of measured K_{BC} values have been reported in the literature, however, and the majority of those are with spiked rather than with historically contaminated sediments. In addition, K_{BC} values reported by different investigators for the same PAH vary significantly, possibly because of procedural differences in determining BC among different laboratories as well as the difficulties in measuring relevant PAH concentrations in water, especially for the less soluble higher-molecular-weight PAHs. Without consistency in K_{BC} values, it is difficult to determine whether combined K_{OC} and K_{BC} partitioning models are useful for improving the prediction of PAH behavior under environmental conditions.

Traditionally, K_{OC} values reported in the literature have been based on total organic carbon (TOC) and more properly could be called K_{TOC} values. For sediments that contain significant BC, K_{TOC} describes the combined partitioning behavior with both natural OC and BC. For the purposes of the present paper, we will use the term K_{TOC} to describe the partitioning based on TOC, K_{OC} to describe partitioning with natural OC, and K_{BC} to describe partitioning with BC.

In Part 1 of this series [2], we measured more than 2,000 K_{TOC} values in 114 historically contaminated and background sediments for all the two- to six-ring PAHs and alkyl PAHs included in the U.S. Environmental Protection Agency (EPA) PAH hydrocarbon narcosis model [19]. The measured values in Part 1 [2] showed that the use of commonly accepted literature K_{OC} values often greatly overpredicts water PAH concentrations and that commonly used K_{OC} values are among the lowest that actually occur in field sediments. Subsequent toxicity studies with *Hyalella azteca* also demonstrated that the bioavailability and resultant toxicity was greatly overpredicted using conventional K_{OC} values for the majority of sediments tested [18,20].

The purpose of the present study was to test the combined $K_{OC}K_{BC}$ partitioning model for the same 114 sediments and for the entire range of parent and alkyl PAHs included in the U.S. EPA hydrocarbon narcosis model for PAH mixtures [19]. The

* To whom correspondence may be addressed (shawthorne@undeerc.org).

Any opinions, findings, conclusions, or recommendations expressed herein are those of the authors and do not necessarily reflect the views of the sponsors.

Published on the Web 7/23/2007.

Table 1. Summary of sediment total organic carbon (TOC) and black carbon (BC) from manufactured gas plant (MGP) and aluminum smelter sites

	TOC (% dry wt)			BC (% dry wt)			Fraction of BC vs TOC (%)			
Site	Low	High	Median	Low	High	Median	Low	High	Median	*n*
MGP	0.37	31.0	4.86	0.25	20.62	2.52	10	87	47	33
MGP	0.73	6.5	2.83	0.23	1.07	0.50	5	65	27	8
MGP	3.05	11.0	4.86	0.43	5.0	2.17	14	48	43	4
MGP	2.46	5.4	3.13	0.38	3.7	1.13	15	71	37	14
MGP	0.55	4.8	3.32	0.22	4.1	1.13	20	84	42	10
MGP, general industry	0.47	13.8	2.39	0.11	7.1	0.49	8	71	22	21
Aluminum smelter	0.30	42.4	5.49	0.15	39.7	4.26	9	100	75	12
Aluminum smelter	0.73	3.9	3.22	0.09	1.2	0.74	13	38	23	12
									Total	114

hope is that a set of suggested K_{BC} values can be determined that can be used in conjunction with conventional K_{OC} values to predict more accurately the partitioning behavior for all of the 18 parent and 16 groups of alkyl PAHs (EPA_{34}) included in the U.S. EPA narcosis model for benthic organisms. As currently applied by several investigators, the combined $K_{OC}K_{BC}$ model is based on the assumption that the K_{OC} value for each PAH is constant for all sediments; that is, accepted literature K_{OC} values for each are used to calculate K_{BC} values based on measured partitioning data [8,11,13–16]. Because accepted measured K_{OC} values required to calculate K_{BC} values are not available for many of the higher-molecular-weight and alkyl PAHs, we suggest K_{OC} values for these species based on the available literature values and from those we measured in Part 1 [2].

Finally, multiple linear regression analysis was performed on the same data set with K_{OC} and K_{BC} as fitted values. This statistical approach tested the ability of the $K_{OC}K_{BC}$ model to fit the experimental partitioning data without requiring the assumption that K_{OC} values for each PAH were constant; in other words, both K_{OC} and K_{BC} were varied in an attempt to best fit the model to the experimental data.

MATERIALS AND METHODS

Sediment samples

The sediment samples used in the present study were described in more detail in Part 1 [2]. Briefly, sediments were collected during the years 2003, 2004, and 2005 at six different sites thought to be impacted by former manufactured gas plant activities and at two sites thought to be impacted by aluminum smelters. All sediments were homogenized in the field, shipped overnight on ice, and stored at approximately 4°C. All sediment and pore-water PAH concentrations were determined in quadruplicate within 28 d of sample collection as described previously [2,21,22].

Total organic and black carbon

Total organic carbon was determined on air-dried sediment (80°C for 2 h) after grinding to a fine powder and acidification with 1 M HCl (2 ml for 1 g of sediment) to remove inorganic carbonates. After acidification, each sediment was observed for bubbles for several minutes. If bubbling occurred, the acidification was repeated to ensure complete removal of carbonates. Black (or soot) carbon was determined using the 375°C oxidation method followed by acidification as described above [16]. A subset of the sample that had been prepared for TOC analysis was oxidized at 375°C in air for 24 h, then acidified to remove any formed carbonates. A gas chromatographic oven (Model 5890 Series 2; Hewlett-Packard, Avondale, PA, USA) was used for the oxidation step to ensure precise temperature control. Elemental analysis before and after oxidation was performed with a Model CE440 elemental analyzer (Leeman Labs, Hudson, NH, USA). All TOC and BC values were determined in triplicate or quadruplicate for each sediment. Natural OC was defined as the difference between TOC and BC.

Statistical data analysis

Multiple linear regression analysis was performed using MINITAB® Release 14 statistical software (Minitab, State College, PA, USA). The response variable was K_D, and the two predictor variables were f_{OC} and f_{BC}. The fitted values (regression coefficients) were K_{OC} and K_{BC}. The ability of the two carbon–type model to fit the experimental data was evaluated using both linear and log-transformed data. The regression equation was $K_D = (f_{OC}K_{OC}) + (f_{BC}K_{BC})$.

RESULTS AND DISCUSSION

Sediment characteristics

The 114 test sediments included both background and highly impacted samples with a broad range of textures and TOC and BC values as summarized in Tables 1 and 2 (more detailed data regarding PAH concentrations and texture, along with details of the sediment collection and preparation, are given in Part I [2]). Sediment textures ranged from gel-like muck to very coarse sand and gravel. Texture analyses (sand/silt/clay) showed that the sediments ranged from nearly pure sand to nearly pure silt and clay. In addition to natural sediment materials, such as gravel and mollusk shells, several sediments showed high amounts of brick fragments and other industrial residue. Total organic carbon values ranged from 0.3 to 42% dry weight. Carbon types ranged from 95% natural OC to 100% BC, and sediment BC values ranged from 0.09 to 40% dry weight (Table 1).

Sediment PAH concentrations vary over a very large range, with total sediment PAH concentrations from as low as 0.2 μg/g as the total U.S. EPA 16 parent PAHs normally reported from U.S. EPA Method 8270 to as high as 8,600 μg/g as total U.S. EPA 16 parent PAHs (EPA_{16}; 17,600 μg/g of total EPA_{34}) (Table 2). Similarly, total pore-water concentrations ranged from 0.02 ng/ml as total EPA_{16} to 9,200 ng/ml as total EPA_{16} (corresponding to 10,900 ng/ml of total EPA_{34}). Pore-water concentrations were not sufficient for a few of the alkyl PAHs (C3-fluorenes and the C2- to C4-chrysenes) to allow quantitation in any of the pore-water samples. It is important to note that none of the individual PAH concentrations determined in pore-water samples exceeded their saturation water solubility,

Table 2. Summary of pore-water and sediment polycyclic aromatic hydrocarbon (PAH) concentrations

	Pore-water concn. (ng/ml)				Sediment concn. (μg/g dry-wt basis)			
	n[a]	Min[b]	Max	Median[c]	n[a]	Min[b]	Max	Median[c]
Naphthalene	73	0.06	8,405	0.32	112	0.02	1,197	0.8
2-Methylnaphthalene	74	0.01	1,288	0.04	112	0.01	559	0.4
1-Methylnaphthalene	87	0.01	1,141	0.16	112	0.003	383	0.3
C2-naphthalenes	85	0.30	952	1.41	112	0.08	1,105	2.1
C3-naphthalenes	89	0.10	851	1.13	112	0.05	651	1.9
C4-naphthalenes	60	0.20	400	0.52	103	0.05	360	1.6
Acenaphthylene	90	0.001	41	0.11	111	0.01	132	1.1
Acenaphthene	107	0.01	462	0.49	112	0.01	675	0.7
Fluorene	99	0.001	143	0.23	112	0.01	547	0.7
C1-fluorenes	84	0.04	69	0.50	112	0.09	391	2.0
C2-fluorenes	68	0.09	51	0.60	103	0.04	383	2.2
Phenanthrene	92	0.009	155	0.48	112	0.05	1,443	6.5
Anthracene	98	0.001	35	0.10	111	0.01	1,202	4.0
C1-phenanthrenes/anthracenes	82	0.03	72	0.48	112	0.12	1,481	10.8
C2-phenanthrenes/anthracenes	69	0.10	45	0.54	112	0.25	2,422	17.6
Fluoranthene	106	0.002	33	0.38	112	0.02	741	13.0
Pyrene	108	0.001	21	0.36	112	0.02	737	13.0
C1-fluoranthenes/pyrenes	85	0.02	25	0.25	112	0.03	1,163	17.5
Benz[*a*]anthracene	85	0.0001	3.1	0.02	111	0.00	373	7.5
Chrysene	89	0.0005	2.9	0.03	111	0.01	396	8.5
C1-chrysenes	49	0.02	5.1	ND	111	0.02	788	15.2
Benzo[*b*+*k*]fluoranthene	52	0.006	2.1	ND	112	0.02	418	10.6
Benzo[*e*]pyrene	50	0.003	2.0	ND	112	0.005	151	4.2
Benzo[*a*]pyrene	48	0.01	2.8	ND	112	0.01	339	8.5
Perylene	30	0.004	0.47	ND	112	0.004	72	2.3
Indeno[1,2,3-*cd*]pyrene	33	0.001	0.16	ND	110	0.01	314	9.4
Dibenz[*a*,*h*]anthracene	16	0.003	0.19	ND	103	0.00	89	1.8
Benzo[*ghi*]perylene	42	0.01	0.26	ND	110	0.01	165	4.6
Total 34 PAHs[d]		ND	10,867	16.0		1.3	17,583	232
Total U.S. EPA 16 PAHs[e]		ND	9,246	5.1		0.2	8,577	92

[a] n = number of detected concentrations out of the 114 sediment samples.
[b] The lowest concentration detected is reported. Detection limits are given in Hawthorne et al. [21,22].
[c] Median concentrations were determined including the nondetect (ND) values.
[d] Sum of all parent and alkyl PAHs in the table using the PAH species defined by the U.S. Environmental Protection Agency (EPA) hydrocarbon narcosis model.
[e] The sum of the 16 parent PAHs as defined in U.S. EPA Method 8270.

because a flocculation step was used before their determination to remove colloidal material from the water samples as previously described [21].

Selection of log K_{OC} *values*

Values of K_{BC} are calculated based on the equation

$$K_D = f_{OC}K_{OC} + f_{BC}K_{BC}C_W^{n-1}$$

where C_W is the water PAH concentration and n is the Freundlich isotherm exponent (typically reported for PAHs as ~0.7 to 1 [13–15]). This equation is easily solved so that

$$K_{BC} = (K_D - f_{OC}K_{OC})/(f_{BC}C_W^{n-1})$$

Values of K_D for each PAH from each sediment are known from the measured pore-water and sediment concentrations of individual PAHs. The term C_W^{n-1} is used to describe the inhibition of sorption at higher concentrations that is observed in spiking studies, and the term must be set to one (n = 1) to compare PAH desorption from a broad range of field (not spiked) sediments. Values of K_{OC} must be assigned a constant number to calculate K_{BC} values; for example, the accepted literature values are used for K_{OC}. Measured K_{OC} values, however, are not available for most of the PAHs on the EPA_{34} list, especially the higher-molecular-weight and alkyl PAHs. Different correlations have been used to predict K_{OC} values based on K_{OW} values [23–25], but measured K_{OW} values also are not available for many PAHs (especially the alkyl PAHs) in the EPA_{34} list. This lack of suitable K_{OC} data could cause significant error in calculating K_{BC} values from our experimental data, because appropriate K_{OC} values are not available for so many of the target parent and alkyl PAHs and predictive correlations (especially regarding those PAHs for which neither measured K_{OW} or K_{OC} values are available) may not be sufficiently accurate.

In Part 1 [2], we noted that the limited number of measured literature K_{OC} values that are available for individual PAHs fit well with the minimum values that we determined for the K_{TOC} of the 114 sediments. Therefore, we investigated whether using the lowest 10% of the K_{TOC} values determined for each PAH from the 114 sediments would give reasonable standard K_{OC} values compared to the K_{OC} values in the literature. Table 3 shows a comparison of the log K_{OC} values that we determined based on the mean of the lowest 10% of the values determined in Part 1 with measured literature log K_{OC} values and those predicted from two commonly used correlations that calculate K_{OC} based on measured K_{OW} values that were developed by Xia [24] and Nguyen et al. [23]. Note that the log K_{OC} values that we propose based on the mean of the lowest 10% of the K_{TOC} values measured in Part 1 generally agree well with those from both the Xia [24] and Nguyen et al. [23] correlations as well as with the experimentally determined log K_{OC} values. This generally good agreement for the low- to high-molecular-weight PAHs in Table 3 indicates that our proposed log K_{OC}

Table 3. Modeled and measured organic carbon–water partition coefficients (K_{OC})

	Modeled log K_{OC}		Measured log K_{OC}			
	Xia[a]	Nguyen et al.[b]	Literature[c]	Mean[d]	SD[e]	*n*
Naphthalene	3.08	2.84	2.94	3.02	0.31	8
2-Methylnaphthalene	3.62	3.46		3.36	0.22	8
1-Methylnaphthalene	3.63	3.47		3.16	0.28	9
C2-naphthalenes				3.63	0.14	8
C3-naphthalenes				3.33	0.20	9
C4-naphthalenes				3.34	0.18	6
Acenaphthylene	3.70	3.55		4.02	0.12	9
Acenaphthene	3.68	3.53		3.37	0.29	11
Fluorene	3.93	3.82	3.45	3.65	0.24	10
C1-fluorenes				3.94	0.07	8
C2-fluorenes				3.67	0.31	7
Phenanthrene	4.21	4.14		4.18	0.27	9
Anthracene	4.20	4.12	4.20	4.60	0.21	10
C1-phenanthrenes/anthracenes				4.38	0.24	8
C2-phenanthrenes/anthracenes				4.64	0.15	7
C3-phenanthrenes/anthracenes				4.74	0.18	6
C4-phenanthrenes/anthracenes				4.77	0.18	2
Fluoranthene	4.68	4.68	4.62	4.74	0.29	11
Pyrene	4.61	4.61	4.80	4.70	0.32	11
C1-fluoranthenes/pyrenes				4.75	0.32	9
Benz[*a*]anthracene	5.37	5.48		5.48	0.26	9
Chrysene	5.37	5.48	5.30	5.50	0.27	9
C1-chrysenes				5.49	0.28	5
Benzo[*b*+*k*]fluoranthene				5.87	0.23	5
Benzo[*e*]pyrene				5.53	0.11	5
Benzo[*a*]pyrene	5.67	5.83		5.68	0.13	5
Perylene				5.88	0.37	3
Indeno[1,2,3-*cd*]pyrene				6.41	0.55	3
Dibenz[*a*,*h*]anthracene			6.31	6.13	0.11	2
Benzo[*ghi*]perylene				6.11	0.39	6

[a] Calculated using the correlation in Xia [24] and suggested octanol–water partition coefficients (K_{OW}) values from the Syracuse Research Corporation CHEMFATE database (Syracuse, NY USA; http://www.syrres.com/esc/chemfate.htrm, accessed January 15, 2007).
[b] Calculated using the correlation in Nguyen et al. [23] and K_{OW} values from the CHEMFATE database.
[c] Measured values taken from the CHEMFATE database.
[d] Mean log K_{OC} values for the lowest 10% of values reported for 114 sediments in Hawthorne et al. [2].
[e] SD = standard deviation.

values for the alkyl PAHs as well as for the parent PAHs are valid. Therefore, the measured log K_{OC} values we report in Table 3 were used to calculate log K_{BC} values discussed below.

Log K_{BC} *values*

As discussed in Part 1 [2], sediment–water partition coefficients as determined for the parent and alkyl PAHs showed nearly four orders of magnitude variation for each individual PAH, although a large range in K_D values might be expected based on the range in sediment characteristics, especially TOC (Table 1). The common practice of describing partitioning behavior using K_{OC} (or K_{TOC}) rather than K_D, however, did little to reduce the range in partition coefficients, and measured K_{TOC} values for these sediments ranged over approximately three orders of magnitude.

Log K_{BC} values determined for the field sediments in the present study are summarized in Table 4, and the entire set of experimental values is shown in Figure 1. Although the models based on two types of carbon sorption assume that log K_{BC} values would be similar among all sediments for a particular PAH, the actual experimental values based on field sediments vary by approximately three orders of magnitude, similar to the variation shown for the same sediments and PAHs for log K_{TOC} values in Part 1 [2]. Thus, even though the concept of combining water partition coefficients for natural OC and black (or soot) carbon has helped to describe PAH partitioning behavior for a few sediments in previous studies, the results presented in Table 4 and in Figure 1 clearly demonstrate that the two carbon–type model cannot be used to predict water concentrations accurately for the range of behavior that occurs in background and historically contaminated field samples.

It might be expected that partitioning behavior at a single site would be more consistent, because sediments from a single site may have more similar carbon types. In Part 1 [2], however, it was shown that log K_{TOC} values at a single site also were highly variable, and K_{TOC} values typically ranged by more than two orders of magnitude at most sites. Unfortunately, log K_{BC} values show a similarly broad range for the individual sites in the present study, thus demonstrating that the combined K_{OC} and K_{BC} model's predictions of pore-water concentrations would not be accurate even for a single site. This likely is a consequence of the multiple carbon sources found at industrial sites, even ones that are relatively isolated from different industries. For example, at one site used in the present study, where only a former manufactured gas plant had significant impact on the sediment, different carbon types, including soot, coal tar pitch, charcoal, wood, coal, and coke have been identified [11].

Interestingly, experimentally determined log K_{BC} values reported by different groups in the literature also show a great deal of variation. Our median log K_{BC} values (Table 4) agree

Table 4. Measured black carbon–water partition coefficients (K_{BC}) for 114 field sediments

	log K_{BC}[a]				
	n	Min	Max	Median	Mean
Naphthalene	73	3.02	6.52	4.62	4.73
2-Methylnaphthalene	72	3.49	6.54	5.00	5.03
1-Methylnaphthalene	84	3.31	6.30	4.79	4.76
C2-naphthalenes	81	3.66	5.95	4.95	4.84
C3-naphthalenes	83	3.55	6.42	4.91	4.85
C4-naphthalenes	58	3.36	6.06	5.05	4.93
Acenaphthylene	88	4.13	7.53	5.56	5.54
Acenaphthene	104	3.57	6.48	4.90	4.89
Fluorene	97	3.85	6.85	5.19	5.17
C1-fluorenes	80	3.95	6.32	5.34	5.23
C2-fluorenes	66	3.83	6.34	5.37	5.25
Phenanthrene	90	4.32	7.40	5.61	5.65
Anthracene	97	4.53	8.35	6.12	6.20
C1-phenanthrenes/anthracenes	81	4.41	7.01	5.93	5.79
C2-phenanthrenes/anthracenes	67	4.84	7.16	6.08	6.04
C3-phenanthrenes/anthracenes	55	4.80	7.54	6.41	6.31
C4-phenanthrenes/anthracenes	13	4.77	7.02	5.63	5.84
Fluoranthene	103	4.94	8.34	6.25	6.28
Pyrene	106	4.89	8.25	6.26	6.32
C1-fluoranthenes/pyrenes	83	4.88	7.68	6.49	6.46
Benz[*a*]anthracene	83	5.60	8.91	7.07	7.03
Chrysene	87	5.54	8.25	7.03	7.08
C1-chrysenes	48	5.85	8.34	7.35	7.30
Benzo[*b*+*k*]fluoranthene	50	5.89	8.97	7.28	7.39
Benzo[*e*]pyrene	46	6.03	8.51	7.18	7.17
Benzo[*a*]pyrene	47	5.70	8.78	7.22	7.22
Perylene	28	6.49	7.91	7.31	7.25
Indeno[1,2,3-*cd*]pyrene	33	6.85	9.50	8.27	8.17
Dibenz[*a*,*h*]anthracene	15	6.32	8.12	7.40	7.37
Benzo[*ghi*]perylene	41	6.12	9.43	7.75	7.73

[a] Log K_{BC} values were calculated as described in the text using the measured log organic carbon partition coefficient (K_{OC}) values presented in Table 3.

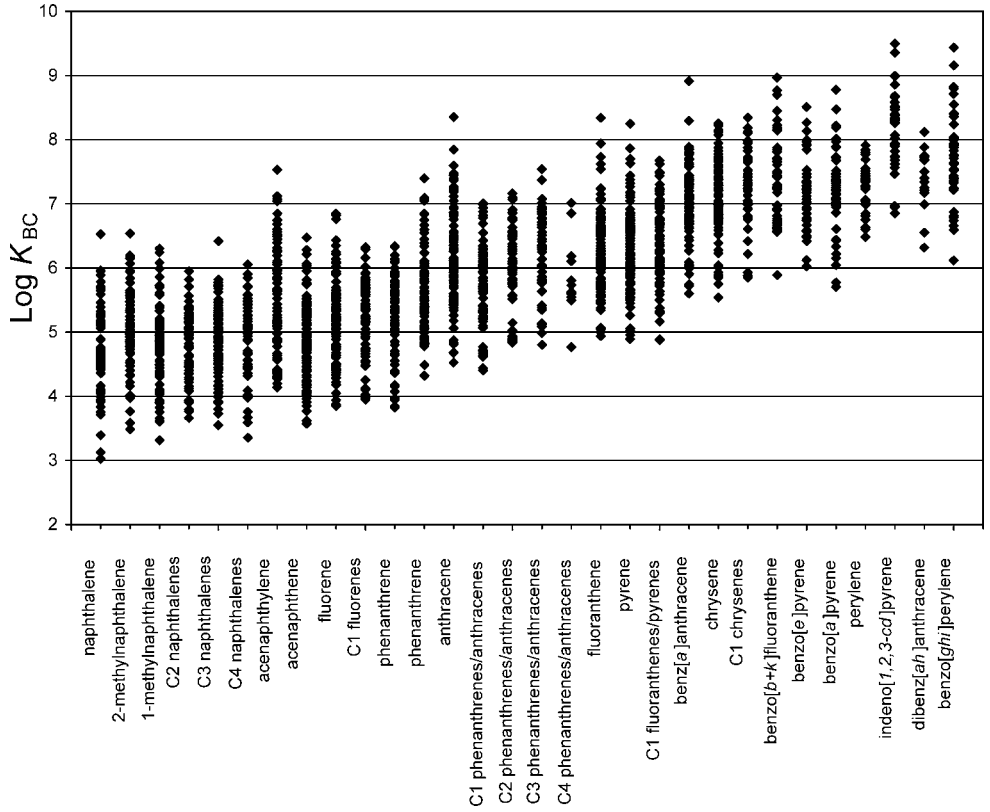


Fig. 1. Log black carbon–water partition coefficient (K_{BC}) values for parent and alkyl polycyclic aromatic hydrocarbons (PAHs) from 114 background and historically contaminated sediments.

Table 5. Literature measured log black carbon–water partition coefficient (K_{BC}) values

	Khalil et al. [11]	Lohmann et al. [13]	Bucheli and Gustafsson [15]	Cornelissen et al. [26]	Jonker and Koelmans [17]		
					Coal soot	Coal	Charcoal
Naphthalene	3.39			4.6, 5.2			
Phenanthrene	5.30	5.9, 6.1	5.4–5.9	5.6–6.8	6.71	8.03	7.57
Anthracene	5.41	6.1, 7.1			6.91	7.56	8.48
Fluoranthene	5.89	6.4, 7.0	5.45		6.90	8.18	7.72
Pyrene	5.97	6.4, 6.8	6.4–6.7	6.2–7.0	6.88	8.41	7.78
Benz[*a*]anthracene	6.45	6.9			7.78	9.41	8.92
Chrysene	6.63				7.68	9.99	8.58
Benzo[*a*]pyrene	6.85	7.1		7.1–7.9	8.58	9.87	10.45
Benzo[*ghi*]perylene	6.94			7.6–8.2	8.81	9.68	9.59

well with those measured by Lohmann et al. [13] and by Cornelissen et al. [26] (Table 5). Both significantly lower and higher log K_{BC} values, however, have been reported for different types of carbon likely to be present in impacted sediments. For example, log K_{BC} values for coal tar pitch often are lower than values reported for sediments [11], whereas log K_{BC} values for other carbon types expected in impacted sediments, such as coal, coal soot, and charcoal, are higher and, typically, are in the range of the highest values we determined in field sediments (Tables 4 and 5). Because previous studies have shown that log K_{BC} values with different carbon types that would be found in our sediments vary so greatly, it is not really surprising that the log K_{BC} values we determined for field sediments show such a broad range of values.

The need for considering more than two carbon types in models used for predicting water–sediment partitioning has been suggested previously [26], but no suitable methods to measure various carbon types on a large number of sediments are available. It also should be noted that the 375°C oxidation method to determine BC has been questioned [7,13] and is a possible contributor to the range of log K_{BC} values we report. When we determined the BC content on the same reference samples as other research groups, however, including the developer of the 375°C oxidation method, we obtain good agreement. For example, for the National Institute of Science and Technology standard reference material 1650 diesel soot, we obtained a value of 77% ± 1% by weight for TOC and 49% ± 1% by weight for BC, which agrees well with those reported by Bucheli and Gustafsson [15] of 77% by weight for TOC and 48% by weight for BC, Jonker and Koelmans [17] of 77%

Table 6. Log black carbon–water partition coefficient (K_{BC}) values for sediments with and without a non–aqueous phase liquid (NAPL) hydrocarbon phase

	No NAPL present				NAPL present			
	Mean log K_{BC}	SD[a]	Median log K_{BC}	*n*	Mean log K_{BC}	SD[a]	Median log K_{BC}	*n*
Naphthalene	4.89	0.57	4.77	49	4.40	0.82	4.36	24
2-Methylnaphthalene	5.15	0.59	5.09	44	4.85	0.65	4.85	28
1-Methylnaphthalene	4.88	0.58	4.82	55	4.54	0.66	4.66	29
C2-naphthalenes	4.90	0.49	4.97	57	4.68	0.50	4.70	24
C3-naphthalenes	4.91	0.55	4.98	57	4.71	0.60	4.69	26
C4-naphthalenes	4.94	0.55	5.05	35	4.91	0.71	5.05	23
Acenaphthylene	5.69	0.86	5.82	60	5.21	0.66	5.17	28
Acenaphthene	4.97	0.62	4.96	74	4.69	0.54	4.79	30
Fluorene	5.23	0.65	5.21	68	5.04	0.68	5.04	29
C1-fluorenes	5.24	0.55	5.34	53	5.23	0.61	5.35	27
C2-fluorenes	5.31	0.57	5.34	39	5.17	0.80	5.46	27
Phenanthrene	5.71	0.68	5.69	61	5.52	0.65	5.53	29
Anthracene	6.34	0.76	6.29	67	5.88	0.68	6.00	30
C1-phenanthrenes/anthracenes	5.87	0.63	5.95	55	5.63	0.60	5.67	26
C2-phenanthrenes/anthracenes	6.01	0.65	6.01	43	6.09	0.68	6.28	24
C3-phenanthrenes/anthracenes	6.32	0.62	6.40	30	6.29	0.72	6.41	25
C4-phenanthrenes/anthracenes	5.86	0.95	5.83	4	5.83	0.43	5.63	9
Fluoranthene	6.35	0.68	6.34	73	6.11	0.51	6.10	30
Pyrene	6.41	0.67	6.46	76	6.11	0.49	6.07	30
C1-fluoranthenes/pyrenes	6.47	0.69	6.45	56	6.42	0.59	6.63	27
Benz[*a*]anthracene	7.04	0.65	6.95	57	6.99	0.45	7.10	26
Chrysene	7.11	0.64	7.16	60	7.00	0.47	7.02	27
C1-chrysenes	7.19	0.63	7.26	30	7.48	0.50	7.58	18
Benzo[*b*+*k*]fluoranthene	7.41	0.74	7.25	32	7.34	0.49	7.43	18
Benzo[*e*]pyrene	7.22	0.60	7.02	28	7.09	0.50	7.20	18
Benzo[*a*]pyrene	7.33	0.65	7.24	32	6.98	0.64	7.21	15
Perylene	7.34	0.36	7.38	17	7.11	0.41	7.28	11
Indeno[1,2,3-*cd*]pyrene	8.21	0.63	8.30	20	8.11	0.66	8.26	13
Dibenz[*a*,*h*]anthracene	7.48	0.39	7.38	8	7.25	0.58	7.40	7
Benzo[*ghi*]perylene	7.83	0.75	7.75	28	7.52	0.62	7.62	13

[a] SD = standard deviation.

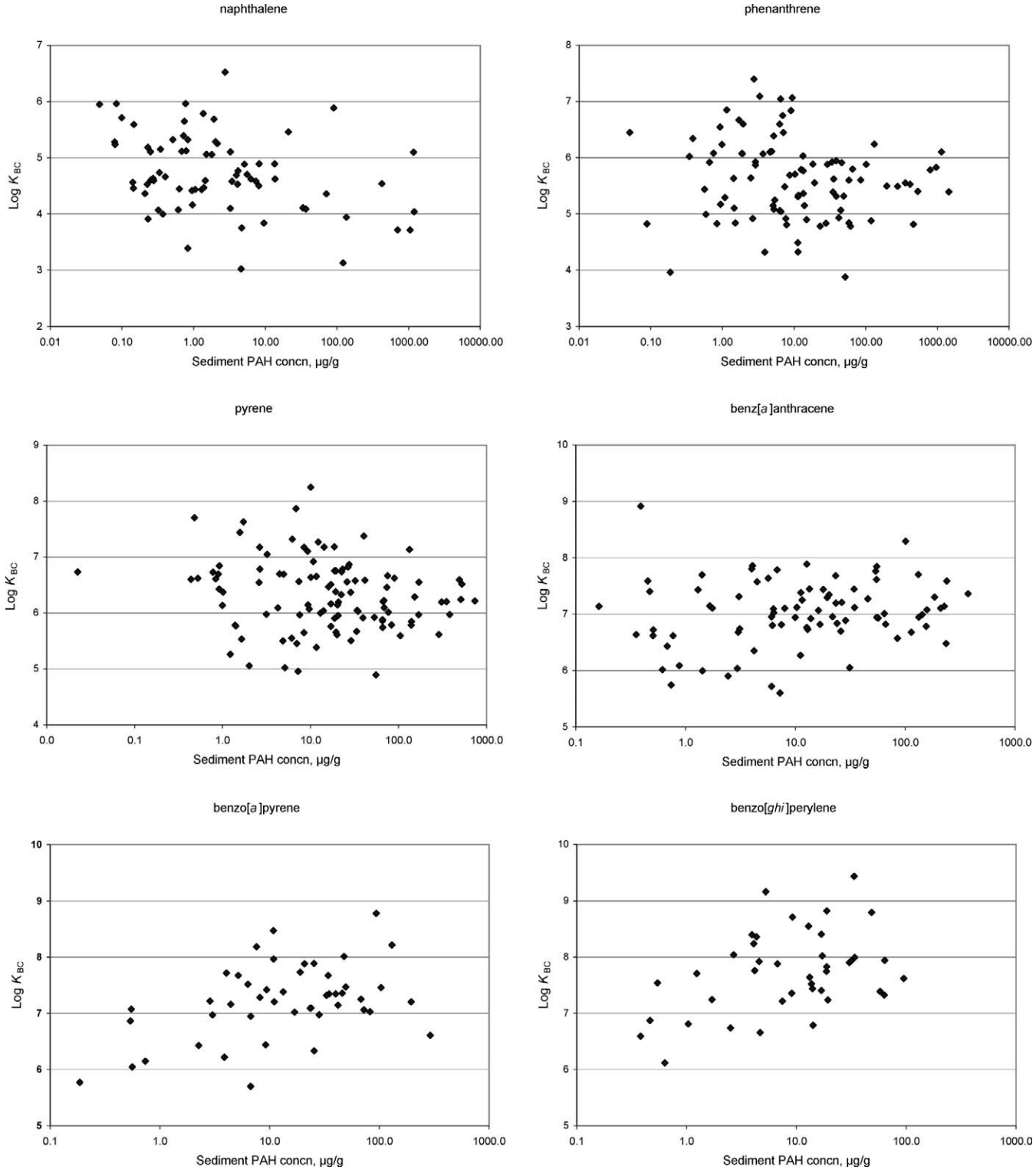


Fig. 2. Log black carbon–water partition coefficient (K_{BC}) values versus sediment polycyclic aromatic hydrocarbon (PAH) concentration for representative two- to six-ring PAHs.

by weight for TOC and 36% by weight for BC, Gelinas et al. [27] of 78% by weight for TOC and 45% by weight for BC, and Nguyen et al. [28] of 46% by weight for BC. Similarly, for standard reference material sediment 1944, we obtained values of 4.5% ± 0.1% by weight for TOC and 1.0% ± 0.1% by weight for BC, similar to the values of 4.4% by weight for TOC and 0.8% by weight for BC reported by Nguyen et al. [28]. Thus, it appears that our determinations of TOC and BC are in accordance with those of other investigators. In any case, some small error in BC determinations cannot account for the three orders of magnitude range in log K_{BC} values that was measured for each PAH in the present study.

Effects of PAH concentration on log K_{BC} *values*

Figure 2 shows a comparison of log K_{BC} values with sediment PAH concentrations for representative two- to six-ring

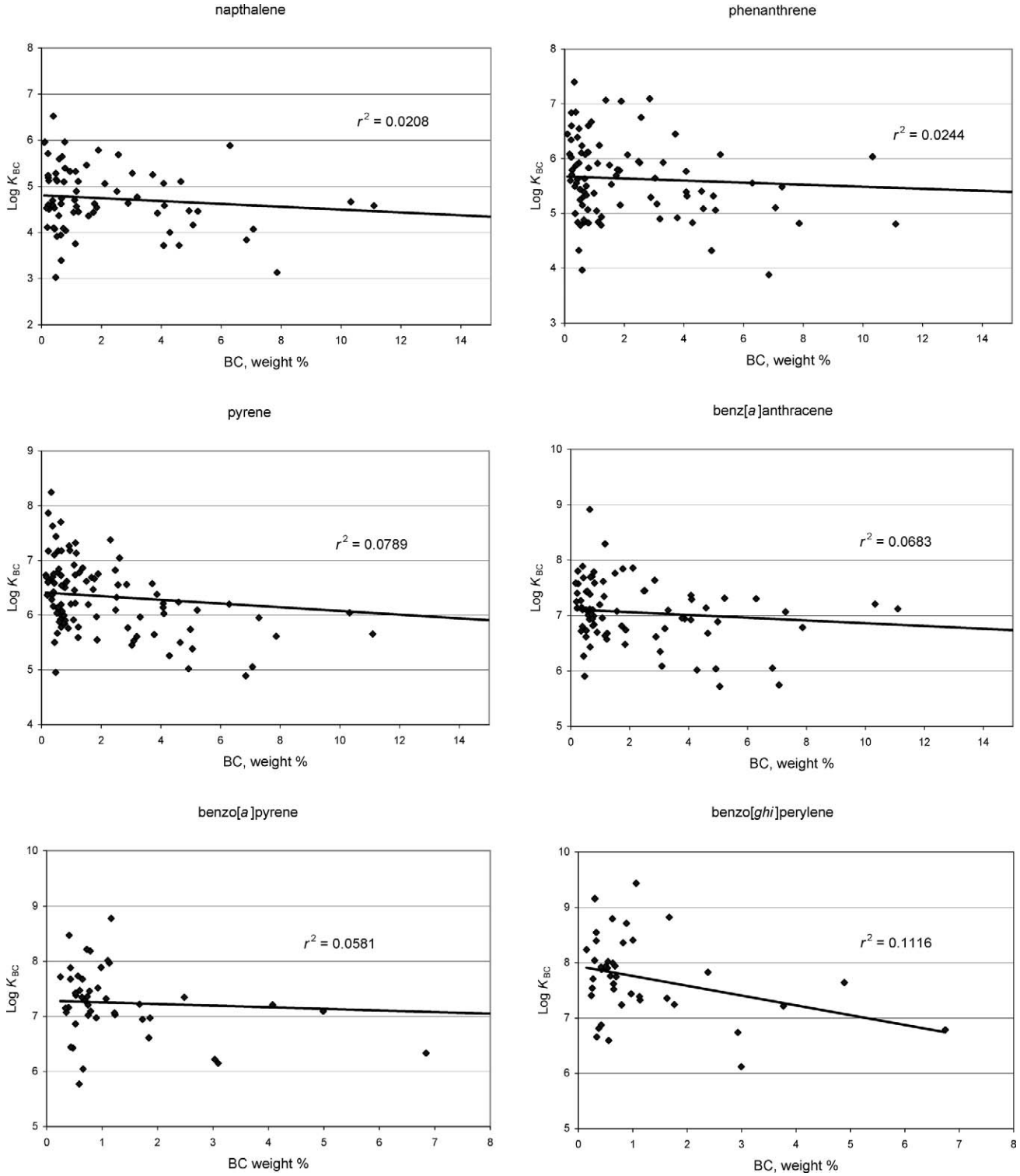


Fig. 3. Log black carbon (BC)–water partition coefficient (K_{BC}) values versus sediment BC content for representative two- to six-ring polycyclic aromatic hydrocarbons (PAHs). Data are not shown for three sediments that had BC contents of 21, 32, and 40% by weight so that the scale could be expanded on the *x* axis; however, the log K_{BC} values for these samples also showed no trend with BC content.

PAHs. Interestingly, there appears to be no effect of PAH concentration on log K_{BC} values, even though the concentrations of each PAH varies by approximately four orders of magnitude. For example, the sediment concentration of pyrene varies from 0.02 to 740 μg/g, yet no trend in K_{BC} values with concentration exists (Fig. 2). Similarly, no effect of water PAH concentrations on log K_{BC} values is apparent (not shown).

At first, these results may appear to conflict with the concept that PAH sorption to BC follows a Langmuir adsorption isotherm [7,14,15,29,30], in which the partitioning of a PAH to the carbon phase is reduced at higher spiked concentrations. Adjusting log K_{BC} values for the Freundlich isotherm only has a small effect, typically a few tenths of a log K_{BC} unit or less; therefore, any such effects are insig-

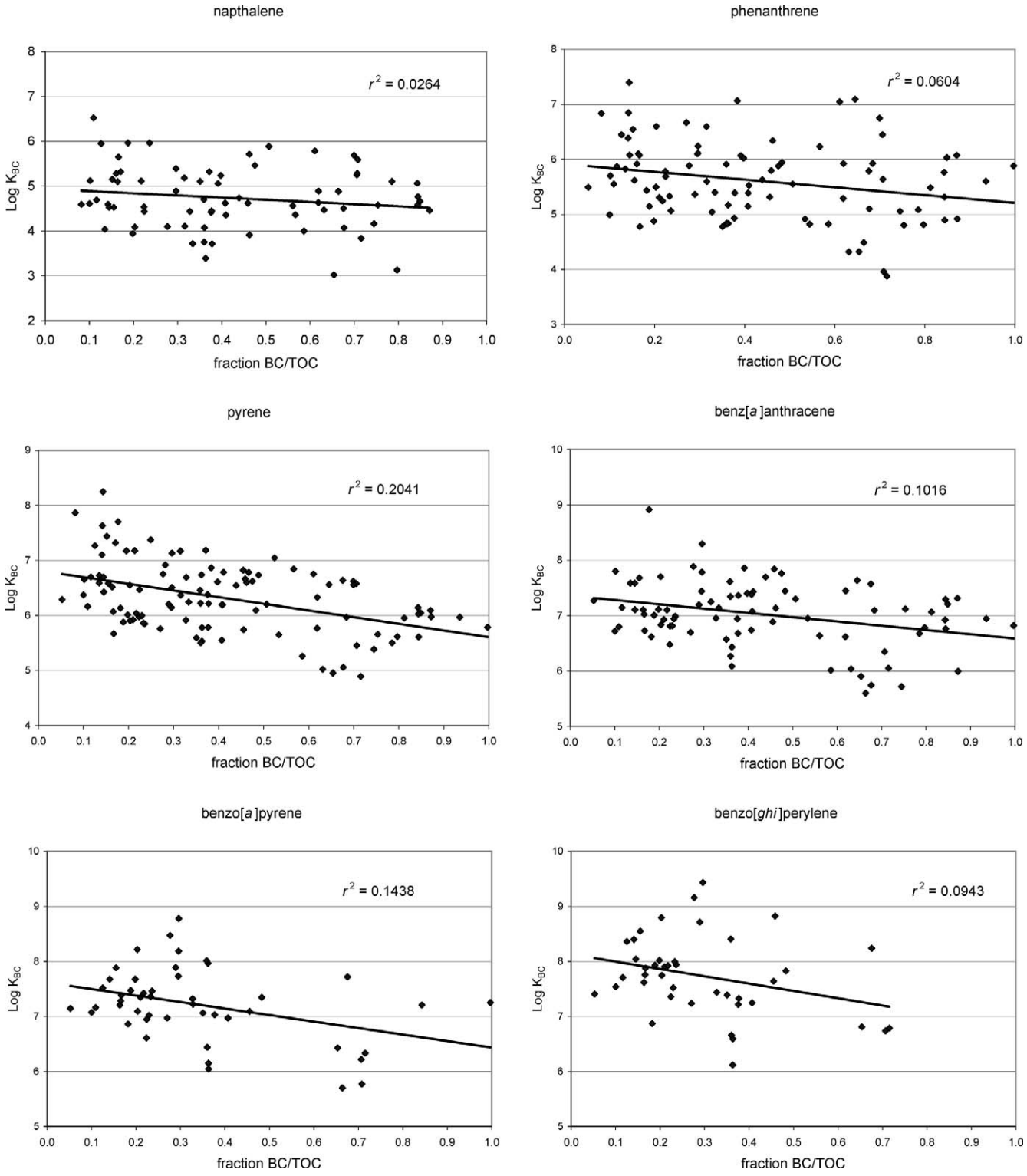


Fig. 4. Log black carbon (BC)–water partition coefficient (K_{BC}) values versus the fraction of BC versus total organic carbon (TOC) for representative two- to six-ring polycyclic aromatic hydrocarbons (PAHs).

nificant compared to the range in log K_{BC} values we measured in the field sediments.

Effects of non–aqueous phase liquids

As is typical in field sediments from industrial waterways, approximately one-third of the impacted sediments in the present study contained a non–aqueous phase liquid (NAPL) phase (based on field observation of a separate phase or sheen and confirmed in the laboratory). Because PAH partitioning could well become dominated by liquid–liquid partitioning (NAPL–water partitioning) rather than being dominated by K_{OC} and K_{BC} partitioning, it seemed to be possible that the large range of log K_{BC} values we measured could be greatly influenced by the presence of a separate hydrocarbon NAPL phase [31]. It was hoped that by removing the samples with NAPL from the data set, the log K_{BC} values would become more consistent for

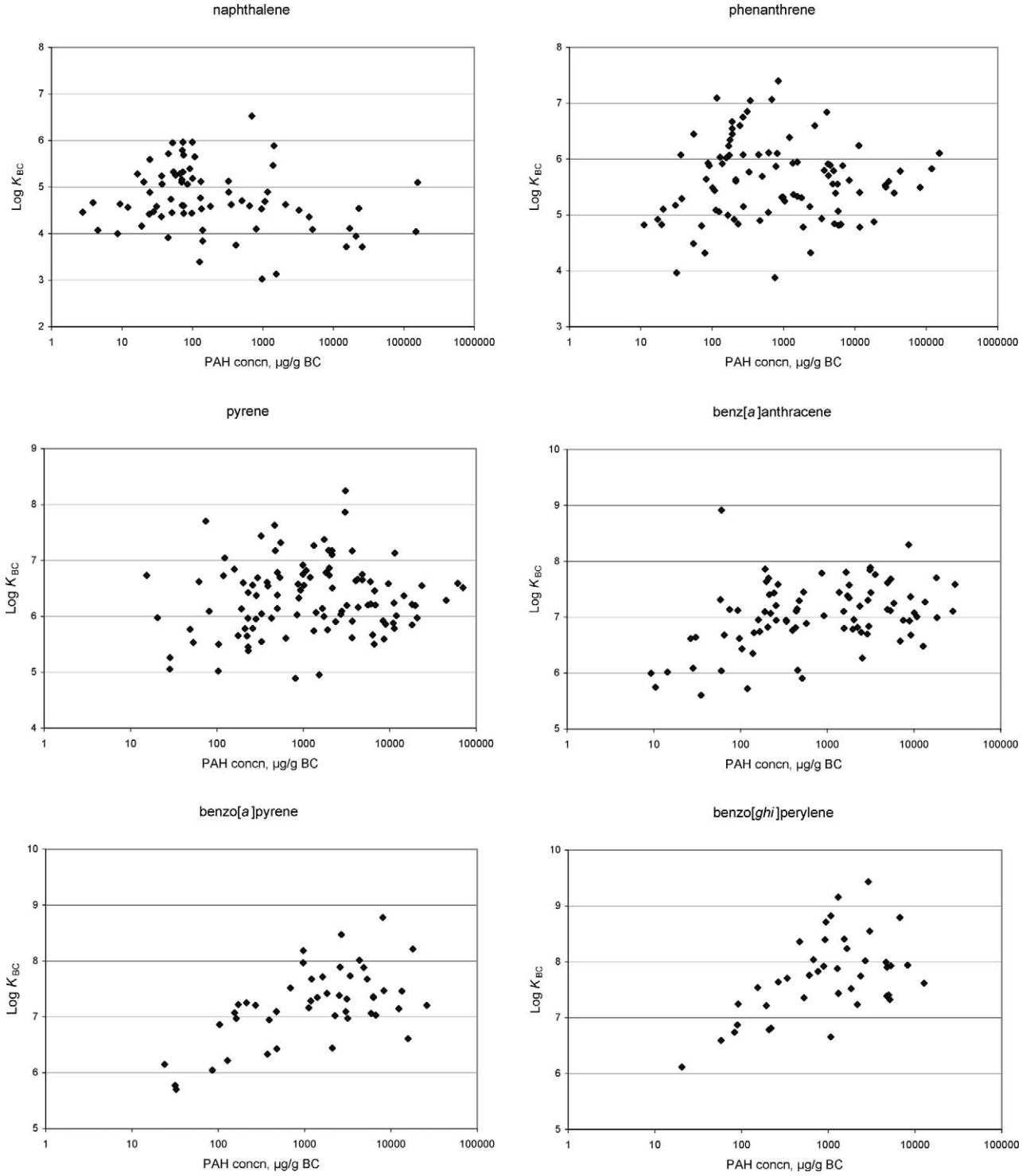


Fig. 5. Log black carbon (BC)–water partition coefficient (K_{BC}) values versus polycyclic aromatic hydrocarbons (PAHs) concentration per gram of BC for representative two- to six-ring PAHs.

all the sediments and, therefore, become more useful in predictive partitioning models.

Table 6 shows a comparison of the log K_{BC} values determined for samples with and without a NAPL phase. Although the mean and median values for log K_{BC} are slightly higher for the sediments with no NAPL phase than for sediments with a NAPL phase, the difference is not significant, and the large variation in log K_{BC} values remains even if the sediments containing NAPL are removed. Therefore, compared to the large range in log K_{BC} values found in our field sediments, the presence or absence of a NAPL phase is not a significant factor.

Effects of carbon type on log K_{BC} *values*

The sediments used in the present study have a much broader range of TOC and BC content as well as in the fraction of total carbon that was BC (Table 1) compared with the sediments used for any previous PAH–BC partitioning studies. Conceivably, the log K_{BC} values could show a trend with either

carbon type or the relative proportions of BC to TOC; however, no such relationships appear to exist. Plots of log K_{BC} for individual PAHs versus either TOC content (not shown) or BC content (Fig. 3) show no significant trend, with linear correlation coefficients (r^2) typically less than 0.1. Log K_{BC} values may show a slight trend toward lower values with a higher fraction of BC versus TOC, but the correlation does not appear to be significant (Fig. 4).

The most likely effect of PAH concentration on log K_{BC} values should be observed when log K_{BC} values are plotted versus the PAH concentration per gram of BC, especially if the competitive sorption mechanism on BC is significant. As shown in Figure 5, however, there appears to be no significant relationship between log K_{BC} values and the measured μg PAH/g BC, even though the range of μg PAH/g BC varies over four orders of magnitude. These results further demonstrate that as discussed above, the Freundlich sorption isotherm has no significant effect on log K_{BC} values compared to the range found in field sediments. Note that the possible apparent increase in log K_{BC} values with PAH concentration per gram of BC for the five- and six-ring PAHs is an artifact of detection limits in water, because PAHs with higher K_{OC} and K_{BC} values must have very high sediment concentrations to yield detectable water concentrations.

Multiple linear regression analysis

As discussed above, calculating K_{BC} values with the $K_{OC}K_{BC}$ model is based on the assumption that K_{OC} for each PAH has a constant value on different sediments [8,11,13–16]. Although previous investigators have reported that K_{OC} values do vary with different carbon types [1,2,32–34], the goal of the $K_{OC}K_{BC}$ model reported by several investigators [8,11,13–16] is to account for these variations to predict partitioning behavior. Unfortunately, the results presented above clearly demonstrate that the model fails to fit the experimental data from the 114 field sediments using constant values of K_{OC} for each PAH.

Multiple linear regression analysis does not require the assumption of a constant K_{OC} value for each PAH but, instead, will yield fitted values for both K_{OC} and K_{BC} for each PAH. Unfortunately, the ability of the $K_{OC}K_{BC}$ model to explain a large percentage of the variance in the data was very poor for all the PAHs on the field sediments investigated in the present study. Both K_{OC} and K_{BC} failed to show any consistent values using either linear or log-linear regressions. For all the PAHs, r^2 values were poor (<0.20) (see *Appendix* [http://dx.doi.org/10.1897/07-087.S1]), clearly showing the limited predictive ability of the two carbon–type model. All the PAHs showed high standard errors and little or no statistical significance ($p > 0.05$), again clearly demonstrating that the $K_{OC}K_{BC}$ model was not capable of predicting PAH partitioning behavior for the 114 field sediments used in the present study.

CONCLUSION

Values of K_{BC} for PAHs on field sediments do not show a narrower range of values than previously reported for K_{OC} values for the same PAHs and field sediments. Therefore, the model combining carbon–water partition coefficients for natural OC and BC is not able to predict water concentrations any better than the simple equilibrium partitioning model based on K_{OC}. Even though the addition of a third carbon type to the model has been proposed [26], it seems unlikely that any such approach will be successful in predicting pore-water concentrations of PAHs based on sediment concentrations for field sediments, especially considering that no agreement exists in the literature regarding how to determine different carbon types, with the possible exception of manually separating sediment particles [11]. This approach simply is not practical, however, for the numbers of samples required in field surveys.

In the past, use of sediment PAH concentrations and standard K_{OC} values, which generally have been based on spiked PAH sorption experiments or estimated from K_{OW} values, have led to greatly overpredicted water PAH concentrations and associated overprediction of toxicity to benthic organisms [19,20]. Based on the wide range of measured K_{BC} values determined in the present study, the use of K_{BC} values that are based on partition coefficients derived by sorption of spiked PAHs onto sediments also clearly will fail to increase the accuracy of predicted water concentrations. Thus, any regulatory framework that uses pore-water concentrations of PAHs to predict environmental effects should use measured pore-water values, not those predicted from current partitioning models.

Although the 114 sediments used in the present study include a range of characteristics (especially PAH concentrations) much broader than those used to develop partitioning models and coefficients, it should be noted that these samples accurately reflect the range of sediments found in field samples. All the sediments used in the present study are from sites that are under regulatory scrutiny and, therefore, represent the types of sites that are of highest concern to regulatory agencies and industry. As noted in Part 1, the conventional use of K_{OC} values to predict water PAH concentrations from sediment concentrations is not adequate to give useful predictions for real-world sediments. Unfortunately, the results of the present study demonstrate that the use of a two carbon–type model including K_{OC} and K_{BC} also does not improve these predictions. Clearly, the application of K_{BC} coefficients is useful for understanding the mechanisms of PAH sorption and partitioning in sediments, but the results of the present study clearly demonstrate that K_{BC} values do not help to predict PAH behavior for the range of sediments that exist in the field.

SUPPORTING INFORMATION

Appendix S1. Residual plots for phenanthrene; correlation coefficients (r^2) values.

Found at DOI: 10.1897/07/087.S1 (98 KB PDF).

Acknowledgement—Financial support of National Grid, New York State Electric and Gas, Alcoa, Northeast Gas Association, and the U.S. Department of Energy under Cooperative Agreement DE-FC26-98FT40321 is gratefully acknowledged. The authors also appreciate helpful discussions with J. Kreitinger (Retec) and E. Neuhauser (National Grid) as well as the assistance of N. Azzolina (Retec) and T. Rielkoff in reducing the data.

REFERENCES

1. Dondelle MM, Loehr RC. 2002. Comparison of estimated and experimentally obtained soil water distribution coefficients. *Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management* 6:218–226.
2. Hawthorne SB, Grabanski CB, Miller DJ. 2006. Measured partitioning coefficients for parent and alkyl polycyclic aromatic hydrocarbons in 114 historically contaminated sediments: Part 1. K_{OC} values. *Environ Toxicol Chem* 25:2901–2911.
3. Jonker MTO, Sinke AJC, Brils JM, Koelmans AA. 2003. Sorption of polycyclic aromatic hydrocarbons to oil contaminated sediment: Unresolved complex? *Environ Sci Technol* 37:5197–5203.
4. Nguyen TH, Sabbah I, Ball WP. 2004. Sorption nonlinearity for organic contaminants with diesel soot: Method development and isotherm interpretation. *Environ Sci Technol* 38:3595–3603.

5. Reeves WR, McDonald TJ, Cizmas L, Donnelly KC. 2004. Partitioning and desorption behavior of polycyclic aromatic hydrocarbons from disparate sources. *Sci Total Environ* 332:183–192.
6. Hong L, Ghosh U, Mahajan T, Zare RN, Luthy RG. 2003. PAH sorption mechanism and partitioning behavior in lampblack-impacted soils from former oil-gas plant sites. *Environ Sci Technol* 37:3625–3634.
7. Cornelissen G, Gustafsson O, Bucheli TD, Jonker MTO, Van Noort PCM. 2005. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: Mechanisms and consequences for distribution, bioaccumulation, and biodegradation. *Environ Sci Technol* 39:6881–6895.
8. Cornelissen G, Haftka J, Parsons J, Gustafsson O. 2005. Sorption to black carbon of organic compounds with varying polarity and planarity. *Environ Sci Technol* 39:3688–3694.
9. McLeod PB, Van Den Heuvel-Greve MJ, Allen-King RM, Luoma SN, Luthy RG. 2004. Effects of particulate carbonaceous matter on the bioavailability of benzo[*a*]pyrene and 2,2′,5,5′-tetrachlorobiphenyl to the clam, *Macoma balthica*. *Environ Sci Technol* 38:4549–4556.
10. Ahn S, Werner D, Karapanagioti HK, McGlothlin DR, Zare RN, Luthy RG. 2005. Phenanthrene and pyrene sorption and intraparticle diffusion in polyoxymethylene, coke, and activated carbon. *Environ Sci Technol* 39:6516–6526.
11. Khalil MF, Ghosh U, Kreitinger JP. 2006. Role of weathered coal tar pitch in the partitioning of polycyclic aromatic hydrocarbons in manufactured gas plant site sediments. *Environ Sci Technol* 40:5681–5687.
12. Chai Y, Kochetkov A, Reible DD. 2006. Desorption resistance of polycyclic aromatic hydrocarbons and duration of exposure. *Environ Toxicol Chem* 25:2827–2833.
13. Lohmann R, MacFarlane JK, Gschwend PM. 2005. Importance of black carbon to sorption of native PAHs, PCBs, and PCDDs in Boston and New York harbor sediments. *Environ Sci Technol* 39:141–148.
14. Accardi-Dey A, Gschwend PM. 2002. Assessing the combined roles of natural organic matter and black carbon as sorbents in sediments. *Environ Sci Technol* 36:21–29.
15. Bucheli TD, Gustafsson O. 2000. Quantification of the soot–water distribution coefficient of PAHs provides mechanistic basis for enhanced sorption observations. *Environ Sci Technol* 34:5144–5151.
16. Gustafsson O, Haghseta F, Chan C, MacFarlane J, Gschwend PM. 1997. Quantification of the dilute sedimentary soot phase: Implications for PAH speciation and bioavailability. *Environ Sci Technol* 31:203–209.
17. Jonker MTO, Koelmans AA. 2002. Sorption of polycyclic aromatic hydrocarbons and polychlorinated biphenyls to soot and soot-like materials in the aqueous environment: Mechanistic considerations. *Environ Sci Technol* 36:3725–3734.
18. Kreitinger JP, Neuhauser EF, Doherty FG, Hawthorne SB. 2007. Greatly reduced bioavailability and toxicity of polycyclic aromatic hydrocarbons to *Hyalella azteca* in sediments from manufactured-gas plant sites. *Environ Toxicol Chem* 26:1146–1157.
19. U.S. Environmental Protection Agency. 2003. Procedures for the derivation of ESBs for the protection of benthic organisms: PAH mixtures. EPA/600/R-02/013. Office of Research and Development, Washington, DC.
20. Bucheli TD, Gustafsson O. 2001. Ubiquitous observations of enhanced solid affinities for aromatic organochlorines in field situations: Are in situ dissolved exposures overestimated by existing partitioning models? *Environ Toxicol Chem* 20:1450–1456.
21. Hawthorne SB, Grabanski CB, Miller DJ, Kreitinger JP. 2005. Solid-phase microextraction measurement of parent and alkyl polycyclic aromatic hydrocarbons in millimeter sediment pore water samples and determination of K_{DOC} values. *Environ Sci Technol* 39:2795–2803.
22. Hawthorne SB, Miller DJ, Kreitinger JP. 2006. Measurement of total polycyclic aromatic hydrocarbon concentrations in sediments and toxic units used for estimating risk to benthic invertebrates at manufactured gas plant sites. *Environ Toxicol Chem* 25:287–296.
23. Nguyen TH, Goss K-U, Ball WP. 2005. Polyparameter linear free energy relationships for estimating the equilibrium partition of organic compounds between water and the natural organic matter in soils and sediments. *Environ Sci Technol* 39:913–924.
24. Xia G. 1998. Sorption behavior of nonpolar organic chemicals on natural sorbents. PhD thesis. Department of Geography and Environmental Engineering, Johns Hopkins University, Baltimore, MD, USA.
25. Karickhoff SW, Brown DS, Scott TA. 1979. Sorption of hydrophobic pollutants on natural sediments. *Water Res* 13:241–248.
26. Cornelissen G, Breedveld GD, Kalaitzidis S, Christanis K, Kibsgaard A, Oen AMP. 2006. Strong sorption of native PAHs to pyrogenic and unburned carbonaceous geosorbents in sediments. *Environ Sci Technol* 40:1197–1203.
27. Gelinas Y, Prentice KM, Baldock JA, Hedges JI. 2001. An improved thermal oxidation method for the quantification of soot/graphitic black carbon in sediments and soils. *Environ Sci Technol* 35:3519–3525.
28. Nguyen TH, Brown RA, Ball WP. 2004. An evaluation of thermal resistance as a measure of black carbon content in diesel soot, wood char, and sediment. *Org Geochem* 35:217–234.
29. Wang X, Sato T, Xing B. 2005. Sorption and displacement of pyrene in soils and sediments. *Environ Sci Technol* 39:8712–8718.
30. Kim HS, Pfaender FK. 2005. Effects of microbially mediated redox conditions on PAH–soil interactions. *Environ Sci Technol* 39:9189–9196.
31. Jonker MTO, Sinke AJC, Brills JM, Koelmans AA. 2003. Sorption of polycyclic aromatic hydrocarbons to oil contaminated sediment: Unresolved complex? *Environ Sci Technol* 37:5197–5203.
32. Wang G, Kleineidam S, Grathwohl P. 2007. Sorption/desorption reversibility of phenanthrene in soils and carbonaceous materials. *Environ Sci Technol* 41:1186–1193.
33. Poerschmann J, Kopinke FD. 2001. Sorption of very hydrophobic organic compounds (VHOCs) on dissolved humic organic matter (DOM). 2. Measurement of sorption and application of a Flory-Huggins concept to interpret the data. *Environ Sci Technol* 35:1142–1148.
34. Karapanagioti HK, Kleineidam S, Sabatini DA, Grathwohl P, Ligouis B. 2000. Impacts of heterogeneous organic matter on phenanthrene sorption: Equilibrium and kinetic studies with aquifer material. *Environ Sci Technol* 34:406–414.