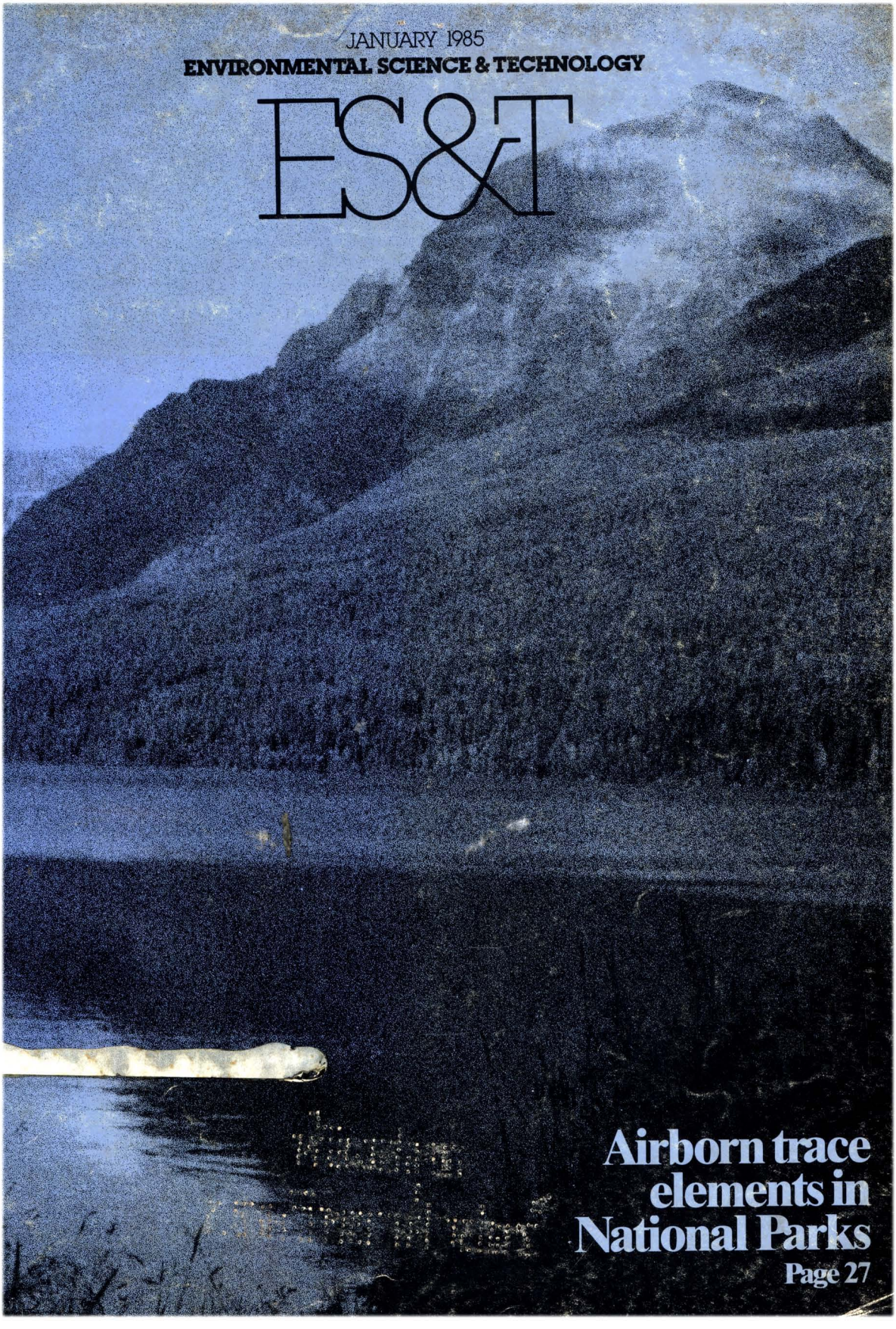
JANUARY 1985
ENVIRONMENTAL SCIENCE & TECHNOLOGY

ES&T



Airborn trace elements in National Parks

Page 27

Results of a fine air analyzer are only as good as its calibration.

...CSI offers a complete family of precision and audit calibrators for NO, NO_2, NO_x, SO_2, H_2S, CO, HC and O_3!

With the addition of the Meloy line of precision calibrators, CSI can now offer the most complete line of audit and precision calibrators found under one roof.

CSI 1800 Audit Calibrator

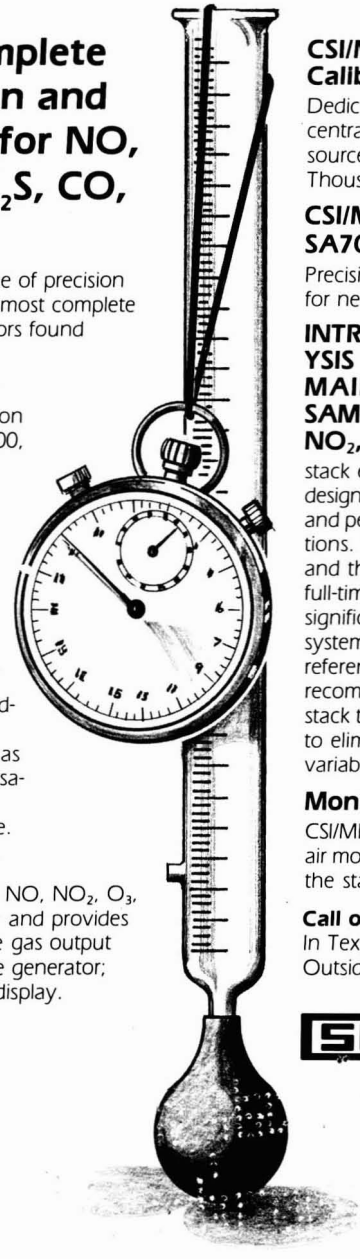
The latest in precision gas calibration equipment, the programmable 1800, provides SO_2, H_2S, NO_2, O_3, CO and CH_4 with manual, automatic or remote operation. Modular design permits economical upgrading. Super stable, patented ozone generator; mass flow controllers and digital display.

CSI 3000 PHOTOCAL™ Primary Ozone Calibrator

Meets EPA requirements as a standard for both manual and field calibration of ozone analyzers. It has temperature and pressure compensation, RS-232 systems compatibility, self-diagnostics and is field portable.

CSI 1700 Audit Calibrator

A gas phase titration calibrator for NO, NO_2, O_3, CO, HC AND SO_2 that is portable and provides manual and remote control of the gas output level. Super stable, patented ozone generator; mass flow controllers, and digital display.



CSI/MELOY CS10-2 Permeation SO_2 Calibrator

Dedicated system produces NBS traceable concentrations of SO_2 using a permeation SO^2 source. Features high reliability and economy. Thousands in use.

CSI/MELOY FS-8 Option for SA700 SO_2 Analyzer

Precision internal span check permeation device for new improved field monitor performance.

INTRODUCING CSI STACK GAS ANALYSIS SYSTEM: A NEW, VIRTUALLY MAINTENANCE-FREE, PRECISION SAMPLING UNIT FOR H_2S, TS, SO_2, NO, NO_2, NO_x, CO_2, and hydrocarbons in

stack effluents is now available. Using established design principles, the DS210 is "Compliance Tested" and performs well within EPA Performance Regulations. The analysis system is performance proven, and the EPA designated ambient monitors insure full-time operation at a startup and an annual cost significantly below existing sampling units and systems. The built-in calibration system using SRM referenced gasses is in full compliance with EPA recommendations, and calibration is carried out at stack temperatures and equivalent flow conditions to eliminate temperature and pressure dependent variables.

Monitor Analyzers & Air Supplies

CSI/MELOY also manufactures a full line of ambient air monitors and air supplies which are now setting the standard for the industry.

Call or write us today for more information:
In Texas - 512/258-5191
Outside of Texas - TOLL FREE 800/531-5003

COLUMBIA SCIENTIFIC INDUSTRIES CORPORATION
P.O. Box 9908 / Austin, TX 78766
TWX 910-874-1364

Photocal™ is a trademark of Columbia Scientific Industries Corporation.

Environmental Science & Technology

Please send *research* manuscripts to Manuscript Reviewing, *feature* manuscripts to Managing Editor. For editorial policy and author's guide, see the January 1985 issue, page 22, or write Janice L. Fleming, Manuscript Reviewing Office, *ES&T*. A sample copyright transfer form, which may be copied, appears on the inside back cover of the January 1985 issue.

Environmental Science & Technology, ES&T (ISSN 0013-936X), is published monthly by the American Chemical Society at 1155 16th Street, N.W., Washington, D.C. 20036; 202-872-4600, TDD 202-872-8733. Second-class postage paid at Washington, D.C., and at additional mailing offices. POSTMASTER: Send address changes to Membership & Subscription Services, P.O. Box 3337, Columbus, Ohio 43210.

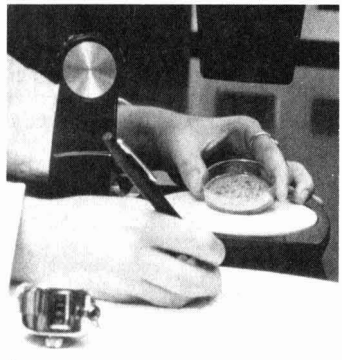
SUBSCRIPTION PRICES 1985: Members, $26 per year; nonmembers (for personal use), $35 per year; institutions, $149 per year. Foreign postage, $8 additional for Canada and Mexico, $14 additional for Europe including air service, and $23 additional for all other countries including air service. Single issues, $13 for current year; $13.75 for prior years. Back volumes, $161 each. For foreign rates add $1.50 for single issues and $10.00 for back volumes. Rates above do not apply to nonmember subscribers in Japan, who must enter subscription orders with Maruzen Company Ltd., 3-10 Nihon bashi 2 chome, Chuo-ku, Tokyo 103, Japan. Tel: (03) 272-7211.

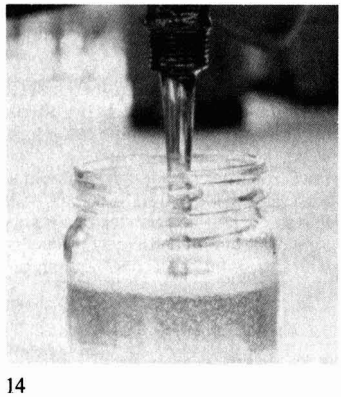
SUBSCRIPTION SERVICE: Orders for new subscriptions, single issues, back volumes, and microfiche and microform editions should be sent with payment to Office of the Treasurer, Financial Operations, ACS, 1155 16th St., N.W., Washington, D.C. 20036. Phone orders may be placed, using Visa, Master Card, or American Express, by calling toll free (800) 424-6747 from anywhere in the continental U.S. Changes of address, subscription renewals, claims for missing issues, and inquiries concerning records and accounts should be directed to Manager, Membership and Subscription Services, ACS, P.O. Box 3337, Columbus, Ohio 43210. Changes of address should allow six weeks and be accompanied by old and new addresses and a recent mailing label. Claims for missing issues will not be allowed if loss was due to insufficient notice of change of address, if claim is dated more than 90 days after the issue date for North American subscribers or more than one year for foreign subscribers, or if the reason given is "missing from files."

ES&T CONTENTS

Volume 19, Number 1, January 1985

FEATURES





REGULATORY FOCUS

DEPARTMENTS

RESEARCH

ESTHAG 19(1) 1-96 (1985)
ISSN 0013-936X

Credits: pp. 8, 11, courtesy New York State Department of Health, Center for Laboratories and Research, Photography Unit; p. 14, courtesy Southern Company Services; p. 16, courtesy Oak Ridge National Laboratory; p. 21, *ES&T*'s Julian Josephson
Cover: Courtesy Cliff Davidson, Carnegie-Mellon University

43
Chemical and biological characterization of organic material from gasoline exhaust particles. Tomas Alsberg,* Ulf Stenberg, Roger Westerholm, Michael Strandell, Ulf Rannug, Annica Sundvall, Lennart Romert, Vibeke Bernson, Bertil Pettersson, Rune Toftgård, Bo Franzén, Maria Jansson, Jan Åke Gustafsson, Karl Erik Egebäck, and Gunnar Tejle

Crude particle extracts are shown to have genotoxic, AHH-inducing, and PAM-cytotoxic components.

51
Impact of tubificid oligochaetes on pollutant transport in bottom sediments. Samuel W. Karickhoff* and Kenneth R. Morris

Results show that sediment pumping by oligochaete worms is a major pollutant transport mode, whereas sediment pelletization severely retards pollutant exchange with water.

57
Partition coefficients of organic compounds in lipid–water systems and correlations with fish bioconcentration factors. Cary T. Chiou

This study identifies the thermodynamic factors affecting the lipid–water partition coefficient (K_{tw}) and evaluates its relationship to K_{ow} and BCF.

63
Mutagenic transformations of dilute wood smoke systems in the presence of ozone and nitrogen dioxide. Analysis of selected high-pressure liquid chromatography fractions from wood smoke particle extracts. Richard Kamens,* Douglas Bell, Andrea Dietrich, Jean Perry, Randall Goodman, Larry Claxton, and Sylvestre Tejada

Chemical fractionation of unreacted and O_3-reacted and NO_2-reacted wood smoke indicates that most of the mutagenicity is contained in the most polar fractions.

70
2,4-Dinitrophenylhydrazine-coated Florisil sampling cartridges for the determination of formaldehyde in air. Frank Lipari* and Stephen J. Swarin

These cartridges provide a low-cost, simple, and sensitive method for ambient HCHO analysis that has many potential environmental applications.

■ 74
Photosensitized transformations involving electronic energy transfer in natural waters: Role of humic substances. Richard G. Zepp,* Patricia F. Schlotzhauer, and R. Merritt Sink

Steady-state concentrations and energy content of the excited species involved in humus-sensitized photoreactions of aquatic pollutants are reported.

82
Surface area and porosity of coal fly ash. Mark R. Schure,* Pat A. Soltys, David F. S. Natusch, and Thad Mauney

The surface area and porosity of fly ash are found to depend on particle size, surface morphology, and carbonaceous particle content.

87
Rate constants for the gas phase reactions of NO_3 radicals with furan, thiophene, and pyrrole at 295 ± 1 K and atmospheric pressure. Roger Atkinson,* Sara M. Aschmann, Arthur M. Winer, and William P. L. Carter

These data indicate that nighttime reaction with the NO_3 radical can be an important, and even dominant, loss process for the three organics studied.

90
On the constancy of sediment–water partition coefficients of hydrophobic organic pollutants. Philip M. Gschwend* and Shian-chee Wu

Microparticles or organic macromolecules released from sedimets and soils to water during sorption tests cause observed partition coefficients to decline with increased solids concentration.

* To whom correspondence should be addressed.

■ This article contains supplementary material in microform. See ordering instructions at end of paper.

ES&T
GUEST EDITORIAL

The road not taken

Two roads diverged in a narrow wood . . . I took the one less traveled by, and that has made all the difference.
—Robert Frost

U.S. environmental policy over the next four years will be strongly shaped by decisions President Reagan and his advisers make during the next few weeks. These decisions obviously include how environmental programs will be treated in the proposed budget and what positions the administration will adopt toward the long list of major environmental statutes that are up for reauthorization. Underlying all these specific issues, however, is a more fundamental choice of direction: Will President Reagan articulate a positive conservative agenda for achieving environmental quality, or will he once again discount it as a low-priority distraction from other issues such as budget reduction, tax changes, and deregulation?

Environmental policy today presents a fragile but important opportunity for creative rethinking and improvement. Existing policies have left many environmental problems unsolved, and some existing policies are themselves part of the problem. The regulatory laws of the 1970s have achieved progress in some areas, but they are poorly coordinated and sometimes costly, and some are quite limited in their environmental effectiveness.

This rethinking could draw substantially on conservative principles and could make a major positive contribution, if it carries a clear administration commitment to the goals of environmental quality. If that commitment is demonstrated, real reforms could be achieved; if it is not, we could easily see another round of frustrating polarization as everyone digs in to protect the status quo.

What might a positive rethinking look like?

Risk priorities. EPA Administrator Ruckelshaus put great effort into developing a management system for setting sensible priorities among risks. This foundation can be built on both within EPA and across the regulatory agencies.

Cross-media pollution. Fragmented laws and programs for air, water, and land are a serious problem both for business and environmental effectiveness. Basic thinking and experiments have been done, and some further steps could be taken administratively; the most ambitious agenda would be to use this problem as a framework for considering all the proposed statutory reauthorizations together, but this clearly would require the strongest possible commitment and credibility to succeed.

Cutting harmful subsidies. Serious budget balancing gains little from cutting environmental regulation and research budgets, but major savings could come from cutting subsidies that are both economically wasteful and environmentally damaging, such as some water and energy projects and agricultural programs. If free-market principles do matter, here is a place to apply them.

Research directions. Environmental research is an essential basis for sensible decisions, but it has been hampered in recent years by short-term regulatory priorities and by budget cuts. The Council on Environmental Quality and the National Science Foundation have recently completed an agenda of long-term research needs, with strong input from the scientific community; if such an agenda is coupled with stable or modestly increased funding, better decisions could result.

A positive conservative agenda to achieve environmental protection remains thus far a road little traveled; President Reagan could make a great difference if he chooses to take it. The choice is his, but the opportunity to make it will be brief.

Richard N. L. Andrews *is professor of environmental sciences and engineering and director of the Institute for Environmental Studies at the University of North Carolina at Chapel Hill.*

0013-936X/84/0916-0003$01.50/0

ES&T LETTERS

Zimmerman Award

Dear Sir: The Central Wisconsin Section of the American Chemical Society, in conjunction with Zimpro, a subsidiary of Sterling Drug, is seeking nominations for the F. J. Zimmerman Award in Environmental Science. The award, which consists of $1000 and a plaque, is given annually to an individual whose research has had a significant influence on environmental protection.

The award announcement and presentation will be made at the ACS 19th Great Lakes Regional Meeting, June 10–12, 1985, at Purdue University in West Lafayette, Ind. The award recipient will be invited to present an overview of the scientific contributions upon which the award is based.

Any scientist residing in the U.S. is eligible for the award. Nomination forms are available from L. A. Ochrymowycz, Department of Chemistry, University of Wisconsin—Eau Claire, Eau Claire, Wis. 54701. Nomination forms and supporting documents must be received no later than Feb. 1, 1985.

Clarence A. Hoffman
Zimpro
Rothschild, Wis. 54474

Editorial opinion

Dear Sir: I have enjoyed *ES&T* for several years and have obtained a good deal of useful information from it, but your editorial in the November 1984 issue (p. 323A) disturbed me very much. Your statement that President Reagan "stands firmly in opposition to the continuity of the nonpartisan environmental progress of the past 14 years," is certainly not the opinion of most of us in the regulated community. During the Carter administration we did not have nonpartisan environmental participation; Congress and EPA, listening primarily to the so-called environmentalists, passed laws and regulations like a runaway train. It's my opinion and certainly that of others, that President Reagan has brought the common sense into the picture that was lacking during the 1970s. It's obvious you have not been out here in the environmental trenches or you would not have written such an absurd editorial.

As *ES&T* is supposed to be nonpartisan and is purchased by the regulated community as well as environmentalists, I suggest you stick to science and technology.

B. M. Beal
Manager, Environmental Activities
Columbia Nitrogen Corporation
Augusta, Ga. 30913

Acid rain research

Dear Sir: I have just finished reading "Red herrings in acid rain research," (June 1984, p. 176A). I found the article very interesting, but I am most concerned about the conclusions.

I realize that feature articles are not subject to the same criteria, accuracy, and objectivity as research papers and that they serve as discussion initiators. I found the conclusions to be inflammatory and not based at all on the facts presented in the body of the discussion.

Am I mistaken in my view of the role of feature articles? I was caught completely off guard when I came to the section on conclusions.

Carlos M. Bowman
Associate Editor
Journal of Chemical Information and Computer Sciences

Dear Sir: As chairman of a company largely devoted to solving environmental problems, I should applaud "Red herrings in acid rain research," because such articles directly or indirectly contribute to my organization's welfare.

However, I do not applaud. I think that the tone and slant of the article are unscientific and therefore inappropriate. The title of the article is emotional and indicates an extreme bias. The basic conclusion by the authors appears to be that all information is in; let's get on with it. Yet they also say: "It is easy to suggest a whole series of alternative, and often unlikely, explanations of the causes and consequences of acid deposition." I suggest that if it is

(*continued on p. 13*)

ES&T
CURRENTS

INTERNATIONAL

An approach to pest management now in use in New Zealand is to find and catalog pest-resistant plants, Douglas Wright of that country's Ministry of Agriculture told a meeting of the Chemical Society of Washington. New Zealand is also making increasing use of pest enemies, such as the milky spore disease. Wright said that more emphasis is being placed on organic methods of agriculture, with less reliance on chemicals than is found in the U.S. or the European Economic Community. He added that conservation of natural resources is now a major public issue in New Zealand.

FEDERAL

EPA has proposed risk assessment guidelines that would set forth the approach the agency will use to estimate the public health risk of pollutants. The guidelines will be used to assess risk of carcinogenicity, mutagenicity, and teratogenicity. Guidelines for systemic toxicity are still under development and will include effects such as liver damage, other organ malfunction, neurobehavioral toxicity, and cardiopulmonary damage. The guidelines were developed for internal use by EPA scientists, but EPA is seeking their review by other scientists in the public and private sectors.

Dioxin (2,3,7,8-TCDD) has been found at Fort A. P. Hill, near Fredericksburg, Va., at levels ranging from 3 to 228 parts per billion (ppb). The maximum safe level is set at 1 ppb. The source of the TCDD is a shed in which the U.S. Army stored liquid herbicides until 1978. One reason for great concern is that Boy Scouts from the U.S. and 28 other nations held a jamboree at the site in 1981, and some camped within a 150-ft radius of the shed where the dioxin was found. A pesticide expert has recommended that those believing themselves to have been exposed to the dioxin seek medical advice. Although Army spokesmen discount any hazard, they say the Army will move aggressively to clean up the area.

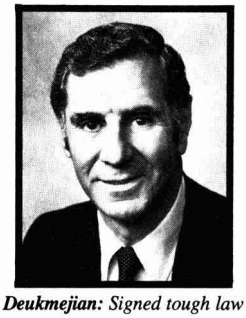
EPA is amending "Guidelines Establishing Test Procedures for the Analysis of Pollutants" (49 *Federal Register,* Oct. 26, 1984, p. 45234). Among the changes are new procedures for testing and quality control for analyzing priority toxic organic pollutants, a new procedure for carbonaceous biochemical oxygen demand (CBOD), certain spectroscopic methods for metal analysis, and requirements for sample holding times and preservation. The CBOD method was published in November; the others will be published Jan. 24, 1985.

EPA has proposed that most non-wood-preservative uses of pentachlorophenol be prohibited on the basis of data showing that the chemical causes birth defects in the offspring of laboratory animals. Pentachlorophenol contaminants, such as hexachlorobenzene and hexachlorodibenzo-*p*-dioxin, are suspected of causing cancer in laboratory animals. Pentachlorophenol is a defoliant, disinfectant, herbicide, moss controller, and antimicrobial agent. Approximately 50 million lb/y is made in the U.S., of which 20% is used as a wood preservative. Use of pentachlorophenol as a wood preservative continues to be allowed, although it is stringently regulated.

As of October, EPA had identified 18,886 potentially hazardous waste sites under its Environmental Response and Remedial Information System (ERRIS). The number of sites could reach 22,000. Preliminary assessments have been conducted at 10,777 of the sites, and initial investigations have started at 4115 ERRIS sites. Based on data collected, risks from these sites are scored for the migration of hazardous substances through groundwater, surface water, and air. Those sites that receive scores above a given level are placed on the National Priorities list after a process of proposal, comments, and possible rescoring after comments have been considered.

STATES

There are widespread threats to groundwater in California's Silicon Valley if preliminary results of an EPA study of the area are borne out. In Santa Clara County alone, there may be as many as 1300 contamination sources, of which about 300 are high-technology manufacturing plants. EPA representatives attribute the groundwater threats from the high-tech sources to solvents and other chemicals that leak from underground storage tanks or are spilled on the ground. EPA has proposed listing 19 toxic waste sites in Santa Clara County for priority cleanup under Superfund. This would be more than the total for any other county in the U.S. The agency is looking into regulatory options for reducing the contamination and preventing its recurrence.



Deukmejian: *Signed tough law*

The Toxic Pits Cleanup Act of 1984, said to be the toughest legislation of its kind in the U.S., has been signed into law by Governor George Deukmejian of California. It extends federal regulations for new ponds to all existing ponds in that state. The law also calls for schedules for closing some types of ponds, establishing leak detection and groundwater monitoring systems, and completion of large-scale geohydrological studies. Moreover, no liquid hazardous waste may be disposed of to an impoundment after Jan. 1, 1989, unless leachate collection and monitoring sys-

0013-936X/84/0916-0005$01.50/0

tems are installed. Deukmejian also signed a law that will require permits for tanks containing certain hazardous substances. The list of those substances covered under the law will be compiled by the state department of health.

New York State has banned commercial fishing for five species in the Hudson River—goldfish, pumpkinseed, brown bullhead, black crappie, and carp. These fish join the striped bass; commercial fishing for this species was prohibited in 1976 when the bass were discovered to have 5 ppm or more of polychlorinated biphenyls (PCBs) in their flesh. The new ban, to take effect March 15, is not because of a higher PCB content in the fish or the river, but because the Food and Drug Administration lowered the permissible PCB content in fish from 5 ppm to 2 ppm last August. Shad, the major commercial fish taken in the Hudson, are not affected by the ban.

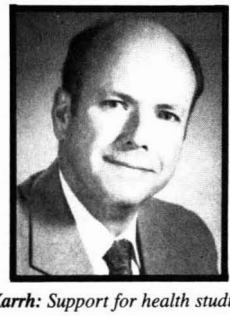
EPA has ordered new monitoring wells for a site at Niagara Falls, N.Y., to test whether the site is indeed leaking. The site is a repository for some of the wastes taken from Love Canal. A draft report from EPA headquarters suggested that the site, operated by Cecos International, was leaking through underground liners beyond its southern boundary. Others at EPA, the New York Department of Environmental Conservation (DEC), and Cecos, however, disputed the report. Some EPA and DEC officials suggested that the contamination came from an adjoining site not operated by Cecos; several EPA Region 2 officials have said that there is no conclusive evidence of leakage from the Cecos site.

VIEWPOINT

Any amended Clean Air Act should contain procedures for accumulating data on health effects of individual pollutants and should focus on pollutants "which, when targeted, will be most likely to reduce health hazards," says the Air Quality Task Force of the American Institute of Chemical Engineers, in a report drafted by Jacoby Scher of Resource Engineering (Houston, Tex.). "The EPA should first address those chemicals which are most widely used and are most generally suspected to cause adverse health effects," according to the task force. Also suggested is a special study of those chemicals with documented toxicity, carcinogenicity, and mutagenicity. The task force has called for up-to-date risk assessment and management language in a reauthorized air law.

SCIENCE

Before a short-term national ambient air quality standard for nitrogen dioxide can be established, more research on the short-term effects of NO_2 is needed, says Morton Lippmann, chairman of EPA's Clean Air Scientific Advisory Committee (CASAC). According to Lippmann, the present standard of 0.053 ppm protects adequately against health effects associated with long-term exposure and protects to some extent against short-term effects. Lippmann added that CASAC members also believe that a more stringent secondary standard to protect public welfare is not needed, because the primary standard offers enough protection against known effects.



Karrh: *Support for health studies*

"There is little conclusive evidence of serious health effects from chemical waste disposal sites," according to a year-long study directed by the Universities Associated for Research and Education in Pathology (UAREP). The Chemical Manufacturers Association (CMA, Washington, D.C.) was one of five sponsors of the UAREP study, which generated a 690-page report. The study's scientific team also called for continued research to identify and eliminate long-term threats to health from chemical waste exposure. "The chemical industry . . . will continue to support such health studies," said Bruce Karrh of Du Pont. Karrh is chairman of a CMA group that will review the study in detail.

How do oceans function as ecosystems? Dale Kiefer of the University of Southern California proposes that satellites, computers, and "old-fashioned mathematical equations" will help to answer that question. For example, biologically productive areas could be identified by remote sensing of color and temperature. Also, because controlled experiments cannot generally be conducted, Kiefer says that "informed guesses [must be made] about the interaction of such factors as turbulence, temperature, and the like." These guesses must be turned into mathematical formulas and then tested in field studies. Kiefer has already conducted such studies in large portions of the Pacific Ocean.

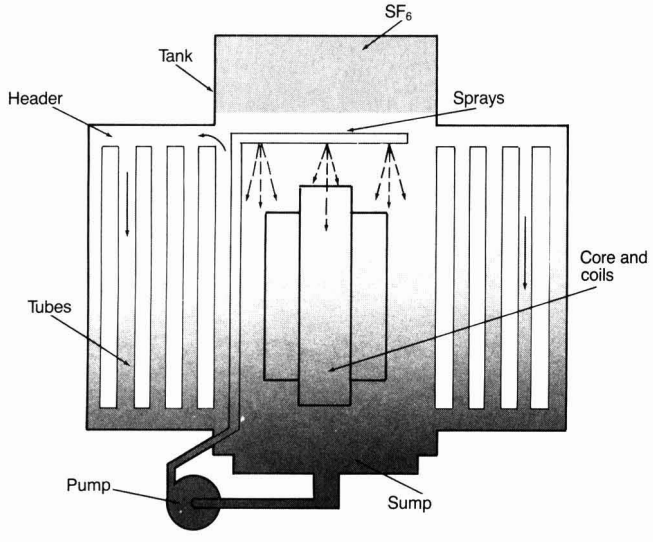
"Pesticide residues in your bloodstream may rival death and taxes as inevitabilities in your life if preliminary results of a supersensitive test prove universal." So says Victor Alexander, medical director of Enviro-Health Systems (New Orleans, La.) about the chlorinated pesticide screening test (CPST) that his company has developed. The CPST can measure 19 of the more common pesticides in blood in concentrations as low as 0.5 ppb. The pesticides include heptachlor, dieldrin, and DDT-related compounds. Alexander suggested that because many of these chemicals are lipophilic, they may be 300–500 times more concentrated in fatty tissue than they are in blood.

TECHNOLOGY

The nation's first fuel cell powered by coal bed methane gas will supply nearly 50% of the energy required by a student recreation center at the University of Alabama at Tuscaloosa. Installed by Southern Company Services (SCS), the cell produces power through an electrochemical process that is said to be virtually pollution free. The coal bed gas, previously considered a waste product of mining, will come from a seam about 2000 ft below the surface of the campus. The combustion process will convert this gas entirely to carbon dioxide, water, and heat, says SCS vice-president S. R. Hart, Jr. The cell, which is about the size of an industrial air conditioner, is being tested under actual operating conditions.

Agricultural potato plants may be protected against insect pests by cross breeding with a species of wild potato, *Solanum berthaultii,* which is found in Bolivia. The wild potato's leaves secrete substances that entrap some insects and are toxic or repellent to others. Researchers at Cornell University hope to develop a potato plant that will retain high-quality tubers while it resists insect pests.

SF_6–fluorocarbon cooling technique



Source: © 1984, *EPRI Journal*, Vol. 9, No. 8, p. 18. Reprinted with permission.

One possible substitute for polychlorinated biphenyls (PCBs) to cool transformers is a two-phase (gas and liquid) approach. This technique uses sulfur hexafluoride (SF_6) gas to do the initial cooling until a liquid, such as Freon, can take over and work through a vaporization and condensation cycle. A second substitute is a three-to-one mixture of perchloroethylene (the coolant) and oil. The oil lowers the freezing point of the coolant. Even with the addition of oil, the coolant's fire resistance is retained because the oil's boiling range (125–150 °C) allows the coolant to limit "hot spots" through a special vaporization and condensation cycle. Both approaches have been tested on prototype units, some at utilities in the eastern U.S.

Dioxin could be removed from industrial wastewater by means of adsorption onto clay, Timothy Nolan of the University of Michigan told the American Institute of Chemical Engineers. The key to the adsorption process is humic acid immobilized on clay pretreated with aluminum compounds. These compounds promote binding between the acid and the clay substrate. In a stream of wastewater, the humic acid–clay compound interacts with and sorbs the dioxin. Nolan said that dioxin concentrations were lowered to below the current detection level and that a more detailed study of this method is planned.

BUSINESS

The Great Plains Coal Gasification Project (Beulah, N.D.), which was begun during the mid-1970s, is now producing pipeline quality gas after completion of the plant at a cost of $2.1 billion. This cost is within the construction budget when one allows for the high rate of inflation of the 1970s. The plant will need federal subsidies because at the time it was planned, a world oil price of $45/bbl or more was expected. Such a high price would have made the plant economical. There are plans to guarantee revenues equivalent to $58/bbl for the first three years of the plant's operation and $43.50/bbl for the following seven years (the current world oil price is $28/bbl). The plant will eventually convert 14,000 tons per day of coal to about 137 million cubic feet per day of gas.

EPA may exempt small chemical manufacturers and importers from many reporting and record keeping requirements of Section 8(a) of the Toxic Substances Control Act. This section mandates record keeping and reporting on specified chemicals and chemical mixtures when EPA believes it is necessary for effective enforcement of the act. A chemical maker or importer is considered small if its total sales are less than $40 million annually and its production in a single plant the maker owns or controls is less than 100,000 lb a year. Alternatively, a manufacturer is small if its total sales are less than $4 million a year, regardless of the amount of production. Changes in the exemption are on a chemical-specific basis only.

The Chemical Transportation Emergency Center (CHEMTREC), a service of the Chemical Manufacturers Association, has been expanded to Canada. Canadian emergency services can now call CHEMTREC using a toll-free number. Previously available only in the U.S., CHEMTREC acts as a relay service for emergency calls from Canada and passes information it receives on to CANUTECH, a Canadian government agency that handles chemical transportation incidents. CANUTECH will notify U.S. shippers if their chemical products are involved in a transportation incident in Canada.

The total number of operable nuclear power plants in the U.S. reached 86 when Pennsylvania Electric Company received a low-power operating license from the U.S. Nuclear Regulatory Commission late in 1984. The utility's Limerick-1 plant will serve approximately 1.3 million customers in southeastern Pennsylvania when it begins commercial operation during the third quarter of 1985. According to Pennsylvania Electric, the new plant will allow the utility to retire oil-fired plants that are costly and inefficient.

Dow Chemical Company has received a draft EPA order to seal over soil at its facility in Midland, Mich., which is reportedly contaminated with up to 52 parts per billion of tetrachlorodibenzo-*p*-dioxin (TCDD). EPA stated that TCDD was being dispersed into the surrounding environment by the wind and on car wheels and pedestrians' feet. Dow answered that TCDD was found in only two areas and that one site had already been sealed over by the company. Company representatives also said that there was no threat to Midland residents and that contamination was not coming from its plant but from other sources, such as an old incinerator.

ES&T FEATURES

Laboratory performance in proficiency testing

The New York State experience

James C. Daly
Kurt E. Asmus
Wadsworth Center for Laboratories and Research
New York State Department of Health
Albany, N.Y. 12201

The New York State Department of Health has responsibility for certifying environmental laboratories (*1*). Since 1978, it has tested these laboratories for proficiency with two bacteriological and thirty-one chemical parameters. In 1977 New York assumed primacy (*2, 3*) for the certification of laboratories involved in the analysis of drinking water under the provisions of the Safe Drinking Water Act of 1974.

Recently, the department was given the authority under state law to certify environmental laboratories for wastewater analysis. This discussion will deal with the proficiency testing aspect of the state certification program and will focus on proficiency tests for drinking-water analysis that have been conducted over the past five years. On average, 91% of the laboratories tested have shown satisfactory performance in the analysis of bacteriological and inorganic chemical parameters, and 80% have had satisfactory performance for the organic parameters.

Quality assurance and the certification of environmental laboratories have been discussed in a number of papers since the enactment of the Clean Water Act and the Safe Drinking Water Act (*4–10*). Only three of these (*8–10*) address proficiency testing, and their scope is limited by the small number of laboratories participating or the short time spans involved. Approximately 140 bacteriology laboratories and 90 chemistry laboratories participate in the

An analyst checks samples under the microscope to ensure compliance with test procedures

0013-936X/84/0916-0008$01.50/0

New York State proficiency testing program. Typically, a bacteriology proficiency test generates some 1600 results, and a chemistry proficiency test produces more than 3500 results. During the five-year period from 1978 to 1982 some 20,000 pieces of data were accumulated. The use of approval categories allows this amount of data to be analyzed manageably and forms the basis for judging the performance of the laboratories that participate in the testing program.

Description of the program

The Department of Health conducts semiannual proficiency tests for the bacteriology and chemistry parameters included in the Drinking Water Analyses Proficiency Testing Program. The parameters in each of the drinking water approval categories are listed in Table 1 with a brief summary of the program in Table 2. The holding medium and procedures used for bacteriology proficiency testing may be of particular interest; they are described elsewhere by Toombs and Connor (*11*).

Certification is given to laboratories by approval category rather than parameter-by-parameter. Meeting two criteria determines satisfactory performance in a particular approval category. Performance is unsatisfactory in a given category if more than one-third of the reported results are outside acceptance limits or if the same parameter is outside acceptance limits on two consecutive proficiency tests. Participants in the proficiency tests are issued individual performance reports for each approval category in which they compete.

Unsatisfactory performance in an approval category does not automatically result in loss of certification. The regulations allow some discretion and an approved laboratory ordinarily is given the opportunity to show cause why it should not lose its approval. On the other hand, a candidate laboratory is automatically denied approval if it performs unsatisfactorily in a proficiency test. Approved laboratories often are able to determine and correct problems that lead to unsatisfactory performance and it is not often that a laboratory has to be decertified.

Proficiency test acceptance limits are determined from a statistical analysis of the data reported by the participants. For chemical parameters, each data base is first screened by using limits of 0.25 times the known value and twice the known value. Results outside these limits are discarded. Following preliminary screening, outliers are rejected using the 99% confidence interval as the limit. Finally, the acceptance limits are determined by using the 95% confidence interval about the mean.

Bacteriology data are handled somewhat differently. Total coliform results derived from multiple-tube data require the use of the mode (the value that occurs with the greatest frequency), with acceptance limits selected from the 95% confidence limit for the most probable number index corresponding to that mode. Coliform results from membrane filter data are handled in much the same way as chemistry data in that a mean and a standard deviation are used to set the acceptance limits. Again, the 95% confidence interval is used to establish the acceptance limits. There is no initial screening for membrane filter data since there are no known values.

Some typical acceptance limits for inorganic parameters are given in Table 3. These acceptance limits range from ±11% for chloride sample (2), to ± 51% for selenium. As one would expect, the acceptance range increases as the concentration decreases, particularly as the detection limit is approached. This is readily apparent in the chloride and fluoride data.

Typical acceptance limits for organic parameters are presented in Table 4. Some of the organic parameters, in particular the pesticides and herbicides, presented more difficulty to the environmental laboratory community. Although the percentage of passing laboratories in the pesticide and herbicide category averaged only 73% over a four-year period, performance in this category did improve, with 83% of the participants in the November 1982 proficiency test showing acceptable performance compared with an average of 59% in the 1979 tests. Acceptance limits range from ±20% for bromodichloromethane to ±70% for low-level silvex. Not surprisingly, the pesticide and herbicide category tends to have the widest acceptance limits.

We have used the 95% confidence interval to set acceptance limits from the start of our proficiency testing program. This confidence interval, coupled with adequate screening and outlier rejection, generally has resulted in reasonable acceptance limits. Occasionally, the acceptance limits for herbicides calculated in this manner turn out to be more generous than we like. There seem to be no standard criteria for setting acceptance limits. EPA has used three-standard-deviation limits in its water pollution performance studies and two-standard-deviation limits in water supply performance studies (*12*). The International Joint Commission on the Great Lakes uses an entirely different approach in setting acceptance limits for the round-robin performance studies conducted by its data quality work group (*13*). Acceptance limits for its studies are empirically determined, based on the level of performance achieved by a group of peer laboratories that give "good" performance. The premise of this method is that all participants should be able to approach the performance level of their peers.

Five-year performance summary

A summary of performance for the five-year period from 1978 to 1982,

TABLE 1
Parameters included in approval categories

Category	Parameters
Water bacteriology	Total coliform, standard plate count
Wet chemistry	Chloride, fluoride, nitrate, sulfate
Trace metals	As, Ba, Cd, Cr, Cu, Fe, Pb, Mn, Hg, Se, Na, Ag, Zn
Volatile haloorganics	Chloroform, bromoform, dichlorobromomethane, dibromochloromethane, carbon tetrachloride, trichloroethylene, tetrachloroethylene, 1,1,1-trichloroethane
Pesticide and herbicide	Endrin, lindane, methoxychlor, toxaphene, 2,4-D, silvex

TABLE 2
Description of proficiency testing program

Approval category	Sample matrix	No. of parameters	No. of samples
Water bacteriology	Holding medium	2	12
Wet chemistry	Ampule concentrate	4	2
Trace metals	Ampule concentrate	13	2
Volatile haloorganics	Ampule concentrate	8	2
Pesticides and herbicides	Ampule concentrate	6	2

TABLE 3
Inorganic acceptance limits for November 1982 proficiency test

Parameter	Sample concentration	Acceptance limit (%)	Parameter	Sample concentration	Acceptance limit (%)
Chloride (1)	4.67 mg/L	±28	Zinc (1)	762	±12
Chloride (2)	32.7	±11	Zinc (2)	2280	±11
Fluoride (1)	2.41	±13	Barium (1)	1020	±16
Fluoride (2)	0.456	±24	Barium (2)	1270	±15
Nitrate (1)	0.410	±26	Chromium (1)	49.1	±23
Nitrate (2)	2.00	±23	Chromium (2)	20.1	±34
Sulfate (1)	84.2	±12	Iron (1)	197	±25
Sulfate (2)	37.4	±14	Iron (2)	570	±11
Arsenic (1)	110 μg/L	±44	Manganese (1)	112	±15
Arsenic (2)	46.1	±44	Manganese (2)	25.6	±23
Selenium (1)	32.5	±51	Mercury (1)	2.39	±30
Selenium (2)	14.1	±48	Mercury (2)	3.76	±34
Cadmium (1)	36.7	±19	Sodium (1)	805	±22
Cadmium (2)	13.8	±38	Sodium (2)	3880	±16
Lead (1)	123	±22	Silver (1)	46.0	±23
Lead (2)	79.1	±22	Silver (2)	81.0	±14
Copper (1)	1510	±8			
Copper (2)	474	±9			

TABLE 4
Organic acceptance limits for November 1982 proficiency test

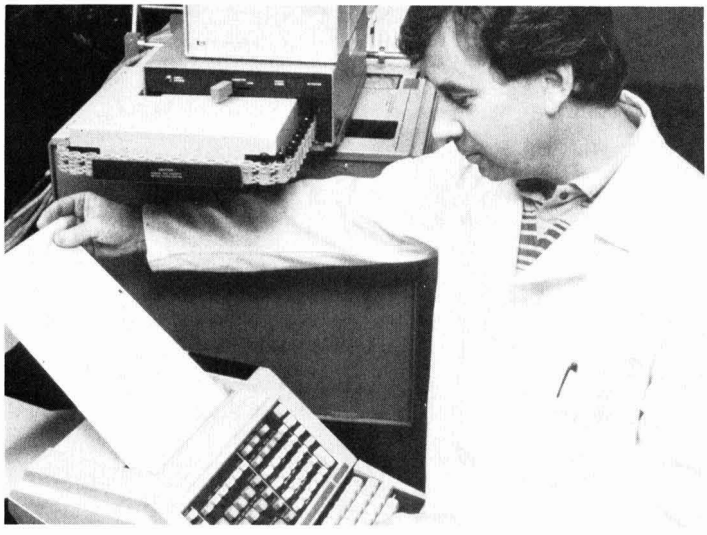
Parameter	Sample concentration (μg/L)	Acceptance limit (%)	Parameter	Sample concentration (μg/L)	Acceptance limit (%)
Chloroform (1)	23.0	±28	1,1,1-Trichloroethane (1)	5.18	±36
Chloroform (2)	33.9	±27	1,1,1-Trichloroethane (2)	2.58	±38
Bromoform (1)	52.3	±42	Endrin (1)	0.119	±46
Bromoform (2)	42.9	±37	Endrin (2)	0.380	±38
Dibromochloromethane (1)	14.9	±33	Lindane (1)	0.395	±38
Dibromochloromethane (2)	73.7	±35	Lindane (2)	0.119	±54
Dichlorobromomethane (1)	50.9	±24	Methoxychlor (1)	3.58	±50
Dichlorobromomethane (2)	26.0	±22	Methoxychlor (2)	1.50	±38
Trichloroethylene (1)	10.7	±33	Toxaphene (1)	2.10	±39
Trichloroethylene (2)	4.35	±31	Toxaphene (2)	6.17	±40
Tetrachloroethylene (1)	3.28	±47	2,4-D (1)	13.5	±56
Tetrachloroethylene (2)	11.2	±33	2,4-D (2)	0.356	±67
Carbon tetrachloride (1)	4.43	±37	Silvex (1)	18.9	±55
Carbon tetrachloride (2)	2.27	±58	Silvex (2)	0.142	±69

based on the regulatory criteria (Figure 1), shows the breakdown of laboratories failing due to overall scores below 67%, those failing because they missed the same parameter on two consecutive tests, and the percentage passing in each test.

In the water bacteriology and wet chemistry categories, more than 90% of the laboratories participating showed satisfactory performance over the five-year period. In the trace metal category, an average of 82% had satisfactory performance during the same period. However, a dip to 68% occurred in November 1978, when a number of failures were attributable to laboratories' missing the same metal parameter on two consecutive tests. Over the five-year period, 58 laboratories failed in the trace metal category for this reason, whereas only 16 failed because of a low score.

Pesticide and herbicide proficiency testing commenced in 1979. Satisfactory performance in this category fluctuated from a low of 50% in the December 1979 test to a high of 83% in the November 1982 test. In the volatile haloorganics category 88% of the laboratories passed in the nine tests.

A comparison of the performance of candidate laboratories vs. approved laboratories in the 1981 and 1982 proficiency tests shows that candidate laboratories have a significantly higher failure rate. This is true in all categories. In wet chemistry, candidate laboratories have a failure rate of 15% for the four tests, compared with 4% for approved laboratories. Similarly, for trace metals the failure rate for candidate laboratories is 34%, compared with 10% for approved laboratories. The difference in the failure rate is less marked for the volatile haloorganics and the pesticides and herbicides, with candidate and approved laboratories having failure rates



An analyst reads a computer printout from an analytical instrument

of 20% and 9% for volatile haloorganics and 29% and 11% for pesticides and herbicides, respectively.

Our experience indicates that proficiency testing is an incentive for laboratories to maintain a high level of performance and that it generally leads to improvement. Those laboratories that cannot meet performance standards simply do not become certified or they lose their approval.

The 1982 tests, for example, resulted in 14 candidate laboratories being denied approval and three approved laboratories having their approval terminated in the inorganic chemistry categories on the basis of their performance in the proficiency tests. During this same one-year period, 18 candidate laboratories were denied approval and two laboratories had their approval terminated in the organic chemistry approval categories. Of the 11 laboratories giving unsatisfactory performance in the 1982 bacteriology proficiency tests, seven were denied approval. The remaining four were approved laboratories, and three had their bacteriology approval terminated.

Performance profiles

The performance summary presented in Figure 1 shows laboratory performance, but it does not indicate whether the laboratories are just meeting minimum criteria or are excelling. Although this is adequate for regulatory purposes, a more comprehensive picture is given by a profile of laboratory scores. Such a profile allows us to see what fraction of laboratories is exceeding, as opposed to just passing or meeting, minimum criteria.

A score of 88% or better on a performance test is defined as excellent, 67–87% is considered satisfactory, and less than 67% constitutes failure. A profile of laboratory performance for water bacteriology and the four chemistry approval categories (Figures 2–6) shows that more than 80% of the laboratories tested in the water bacteriology and wet chemistry categories routinely exceeded minimum standards. In the trace metal category 65–85% were rated excellent. For these three categories the failure rate generally was less than 10% during the five-year period. For the volatile haloorganics the percentage of laboratories in the excellent range was 56–88%, with a failure rate of less than 15%. Figure 6 shows a range from 42% to 74% excelling in the pesticide and herbicide category. The failure rate for this approval category initially was 33%; that fell to around 17% in the next four tests and leveled off to a respectable 7% in the last two tests.

Conclusions

The proficiency testing program has grown substantially since it began in 1978. Participation in water bacteriology testing, starting with 104 laboratories, is now about 140. Participants in the wet chemistry category have almost doubled; those in the trace metal category have more than doubled. From only eight participants in the volatile haloorganic category in 1979 and only six in the pesticide and herbicide category, the number of participants has increased to about 40 in each category. More important than the increase in numbers is the level of performance or improvement in performance during the study period. Averaged over five years, 88% of the laboratories tested were in the excellent range for water bacteriology, 86% for wet chemistry, 75% for both the trace metals and the volatile haloorganics, and 56% for pesticides and herbicides. The performance level for water bacteriology and the inorganics generally has been at a high level and has been fairly constant from the outset of the program. For trace metals and volatile haloorganics, however, it has been somewhat lower and has fluctuated more.

Only the pesticide and herbicide category (Figure 6) shows a definite trend toward improved performance as evidenced by the marked decrease in failure rate. These patterns probably reflect the fact that most environmental laboratories were at a fairly high level of competence in the bacteriology and inorganic categories when the proficiency testing program began in 1978, whereas the majority of laboratories participating in the organic categories were novices in the early proficiency tests.

There is little doubt that proficiency testing provides a strong incentive for environmental laboratories to maintain high standards of quality. Proficiency testing provides the Department of Health and other interested agencies with a tangible record of laboratory performance. The improvement in performance in the pesticide and herbicide category from 1979 to 1982 is testimony to the usefulness and efficacy of the proficiency testing program. It is noteworthy that in every approval category other than pesticides and herbicides the majority of laboratories have excelled in all of the proficiency tests.

An argument sometimes put forth by detractors of proficiency testing is that laboratories concentrate their best efforts on proficiency test samples (*8*). Although this may be true, their competency is nonetheless tested, even if it is not truly representative of their day-to-day operation. If a laboratory is indeed incompetent, it is not likely to do well on a proficiency test no matter what special efforts are made. The primary purpose of proficiency testing is to weed out incompetent laboratories; internal practices should document the day-to-day quality of laboratory measurements. Proficiency testing, coupled with on-site laboratory inspections and backed up by statutory authority, provides the best assurance of high-quality environmental data.

Acknowledgment

Before publication, this article was reviewed for suitability as an *ES&T* feature

by Lawrence Keith, Radian Corporation, Austin, Tex., and William H. Glaze, UCLA School of Public Health, Los Angeles, Calif.

References

(1) "Official Compilation of Codes, Rules and Regulations, Title 10, Health, Subpart 55-2"; New York State Department of Health: Albany, N.Y., January 1983.
(2) *Fed. Regist.* **1975,** 59566–88.
(3) "Manual for the Certification of Laboratories Involved in Analyzing Public Drinking Water Supplies, Criteria and Procedures," EPA 600/8-78-008; NTIS No. PB 287118; Office of Monitoring and Technical Support, Washington D.C., 1978
(4) Oatess, W. E. *Environ. Sci. Technol.* **1978,** *12,* 1124–27.
(5) Seeber, A. J. *Environ. Sci. Technol.* **1978,** *12,* 1128–30.
(6) Greenberg, A. E.; Hausler, W. J., Jr. *Environ. Sci. Technol.* **1981,** *15,* 520–22.
(7) Kirchmer, C. J. *Environ. Sci. Technol.* **1983,** *17,* 174–81A.
(8) Edwards, R. R.; Schilling, D. L., Jr.; Rossmiller, T. L. *J. Water Pollut. Control Fed.* **1977,** *49,* 1704–12.
(9) Regnier, J. E.; Gable, J. W., Jr.; Marsh, J. L. *Environ. Sci. Technol.* **1979,** *13,* 40–45.
(10) Marsh, J. L.; Gable, J. W.; Regnier, J. E. *Am. Lab.* **1980,** *12*(12) 55–59.
(11) Toombs, R. W.; Connor, D. A. *Appl. Environ. Miocrobiol.* **1980,** *40,* 883–87.
(12) "Updated Performance Evaluation Statistics for Estimating Acceptance Limits," memorandum from Paul W. Britton, EMSL Quality Assurance Branch to EPA Regional Quality Assurance Coordinators, April 25, 1983.
(13) "Report of the Data Quality Work Group"; International Joint Commission on the Great Lakes; Windsor, Ont., Canada, November 1980.

James C. Daly *(l.) is a principal chemist at the Wadsworth Center for Laboratories and Research of the New York State Department of Health. He heads the proficiency testing program for the Environmental Laboratory Accreditation Program. Daly received a B.S. in chemistry from Siena College and an M.S. in radiation biology from the University of Rochester. He has been with the Department of Health since 1960 and has been a quality assurance officer for the Division of Environmental Sciences in that department since 1975.*

Kurt E. Asmus *(r.) is a chemist at the Wadsworth Center for Laboratories and Research of the New York State Department of Health. He is a supervisor of the quality control laboratory and oversees the environmental laboratory proficiency testing operation. He received a B.S. in biochemistry from Syracuse University in 1968 and has been with the Department of Health since 1978.*

FIGURE 1
Performance summary based on regulatory criteria, 1978–1982

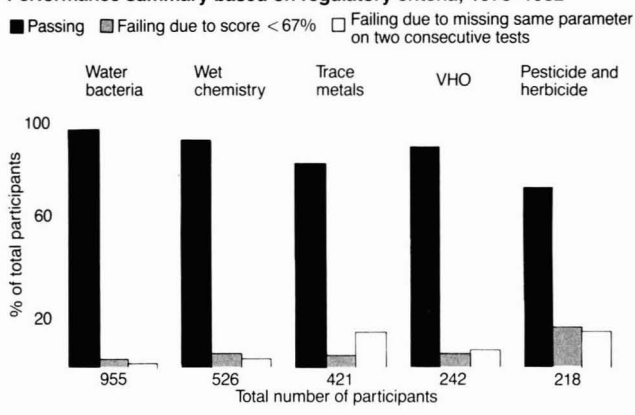


FIGURE 2
Water bacteriology performance profile

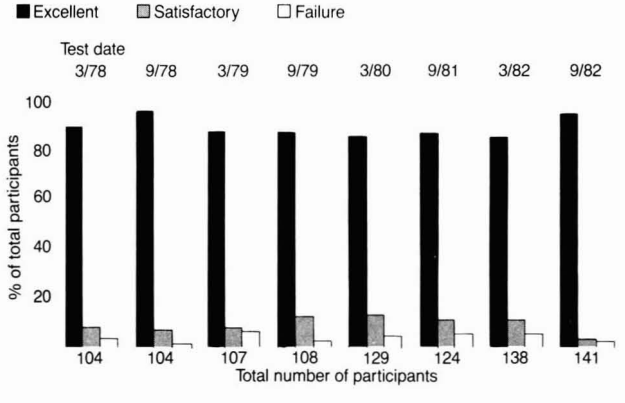


FIGURE 3
Wet chemistry performance profile

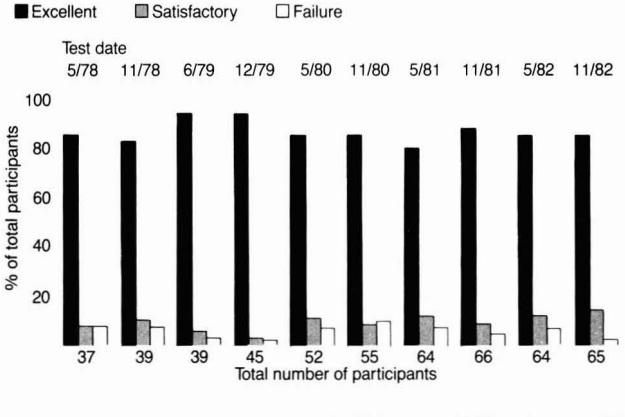


FIGURE 4
Trace metal performance profile

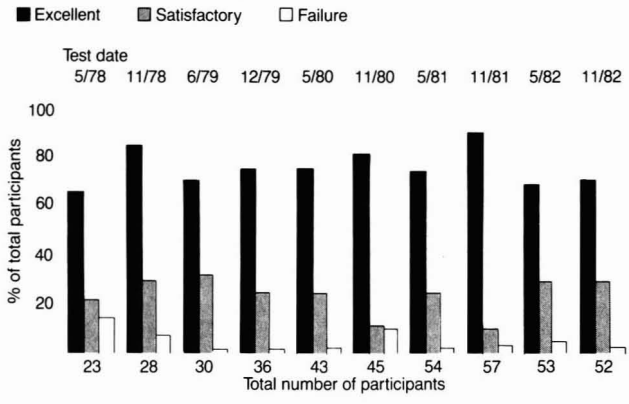


FIGURE 5
Volatile haloorganic performance profile

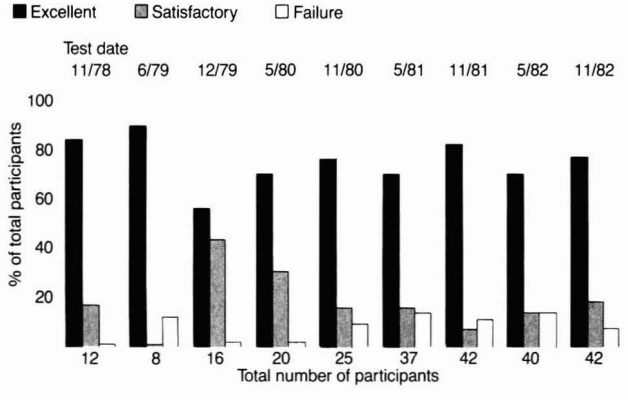
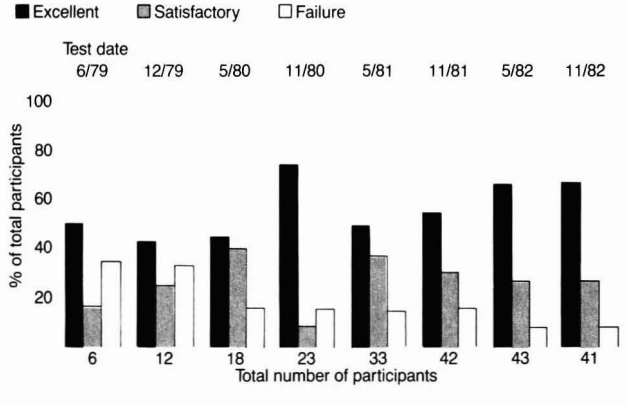


FIGURE 6
Pesticide–herbicide performance profile



(Letters, *from p. 4*)

all that easy and it takes scientists years "to conclude that the explanation is not valid" that the authors have indicated that the questions are valid, even if they are ultimately disproved.

It appears to me that before billions of dollars are spent to reduce sulfur emissions from stack gases all alternative explanations should be examined. And I also suggest that such research would best be done by people who do not have emotional attachments to their preconceptions.

James R. Dunn
Chairman
Dunn Geoscience Corporation
Latham, N.Y. 12110

Dear Sir: The authors of "Red herrings in acid rain research," have confused scientific facts with their desire to advocate a policy prescription. Let me highlight a few examples.

It is an accepted scientific fact that bog lakes are naturally acidic; it is also generally accepted that many lakes are not naturally acidic. It is agreed that some lakes have become acidified by land use practices and that some other lakes have been acidified through acidic deposition. Likewise, some fish populations have declined from acidification and other fish populations have declined for other reasons, both human-induced and natural.

More specifically, the Integrated Lake–Watershed Acidification Study (ILWAS), sponsored by the Electric Power Research Institute (EPRI), attempted to relate water chemistry to a mechanistic understanding of the processes including deposition. It is unfortunate (if it is true) that some may have concluded "largely as a result of [ILWAS]" that ". . . regional lake acidification cannot be due to acid precipitation." But it is equally unfortunate that some ignore the findings of ILWAS as to the importance of other factors in addition to deposition.

It is too bad that the authors feel it necessary to denigrate the work of others because the findings differ from what these authors would wish them to be. Such findings are neither red herrings nor smoke screens. Both those people who advocate reducing emissions and those who advocate waiting for the results of more research have argued their scientific cases as would good defense lawyers. That is, they display only the evidence supporting pre-

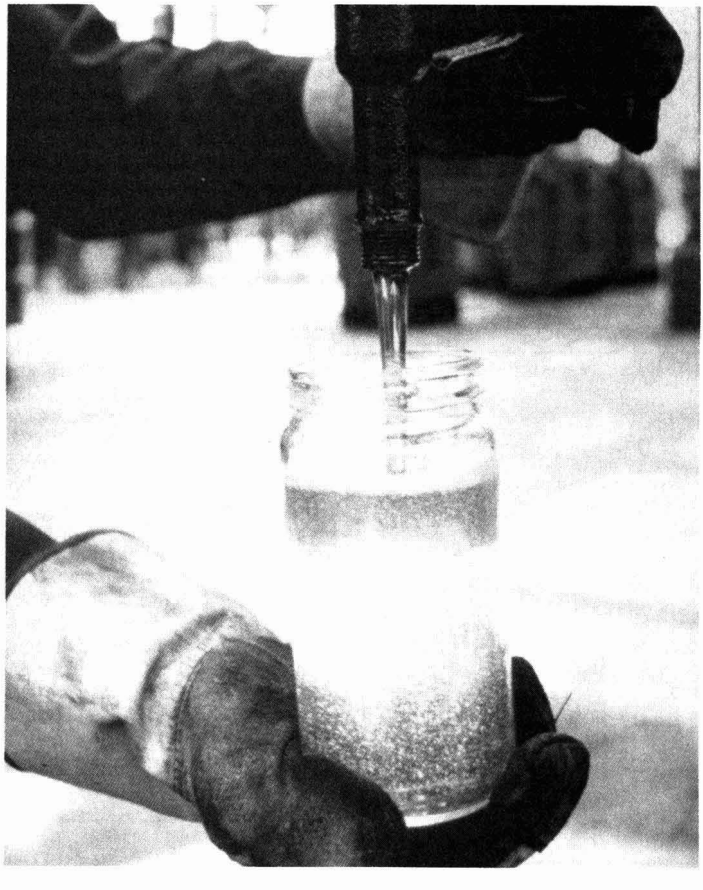
(*continued on p. 20*)

Coal liquefaction products

What are the environmental consequences when they are released to inland waters?

Jeffrey M. Giddings
Stephen E. Herbes
Carl W. Gehrs
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, Tenn. 37830

The development of a commercial synfuels industry will probably be accompanied by accidental releases of liquid products into freshwater environments. Based on the record of the petroleum industry, we estimate that a major spill (greater than 189 m^3 or 50,000 gallons) of coal-derived oil into freshwater is likely to occur approximately every 28 years, and smaller releases will occur more frequently (*1*). Because such releases pose potential damage to natural ecosystems and because information about the environmental properties of synthetic oils will be needed for compliance with the Toxic Substances Control Act and other laws, ecologists and environmental chemists have been investigating coal-derived liquids since the late 1970s. Although questions remain, enough has been learned to allow preliminary assessment of the environmental hazards of synthetic oils—especially coal liquefaction products—in freshwater systems.



Crude synthetic fuels produced by direct coal liquefaction generally differ chemically from petroleum in several respects, including greater aromaticity; higher concentrations of polar polycyclic aromatic hydrocarbons (PAHs), phenols, and nitrogen-containing compounds; and a greater number of (and shorter) alkyl side chain substituents on aromatic rings (*2*).

The environmental properties of synthetic oils depend on their chemical composition and can be analyzed according to the characteristics of the major classes of synfuel constituents. For each constituent class, information is needed both about environmental fate (dissolution, evaporation, sorption to particles, photodegradation, biodegradation, and uptake and transformation by organisms) and potential ecological effects (acute and chronic toxicity and indirect effects). In this paper we describe the forms and concentrations of oil constituents that might be found in the aquatic environment after accidental releases of coal liquids under various conditions, and we try to forecast the ecological effects that these contaminants might cause.

Environmental partitioning

When oil is released onto water, environmental processes immediately begin to partition the oil constituents according to their physical and chemical properties (Figure 1). Volatile components quickly evaporate from a floating slick; soluble components dissolve into the underlying water; and insoluble components emulsify, sink directly, or sorb to particles and eventually accumulate in bottom sediments.

The volatile components have a minimal effect on the aquatic system. Nevertheless, the water-soluble fraction (WSF) can contaminate drinking water supplies and have toxic effects on aquatic life. But because most water-soluble compounds in coal liquids are readily degraded by natural bacterial

0013-936X/84/0916-0014$01.50/0

communities, they do not persist long enough to pose long-term hazards unless oil releases are frequent or continuous.

The insoluble residue of the oil can cause physical damage to water birds and to shoreline and bottom communities. Unlike WSFs, residues incorporated into sediments are likely to persist for months or years, accumulating in the tissues of plants and animals. The result is chronic ecological damage.

Dissolved coal liquids

WSF composition. WSFs of petroleum and synthetic oils contain light aromatic hydrocarbons such as benzene, toluene, and naphthalene (*3–6*). In WSFs of many crude coal liquids—but not those of petroleum—phenols and anilines occur in concentrations that far exceed those of the aromatic hydrocarbons (*5, 6*). Because most one-ring phenols and anilines have boiling points in the range of 180–220 °C, they are especially abundant in middle distillates; most are too high boiling to be present in naphthas, and too low boiling to be present in heavy fuel oils.

The concentrations of these highly soluble chemical classes in coal liquids are therefore affected by distillation. They are also likely to change during refining. For example, hydrotreatment, a probable first step in upgrading coal liquids to transportation fuels, breaks down phenols and anilines (*7*). Thus, WSF components are most likely to be important after releases of unrefined materials that contain a significant mass fraction in the 180–220 °C boiling range.

Dissolution and evaporation. Kinetics of dissolution and evaporation of phenols, anilines, and light aromatic hydrocarbons from oil slicks have been measured under laboratory conditions, and mathematical models have been formulated to describe these processes specifically for coal liquids (*6, 8*). Phenols and anilines dissolve more rapidly and evaporate more slowly than light aromatics (Figure 2). This suggests an important difference between petroleum and coal liquids. When petroleum is spilled, evaporation removes much of the soluble material before it dissolves into the underlying water; thus, concentrations of dissolved oil under petroleum slicks in the water are usually in the μg/L range (*9*). In contrast, most of the soluble components of a spilled coal liquid dissolve faster than they evaporate, and concentrations of dissolved oil become much higher than those of petroleum products.

Releases in standing water. The highest concentrations of dissolved coal liquid components will be found after releases into isolated or enclosed bodies of water, including riverine embayments (such as coves) and backwaters, where the oil remains in contact with the same mass of water long enough (several days) for virtually all soluble material to be removed. Without dilution and dispersion, dissolved oil will persist until other processes, especially microbial degradation, begin to remove the contaminants.

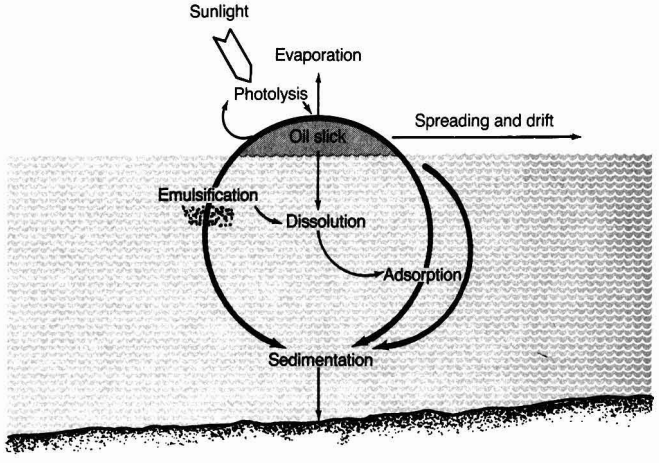
We used outdoor ponds and aquarium microcosms as physical models to investigate this situation (*10–13*). When coal-derived crude oil containing 3% phenolics was added to microcosms and ponds, dissolved oil concentrations reached maxima within 24 h and then declined. Environmental conditions influenced the fate of the oil: Maximum concentrations were higher and loss rates were slower in the microcosms than in the ponds, because lower temperatures and slower oxygen diffusion in the smaller systems retarded microbial degradation rates. In both systems, unsubstituted phenol and cresols were degraded most rapidly, whereas more highly alkylated phenols were more persistent. Dissolved oil concentrations fell below the detection limit of 5 μg/L within one to five weeks, depending on the volume of oil released (*1*).

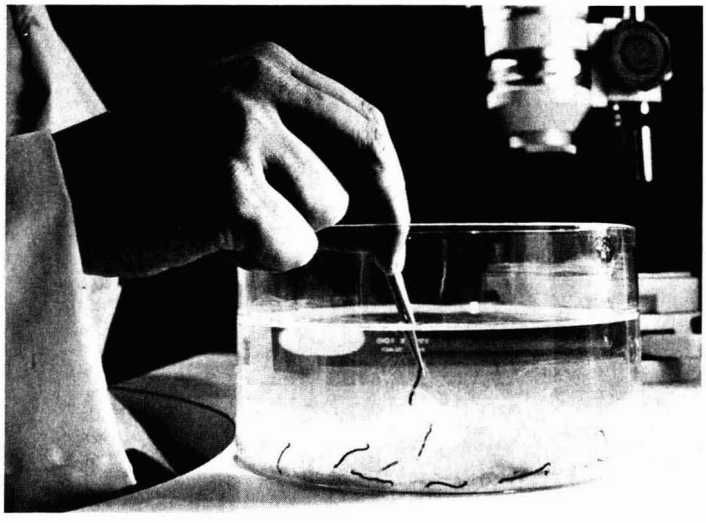
Results of the microcosm and pond experiments were consistent with mathematical models of dissolution and evaporation (*6, 8*). Virtually all of the soluble material in the oil entered the water. The maximum concentration of dissolved oil expected after a spill in standing water therefore can be estimated from the relative volumes of oil and water and the percentage of water-soluble material in the oil. For example, release of 300 m^3 (80,000 gal) of oil containing 3% phenolics into a typical embayment 1 km^2 in area and 1 m deep (volume = 10^6 m^3) would result in a theoretical maximum concentration of $(300 \times 0.03)/(10^6) = 9$ ppm dissolved phenolics.

Releases in large rivers. Oil dissolution is more complicated in a riverine environment than in standing water, and experimental releases in rivers are not feasible. To determine concentrations of dissolved oil under such conditions, mathematical formulations describing dissolution, evaporation, and oil slick spreading were integrated with a one-dimensional model of river hydrology to create SOPTRAN, a synthetic oil pollutant transport model (*1*).

This model was used to simulate a release of 300 m^3 of a crude, coal-derived middle distillate containing 3% phenols into a navigable river similar in size and flow to the lower Ohio River. The model predicted that most phenolics would dissolve from the oil within 24 h. Maximum concentrations of total dissolved phenolics (about 0.7 mg/L) would occur 8 h after the spill, about 14 km downstream. As the pulse of contaminated water moved downstream, phenolic concentrations would decline because of dispersion and dilution; after 16 h, the maximum concentration would be 0.5 mg/L. Water that contained the highest concentrations of dissolved phenolics would pass a given point on the river in less than 6 h.

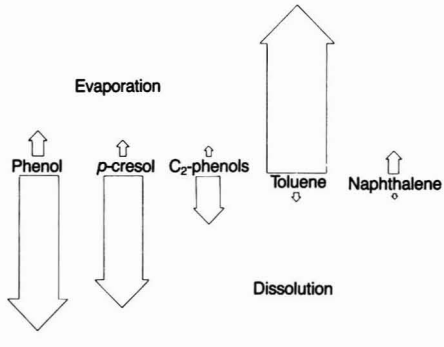
FIGURE 1
Environmental processes affecting coal liquids released into an inland water body





Bioassay tests the effects of synthetic oil on midge fly larvae

FIGURE 2
Relative mass fluxes from a coal liquid layer [a,b,c,d]



[a]Mass flux is proportional to width of arrow
[b]Extent of evaporation and dissolution is proportional to length of arrow
[c]Coal liquid layer is 1.4 mm thick
[d]Wind speed 2 cm above the oil (coal liquids) is 0.2 m/s

Thus, a coal liquid release into a large river would produce patterns of dissolved oil concentrations much different from those occurring after a spill in a backwater. Maximum concentrations from a 300-m^3 spill would be more than 10 times higher in the hypothetical 1-km^2 embayment than in the river. Organisms in the embayment would experience high concentrations of dissolved oil for many days, whereas exposures in the river would last for less than 6 h. The next step in our analysis will be to determine the potential ecological effects of both types of WSF exposure.

WSF toxicity. As a first approximation, the ecological effects of dissolved oil can be estimated from bioassay data for individual species. Reviewing published data on petroleum WSF toxicity to marine organisms, Moore and Dwyer (*4*) concluded that acute toxicity to adults generally occurs at concentrations between 1 mg/L and 100 mg/L, whereas larval stages of some species are sensitive to as little as 0.1 mg/L. Results for WSFs of coal liquids are comparable (Table 1). Concentrations of about 10 mg/L are lethal to fish in acute toxicity tests (48-h to 96-h exposures). *Daphnia magna,* a crustacean that is more sensitive to many toxicants than other bioassay species, is killed or immobilized by short-term exposure to 1 mg/L, and its long-term survival and reproductive capacity can be reduced by concentrations below 0.1 mg/L. Snails and algae are relatively resistant to coal liquid WSFs.

The toxicity values shown in Table 1 are similar to those for the multialkylated phenolics that make up the majority of the WSFs (*24*). The toxicity of phenolics increases with increasing alkylation and coal liquid WSFs are more toxic, in general, than unsubstituted phenol. Some of the variability in toxicity values among WSFs reflects differences in proportions of alkyl phenols.

The results of WSF bioassays indicate that milligram for milligram, dissolved petroleum and dissolved coal liquid constituents are approximately equal in toxicity. However, because crude coal liquids contain a much greater mass of soluble material than does petroleum, water contaminated with coal liquid becomes more toxic than water contaminated with the same volume of petroleum. When bioassay results are expressed as percent dilutions of full-strength WSFs, coal liquids are several orders of magnitude more toxic than petroleum (*7, 25–27*). Although bioassay results are useful for comparisons among WSFs, they are unavailable for most aquatic species; moreover, they are obtained under laboratory conditions that cannot reflect the organisms' natural environment.

Microcosm and pond experiments have been conducted to give a more complete picture of the potential effects of coal liquids in nature (*10, 11, 28*). These experiments involved a middle distillate product with a toxicologically inert insoluble fraction that indicated that the observed effects may be attributed primarily to dissolved oil. The components of the experimental ecosystems that were most sensitive to the oil were cladoceran crustaceans (taxonomically related to *D. magna*), fish, mayflies, and rooted aquatic plants. As the abundance of these organisms declined, other, more resistant species (including algae, bacteria, rotifers, and chironomid midges) came to dominate the ecosystems. At the highest exposure levels tested, even many resistant organisms were eliminated, and ecological recovery was extremely slow.

In these closed systems, the threshold for ecological damage occurred at exposures equivalent to single releases of 15 $m^3/10^6$ m^3 (oil volume/water volume), or chronic releases of 2 $m^3/10^6$ m^3 per day. Dozens of petroleum spills of this magnitude or greater are reported each year in inland waters (*24*). In contrast, the SOPTRAN simulation demonstrated that release of as much as 300 m^3 of coal liquid in a large river would expose organisms only briefly to dissolved oil, with concentrations remaining below the acute toxicity level for most species. Thus, the ecological effects of dissolved oil would probably

TABLE 1
Acute and chronic toxicities of three coal liquid WSFs to aquatic organisms[a,b]

Species	Exposure	Toxic concentration, mg/L TOC		
		WSF A[c]	WSF B[d]	WSF C[e]
Fish				
Pimephales promelas	Acute	11 *(5)*	9.5–10.3 *(14)*	—
	Chronic	0.4–1 *(15)*	—	—
Salmo gairdneri	Chronic	0.3–3 *(15)*	—	1.4 *(16)*
Ictalurus punctatus	Acute	—	8.3 *(17)*	—
	Chronic	—	—	3.4 *(16)*
Lepomis macrochirus	Chronic	—	—	9.8 *(16)*
Gambusia affinis	Acute	—	9.5 *(14)*	—
Invertebrates				
Daphnia magna	Acute	3–5 *(5)*	1–2 *(14)*	9.6 *(18)*
	Chronic	0.3–0.9 *(19)*	0.03 *(14)*	0.12 *(20)*
Chironomus tentans	Acute	14 *(5)*	11 *(14)*	31 *(21)*
	Chronic	0.5–5 *(19)*	—	—
Tanytarsus dissimilis	Chronic	0.1–3.4 *(19)*	—	—
Physa gyrina	Acute	—	—	72 *(22)*
	Chronic	—	—	26 *(22)*
Helisoma trivolvis	Acute	—	—	130 *(22)*
	Chronic	—	—	3 *(22)*
Algae				
Selenastrum capricornutum	Acute	—	6.5 *(14)*	56 *(23)*
	Chronic	15–50 *(19)*	—	—
Six other species	Acute	—	—	25–166 *(23)*

[a]Toxic concentrations are expressed as mg/L total organic carbon (TOC), [b]Reference numbers are given in parentheses, [c]WSF A = SRC-II fuel oil blend, [d]WSF B = H-coal middle distillate, [e]WSF C = H-coal fuel oil blend

be minor in this situation. This assessment does not account for the potential effects of insoluble oil components, to which we next direct our discussion.

Insoluble oil components

WSF components, although highly toxic to aquatic organisms, constitute less than 15% of the mass of most coal liquids. The remainder is a complex mixture of aliphatic, aromatic, and naphthenic hydrocarbons, with lesser amounts of nitrogen-, oxygen-, and sulfur-containing polycyclic compounds. Because some of these compounds, notably PAHs and polycyclic aromatic amines (PAAs), are responsible for much of the mammalian carcinogenicity and Ames test mutagenicity of the direct coal liquefaction products that have been studied (*29, 30*), their long-term fate in aquatic systems is of considerable concern.

Although far from complete, data are available that address the environmental transport of these coal liquid constituents. In our pond studies we observed evaporative loss of 75% of the mass of one test spill during one week of weathering. As the lighter hydrocarbons evaporated, they left a floating, viscous, waxy residue enriched in long-chain aliphatic naphthalenes, phenanthrenes, fluorenes, pyrenes, and other PAHs, as well as some nitrogen-containing polycyclic compounds (*31*). The residue sank and was incorporated into pond sediments. Microbial degradation and dissolution into overlying water removed lighter two- and three-ring PAHs over the five-month duration of the study, but the larger PAHs remained virtually unchanged.

The pattern of residue alteration in the sediment closely resembled compositional changes observed in petroleum residues in marsh sediment after an ocean spill (*32*). Our limited data suggest that once coal liquid residues sink and accumulate in depositional zone sediments of rivers and impoundments, their components will persist in a manner resembling that of petroleum residues.

At least some of the components of coal liquid residues are readily available for uptake by aquatic organisms. In recent studies at our laboratory, aquatic insects (*Chironomus tentans*) inhabiting sediment that contained coal liquid residues accumulated PAHs to levels exceeding sediment concentrations (*33*). Benthic organisms are also known to accumulate PAHs from other materials such as creosote (*34*).

The ecological effects of PAHs, however, are not clear. Some marine studies have demonstrated a correlation between the incidence of hepatic lesions and tumors in bottom-dwelling flatfish and levels of sediment-associated contaminants including PAHs (*35, 36*).

Coal liquid residues in our pond studies contained a higher proportion of PAHs than did sediment residues from petroleum contamination (*37*), reflecting the greater aromaticity of coal liquids. Exposure of fish and other aquatic organisms to polycyclic compounds, and the potential for deleterious effects, may thus be greater for coal liquid residues in sediments than for petroleum residues. Until more information is obtained on chronic effects caused by exposure to weathered coal liquid residues, however, potential long-term ecological hazards will remain difficult to assess.

Research needs

A variety of advanced fossil energy technologies have arisen in recent years, and it is impossible to predict the eventual configuration of the mature industry—what processes will be used, what types of products will be selected, and where and how the products will be consumed. Commercial coal-derived oils may differ from the materials that have been available for environmental research. However, much has been learned about the major components of synthetic oils, and on this basis a preliminary environmental assessment is possible.

Water-soluble compounds, especially phenolics, are of greatest concern in crude coal liquids in the middle-distillate range. Simulation modeling, bioassay results, and field studies indicate that even high-volume releases of such

products in large navigable rivers—the most probable areas for spills—are unlikely to result in hazardous concentrations of dissolved oil constituents. Releases in standing water, however, could be more serious, depending on the volume of oil spilled and the size of the affected water body. The water solubility of coal liquids is reduced by hydrotreatment, distillation, or extraction.

Insoluble oil residues containing PAHs, PAAs, and other polycyclic aromatic compounds constitute a potential long-term environmental hazard associated with coal liquid releases, especially for products with higher boiling points. Unfortunately, there is almost no quantitative information on the effects of these materials on aquatic organisms and therefore no way to predict the ecological effects of oil residues in specific situations.

Research is needed in several areas. The highest priority should be given to determining the chronic effects of coal liquid residues on organisms living in or near contaminated sediments. The persistence of sediment-bound residues and the ability of sediment-dwelling organisms to concentrate residue components within their tissues are also of concern from the standpoint of protecting the public from exposure to carcinogens and mutagens. Models of coal liquid transport and transformation in aquatic environments are still rudimentary and should be refined to include processes of emulsification, sedimentation, photodegradation, and biodegradation. The fate and effects of coal liquids that are released under extreme environmental conditions (below the ice, for example) must be investigated before a complete hazard assessment can be made.

When these topics have been more fully explored, the information can be applied to comparisons of the environmental properties of oils from different synfuels processes, feedstocks, and product fractions. The influence of hydrotreatment and other upgrading processes on the environmental properties of products can then be determined, and the environmental risks associated with different synfuels market patterns, product end uses, and transportation routes and methods can be evaluated.

Acknowledgment

Research for this feature was sponsored by the Ecological Research Division, Office of Health and Environmental Research, U.S. Department of Energy, under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc. Publication No. 2437, Environmental Sciences Division, ORNL.

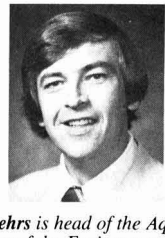
Before publication, this article was reviewed for suitability as an *ES&T* feature by D. F. Charles Menzie, EG&G Environmental Consultants, Waltham, Mass. 02154, and Jean K. Whelan, Department of Chemistry, Woods Hole Oceanographic Institution, Woods Hole, Mass. 02543.

References

(1) Herbes, S. E.; Yeh, G. T. *Environ. Toxicol. Chem.*, in press.
(2) Crynes, B. L. In "Chemistry of Coal Utilization," 2nd Supplementary Vol.; Elliot, M. A., Ed.; Wiley-Interscience: New York, N.Y., 1981; pp. 1991-2070.
(3) Anderson, J. W. et al. *Mar. Biol.* **1974,** *27,* 75-88.
(4) Moore, S. F.; Dwyer, R. L. *Water Res.* **1974,** *8,* 819-27.
(5) Becker, C. D. et al. *Water Air Soil Pollut.* **1983,** *19,* 171-84.
(6) Herbes, S. E.; Southworth, G. R.; Allen, C. P. *Water Res.* **1983,** *17,* 1639-46.
(7) Griest, W. H.; Giddings, J. M.; Klein, J. A. "Effects of Hydrotreatment on the Properties of Coal-Derived Liquid Products: A Status Report," ORNL/TM-8836, Oak Ridge National Laboratory: Oak Ridge, Tenn., 1983.
(8) Southworth, G. R.; Herbes, S. E.; Allen, C. P. *Water Res.* **1983,** *17,* 1647-51.
(9) McAuliffe, C. D. In "Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms"; Wolfe, D. A., Ed.; Pergamon: New York, N.Y., 1977; pp. 238-45.
(10) Franco, P. J. et al. *Environ. Toxicol. Chem.* **1984,** *3,* 447-63.
(11) Giddings, J. M. et al. *Environ. Toxicol. Chem.* **1984,** *3,* 465-88.
(12) Herbes, S. E. In "Environmental Hazard Assessment of Effluents"; Bergman, H. L.; Kimerle, R. A.; Maki, A. W., Eds.; Pergamon: Elmsford, N.Y., in press.
(13) Southworth, G. R. et al. *Water Air Soil Pollut.*, in press.
(14) Franco, P. J. In "Summary Report on H-Coal Process Health and Environmental Studies"; Cowser, K. E., Ed.; Oak Ridge National Laboratory: Oak Ridge, Tenn., in press.
(15) Dauble, D. D. et al. *Am. Fish. Soc. Trans.* **1983,** *112,* 712-19.
(16) Birge, W. J. et al. "Aquatic Toxicity Tests on Organic Contaminants Originating from Coal Conversion. Phase II. Fuel Oils and Fuel Oil Water Soluble Fractions"; Oak Ridge National Laboratory: Oak Ridge, Tenn., 1983.
(17) Franco, P. J., Oak Ridge National Laboratory, unpublished data.
(18) Ullrich, S. O., Jr.; Millemann, R. E. *Can. J. Fish. Aquat. Sci.* **1983,** *40,* 17-26.
(19) Dauble, D. D. et al. *Arch. Environ. Contam. Toxicol.* **1982,** *11,* 553-60.
(20) Cada, G. F. "Environmental Studies of Materials from the H-Coal Liquefaction Process Development Unit," ORNL/TM-8466, Oak Ridge National Laboratory: Oak Ridge, Tenn., 1982.
(21) Franco, P. J. et al. *Environ. Pollut. Ser. A* **1984,** *34,* 321-31.
(22) Millemann, R. E. et al. *Environ. Pollut. Ser. A* **1984,** *33,* 23-38.
(23) Giddings, J. M. et al. In "Aquatic Toxicology and Hazard Assessment: Sixth Symposium," ASTM STP 802; Bishop, W. E.; Cardwell, R. D.; Heidolph, B. B., Eds.; American Society for Testing and Materials: Philadelphia, Pa., 1983; pp. 445-59.
(24) Giddings, J. M.; Herbes, S. E.; Gehrs, C. W. "Coal Liquefaction Product Releases in Inland Waters: A Preliminary Environmental Assessment"; Oak Ridge National Laboratory: Oak Ridge, Tenn., in press.
(25) Giddings, J. M. et al. *Bull. Environ. Contam. Toxicol.* **1980,** *25,* 1-6.
(26) Giddings, J. M.; Washington, J. N. *Environ. Sci. Technol.* **1981,** *15,* 106-8.
(27) Gray, R. H. et al. *Environ. Sci. Technol.* **1982,** *16,* 225-29.
(28) Cushman, R. M.; Goyert, J. C. *Environ. Pollut. Ser. A* **1984,** *33,* 163-86.
(29) Guerin, M. R. et al. *Environ. Res.* **1980,** *23,* 42-53.
(30) Wilson, B. W. et al. *Fuel* **1981,** *60,* 289-94.
(31) Backhus, D. A.; Herbes, S. E., Oak Ridge National Laboratory, unpublished data.
(32) Teal, J. M.; Burns, K. A.; Farrington, J. W. *J. Fish. Res. Board Can.* **1978,** *35,* 510-20.
(33) Backhus, D. A.; Franco, P. J.; Herbes, S. E., Oak Ridge National Laboratory, unpublished data.
(34) Neff, J. M. "Polycyclic Aromatic Hydrocarbons in the Aquatic Environment"; Applied Science: London, U.K., 1979.
(35) Malins, D. C. *Environ. Sci. Technol.* **1980,** *14,* 32-37.
(36) Malins, D. C.; Hodgins, H. O. *Environ. Sci. Technol.* **1981,** *15,* 1272-78.
(37) Wakeham, S. G.; Farrington, J. W. In "Contaminants and Sediments, Volume 1. Fate and Transport Case Studies, Modeling, Toxicity"; Baker, R. A., Ed.; Ann Arbor Science: Ann Arbor, Mich., 1980; pp. 3-32.

Jeffrey M. Giddings *(l.) is a research staff member in the Environmental Sciences Division of Oak Ridge National Laboratory. He received his Ph.D. in aquatic ecology from Cornell University. During the past six years he has investigated the effects of synthetic fuel products and wastes on aquatic organisms and ecosystems.*

Stephen E. Herbes *(r.), a research staff chemist in the Environmental Sciences Division of Oak Ridge National Laboratory, received a B.S. in chemistry from Muhlenberg College and an M.S. and a Ph.D. in environmental health sciences from the University of Michigan. His research is on environmental aspects of synthetic fuel processes and surface and groundwater transport of organic contaminants.*



Carl W. Gehrs *is head of the Aquatic Ecology Section of the Environmental Sciences Division of Oak Ridge National Laboratory and has served on several interagency committees concerned with environmental effects of synthetic fuels. He was responsible for the environmental research related to coal conversion conducted at Oak Ridge National Laboratory. His current research is on environmental toxicology and the development of systems for increasing the ability to predict the effects of human activities on the environment.*

The new RCRA

Richard M. Dowd

The 98th Congress passed only one major pollution control law, the reauthorization of the 1976 Resource Conservation and Recovery Act (RCRA), which governs hazardous waste disposal. Many of the revisions in the legislation appear to reflect congressional response to public concern over EPA's implementation of the original law. The new provisions are often so detailed that the act reads as if it were a series of regulations rather than an authorizing statute.

Major changes

The new RCRA removes a great deal of EPA's flexibility in both discretion and timing to deal with case-by-case situations—including decisions about which chemical substances to regulate. It details tight schedules that the agency must meet to forestall the implementation of mandatory listings and prohibitions on disposal. And because the bill contains major changes governing small generators, land disposal practices, and underground storage of chemicals, many companies and individuals not previously affected are now subject to the law's requirements.

The limitation that exempts small generators from disposal and reporting requirements has been reduced from 1000 kg of listed hazardous wastes per month to 100 kg. By including all generators of 100 kg or more per month, it is estimated that roughly 100,000 additional businesses will now be covered by RCRA. This will have two immediate consequences: It will require the establishment, within the act's mandated 270 days, of a manifest system for these small generators to report their generation and transport of wastes. It also will increase the need for disposal capacity to accept these wastes, which must go either to interim or to fully permitted hazardous waste facilities.

Land disposal: Going, going . . .

The new provisions for land disposal incorporate the act's most sweeping changes. RCRA states that it is national policy to minimize or eliminate land disposal of hazardous wastes and that land disposal technologies should be used as the least favored or last resort. Many of the act's detailed instructions and accompanying deadlines for EPA are designed to lend impetus to this major policy shift, which accords preference to almost all alternatives—advanced technology, recycling, and incineration—over land disposal.

To further impel this shift to alternative disposal technologies, Congress has established a very specific schedule of tiered prohibitions that restricts certain wastes from landfills and requires EPA to evaluate other wastes not specifically banned in the law. Within 32 months, EPA must evaluate 50–75% of the hazardous wastes now disposed of on land, to determine whether continued land disposal is acceptable.

Underground storage permitting

Congress also has addressed the issue of underground storage of various materials, including hazardous wastes and petroleum products. RCRA requires that the owners of storage tanks that are more than 10% underground and that contain either hazardous wastes or petroleum products must report to an appropriate state agency within 12 months. (The agency will be designated by each governor within six months.) In addition, EPA must promulgate regulations requiring appropriate systems for leak detection, record keeping, and reporting of tank releases.

The act also changes some of the permitting operations of the agency. It establishes a set of minimum technological requirements for land disposal facilities that generally will require double-lined landfills, along with groundwater monitoring and leachate collection systems. Permeating the sections on disposal facility standards is a sense that not enough monitoring data exist, and requirements for groundwater measurement are specified.

Even a cursory examination of the major requirements in the new act makes it clear that hazardous waste treatment companies will have to meet substantially stricter requirements and that EPA must meet tough schedules in establishing many entirely new sets of criteria. Even with RCRA's greater emphasis on delegation of responsibilities to state and local governments wherever possible, it seems unlikely that this act can be implemented without a substantial increase in funding and personnel resources for EPA. And although the administration's fiscal 1986 budget is yet to be released, it also seems unlikely that any great increase in resources will be proposed. This likely gap may give additional clout to RCRA's expanded authorization for citizen suits against generators and disposers, as well as against governments, for noncompliance with the act's terms.

Richard M. Dowd, Ph.D., is a Washington, D.C., consultant to Environmental Research and Technology, Inc.

0013-936X/84/0916-0019$01.50/0

(Letters, *from p. 13*)

determined policy conclusions. This may be acceptable in a legal forum, but it is not the scientific process.

Moreover, the authors do not make clear (and perhaps do not consider) that a policy decision rests on more than the scientific evidence. Beyond the scientific facts are the costs imposed on society of either taking remedial action or continuing on the present path. This involves the evaluation of both the scientific evidence as to the cost of damage and of the technical information as to the cost of emissions control. Also, policy decisions must reflect societal values. This is a much softer area of information on which persons of equally upstanding character may differ substantially. To some, ecological risks should be avoided at nearly all costs; to others the value of economic development makes it worth accepting substantial ecological risks.

Finally, the authors have surprisingly discounted "cooperation between industry, government, and universities to solve the problem of acid rain." For example, EPRI (representing the interests of the scientific and industrial communities), along with federal and state organizations (representing interests of government), is working with universities and other groups in a closely coordinated program of acid rain scientific research. EPRI alone has scheduled $75-million worth of such research for the five years between 1984 and 1988. These same groups also are developing technologies for ultimate control of emissions. Again EPRI is investing $100 million over this period on SO_2 and NO_x emission control technologies. Beyond this, EPRI is investing nearly $200 million in the study of cleaner ways of using coal: fluidized bed combustion and gasification prior to combustion.

Clearly, many people with different points of view on what policy prescription should be followed are concerned about the acid rain issue. We at EPRI have found it helpful to focus on developing sound scientific and technological information while allowing others to debate how to interpret this information into advocacy for legislative and regulatory remedies. We believe that this distinction has helped identify the true red herrings.

René H. Malès
Vice-President
Energy Analysis and
Environment Division
Electric Power Research Institute
Palo Alto, Calif. 94303

Dear Sir: Magda Havas and coauthors ("Red herrings in acid rain research") attribute "lake acidification" to "acid precipitation," although neither term is defined or quantified. The authors note that a variety of factors "can all influence the sensitivity of lakes to acidification," and they cite data from the Integrated Lake-Watershed Acidification Study (ILWAS) to support their argument. A more extensive examination of the ILWAS data shows that the connection between the acidity of precipitation and of lake waters is weaker than generally realized.

Of the two most extensively studied ILWAS lakes, Woods Lake receives about 6.6% more hydrogen ion input, based on an annual average of pH 4.2, than Panther Lake, and the Woods watershed receives about 39% more hydrogren ion input than Panther. These differences in precipitated acidity cannot account for the marked differences in the acidities of the two lakes. Woods Lake, at pH 4.5–5.1, has about 450% greater hydrogen ion concentration than Panther at pH 5.3–7.8. (Another report noted pH values of 4.6 and 6.04 for Woods and Panther Lakes, respectively, or 220% greater hydrogen ion concentration at Woods.)

The leaf canopy can have a marked effect on the acidity of the throughfall. Both Woods and Panther watersheds are more than 90% forested, and the known effects of canopies of different kinds of trees suggest that through-fall at Panther should be more acidic than at Woods, confounding the role of pH 4.2 precipitation.

The decaying organic matter (O horizon) on the forest floor at Panther watershed is twice as acidic as that at Woods, reflecting the greater proportion of conifers in the Panther forestation. But this seems to enlarge the anomaly in the relationship of precipitation and lake water acidity.

The resolution of the anomaly lies in the chemistry of the mineral soil. The deepest of the soil layers (C horizon) at Panther has about half the acidity (pH 4.92) of that at Woods (pH 4.66). Moreover, the till (soil) of the Panther watershed has about 4.5 times as great a volume as that at Woods, and it is more permeable. ILWAS researchers were led by these facts to conclude that the differences in the soil characteristics explain the differences in the acidities of the two lakes.

It is noteworthy that the Office of Technology Assessment drew a similar conclusion in its June 1984 report, stating that "the amount of acidifying material that actually enters a given lake or stream is determined primarily by the soil and geologic conditions of the surrounding watershed."

Citing differences in the acidities of lakes that receive essentially identical precipitation is not a red herring. When all of the data are examined, the answer emerges, with the soil rather than precipitation explaining the acidities of lakes.

Alan W. Katzenstein
Katzenstein Associates
Larchmont, N.Y. 10538

The authors reply:

We have received four letters regarding our article and wish to respond to one of these in detail. The letters from Mr. Bowman, Mr. Dunn, and Mr. Malès do not challenge the scientific basis of our paper and therefore do not warrant a detailed reply.

In response to Mr. Malès, we only wish to say that we basically agree with his comments. We recognize that the solution to the acid rain problem is not simple and that the policy decisions of well-intentioned men and women *can* differ on such a complex environmental problem. Our intention was not to denigrate ILWAS or EPRI's efforts in SO_2 and NO_x control technologies—which are commendable. Our criticism is directed solely toward those who *deliberately* misinterpret scientific data with the intent to create confusion.

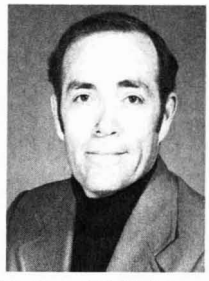
Mr. Katzenstein's letter is not so much a rebuttal of our paper as it is a detailed review of ILWAS, followed by some erroneous conclusions. We have already presented the highlights of that study in our paper and will not repeat them here. We wish only to comment on a few points.

The discussion of throughfall chemistry vs. precipitation chemistry and that of the soil-neutralizing capacity and natural soil acidity are interesting, but lead to impasse. For example:

It is a fact that the precipitation under a deciduous tree (throughfall) usually has a higher pH than precipitation in an open area, with the exception of light rainfall preceded by drought. In this case there is an accumulation of acidic dry deposition on leaf surfaces and correspondingly acidic throughfall when this is washed off. Although leaves from deciduous trees can buffer acidic rainfall during the summer and fall by allowing exchange of ions at the leaf surface, they provide no buffering during the winter and spring when leaves are absent. Also the buffering potential of the leaves is limited. The conse-

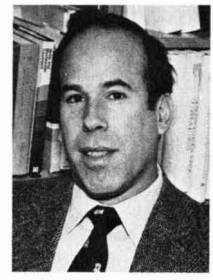
(*continued on p. 26*)

ES&T's 1985 Advisory Board



Dr. Russell F. Christman
Editor
University of North Carolina

Dr. John H. Seinfeld
Associate Editor (air)
California Institute of Technology



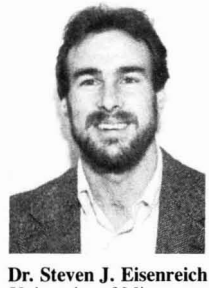
Dr. Philip C. Singer
Associate Editor (water)
University of North Carolina

Dr. Russell F. Christman, editor, has announced the appointment of three new members to the *ES&T* advisory board. **Marcia C. Dodge** is a physical chemist in the Atmospheric Sciences Research Laboratory at EPA's Environmental Research Center, Research Triangle Park, N.C. Her research interests include the development of chemical models to describe the formation of photochemical oxidants and acid rain. **Jarvis L. Moyers** is program director for atmospheric chemistry in the National Science Foundation's Division of Atmospheric Sciences. His research interests emphasize chemical and physical processes responsible for atmospheric gas-to-particle and particle-to-gas conversion processes. **Kathleen C. Taylor** is head of the Environmental Science Department at General Motors Research Laboratories. Her research involves atmospheric chemistry and modeling, offsite atmospheric monitoring, acid deposition, exhaust emissions characterization, toxic and hazardous waste control, and water pollution.

Board members serve three-year terms. The last year of each member's term is noted in parentheses.

Dr. Julian B. Andelman
University of Pittsburgh
(1985)

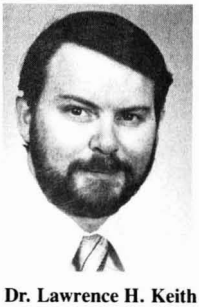
Dr. Marcia C. Dodge
EPA Environmental Research Center
(1987)



Dr. Steven J. Eisenreich
University of Minnesota
(1986)

Dr. William H. Glaze
University of California at Los Angeles
(1986)

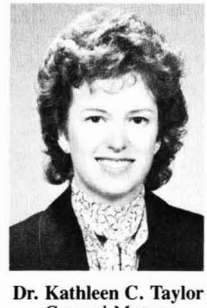
Dr. Michael R. Hoffmann
California Institute of Technology
(1986)



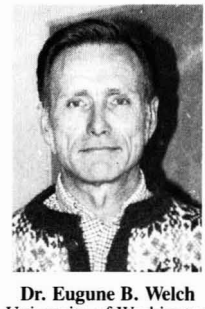
Dr. Lawrence H. Keith
Radian Corporation
(1985)

Dr. Donald Mackay
University of Toronto
(1986)

Dr. Jarvis L. Moyers
National Science Foundation
(1987)



Dr. Kathleen C. Taylor
General Motors Research Laboratories
(1987)



Dr. Eugune B. Welch
University of Washington
(1985)

Editorial policy

Environmental Science & Technology reports on aspects of the environment and its control by scientific, engineering, and political means. Contributed materials may appear as feature articles, critical reviews, current research papers, research notes, and correspondence. Central to the evaluation of all contributions is a commitment to provide the readers of *ES&T* with scientific information and critical judgments of the highest quality. For the convenience of authors, the specific nature of each type of contribution is outlined below.

Feature articles. A manuscript submitted for publication as a feature article should present useful discussion and opinion on important research directions in environmental science, developing technology, environmental processes, and social, political, or economic aspects of environmental issues. Each manuscript undergoes review by qualified peers as well as by the editors for the purpose of balance and elimination of inappropriate bias. Review criteria include significance of the scientific issue or process described, quality and succinctness of the text, and identification of potential research needs. Strict requirements for documentation of results, completeness of data, and originality, such as those applicable to research manuscripts, are not included in the review criteria for feature articles.

Critical reviews. Critical reviews are thoroughly documented, peer-reviewed assessments of selected areas of the environmental science research literature for the purpose of identifying critical research needs. Criteria for acceptability include current importance of the field under review, thoroughness of the literature coverage, clarity of text, and adequacy of research need identification.

Current research papers. The research pages of *ES&T* are devoted to the publication of critically reviewed papers concerned with the fields of water, air, and waste chemistry, and with other scientific and technical fields that are relevant to the understanding and management of the water, air, and land environments. Contributed research papers, in general, describe complete and fully interpreted results of original research.

Environmental Science & Technology seeks to publish papers of an original and significant nature. Originality should be evidenced by new experimental data, new interpretations of existing data, or new theoretical analysis of environmental phenomena. Significance will be interpreted with respect to the breadth of impact of the reported findings. Manuscripts reporting data of a routine nature that do not offer heretofore unavailable important information or do not substantially augment already available data will be declined publication in *ES&T*. The scope of the reported data in ambient monitoring studies should be such that broad conclusions applicable to more than the particular local scale are possible.

All research articles emphasizing analytical methodology for air or water analysis must include substantial application to environmental samples. *ES&T* faces some overlap with other journals in this area, and articles that do not contain, in the editors' judgment, a significant emphasis on environmental analysis will be returned to the authors for submission elsewhere.

Manuscripts should be prepared with strict attention to brevity. The vast majority of articles are expected to be fewer than four published pages. Processing time will be shortened if the editors do not have to return manuscripts to be condensed.

Notes and correspondence, as well as full-length papers, will be published in the research section. **Notes** are shorter research reports describing preliminary results of unusual significance or studies of small scope. Authors of Notes should be able to justify why it is not desirable to wait for a more complete report to be published as a full-length paper. **Correspondence** is a significant comment on work published in the research section of *ES&T*. Comments should be received within six months of date of publication of the original article. The authors of the original article ordinarily will be allowed to reply.

Send manuscripts to *Environmental Science & Technology*, 1155 16th St., N.W., Washington, D.C. 20036. Address feature manuscripts to Managing Editor; research manuscripts to Manager, Manuscript Reviewing Office. Include a signed copyright transfer form, a copy of which appears on the inside back cover of this issue.

Current research author's guide

This manuscript preparation guide is published to aid authors in writing, and editors and reviewers in expediting the review and publication of research manuscripts in *Environmental Science & Technology*. For a detailed discussion with examples of the major aspects of manuscript preparation, please refer to "Handbook for Authors of Papers in American Chemical Society Publications" (1978).

Title

Use specific and informative titles. They should be as brief as possible, consistent with the need for defining the subject of the paper. If trade names are used, give generic names in parentheses. Key words in titles assist in effective literature retrieval.

Authorship

List the first name, middle initial, and last name of each author. Omit professional and official titles. Give the complete mailing address where work was performed. If present address of author is different, include the new information in a footnote. In each paper with more than one author, the name of the author to whom inquiries should be addressed carries an asterisk. The explanation appears on the contents page.

Abstracts

An abstract, which will appear at the beginning of each paper, must accompany each manuscript. Authors' abstracts frequently are used directly for *Chemical Abstracts*. Use between 100 and 150 words to give purpose, methods or procedures, significant new results, and conclusions. Write for literature searchers as well as journal readers.

Text

Consult a current issue for general style. Assume your readers to be professionals not necessarily expert in your

particular field. Historical summmaries are seldom warranted. However, documentation and summary material should be sufficient to establish an adequate background. Divide the article into sections, each with an appropriate heading, but do not oversectionalize. The text should have only enough divisions to make organization effective and comprehensible without destroying the continuity of the text. Keep all information pertinent to a particular section within that section. Avoid repetition. Do not use footnotes; include the information in the text.

Introduction. Discuss relationship of your work to previously published work, but do not repeat. If a recent article has summarized work on the subject, cite the summarizing article without repeating its individual citations.

Experimental. Apparatus: List devices only if of specialized nature. Reagents: List and describe preparation of special reagents only. Procedure: Omit details of procedures that are common knowledge to those in the field. Brief highlights of published procedures may be included, but details must be left to literature cited. Describe pertinent and critical factors involved in reactions so that the method can be reproduced, but avoid excessive description. Results and discussion: Be complete but concise. Avoid nonpertinent comparisons or contrasts.

Manuscript requirements

Three complete legible copies of the manuscript are required. They should be typed double or triple spaced on 22 × 28-cm paper, with text, tables, and illustrations of a size that can be mailed to reviewers under one cover. Duplicated copies will be accepted only if very clear.

If pertinent references are unpublished, furnish copies of the work or sufficient information to enable reviewers to evaluate the manuscript.

In general, graphs are preferable to tables if precise data are not required. When tables are submitted, however, they should be furnished with appropriate titles and should be numbered consecutively in Roman numeral style in order of reference in the text. Double space with wide margins, and prepare tables in a consistent form, each on a separate 22 × 28-cm sheet.

Submit original drawings (or sharp glossy prints) of graphs, charts, and diagrams prepared on high-quality inking paper. All lines, lettering, and numbering should be sharp and unbroken. If coordinate paper is used, use blue crosshatch lines because no other color will "screen out."

Typed lettering does not reproduce well: Use black India ink and a lettering set for all letters, numbers, and symbols. On 20 × 25-cm copy, lettering should be at least 0.32 cm high—for example, with a Leroy lettering set, use template 120C and pen No. 0. Lettering on copy of other sizes should be in proportion. Label ordinates and abscissas of graphs along the axes and outside the graph proper. Do not use pressed wax for numbering or lettering; it rubs off in all the mailings and handlings necessary before receipt by the printer.

Photographs should be supplied in glossy print form, as large as possible, but preferably within the frame of 20 × 25 cm. Sharp contrast is essential.

Number all illustrations consecutively using Arabic numerals in the order of reference in the text. Include a typed list of captions and legends for all illustrations on a separate sheet.

If drawings are mailed under separate cover, identify by name of author and title of manuscript. Advise editor if drawings or photographs should be returned to the author.

Nomenclature

The nomenclature should correspond, as closely as possible, to that used by other ACS primary publications (refer to "Handbook for Authors").

Use consistent units of measure (preferably SI).

If nomenclature is specialized, include a "Nomenclature" section at the end of the paper, giving definitions and dimensions for all terms. Write out names of Greek letters and special symbols in margin of manuscript at point of first use. If subscripts and superscripts are necessary, place them accurately. Avoid trivial names. Trade names should be defined at point of first use (registered trade names should begin with a capital letter). Identify typed letters and numbers that could be misinterpreted, for example, one and the letter "I," zero and the letter "O."

Formulas and equations

Chemical formulas should correspond to the style of ACS publications. Chemical equations should be balanced and numbered consecutively along with mathematical equations. The mathematical portions of the paper should be as brief as possible, particularly where standard derivations and techniques are commonly available in standard works.

Safety

Authors are requested to call special attention—both in their manuscripts and in their correspondence with the editors—to safety considerations such as explosive tendencies, precautionary handling procedures, and toxicity.

Acknowledgment

Include essential credits in an "Acknowledgment" section at the end of the text, but hold to an absolute minimum. Give meeting presentation data or other information regarding the work reported (for example, financial support) in a note following Literature Cited.

References

Literature references should be numbered and listed in order of reference in text. They should be listed by author, patentee, or equivalent. In the text, just the number should be used, or the name should be followed by the number. "Anonymous" is not acceptable for authorship. If the author is unknown, list the reference by company, agency, or journal source. Do not list references as "in press" unless they have been formally accepted for publication. Give complete information, using abbreviations for titles of periodicals as in the Chemical Abstracts Service Source Index, 1975.

For periodical references to be considered complete, they must contain authors' surnames with initials, journal source, year of issue, volume number, issue number (if any), and the first and last page numbers of the article. Consult the "Handbook for Authors" for reference style.

Supplementary material

Extensive tables, graphs, spectra, calculations, or other material auxiliary to the printed article will be included in the microfilm edition of the journal. Identify supplementary material as to content, manuscript title, and authors. Three copies of the supplementary material, one in a form suitable for photoreproduction, should accompany the manuscript for consideration by the editor and reviewers. The material should be typed on white paper with black typewriter ribbon. Computer printouts are acceptable if they are clearly legible. If individual characters for any of the material, computer or otherwise, are broken or disconnected, the material is definitely unacceptable.

Figures and illustrative material should preferably be original India ink drawings or matte prints of originals. Optimum size is 22 × 28 cm. Minimum acceptable character size is 1.5 mm. The caption for each figure should appear on the same piece of copy with the figure. Be sure to refer to supplementary material in text where appropriate.

Supplementary material may be obtained in photocopy or microfiche form at nominal cost. Material of more than 20 pages is available in microfiche only. Photocopy or micro-

fiche must be stated clearly in the order. Prepayment is required. See instructions at the end of individual papers.

The supplementary material is abstracted and indexed by Chemical Abstracts Service.

Subscribers to microfilm editions receive, free, the supplementary material in microfiche form from individual papers in any particular issue. For information, contact Microforms Program at the ACS in Washington, D.C., or call (202) 872-4554.

Peer review in *ES&T*

Characteristics of *ES&T*

ES&T stands out among American Chemical Society journals in that it combines both a magazine and a journal. Only one other ACS publication contains this combination—our sister publication, ANALYTICAL CHEMISTRY. Because of the hybrid nature of our publication, it serves a large and diverse audience.

Central to the evaluation of all contributions to *ES&T* is a commitment to provide our readers with scientific information of the highest quality. The publication seeks the most significant, original, and broadly applicable types of articles for its current research section. A vast number of persons review original manuscript contributions and indicate in their evaluations the originality and scientific validity of the work, as well as the appropriateness of the material for our publication.

The editor and associate editors, who are located at the University of North Carolina and the California Institute of Technology, are fully responsible for all material published in *ES&T*. This policy is a general one applicable to all editors of American Chemical Society publications. The 10 members of the Advisory Board are chosen by the editor to provide input to *ES&T*'s operation. The members are chosen to represent various constituent groups in the research and reader communities and serve three-year terms. Although the editors seek advice and help from individuals in the scientific community and from advisory groups, it is ultimately the editors' responsibility to provide editorial direction, set editorial policies, and make individual publication decisions.

The Washington editorial staff handling the current research section is responsible for the day-to-day operation of the peer review system. All editorial staff members have chemistry or related science degrees.

General guidelines and overall editorial policies set by the editor form the basis for evaluating reviewers' comments on research articles submitted for the current research section.

A look at peer review

Each manuscript submitted to the current research section is assigned to a particular staff member who is then responsible for the manuscript—from choosing reviewers to communicating ultimate acceptance or rejection. The staff editor screens manuscripts to determine whether the papers may fall outside of *ES&T*'s scope. If there seems to be a question, the manuscript is immediately referred to the editor or an associate editor.

Reviewers are carefully selected, based on the subject matter of the paper, the experts available in a given area, and the editorial staff member's knowledge of the habits of proposed reviewers. Thus, known slow reviewers are avoided when possible. Potential reviewers for each paper are identified through various means, one of which involves a computer search of subjects that reviewers have indicated are their areas of expertise. Reviewers are normally asked to respond within three weeks, and if they are late, reminders are sent. Late review notifications are generated and dispatched as mailgrams on a weekly basis.

When the reviews come in, they are examined and evaluated by the staff editor. If the first two reviewers do not agree on the disposition of the paper, a third reviewer is selected. If a review is deemed lacking in critical quality, that is, if the technical and scientific strengths or shortcomings of the work have not been adequately addressed, then a third review is also sought.

Copies of the reviews (at least two) and the manuscripts are always sent to the editor or an associate editor, who provides oversight for the entire operation and makes final decisions about manuscript disposition. The subject matter of the manuscript determines which editor will receive the file. Although all letters are writen in the editorial office, the editors examine all materials and decide on the course of action, which is then conveyed to the staff editor.

If the editor or associate editor has recommended revision of the manuscript, the staff editor goes over the paper carefully in a "pre-edit" check to aid the author in revising the manuscript.

Tips for authors of papers submitted to *ES&T*

- Prepare your paper with the audience of the publication in mind. Papers prepared for other journals are likely to need some revision to make them suitable for *ES&T*.
- Clearly state in the introduction the purpose of the work and put the work in perspective with earlier work in the area. This may appear obvious, but authors often fail to clearly state the purpose and significance of their work.
- Write concisely. The vast majority of articles are expected to be fewer than five published pages. Long manuscripts are looked at much more closely and critically both by reviewers and editors. Do not repeat information or figures or tables that have appeared elsewhere. Use illustrative data rather than complete data where appropriate.
- Suggest names of possible reviewers for your paper. You may also suggest the names of persons who you do not want to review the paper. The editors try to use at least one reviewer who has been suggested by authors. This cannot be assured, however, since specific reviewers may not be available for reviewing or may already be overloaded.
- Follow the Current Research Author's Guide, published in every January issue.

If your manuscript is rejected

- Read the reviews carefully. If the reviewers have "missed the point," as authors often claim, consider how the presentation can be clarified and improved to make the point clear. If reviewers have not understood, it is unlikely that readers will understand.
- Is the manuscript, after all, more suitable for another journal?
- Is the work sufficiently complete, or do you need to do more work before seeking publication?
- If you feel strongly that the paper has not been judged fairly, then carefully revise the manuscript taking into account the reviewers' criticisms and send the manuscript to the editorial office with a rebuttal letter asking that the manuscript be reconsidered. Provide an itemized list of changes made in the manuscript in response to reviewer comments, as well as objective rebuttals to the criticisms with which you do not agree.

CLASSIFIED SECTION ■ POSITIONS OPEN

ENVIRONMENTAL SCIENTISTS AND ENGINEERS

ENVIRON is a fast-growing, multi-disciplinary health and environmental science consulting firm with its headquarters in Washington, D.C. and an office in Princeton, N.J. We are seeking qualified candidates to serve our expanding base of private and public sector clients as follows:

• **CHEMICAL/ENVIRONMENTAL ENGINEERS** with experience with EPA regulatory issues, hazardous waste problems or environmental assessments of industrial sites.

• **HEALTH SCIENTISTS/TOXICOLOGISTS** with experience in risk and safety chemical assessments and preferably experience with scientific regulatory issues.

Successful candidates should have an M.S./Ph.D., at least 5 years experience, and possess strong analytical and communication skills. Prior consulting or project management experience desirable.

Other positions requiring less experience exist for scientists and engineers with expertise in the above areas.

ENVIRON offers competitive salaries and excellent benefits. Equal opportunity employer, M/F. Send resume and salary requirements to Personnel, Dept. No. 18, ENVIRON Corporation, 777 Fourteenth Street, N.W., Suite 1000, Washington, D.C. 20005.

ENVIRON
Counsel in Health and Environmental Science

ANALYTICAL CHEMISTRY: Assistant or associate professor, tenure-track position; appointment for August 1985. Ph.D. required. Primary teaching responsibility in Instrumental Analysis. Active research program expected. Qualified candidates in physical, inorganic, or environmental chemistry will also be considered. Research analytical instrumentation including nmr, HPLC, and capillary gc-mass spec available. Send vita, transcripts, and three letters of reference to: **L. Keller, Department of Chemistry, Florida International University, Miami, FL 33199.** Closing date is January 10, 1985. FIU is a member of the State University System of Florida with an enrollment of 16,000 students and with anticipated expansion in science and engineering. FIU is an equal opportunity/affirmative action employer.

ANAEROBIC DIGESTION RESEARCH STAFF POSITION

The Renewable Resources Section of Argonne National Laboratory Energy and Environmental Systems Division seeks to fill a staff position in the field of applied anaerobic microbiology. The person qualified for the position will evaluate methods for converting high-strength organics of industrial wastewaters to methane in anaerobic digestion. The candidate must have a Ph.D. in chemical or environmental engineering or microbiology, with laboratory research experience in anaerobic digestion, wastewater treatment, a working knowledge of selective pretreatment with physical-chemical methods, and an understanding of biochemical kinetics. Highly developed, proven, written and oral English language technical communication skills are required. The position requires the person to independently design laboratory experiments according to the goals and objectives of the program, conduct the experiments in a scientific manner, communicate the results of the research to supervisory personnel and sponsors, and publish the results in refereed journals and at technical conferences.

For confidential consideration, send resume to: **Rosalie L. Bottino, Box-J-EES-15607-28, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, IL 60439.** An affirmative action/equal opportunity employer.

CHEMICAL LIMNOLOGY TECHNICIANS

Openings available for MS or BS level technicians with backgrounds in limnology or environmental chemistry. Three positions are presently available in a water quality monitoring program. Additional openings are anticipated in an ecosystem development study of a new cooling lake. Experience in basic limnology, water chemistry, productivity/nutrient studies, or phycology is preferred. Applicant must be a U.S. citizen. Send resume, transcripts and three letters of reference to: **Dr. M. C. Newman, Savannah River Ecology Laboratory, University of Georgia, Drawer E, Aiken, SC 29801.** Applications accepted until positions are filled. The University of Georgia is an Equal Opportunity/Affirmative Action Institution.

Chairperson. The Department of Chemical Engineering and Environmental Engineering, Rensselaer Polytechnic Institute, invites applications for the position of Chairperson. The candidate should hold the Ph.D. degree and have made significant research and teaching contributions to chemical and/or environmental engineering. The department has excellent undergraduate and graduate programs, and numerous active research programs. Research support includes a substantial industrial component. The department currently has 35 active Ph.D. candidates. During the last few years, the Engineering School as a whole has expanded its graduate programs significantly; at the present time it is ranked in the top ten nationally in total external funding (>$20 million). Resume and four references should be sent to **Elmar R. Altwicker, Chairman, Search Committee, Department of Chemical Engineering and Environmental Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180-3590; telephone: (518) 266-6927. RPI is an EO/AA employer.**

Academic Position Environmental Chemist

Post at Environmental Health Laboratory, rank of Lecturer (non-tenure) to Senior Lecturer or higher (tenure track) depending on individual qualifications. Ph.D. and independent research experience required. Teaching areas: Water and wastewater chemistry; industrial hygiene; toxicology; analytical methods. Knowledge of Hebrew desirable. Send resume, list of publications and names of three referees to Director, School of Public Health of the Hebrew University and Hadassah, POB 1172, Jerusalem, Israel, by March 15, 1985.

ENVIRONMENTAL SCIENTIST

The National Wildlife Federation (NWF) has an immediate opening for a Staff Scientist at its Great Lakes Natural Resource Center in Ann Arbor, Michigan. The Staff Scientist works with NWF's legal and administrative staff to affect government policy relating to toxic substances and the Great Lakes ecosystem. NWF is the nation's largest nongovernmental, conservation edcation organization. Candidates should have a Ph.D. in Toxicology or related field, interest and experience in the environmental fate and transport of toxicants, and excellent writing and speaking skills. Salary commensurate with qualifications and experience, excellent benefits. Send resumes and copies of published work to: **Great Lakes Natural Resource Center, 802 Monroe, Ann Arbor, MI 48104.**

ENVIRONMENTAL ENGINEERING UNIVERSITY OF SOUTHERN CALIFORNIA

The Environmental Engineering Program of the Civil Engineering Department at the University of Southern California invites applications for a tenure-track faculty position at the assistant professor level. Individuals with background in hazardous waste management will be given preference. Candidates will be expected to develop vigorous research programs and to teach undergraduate and graduate classes.

The appointment is expected in September of 1985. Applications will be accepted until the position is filled. A resume and a list of three references should be sent to:

Dr. Ronald C. Henry
Biegler Hall of Engineering 213L
University of Southern California
Los Angeles, CA 90089-0231

GRADUATE STUDY IN SOCIAL ECOLOGY

Coursework and research opportunities are offered in water quality, microbacteriology, air pollution monitoring and health risks, and elemental baseline biogeochemistry leading to the M.A. or Ph.D. degrees in Social Ecology. An internationally-recognized interdisciplinary program which includes scientists and specialists in public and environmental law. Apply: **Jan Martin, Program in Social Ecology, University of California, Irvine, CA 92717.** EO/AA Employer.

Advertising Management for the American Chemical Society Publications

CENTCOM, LTD

President
Thomas N. J. Koerwer

Executive Vice President **James A. Byrne**
Senior Vice President **Benjamin W. Jones**

Alfred L. Gregory, *Vice President*
Clay S. Holden, *Vice President*
Robert L. Voepel, *Vice President*
Joseph P. Stenza, *Production Director*

25 Sylvan Road South
P.O. Box 231
Westport, Connecticut 06881
(Area Code 203) 226-7131.
Telex No. 643310

ADVERTISING SALES MANAGER
James A. Byrne, *VP*

ADVERTISING PRODUCTION MANAGER
Geri Anastasia

(Letters, *from p. 20*)

quences are that acidic spring showers remain acidic irrespective of the forest type and that summer and fall showers may or may not produce acidic throughfall, depending on the amount of acidic dry deposition already on the leaves.

It is a fact that some soils can neutralize precipitation acidity. To do so the water must come in contact with the soil and remain in contact long enough for chemical reactions to occur. During the winter and early spring, surface soils are frequently frozen, in which event they do not participate in the buffering of acidic meltwater. During heavy showers the movement of water through the soil is so rapid that little buffering occurs. The consequences are the acidic pulses we see in the spring and autumn, with elevated levels of SO_4 in the runoff. Because fish and aquatic insects are particularly vulnerable during these seasons, the effects on the biota can be profound, despite "safe" pH levels during the summer months.

Finally, it is a fact that soils can be acidic due to naturally occurring organic acids. Podzols, which are commonly found in areas exposed to acidic deposition, are naturally acidic soils. The humic and fulvic acids, which are associated with soluble aluminum and iron compounds, are deposited in deeper soil layers, especially the B horizon, giving a characteristic podzolic profile. As we stated (red herring number one) there *is* a difference between organic acids and inorganic acids. If organic acids are leached from soils the accompanying aluminum is organically bound. A number of streams in areas exposed to acid rain have low pH levels, excess levels of SO_4, very low levels of dissolved organic carbon, and elevated levels of inorganic aluminum. Acidification in these streams cannot be due to the leaching of naturally occurring organic acids.

If the hypothesis of Katzenstein is correct (that is, that soil and canopy processes predominate), then why are not all lakes on the Precambrian shield in watersheds with acid soils (i.e., podzols) not acidic? Why has a recent trend of increased lake acidification spontaneously accelerated in recent years, coinciding (and confusing us) with parallel increases in acid precipitation inputs? It seems most unlikely.

Magda Havas
Thomas C. Hutchinson
Gene E. Likens

ES&T RESEARCH

Airborne Trace Elements in Great Smoky Mountains, Olympic, and Glacier National Parks

Cliff I. Davidson,* William D. Goold, and Thomas P. Mathison

Departments of Civil Engineering and Engineering & Public Policy, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213

G. Bruce Wiersma

Earth and Life Sciences, E G & G Idaho, Idaho Falls, Idaho 83415

Kenneth W. Brown

Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Las Vegas, Nevada 89114

Michael T. Reilly

Department of Chemical Engineering, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213

■ Airborne trace elements were studied at remote sites in three U.S. National Parks where crustal weathering, sea spray, and long-range transport of anthropogenic emissions were likely to influence concentrations. Levels of all elements studied except Pb were smaller in Great Smoky Mountains National Park than in Olympic or Glacier Parks. Size distribution and Teflon plate dry deposition data showed that elements derived from crustal weathering were associated with larger particles and had greater dry deposition velocities than elements that were enriched relative to crustal composition. The bulk of the mass deposition of each element resulted from the small fraction of large airborne particles. On the basis of the dry deposition data, as well as concentration data obtained within and above the forest canopy, it is hypothesized that airborne particles may undergo successive deposition/resuspension processes during transport from source to ultimate sink, complicating the measurement of net influx of a species into a region from upwind areas.

Introduction

Over the past several years, a plethora of data has been published on atmospheric trace elements. We now have information on sources and transport pathways of several elements on a global scale. For example, we know that anthropogenic activities contribute far more Pb, Cd, and Zn to the atmosphere than emissions from natural sources (*1–3*). Airborne concentrations of most elements range over factors of $>10^3$ from the most remote regions of the world to industrial urban areas (*4, 5*). The physical and chemical forms vary from one location to another, although airborne size distributions of certain species show surprising uniformity (*6*).

There have been few studies of atmospheric trace elements in remote areas of the continental United States. Such data are nevertheless important: some of the elements are toxic and may cause biological change in wilderness ecosystems. Other elements can be used to trace long-range atmospheric transport of gaseous and particulate pollutants (*7*). Establishing a remote area data base is thus important for identifying future degradation of pristine environments caused by anthropogenic activities.

In this paper, we present the results of a pilot study conducted as part of the International Man and Biosphere (MAB) Program. The primary objective of the pilot study was to characterize several airborne trace elements, and to identify possible sources and transport processes influencing these elements, at sites in three remote areas of the U.S. A secondary objective was to establish suitable sampling locations and experimental methods for conducting future long-term monitoring studies as part of the MAB Program. The data discussed in this paper include airborne concentrations, size distributions, and dry deposition rates; it must be recognized that the short duration and limited number of sampling sites prevent generalization to other remote areas or to times other than those reported here.

Experimental Methods

Laboratory Procedures. All of the preparatory lab work for the project was conducted in the Carnegie-Mellon University (C-MU) class 100 clean laboratory (*8*). Modeled after the California Institute of Technology clean lab designed by Patterson and Settle (*9*), the C-MU facility is accessible through a change room and is maintained at positive pressure with filtered air. Only trained personnel wearing special garb are permitted to enter.

Several types of samplers were used to collect airborne particles for later analysis. Cellulose acetate filters of 47-mm diameter and 0.45-μm pore size (Millipore Corp. HAWP) were used at all of the remote locations. The minimum collection efficiency at flow rates used in this study, which occurs in the size interval 0.1–0.5 μm, is greater than 95% for these filters (*10*). Eight-stage Andersen 20-800 impactors with modified upper stages (*11*) were used to obtain size distribution data in Great Smoky

0013-936X/85/0919-0027$01.50/0

Mountains and Olympic National Parks. This impactor was chosen for the large number of size intervals and because sufficient aerosol could be collected in a reasonable time period. A high volume five-stage impactor (Sierra 235) was run simultaneously with the Andersen sampler in Olympic National Park to provide comparative data. An additional run comparing the two impactors was conducted 200 km SE of the Park. Finally, deposition plates were used to measure dry deposition at several sites in Olympic National Park. The plates were similar to those used in previous dry deposition studies (*12*, *13*) consisting of a Teflon disk attached to a stainless steel base.

Stringent contamination control procedures were implemented to prepare the various samplers for the field experiments. The filters were soaked in 0.1 N HNO_3 (National Bureau of Standards) for 30 min at room temperature, rinsed thoroughly with purified water, oven-dried, and loaded into stainless steel cartridges (Millipore XX 50-047). The cartridges had been soaked in 16 N HNO_3 (G. Frederick Smith, redistilled). Dilution water for washing the filters, and for diluting samples and standards, was provided by a Corning LD2-A demineralizer and MP3-A still followed by a final purification step with a subboiling quartz still. The final purification step was omitted when general purpose wash water was being produced for the laboratory.

The impactors were washed with acetone and water. Impactor and deposition plate substrates consisted of 20 mil FEP Teflon film which was soaked according to the following schedule: one room temperature 16 N HNO_3 bath for 24 h, two successive 80 °C 16 N HNO_3 baths for 24 h each (G. Frederick Smith, redistilled), and a 24-h 80 °C water bath.

Other investigators have shown that the use of adhesive material on impactor substrates is essential to control particle bounce-off (*14*, *15*). To minimize this problem, a mixture of 1 part commercially available Vaseline dissolved in 10 parts hexane (Baker Instra-analyzed) was pipetted onto the impactor substrates. Application of 2 mL of this mixture onto each Andersen substrate, and 3 mL onto each Sierra substrate, yielded a film of Vaseline several micrometers thick after the hexane evaporated. Optical microscope examination of ambient aerosol collected on the substrates verified that bounce-off was effectively controlled for the Andersen impactor, provided that the particle loading on each stage was small. Bounce-off was minimized for the Sierra impactor as long as the particle loading was small and the flow rate was relatively low.

Both impactors used backup filters to collect particles smaller than the cutoff diameters of their lowest stages. The Andersen impactor incorporated a cellulose acetate backup filter identical with those described above, while the Sierra impactor used a 20 cm × 25 cm HAWP cellulose acetate Hi-Vol filter for backup.

After preparation in the clean lab, the samplers were triple bagged in cleaned polyethylene (Clean Room Products UCF-POLY) and shipped to the field locations. The bags remained sealed until just prior to use. Upon concluding each run, the filters, impactor substrates, and deposition plate substrates were transferred at the field sites into airtight PFA Teflon digestion vessels (Savillex 01-350) to prevent loss of particles. The samples were then triple bagged for shipment to C-MU.

Once returned to the clean lab, a solution of 1.75 mL of 16 N HNO_3 (National Bureau of Standards), 0.75 mL of 16 N HF (Ultrex), and 2.5 mL of purified water was added to each vessel. The mixtures were then heated for several hours at 100 °C, a procedure which dissolved the cellulose acetate filters and which dissolved the particles deposited on the impactor and deposition plate substrates. The substrates were removed by using Teflon rods, and the remaining solutions were diluted to 4 N by adding purified water. The final samples were poured into 30-mL FEP Teflon jars (Nalge 1600-001) for analysis. These final samples included blanks which were prepared, transported to and from the field, and digested in an identical manner as the samples. All of the Teflon vessels and tools described above were washed by using the same 4-day cleaning procedure applied to the substrates.

Analyses were conducted by flameless atomic absorption spectrophotometry with background correction using a Perkin-Elmer 703 AA unit and HGA 2200 graphite furnace. Each sample was analyzed a minimum of 3 times, the standard deviation being used to assess analytical uncertainties. These errors were usually less than ±10%. The samples were stored in a freezer between analyses.

An uncertainty based on AA analytical errors and variations in the field blanks has been determined for each sample, using previously established methods (*16*, *17*). The net mass of an element in each atmospheric sample is calculated from

$$N = S - \bar{B} \tag{1}$$

where S is the total mass of an element in a given sample and $\bar{B}$ represents the arithmetic average of several corresponding field blanks. Note that each field blank has a value B_i which is itself an average of at least three separate AA determinations. The standard deviation of N, denoted by σ_N, is determined by compounding two types of uncertainties: the analytic errors in determination of S and each B_i, and the true variability in the set of B_i values caused by impure filter or substrate material, impure acids, and contamination during handling.

In some of the analyses, the value of N was less than $1.64\sigma_N$. For these cases, the value is quoted as an upper limit:

$$\text{net sample mass} < N + 1.64\sigma_N \tag{2}$$

This corresponds to the 95% confidence level (*18*). Other errors associated with the measurements in this study such as AA interferences and uncertainties in calibrating the flowmeters are considered systematic rather than random. Hence, these additional errors cannot be merely compounded with σ_N.

None of the data in this paper have been corrected for AA interference. However, the method of standard additions (*19*) was used on several of the samples to investigate possible interferences. The procedure of Waughman and Brett (*20*) was also used to check for interferences from Al, Ca, Fe, Mg, and Na on all elements examined; these species were chosen because of their relatively high concentrations in some samples. The technique involved measuring absorbances of the interfering agents at the wavelength of each element of interest. Results of these tests suggested that interferences were less than 10% in nearly all cases. A notable exception was Zn, where a possible interference from Ca was observed in some samples.

Field Sampling Procedures. Each of the three remote areas was selected after considering a number of alternate sites. Great Smoky Mountains National Park, TN/NC, was chosen as a relatively clean region in the eastern part of the country. Although surrounded by populated areas, this park represents one of the few large undeveloped tracts of land in the East. Olympic National Park, WA, was selected as a less polluted location, receiving winds

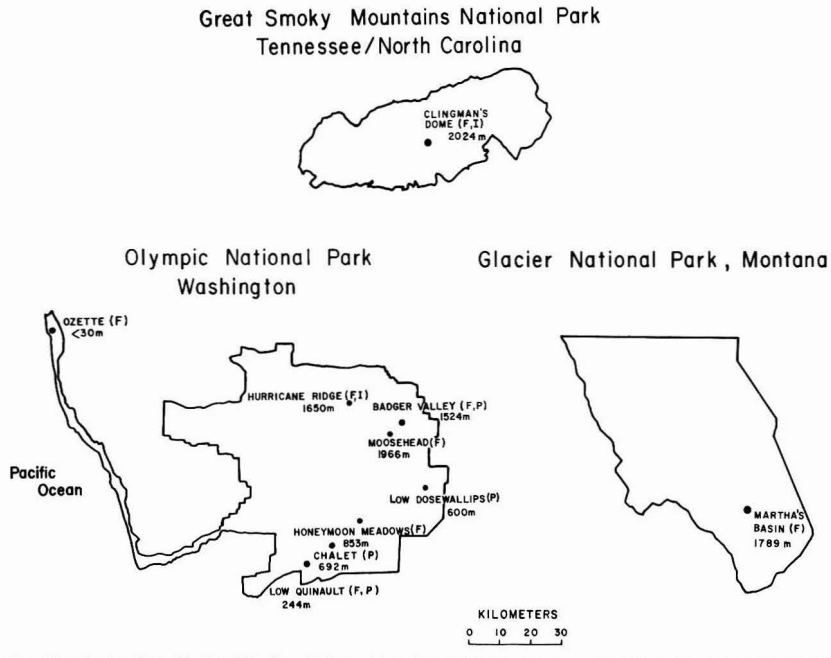


Figure 1. Sampling sites in the three National Parks. F, I, and P refer to filter, impactor, and Teflon plate sampling, respectively.

directly off the Pacific Ocean much of the time. Marine as well as continental influence was expected. Glacier National Park, MT, was chosen as a remote continental location in Western U.S., surrounded by sparsely populated agricultural land over extensive distances. All three of these parks are suitable candidates for monitoring anthropogenic pollutants transported over considerable distances.

Sampling in Great Smoky Mountains National Park took place during Oct 15–26, 1979. The experiments were conducted in a coniferous forest near Clingman's Dome, the highest point in the Park (see Figure 1). Electric power was available at a small shed approximately 100 m from the lookout tower on the peak, accessible by a footpath leading up from a dead-end paved road. The total distance from the road to the samplers was 700 m. To minimize the effects of traffic, the equipment was operated only during nighttime hours. Park records indicated that less than 10 vehicles/h used this road during the night.

The samplers included two cellulose acetate filters and two Andersen impactors; one of the impactors incorporated a rotating cowl/windvane assembly for proper directional alignment with the wind (*21*). The equipment was set up 1 m above the ground in a forest clearing. Additional airborne concentration measurements were conducted on a nearby radio tower just above the treetops at a height of 25 m. These measurements involved two cellulose acetate filters sampling sequentially, and operation was nearly simultaneous with the filters at 1 m. Power limitations prevented operation of the impactors simultaneously with that of the filters, although there was considerable overlap in sampling. Air flow rates were measured by using rotameters which were calibrated with Singer dry test meters. Flow rates through each filter and Andersen impactor averaged 7 and 10 L/min STP, respectively (STP = standard temperature and pressure of 0 °C and 760 mmHg). Calibration of the Andersen impactor with monodisperse aerosols at a similar flow rate was reported previously (*22*).

Sampling sites in Olympic National Park are shown in Figure 1. The most detailed runs took place near Hurricane Ridge, where electric power was available in a meadow 300 m west of the visitor center, approximately 200 m from a paved park road. Sampling at this site took place during July 28–Aug 7, 1980. Two cellulose acetate filters, one Andersen impactor, and one Sierra impactor, all positioned 1 m above the ground, were run simultaneously during nighttime hours when the visitor center was closed. Both impactors used rotating cowl/windvane assemblies. To determine the effect of automobile traffic, an additional cellulose acetate filter was run during daylight hours. Average flow rates through each filter, the Andersen impactor, and the Sierra impactor averaged 13, 13, and 230 liters per minute STP, respectively. Park records indicated that less than 4 vehicles per hour used the road during the periods of nighttime sampling, although traffic during daytime sampling averaged over 20 vehicles per hour.

Figure 1 shows that five sites besides Hurricane Ridge were used for filter sampling. Because electric power was not available, solar cells and nickel-cadmium batteries were used to drive a DuPont P4000A vacuum pump connected to two cellulose acetate filters at each location. Flow rates averaged one liter per minute STP through each filter. Details of this remote sampling system are given by van Ee (*23*). Four sites in the Park involved dry deposition measurement with a Teflon plate, as indicated in Figure 1. Two of these sites, Low Quinault and Badger Valley, included filters operated simultaneously with the plate exposures.

Sampling runs at these seven remote sites were not conducted simultaneously, but involved overlapping time periods. Setting up the experiments took place during Aug 5–9; concluding the runs occurred during Aug 12–17, 1980. Each experiment was run continuously for an average of 7 days. The plates and filters were set up approximately 0.5–1 m above the ground. All of the locations were at least 2 km from the nearest road; Chalet and Honeymoon Meadows were more than 10 km from a road.

Table I. Airborne Trace Element Data for Great Smoky Mountains National Park

elements	airborne concentration, ng/m³ STP: within canopy	above canopy	av	crustal enrichment factor, av
crustal				
Al	86	65	76	1.0
Ba	0.28	0.26	0.27	0.69
Ca	26	22	24	0.63
Fe	ND[a]	250	250	4.8
Mg	57	50	54	2.5
Na	40	30	35	1.6
Ti	4.1	1.4	2.7	0.52
enriched				
Ag	0.025	0.012	0.018	280
As	1.9	<1.2	<1.6	<960
Cd	0.17	0.070	0.12	650
Cu	ND	1.6	1.6	31
Pb	23	6.8	15	1300
Zn	4.4	2.3	3.3	51

[a] ND, no data.

Sampling in Glacier Park was conducted during Aug 18–Sep 14, 1981, at Martha's Basin, near Eaglehead Mountain. A cellulose acetate filter was set up in the center of a meadow and operated at 1 L/min STP with the solar cell/battery pack system (*23*). This site was approximately 16 km from the nearest road.

Data Presentation and Discussion

Table I lists airborne concentrations of 13 trace elements in Great Smoky Mountains National Park. Average in-canopy and above-canopy concentrations, as well as the overall average concentration, are given for each element. In a few instances, upper limit values (see eq 2) have been included in the averaging process. Above-canopy concentrations for Ba and Ti are based on confidence levels of 80–90% rather than 95% due to variability in the field blanks; above-canopy concentrations for As are upper limits due to poor sample:blank ratios. The elements are categorized as crustal or enriched, based upon values of the enrichment factor, EF (*4*):

$$\mathrm{EF} = \frac{C_X/C_{Al}}{C_{X,crust}/C_{Al,crust}} \quad (3)$$

where C_X refers to the airborne concentration of any element X, C_{Al} is the airborne concentration of aluminum, $C_{X,crust}$ is the average concentration of element X in the earth's crust, and $C_{Al,crust}$ is the average concentration of aluminum in the earth's crust. In the present study, the relative crustal abundances have been taken from Taylor (*24*). Values of EF corresponding to each average airborne concentration are shown in Table I. In general, these values agree with other remote area enrichments reported in the literature (*4*, *5*).

The data show that the airborne concentrations at 1 m are somewhat greater than those at 25 m. This negative gradient suggests that vegetation debris, soil dust, and other material originating in the forest may be largely responsible for the trace element concentrations listed in the table. However, the fact that airborne Pb concentrations within the canopy are especially high suggests a more complex interpretation.

Significant amounts of Pb are emitted from motor vehicles in the populated areas surrounding the Smokies; the location of Clingman's Dome remote from local traffic implies that most of the Pb measured at the site was transported over distances of tens of kilometers or greater.

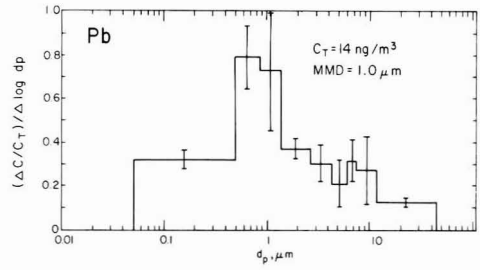


Figure 2. Distributions of airborne Pb mass with respect to particle size in Great Smoky Mountains National Park. The total airborne Pb concentration in all size ranges is denoted by C_T, while the mass median diameter is represented by MMD.

Most likely, Pb carried previously to the site had deposited and accumulated on surfaces in the forest and had later become resuspended due to in-canopy air flow and the movement of plants and animals. It is conceivable that such deposition and resuspension processes might occur several times before airborne material is eventually incorporated into the forest floor. Many of the elements in Table I in addition to Pb may have been influenced by these processes.

The presence of a negative concentration gradient in the Smokies is consistent with data obtained earlier in the Sierra Mountains of California (*25*). Dry, windy conditions conducive to soil resuspension resulted in negative airborne concentration gradients of Pb and six crustal elements, although positive gradients were measured for all of these species when the ground was covered with snow. The resuspended Pb was shown to be derived from anthropogenic sources, particularly motor vehicle emissions, which had been transported previously into the Sierras and had deposited on soil and vegetation (*26*).

Figure 2 shows the mass concentration distribution function for Pb measured at Clingman's Dome during these experiments. The plot is normalized to the total airborne Pb concentration. Minimum and maximum diameters of 0.05 and 40 μm, respectively, have been assumed on the basis of scanning electron microscopy data reported in the literature (*27*) and on optical microscopy examination of the impactor stages as part of the present study. The graph represents average data from the two Andersen impactors operated during overlapping time intervals; the error bars indicate the range of the two values for each size range. The distribution illustrates two features of well-aged aerosol. First, very small particles have grown into the stable size range 0.5–1 μm. Urban area data, in contrast, often show a greater fraction of Pb below 0.5 μm (*6*). Second, the weak upper mode suggests that much of the large Pb-containing aerosol emitted from motor vehicles and industrial sources has deposited en route to Clingman's Dome. One complication is that successive deposition and resuspension processes can influence the shape of the distribution. Such processes may increase the sizes of particles associated with airborne Pb because of attachment to soil particles and biological material, but quantification of this effect is difficult. The impactor samples collected at Clingman's Dome were analyzed only for Pb.

Weather data from the National Oceanic and Atmospheric Administration (*28*) suggest that material transported into the Smokies from surrounding areas during the sampling period probably came from sources to the west. Winds at the 850-mbar level for stations in several states around the park were in the range 5–10 m/s. For

Table II. Airborne Trace Element Data for Olympic National Park

elements	airborne concentration, ng/m³ STP: Badger Valley	Honeymoon Meadows	Hurricane Ridge	Low Quinault	Moosehead	Ozette	av	crustal enrichment factor, av
crustal								
Al	100	120	120	60	110	150	110	1.0
Ba	0.91	<1.1	0.43	<1.1	0.94	<1.8	1.1	1.8
Ca	21	32	27	<31	28	38	30	0.53
Fe	300	350	220	200	340	480	310	4.1
Mg	310	380	340	200	320	510	340	11.
Na	290	240	190	180	280	380	260	8.2
Ti	3.6	5.2	4.3	<6.5	3.6	5.2	4.7	0.61
enriched								
Ag	0.084	0.15	0.13	<0.12	0.10	0.15	0.12	1300
As	1.7	2.0	2.0	<3.4	2.0	3.0	2.3	960
Cd	0.51	0.47	0.57	0.30	0.61	0.76	0.54	2000
Cu	5.4	6.4	6.0	3.3	6.7	6.3	5.6	76
Pb	1.7	2.5	2.6	1.4	1.9	3.0	2.2	130
Zn	6.9	10	11	4.6	7.4	13	8.9	94

the first few days of the experiment, these winds were from the southwest, gradually shifting to northwest by the end of the run. Such westerly winds are typically encountered in the Smokies during October (*28*).

Airborne concentrations in Olympic National Park are given in Table II. Each value represents the average of two filters run simultaneously; the variability, as denoted by half the difference between the two airborne concentrations, averages 4% of the Table II values for Hurricane Ridge and 8% of the Table II values for the other sites. The smaller dispersions for Hurricane Ridge reflect the greater air sampling volumes and hence lesser sensitivity to variations in field blanks.

The Hurricane Ridge values in Table II are for nighttime sampling. The filter operated at this location during daylight hours yielded only slightly greater concentrations: the ratio daytime/nighttime values averaged 1.20 ± 0.20 for the 13 elements, with no significant differences in the ratio for crustal as opposed to enriched species. The fact that this ratio is near unity suggests that the diurnal cycle in meteorological conditions, as well as daytime activity at the nearby visitor center, had little influence on airborne trace element levels during the experiment.

Values of the enrichment factor in Table II suggest the importance of sea spray in influencing Mg and Na levels in the park; values of EF for the other crustal elements are closer to unity, implying crustal weathering as the source. Sea spray and soil dust also may have affected concentrations of the six enriched elements in the table. Duce et al. (*29*) have shown that the ocean surface microlayer contains much greater concentrations of these elements than bulk seawater, implying that sea spray aerosol may be enriched.

Winds at the 850-mbar level measured at Quillayute and Olympia (*28*), the two stations closest to the park, were predominantly northwesterly averaging 5 m/s for the period July 29 to Aug 17. Air reaching the park during these experiments was thus directly off the Pacific Ocean. However, this does not imply the complete absence of natural or anthropogenic material emitted on the continent, since such material may have been previously transported out to sea during offshore breezes and then sampled upon return to land.

Airborne size distributions measured with the Andersen impactor at Hurricane Ridge are presented in Figure 3. Representative data are given for three crustal and four enriched elements. Standard deviations are indicated by the error bars, and size ranges with shading rather than

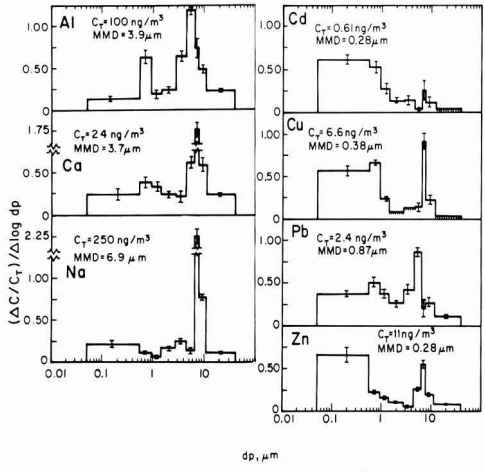


Figure 3. Distributions of airborne trace element mass with respect to particle size in Olympic National Park.

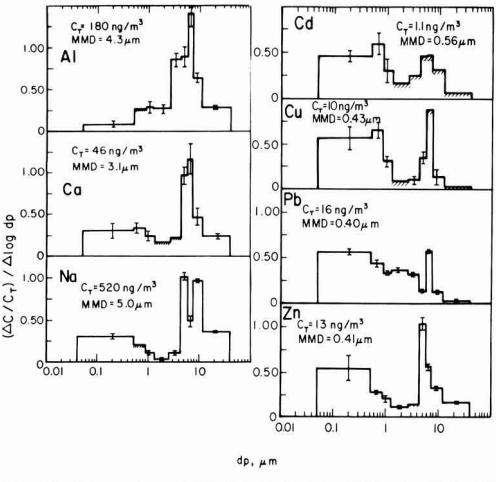


Figure 4. Distributions of airborne trace element mass with respect to particle size in Packwood, WA.

error bars denote upper limit values as defined by eq 2. Note that the total airborne concentrations measured with

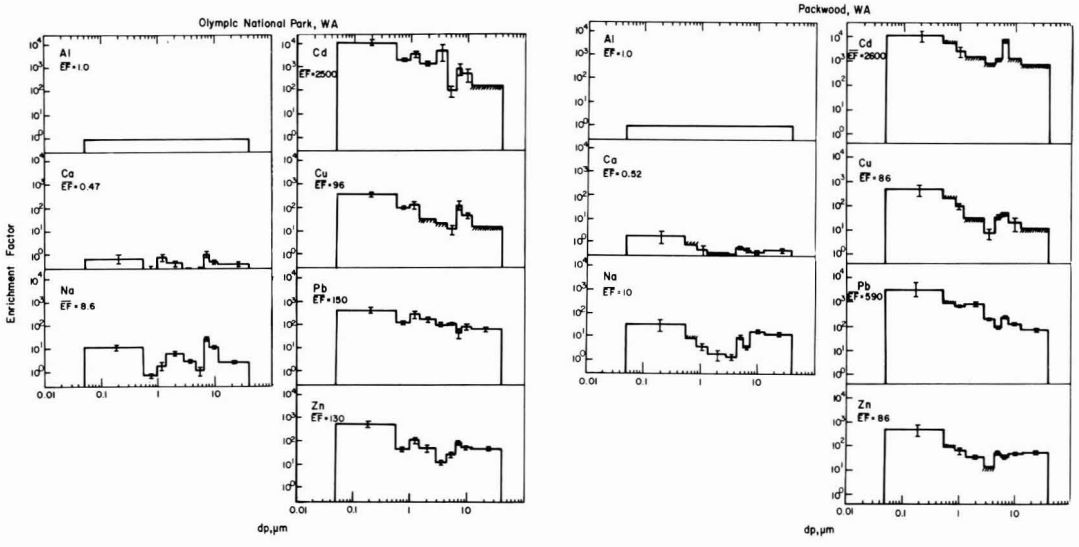


Figure 5. Distributions of enrichment factors with respect to particle size, based on the mass distributions of Figures 3 and 4.

the impactor and backup filter are similar to the Hurricane Ridge values of Table II, obtained simultaneously with cellulose acetate filters.

Figure 4 shows additional size distribution data obtained with the same equipment at Packwood, WA, for comparison with the Olympic Park data. This rural site is 200 km southeast of the park and 50 km northeast of Mt. St. Helens volcano. Sampling was conducted during nighttime hours on July 22–27, 1980. A paved road 500 m north of the site carried an average of 10 cars per hour during operation of the samplers. Light westerly winds and dry surface conditions predominated throughout the experiment. A minor eruption of the volcano on July 22 preceded the run, although winds carried the plume south of the sampling site. Deposited volcanic debris from the May 18 eruption was still abundant in the area, however, and it is possible that resuspended ash influenced the data.

Size distributions measured with the Sierra impactor at Hurricane Ridge and at Packwood resembled the Andersen data of Figures 3 and 4, in terms of total airborne concentrations, mass median diameters, and overall shapes of the distributions. The agreement suggests that operation of the Sierra with adhesive coated substrates at the reduced flow rate of 230 L/min effectively minimizes particle bounce problems. The same conclusion was reached by Knuth (*30*), who calibrated the Sierra impactor with monodisperse aerosols at a flow rate comparable to that used in this study.

The distributions at Hurricane Ridge appear quite similar to those at Packwood, in spite of greater airborne concentrations at the latter site. Most of the elements show bimodal spectra. The crustal elements have a pronounced supermicron peak, while the enriched elements have a large fraction of mass on the backup filter. Other investigators have shown that erosion of the earth's crust is a major source of supermicron particles; a variety of natural and anthropogenic sources, particularly combustion processes, are responsible for production of submicron particles (*4*, *31*, *32*).

The large peaks at 6–8 μm may be partially due to biases in the Andersen impactor. Most of the peaks occur on stage 2, which has the narrowest size range of any of the stages. If the true size range is not as narrow as indicated, due to the nonideal nature of the sampler, then the peaks may be exaggerated. The Sierra impactor data showed smaller, broader peaks in this size range, however, suggesting that the Andersen data are at least qualitatively correct.

Additional information can be obtained by using the distributions of Figures 3 and 4 to construct plots of crustal enrichment vs. particle size. Results are shown in Figure 5. Note that the enrichment factor decreases with increasing particle diameter for Cd, Cu, Pb, and Zn, indicating that the larger particles more closely resemble the earth's crustal composition than do the submicron fractions of these distributions. This is consistent with other data in the literature showing preferential emission of volatile elements from combustion processes, resulting in highly enriched submicron aerosol (*33–35*). Even the supermicron particles for these elements show appreciable enrichment, however; this may reflect attachment of submicron particles to crustal material during atmospheric transport and deposition/resuspension processes, or condensation of volatile species on the surface of large particles immediately following combustion (*36*, *37*).

Table III lists fluxes and deposition velocities (computed as the ratio flux/airborne concentration) for Olympic National Park. Because no airborne concentration data were obtained at Chalet or Low Dosewallips, the park average values in Table II have been used to compute deposition velocities for these two sites. The value of σ_N for the fluxes in Table III averages 6% of the values listed in the table.

Note that the deposition velocities of the crustal elements onto the Teflon plates are considerably greater than those of the enriched elements. Similar findings have been reported for deposition into dustfall buckets on the Pacific coast (*38*) and for deposition onto filter paper in the United Kingdom (*39*, *40*).

The size distributions of Figure 3 can be used to gain insight into mechanisms of trace element dry deposition onto the Teflon plates. The overall dry deposition velocity v_d can be calculated as

$$v_d = \sum_{i=1}^{n} v_{di} C_i / \sum_{i=1}^{n} C_i \qquad (4)$$

All forward thinking environmental scientists depend on ES&T. They get the most authoritative technical and scientific information on environmental issues—and so can you! Have *your own* subscription delivered directly to you each month!

☐ **YES!** Enter my own subscription to *ENVIRONMENTAL SCIENCE & TECHNOLOGY* at the rate I've checked below: 1985

One Year	U.S.	Mexico & Canada	Europe	All Other Countries
ACS Members *	☐ $ 26	☐ $ 34	☐ $ 40	☐ $ 49
Nonmembers—Personal *	☐ $ 35	☐ $ 43	☐ $ 49	☐ $ 58
Nonmembers—Institutional	☐ $149	☐ $157	☐ $163	☐ $172

☐ Payment Enclosed (Payable to American Chemical Society)
☐ Bill Me ☐ Bill Company Charge my: ☐ VISA ☐ MasterCard

Card No. _____

Exp. Date _____ Interbank # _____ (Mastercard Only)

Signature _____

Name _____

Title _____ Employer _____

Address _____

City, State, Zip _____

Employer's Business: ☐ Manufacturing, type _____

☐ Academic ☐ Government ☐ Other _____

***Subscriptions at these rates are for personal use only.**

All foreign subscriptions are now fulfilled by air delivery. Foreign payment must be made in U.S. currency by international money order, UNESCO coupons, U.S. bank draft, or order through your subscription agency. For nonmember subscription rates in Japan, contact Maruzen Co., Ltd.

Please allow 45 days for your first copy to be mailed. Redeem until December 31, 1985.

MAIL THIS POSTAGE-PAID CARD TODAY!

DEPEND
ES&T
ON IT!

All forward thinking environmental scientists depend on ES&T. They get the most authoritative technical and scientific information on environmental issues—and so can you! Have *your own* subscription delivered directly to you each month!

☐ **YES!** Enter my own subscription to *ENVIRONMENTAL SCIENCE & TECHNOLOGY* at the rate I've checked below: 1985

One Year	U.S.	Mexico & Canada	Europe	All Other Countries
ACS Members *	☐ $ 26	☐ $ 34	☐ $ 40	☐ $ 49
Nonmembers—Personal *	☐ $ 35	☐ $ 43	☐ $ 49	☐ $ 58
Nonmembers—Institutional	☐ $149	☐ $157	☐ $163	☐ $172

☐ Payment Enclosed (Payable to American Chemical Society)
☐ Bill Me ☐ Bill Company Charge my: ☐ VISA ☐ MasterCard

Card No. _____

Exp. Date _____ Interbank # _____ (Mastercard Only)

Signature _____

Name _____

Title _____ Employer _____

Address _____

City, State, Zip _____

Employer's Business: ☐ Manufacturing, type _____

☐ Academic ☐ Government ☐ Other _____

*Subscriptions at these rates are for personal use only.

All foreign subscriptions are now fulfilled by air delivery. Foreign payment must be made in U.S. currency by international money order, UNESCO coupons, U.S. bank draft, or order through your subscription agency. For nonmember subscription rates in Japan, contact Maruzen Co., Ltd.

Please allow 45 days for your first copy to be mailed. Redeem until December 31, 1985.

MAIL THIS POSTAGE-PAID CARD TODAY!

(800) 424-6747 (U.S. only)

NO POSTAGE
NECESSARY
IF MAILED
IN THE
UNITED STATES

BUSINESS REPLY CARD

FIRST CLASS PERMIT NO 10094 WASHINGTON D C

POSTAGE WILL BE PAID BY ADDRESSEE

American Chemical Society
Periodicals Marketing Dept.
1155 Sixteenth Street, N.W.
Washington, D.C. 20036

(800) 424-6747 (U.S. only)

NO POSTAGE
NECESSARY
IF MAILED
IN THE
UNITED STATES

BUSINESS REPLY CARD

FIRST CLASS PERMIT NO 10094 WASHINGTON D C

POSTAGE WILL BE PAID BY ADDRESSEE

American Chemical Society
Periodicals Marketing Dept.
1155 Sixteenth Street, N.W.
Washington, D.C. 20036

Table III. Dry Deposition Data for Olympic National Park

elements	dry deposition flux, ng/(cm² day)				dry deposition velocity, cm/s				
	Badger Valley	Low Quinault	Chalet	Low Dosewallips	Badger Valley	Low Quinault	Chalet	Low Dosewallips	av
crustal									
Al	86	52	67	49	9.7	9.9	6.9	5.1	7.9
Ba	0.32	0.21	0.23	0.12	3.2	>2.4	2.5	1.3	2.3
Ca	18	22	15	11	9.6	>8.3	5.8	4.4	7.0
Fe	230	150	190	160	8.8	8.4	7.1	5.9	7.5
Mg	330	330	250	115	12	20	8.4	3.9	11
Na	220	150	140	97	8.9	10	6.3	4.3	7.4
Ti	2.3	1.5	3.5	1.6	7.2	>2.7	8.5	4.0	5.6
enriched									
Ag	0.0046	0.0031	0.0015	0.0015	0.63	>0.30	0.15	0.15	0.30
As	0.057	0.024	0.021	0.030	0.39	>0.081	0.10	0.15	0.18
Cd	0.015	0.0060	0.0057	0.0057	0.34	0.23	0.12	0.12	0.20
Cu	0.31	0.071	0.072	0.047	0.67	0.25	0.15	0.097	0.29
Pb	0.0072	0.023	0.027	0.040	0.048	0.20	0.14	0.21	0.15
Zn	0.30	0.30	0.097	0.16	0.51	0.75	0.13	0.21	0.40

where v_{di} and C_i represent the deposition velocity and airborne concentration, respectively, corresponding to impactor stage i. Previous field work with Teflon plates has shown that sedimentation of the small fraction of large airborne particles dominates the mass deposition of Cd, Pb, and Zn in the Los Angeles Basin (*13*). The large crustal element deposition velocities in Table III suggest the importance of sedimentation at the Olympic Park sites. When sedimentation is used for v_{di} in eq 4, deposition velocities as great as those in Table III are calculated only if one assumes very large particles sizes associated with the top impactor stage. For example, v_d values of 8.1, 7.7, and 4.1 cm/s, respectively, are calculated for Al, Ca, and Na if all of the mass on the top impactor stage is associated with particles of 200-μm aerodynamic diameter. For the enriched elements, agreement between measured and calculated v_d requires smaller maximum aerodynamic diameters; a value of 50 μm yields deposition velocities of 0.19, 0.16, 0.43, and 0.40 cm/s for Cd, Cu, Pb, and Zn, respectively.

It is likely that the values of C_i for each element on the uppermost impactor stage are underestimates, due to deviations from isokinetic sampling and losses in the rotating cowl inlet (*41*, *42*). Greater values of C_i would require particle diameters smaller than 50 or 200 μm in order to obtain agreement with the measured deposition velocities. Nevertheless, the large values of v_d for the crustal elements measured with the Teflon plates imply that large particles are indeed responsible for the observed deposition data. The values of v_d for the enriched elements, although smaller, are comparable to deposition velocities reported for Pb and SO_4^{2-} in other studies where the influence of large particles was demonstrated (*43–45*). Such large particles probably include soil dust, vegetation debris, and resuspension of previously deposited material from a variety of natural and anthropogenic sources.

Table IV lists airborne concentrations and enrichment factors for nine trace elements in Glacier National Park. The concentrations of the first five elements reflect the crustal composition at the sampling site: siltite, quartzite, red argilite, and limestone are found in the vicinity of Martha's Basin (*46*). These rocks contain large amounts of the crustal elements listed in the table, with the exception of Al. The high concentrations relative to Al are partially responsible for the large enrichments observed for many of the elements in Table IV.

Winds at the 850-mbar level measured at several stations surrounding the park were in the range 5–10 m/s, predominantly westerly but showing some variability (*28*). Such wind conditions are typical of this area. Although the entire region is sparsely populated, a few large point sources in western Montana and motor vehicle traffic throughout the region may have affected air quality in the park.

Table IV. Airborne Trace Element Data for Glacier National Park

elements	airborne concentration, ng/m³ STP	crustal enrichment factor
crustal		
Al	75	1.0
Ba	7.8	20
Ca	230	6.0
Fe	360	7.0
Mg	43	2.0
enriched		
Ag	<0.19	<2900
As	1.3	780
Cd	0.99	5400
Pb	4.6	400

Comparing the data in Tables I, II, and IV shows that airborne trace element concentrations in the Smokies were generally smaller than those in Olympic or Glacier Park. Greater vegetation cover and soil moisture in the Smokies, compared with the large amounts of exposed rock and soil in the other parks, may be partially responsible. It is noteworthy that precipitation was well below normal for several weeks during and preceding the Olympic Park runs (*28*), resulting in dry conditions which may have contributed to airborne soil dust levels. Enriched as well as crustal element concentrations may have been affected due to resuspension of previously deposited material.

In contrast to the other elements, the concentration of Pb was greatest in the Smokies. High traffic densities in upwind areas are most likely responsible. Note that Stevens et al. (*47*) measured an average nighttime Pb concentration of 74 ng/m³ 1 year earlier, at a lowland site in the Smokies closer to populated areas.

It is important to recognize the limitations of the data presented here. Flocchini et al. (*48*, *49*) have shown that airborne concentrations of many trace elements in Western U.S. vary with season of the year and that there also may be considerable day-to-day and site-to-site variation. The National Park data obtained in this pilot study involved sampling over periods of 1–4 weeks at a limited number

of locations. Nevertheless, the small airborne concentrations of some elements reported here (especially Pb), compared with published data from less remote sites in the continental U.S. (*5*), suggest the importance of site selection when one is attempting to measure representative concentrations in remote regions.

Conclusions

Airborne trace elements in Great Smoky Mountains, Olympic, and Glacier National Parks result from a variety of sources such as crustal weathering, sea spray, and anthropogenic activities. Differences in concentration among the three parks in part reflect the complexity of sources influencing these locations.

Concentrations of most elements within the forest canopy in the Smokies were greater than those above the treetops, suggesting the forest as a source. Resuspension of material that had been previously transported into the park and had deposited on surfaces, as well as soil dust and vegetation debris, may have been significant.

Trace element size distributions in the Olympics and in a rural area of central Washington were bimodal. Crustal elements were associated primarily with supermicron particles, while elements with high enrichment relative to crustal composition were predominantly submicron. The enrichment factors decreased with increasing particle size.

Dry deposition data obtained with Teflon plates in Olympic National Park showed consistently large deposition velocities for the crustal elements, with smaller values for the enriched elements. Calculating deposition rates from the size distribution data indicated that the small fraction of large airborne particles was most likely responsible for the bulk of the observed Teflon plate dry deposition for both categories of elements. Results of this research thus suggest that surrogate surfaces may be of value when studying species whose mass deposition onto natural surfaces is dominated by large particles. Use of such surfaces to assess dry deposition may be difficult in areas where particle transport is characterized by successive deposition and resuspension processes, rather than by direct atmospheric transport from source to sink.

Acknowledgments

The assistance of several National Park Service personnel is gratefully acknowledged: G. Larsen and R. Matthews (Smokies); J. Aho, P. Crawford, R. Olson, W. Reynolds, J. Rockwell, and L. Whitney (Olympics); J. DeSanto, C. Fauley, and C. Martinka (Glacier). Other individuals assisting in this research include R. Schonbrod, J. van Ee (EPA–Las Vegas), R. Binda, R. Breckenridge, C. Frank (EG&G Idaho), T. Hinckley, R. Earhart (USGS), L. Cartwright, M. Nasta, and S. Santhanam (C-MU). The solar cell-battery pack units were provided by the Environmental Monitoring Systems Laboratory, EPA, constructed under the Federal Photovoltaic Utilization Program of DOE. The manuscript was prepared by G. Blake and M. LaBella.

Literature Cited

(1) Patterson, C. C. In "Lead in the Human Environment"; National Academy of Sciences: Washington, DC, 1980; pp 265–349.
(2) Nriagu, J. O. In "Cadmium in the Environment"; Nriagu, J. O., Ed.; Wiley: New York, NY, 1980; pp 71–114.
(3) Nriagu, J. O.; Davidson, C. I. In "Zinc in the Environment"; Nriagu, J. O., Ed.; Wiley: New York, NY, 1980; pp 113–159.
(4) Rahn, K. A. "The Chemical Composition of the Atmospheric Aerosol". Graduate School of Oceanography, University of Rhode Island, Kingston, RI, 1976, Technical Report.
(5) Wiersma, G. B.; Davidson, C. I. In "Toxic Metals in the Air"; Nriagu, J. O.; Davidson, C. I., Eds.; Wiley: New York, NY, 1985; in press.
(6) Davidson, C. I.; Osborn, J. F. In "Toxic Metals in the Air"; Nriagu, J. O.; Davidson, C. I., Eds.; Wiley: New York, NY, 1985; in press.
(7) Rahn, K. A.; Shaw, G. E. *Nav. Res. Rev.* **1982**, *34*, 3–26.
(8) Shaeffer, M. D.; Davidson, C. I. *Heat./Piping/Air Cond.* **1979**, *51*, 61–62.
(9) Patterson, C. C.; Settle, D. M. *NBS Spec. Publ. (U.S.)* **1976**, *No. 422*, 509–539.
(10) Spurny, K. R.; Lodge, J. P., Jr.; Frank, E. R.; Sheesley, D. C. *Environ. Sci. Technol.* **1969**, *3*, 453–464.
(11) McFarland, A. R.; Wedding, J. B.; Cermak, J. E. *Atmos. Environ.* **1977**, *11*, 535–539.
(12) Huntzicker, J. J.; Friedlander, S. K.; Davidson, C. I. *Environ. Sci. Technol.* **1975**, *9*, 448–457.
(13) Davidson, C. I.; Friedlander, S. K. *J. Geophys. Res.* **1978**, *83*, 2343–2352.
(14) Dzubay, T. G.; Hines, L. E.; Stevens, R. K. *Atmos. Environ.* **1976**, *10*, 229–234.
(15) Lawson, D. R. *Atmos. Environ.* **1980**, *14*, 195–199.
(16) Maenhaut, W.; Zoller, W. H. *J. Radioanal. Chem.* **1977**, *37*, 637–650.
(17) Davidson, C. I.; Chu, L.; Grimm, T. C.; Nasta, M. A.; Qamoos, M. P. *Atmos. Environ.* **1981**, *15*, 1429–1437.
(18) Currie, L. A. *Anal. Chem.* **1968**, *40*, 586–593.
(19) Timm, J.; Diehl, H.; Harbach, D. *Fresenius' Z. Anal. Chem.* **1980**, *301*, 199–202.
(20) Waughman, G. J.; Brett, T. *Environ. Res.* **1980**, *21*, 385–393.
(21) Wedding, J. B.; McFarland, A. R.; Cermak, J. E. *Environ. Sci. Technol.* **1977**, *11*, 387–390.
(22) Davidson, C. I. Ph.D. Thesis, California Institute of Technology Pasadena, CA, 1977.
(23) van Ee, J. J. "The EPA Solar Pack Manual". Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, 1981, unpublished report.
(24) Taylor, S. R. *Geochim. Cosmochim. Acta* **1964**, *28*, 1273–1285.
(25) Davidson, C. I.; Elias, R. W. *Geophys. Res. Lett.* **1982**, *9*, 91–93.
(26) Elias, R. W.; Hirao, Y.; Patterson, C. C. *Geochim. Cosmochim. Acta* **1982**, *46*, 2561–2580.
(27) Hidy, G. M. "Characterization of Aerosols in California"; State of California Air Resources Board: Sacramento, CA, 1974; Vol. II, Chapter 3.
(28) National Oceanic and Atmospheric Administration, Asheville, NC, Oct 1979; July–Aug 1980; Aug–Sept 1981, unpublished climatological data.
(29) Duce, R. A.; Hoffman, G. L.; Ray, B. J.; Fletcher, I. S.; Wallace, G. T.; Fasching, J. L.; Piotrowicz, S. R.; Walsh, P. R.; Hoffman, E. J.; Miller, J. M.; Heffter, J. L. In "Marine Pollutant Transfer"; Windom, H. L.; Duce, R. A., Eds.; D. C. Health: Lexington, MA, 1976; pp 77–119.
(30) Knuth, R. H. "Calibration of a Modified Sierra Model 235 Slotted Cascade Impactor". U.S. Department of Energy, New York, NY, 1979, Environmental Measurements Laboratory Report EML-360.
(31) Johansson, T. B.; Van Grieken, R. E.; Nelson, J. W.; Winchester, J. W. *Anal. Chem.* **1976**, *47*, 855–860.
(32) Winchester, J. W.; Ferek, R. J.; Lawson, D. R.; Pilotte, J. O.; Thiemens, M. H.; Wangen, L. E. *Water, Air, Soil. Pollut.* **1979**, *12*, 431–440.
(33) Zoller, W. H.; Gladney, E. S.; Duce, R. A. *Science (Washington, D.C.)* **1974**, *183*, 198–200.
(34) Lee, R. E., Jr.; Crist, H. L.; Riley, A. E.; MacLeod, K. E. *Environ. Sci. Technol.* **1975**, *9*, 643–647.
(35) Davidson, C. I.; Santhanam, S.; Stetter, J. R.; Flotard, R. D.; Gebert E. *Environ. Monit. Assess.* **1982**, *1*, 313–335.
(36) Linton, R. W.; Loh, A.; Natusch, D. F. S.; Evans, C. A., Jr.; Williams, P. *Science (Washington, D.C.)* **1976**, *191*, 852–854.
(37) Van Craen, M. J.; Denoyer, E. A.; Natusch, D. F. S.; Adams, F. *Environ. Sci. Technol.* **1983**, *17*, 435–439.
(38) Crecelius, E. A.; Robertson, D. E.; Abel, K. H.; Cochran, D. A.; Weimer W. C. "Atmospheric Deposition of ^{7}Be and Other Elements on the Washington Coast". Richland, WA,

1978, Battelle Pacific Northwest Laboratory Report PNL-2500, Part 2, pp 7.25-7.26.
(39) Peirson, D. H.; Cawse, P. A.; Salmon, L.; Cambray, R. S. *Nature (London)* **1973**, *241*, 252-256.
(40) Peirson, D. H.; Cawse, P. A.; Cambray, R. S. *Nature (London)* **1974**, *251*, 675-679.
(41) Liu, B. Y. H.; Pui, D. Y. H. *Atmos. Environ.* **1981**, *15*, 589-600.
(42) Shaw, R. W.; Stevens, R. K.; Lewis, C. W.; Chance, J. H. *Aerosol Sci. Technol.* **1983**, *2*, 53-67.
(43) Davidson, C. I.; Miller, J. M.; Pleskow, M. A. *Water, Air, Soil Pollut.* **1982**, *18*, 25-43.
(44) Davidson, C. I.; Lindberg, S. E.; Schmidt, J. A.; Cartwright, L. G.; Landis, L. R. *J. Geophys. Res.*, in press.
(45) Lindberg, S. E.; Lovett, G. M. *Environ. Sci. Technol.*, in press.
(46) (a) Raup, O. B.; Earhart, R. L.; Whipple, J. W.; Carrara, P. E. "Geology Along Going-to-the-Sun Road, Glacier National Park"; Falcon Press: Helena, MT, 1983; (b) R. L. Earhart, personal communication, 1983.
(47) Stevens, R. K.; Dzubay, T. G.; Shaw, R. W., Jr.; McClenny, W. A.; Lewis, C. W.; Wilson, W. E. *Environ. Sci. Technol.* **1980**, *14*, 1491-1498.
(48) Flocchini, R. G.; Cahill, T. A.; Ashbaugh, L. L.; Eldred, R. A.; Pitchford, M. L. *Atmos. Environ.* **1981**, *15*, 315-320.
(49) Flocchini, R. G.; Cahill, T. A.; Pitchford, M. L.; Eldred, R. A.; Feeney, P. J.; Ashbaugh, L. L. *Atmos. Environ.* **1981**, *15*, 2017-2030.

Received for review July 25, 1983. Revised manuscript received June 30, 1984. Accepted August 30, 1984. This work was funded by the Water Resources Field Support Laboratory of the U.S. National Park Service and by the Claude W. Benedum Foundation. A preliminary version of this paper was presented at the Fourth International Conference on Precipitation Scavenging, Dry Deposition, and Resuspension, Santa Monica, CA, Nov 29-Dec 3, 1982.

Statistical Considerations in the Evaluation of Chronic Aquatic Toxicity Studies†

Thomas Caplzzi,* Leonard Oppenheimer, Hina Mehta, and Hussein Naimie

Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065

Jenny L. Fair

Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

■ Statistical methodological issues for estimating the no observable effects concentration (NOEC) in chronic aquatic toxicity tests are addressed. The proposed approach utilizes a two-stage estimation procedure which first examines survival and then evaluates sublethal effects on those concentrations which do not affect survival. At each stage, a one-sided stepwise trend test is employed to test for progressiveness of response with increasing concentration. The log rank test is employed for the analysis of censored survival and time to maturation variables. Adjustments are made for the period of reproductive potential, and transformations are applied to the reproductive variables. Theoretical arguments and empirical results from four previously described *Daphnia magna* assays show that the proposed procedure should provide more meaningful and sensitive NOEC estimates than presently used methods. The necessity of providing some statement on the sensitivity of the study design and estimation procedure to detect environmentally important differences is also discussed.

Introduction

A chronic aquatic toxicity test examines the toxic effects of long-term exposure of aquatic organisms to different concentrations of a test substance including one or more control groups. Replicate vessels of either individual, or groups of organisms, are maintained under static renewal or flow-through conditions. It is recommended that the highest concentration employed in the test be toxic, e.g., a concentration equal to the 96-h LC50 (*1*). The study runs for a predetermined length of time, and indexes representing lethality (e.g., lifetime of parent) and sublethal effects (e.g., growth, maturation, and reproduction) are measured and evaluated.

The major objective of these studies is to determine whether, from the chosen set of concentrations, there is evidence of a toxic effect and to estimate an "acceptable concentration", i.e., the highest *observable* concentration of the substance at which there will be no detectable effects of biological importance on survival, maturation, or reproduction (*2*). Maki (*3*) preferred to call this concentration the no observable effects concentration (NOEC). The process of obtaining a meaningful estimate first involves consideration of what are deleterious biological effects. Appropriate statistical methodology then provides direct and sensitive assistance in interpreting the assay results in light of the biological issues. This report concentrates on the statistical aspects of the NOEC estimation process; however, it should be emphasized that this is a sterile exercise unless the underlying biological issues are also addressed.

As Gelber et al. (*4*) discussed, the usual practice for analyzing chronic toxicity studies is some variant of the following procedure. For each parameter, an analysis of variance is performed to test for an overall concentration effect. A multiple comparison procedure is then employed to determine which concentrations are statistically significantly different from the controls at some error rate (usually 5%). For example, Parkhurst et al. (*5*, *6*) and Winner and Farrell (*7*) used Duncan's (*8*) multiple range test while the ASTM guidelines (*9*) recommend Dunnett's procedure (*10*). The NOEC estimate for a single parameter is then defined as the highest observed concentration which gives results which are not statistically significantly different from the control group (*3*, *5*, *6*). The NOEC for the test substance is the minimum NOEC over all the parameters examined.

The purpose of this report is to provide a comprehensive approach that offers several potential improvements over the standard NOEC estimation procedures. With the usual procedures all of the indexes are considered to be equally important. It would seem preferable to distinguish between indexes concerned with survival from those con-

† Portions of this paper were presented at the 1983 Eastern North American Region of the Biometric Society Spring Meeting, Nashville, TN, March 20-23, 1983.

0013-936X/85/0919-0035$01.50/0

cerned with sublethal effects. Such a procedure is a two-stage approach (*4*) that first examines survival and then evaluates the sublethal indexes only for those concentrations for which an adverse effect on survival was not demonstrated. This scheme has the added advantage that, in the second stage analysis, statistical procedures can be applied with less concern for treatment-related lethality.

The usual analysis of variance and "comparison with control" procedures which compare each concentration separately with the control ignore the ordering expected with increasing concentration when effects are actually present.

They lack selectivity and sensitivity since they do not take into account the dependence of response with concentration (*11–14*). Preferred alternatives are procedures such as a trend test, which address the real question of interest, i.e., progressiveness of response with increasing concentration in the direction of toxicity. The trend test is described in ref 15 and is applied in a stepwise manner (*16*), dropping out the higher concentrations, one at a time until no concentration–response relationship is apparent, in order to estimate the NOEC.

Additional consideration is given to the format of the data used in the analysis. When an assay on individually maintained organisms is terminated after a specific number of days, there will be organisms for which the exact lifetime or maturation time is not known exactly (right censored data). Invalid results may be obtained if the length of the study is mistakenly used as the lifetime of the organisms still alive at study termination. Transformations are also employed on the reproductive variables so as to more nearly satisfy the underlying assumptions required for the statistical procedures employed. In addition, for organisms not surviving to the end of the study an adjustment for the length of their reproductive potential period may prove useful.

This new methodology is applied to four previously discussed *Daphnia magna* studies (*5*, *6*). The two-stage, stepwise, one-sided, trend test estimates are compared with the two-stage Dunnett's test estimates as well as the estimates obtained by using the standard one-step procedures discussed in ref 6.

The necessity of providing some statement about the sensitivity of the estimation procedure and study design to detect environmentally important differences when NOEC estimates are reported is also discussed. The results of a limited sensitivity analysis are reported.

Experimental Section

Data Description. The statistical methodology is illustrated on data from four 28 day static renewal studies that examined the chronic toxicity of acridine to *Daphnia magna* (*5*, *6*). In these studies approximately 20 individually maintained first instar organisms were exposed to each of the following test solutions and controls: five acridine concentrations (0.2, 0.4, 0.8, 1.6, and 3.2 mg/L), well water, and well water with methanol. The methanol was used to help dissolve acridine prior to dilution, and well water served as the dilution medium. For each organism, the following information was determined:

(1) Survival: lifetime of test organism in days (LTIME).

(2) Reproduction: (a) the number of broods produced (BROOD); (b) the total number of young produced (YOUNG); (c) average number of young per brood (AYOUNG).

(3) Maturation: (a) the day of occurrence of the primiparous instar; (b) the age of the organism when young were first released, i.e., appearance time, (ATIME), i.e., the day of onset of reproduction.

Note that the survival times, LTIME, are right censored at 28 days: i.e., termination of the study precludes knowing the exact time to death for organisms alive at the end of the study. The maturation parameters are also subject to censoring (being less than the minimum of either 28 days or the lifetime of the organism). Note when there are no young produced, YOUNG = 0, BROOD = 0, but AYOUNG is mathematically undefined (division by zero) and should be considered as a missing value, not a zero. The evaluation of this variable then represents the average number of young per brood only for those organisms that produce offspring. If more than a few experimental organisms do not produce offspring, the young per brood results should be cautiously interpreted. (For instance, concentrations of a substance that inhibit reproduction might have higher mean young per brood than concentrations for which most organisms produce young.)

Proposed Methodology for NOEC Estimation

The general two-stage procedure employing a one-sided sequential trend test for NOEC estimation is described in this section. In addition, specific issues arising in the evaluation of the four *Daphnia magna* toxicity studies are addressed. These issues include (i) methods for examining censored data, (ii) adjusting reproductive data for period of exposure, and (iii) data transformations. Chart I provides a summary of the subsequent discussion.

Two-Stage Approach. A two-stage approach as initially proposed by Gelber et al. (*4*) is utilized for the purpose of NOEC estimation. The NOEC for survival is estimated first, and the reproduction and maturation indexes are evaluated only for concentrations at or below the survival NOEC. This approach is appealing since it recognizes lethality as the most important toxicity parameter and then considers the additional sublethal effects. In addition, misleading effects may ensue if sublethal indexes are evaluated when there are differences in survival among the organisms tested at the higher concentrations.

Censored Data. Observations on individually maintained daphnids such as lifetime and time to maturation for which results are not available because the study ended or the organism died are said to be (right) censored. Data analytic methods that take into account this censoring must be used for the evaluation, e.g., the log rank test (*17*, *18*). This test compares differences between survivor curves (or age of onset curves) and can be thought of as a censored data generalization of nonparametric tests such as Wilcoxon's procedure (*19*). Another advantage of the log rank test is that it is an efficient procedure for evaluating time to response data even when there is no censoring; i.e., the exact lifetimes of all the organisms are known (as in ref 7) or the time to maturation is known for each organism. (A recent IARC monograph (*18*) presents an excellent nontechnical presentation of the philosophy and computational scheme of the procedure as used for long-term animal carcinogenicity assays.)

Adjusting Reproductive Data for Period of Exposure. In the second stage analysis, the test concentrations are assumed to be nonlethal; i.e., deaths among test animals are assumed not to be treatment related. Hence, the responses for the reproductive indexes of organisms that die before the end of the study are adjusted for the actual period of reproductive potential (relative to the maximal period of reproductive potential).

The following procedure was adopted to adjust for reproductive exposure time for the *Daphnia magna* studies under consideration:

(i) For each experiment, the maximal period of reproductive potential is the duration of the study minus the

Chart I. Modifications in Statistical Methodology

proposed methodology	previous practice	brief description
(1) two-stage approach	one stage	lethal effects (survivorship) are evaluated first and sublethal effects are only examined for those concentrations which exhibit no significant survival effects
(2) log rank test for censored data	censored data treated as if it were not censored	observations (LTIME, ATIME) for which readings may be curtailed because the study ends or an organism dies must be handled by using techniques that take this into account; ignoring the censoring may result in misleading conclusions
(3) adjusting reproductive data for period of exposure	no adjustment made	analysis of reproductive results (BROOD, YOUNG) for organisms that die early must account for the limited reproductive potential and adjustments must be made; assumption is that early deaths for stage two analysis are *not* related to the test substance
(4) data transformations	no transformations	required to meet homogeneity of variance and Gaussian distribution assumptions needed for the statistical procedures employed, i.e., $(BROOD)^{1/2}$, log (YOUNG + 1), and log (AYOUNG)
(5) test for multiple control groups	varied	test for differences among control groups; if no differences exist then they are pooled; if differences exist, then the more relevant control group is employed or results are applied to each group separately
(6) one-sided tests	two-sided tests	in chronic toxicity studies the toxic effect is unidirectional, and a one-sided test is thus appropriate
(7) trend test	heterogeneity among concentrations	a trend test addresses the primary question of interest i.e., progressiveness of response with increasing concentration; a test for concentration heterogeneity, (e.g., one-way ANOVA) examines all differences and does not focus on the specific hypothesis
(8) stepwise trend tests	separate comparison with control	used to obtain the NOEC estimate, i.e., determine the highest concentration at which not deleterious effect is detected; based on highest concentration such that no trend exists or such that no differences exist when that concentration is compared with the control
(9) sensitivity analysis	none	an assessment of the ability of the study design and statistical methodology to detect a biologically important effect

minimum ATIME (i.e., time at which the first offspring appears).

(ii) For each organism, the actual reproduction exposure period is the lifetime of the organism minus the minimum ATIME. Organisms that die before the minimum onset age of reproduction (i.e., LTIME < min (ATIME)) are not included in the analysis.

(iii) The proposed adjustment for the BROOD and YOUNG indexes is to divide each response by the following weight:

$$\text{weight} = \text{minimum } (W,1)$$

where

$$W = \frac{\text{LTIME} - \text{min (ATIME)}}{28 - \text{min (ATIME)}} + C \qquad (1)$$

where $C = 1/[2 \text{ min (ATIME)}]$. Note that AYOUNG is not adjusted.

This adjustment attempts to provide a more appropriate measure of mean reproductive ability and experimental variability in that it accounts for the length of time the organism was able to reproduce. (Note that the arbitrary constant (C) is added so as not to overcompensate for organisms that die just after the date of the minimum ATIME and is based on our experience with this type of data. Also, the minimum of W and 1 is used so as not to unduly adjust the responses for organisms which are alive during most of the study.)

For example, suppose the minimum ATIME for a 28-day study was 8 days; then we would adjust the number of broods as follows:

LTIME	broods	weight	adjusted broods
7	0		organism not included
9	1	0.11	8.9
14	0	0.36	0
16	3	0.46	6.5
27	7	1	7

Data Transformations for Reproduction Data. Assumptions required for the statistical analysis of the reproduction variables are (i) homogeneity of variance among the concentration groups and (ii) a Gaussian distribution. The data should be checked to see if transformations are needed. On a theoretical basis, for count data (such as the number of broods or the total number of young) and for ratio data (such as average number of young per brood), transformations are usually employed in order to ensure that the required assumptions hold (*20*). Our best judgment recommendations based on our experience with these data are to use (i) the square root transformation for number of broods and (ii) a log transformation for the number of young, of the form log (YOUNG + 1), and also for young per brood, of the form log (AYOUNG).

Multiple Control Groups. Often, as in the studies described above, there is more than one control group (e.g., dilution water control and vehicle control). In these cases, tests for differences among the control groups are recommended. If there are no statistically significant differences among the control groups, then all control data can be pooled for the remaining analysis. If statistically significant differences are observed, then the effects of the toxicant are compared (i) with the most appropriate control group (e.g., the vehicle control) or (ii) if there is no natural choice, to each control group separately.

One-Sided vs. Two-Sided Tests. In evaluating the null hypothesis of no concentration effect, the alternative hypothesis of interest is whether there is progressiveness of response with increasing concentration in the direction of toxicity. In *Daphnia magna* chronic toxicity studies, a toxic response is unidirectional, i.e., lower survival, longer time to maturation, and lower reproduction results with increasing concentration. Thus, for each parameter, a one-sided test of significance is employed in order to estimate the NOEC. In other situations, where either an increasing or decreasing response with concentration would be considered as evidence of toxicity, a two-sided test

Table I. Summary of Lifetime, Study 2 (*6*): Cumulative Proportion of Organisms Surviving by Day (When at Least One Death Occurred) and Concentration

			acridine, mg/L				
day	methanol control	well water control	0.2	0.4	0.8	1.6	3.2[a]
0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
5	1.0	1.0	1.0	1.0	1.0	1.0	0.4
8	1.0	1.0	1.0	1.0	1.0	1.0	0.0
12	0.95	0.95	1.0	1.0	1.0	1.0	0.0
15	0.95	0.95	1.0	1.0	1.0	0.95	0.0
22	0.90	0.95	1.0	1.0	0.95	0.95	0.0
24	0.90	0.85	1.0	1.0	0.95	0.95	0.0
26	0.90	0.75	0.95	0.95	0.95	0.95	0.0
28	0.90	0.75	0.95	0.95	0.95	0.95	0.0
N[b]	18	19	20	20	20	20	20

[a] Concentration was statistically significantly different from combined controls when the log rank test (*17*) and the stepwise trend test procedure (*16*) were used at a one-sided 5% level of significance. [b] N denotes number of organisms on test for each treatment group.

would be appropriate. Thus, a two-sided test is used in comparing multiple control groups since the hypothesis of no difference is tested against the alternative that there is a difference in either direction.

Taking Concentrations into Account. The usual comparison with control techniques for estimating the NOEC attempt to answer too many questions (e.g., all pairwise comparisons among concentration groups) and do not focus in on the primary question of progressiveness of response with increasing concentration (*11–14*). An alternative procedure that offers advantages in terms of selectivity and sensitivity is a one-sided linear trend test (*15, 16*). This procedure tests whether there is no concentration–response relationship (a zero regression slope) vs. the alternative that there is a trend in the direction of toxicity. Since the concentration scaling affects the results of any trend test, three scalings are utilized that span a wide range of expected biological response patterns. Let $C_0, C_1, C_2, \ldots C_K$ denote $K + 1$ concentrations including the control (C_0). The scalings chosen are as follows:

(1) Arithmetic (trend test 1): use the actual concentrations, C_i, $i = 0, \ldots K$.

(2) Ordinal (trend test 2): equal step scaling of the concentrations 0, 1, 2, ... K, where C_0 becomes zero, C_1 becomes 1, and so forth.

(3) Arithmetic–logarithmic (trend test 3): this scaling preserves arithmetic spacing among the lowest concentrations and logarithmic spacing among the higher concentrations; i.e., the scalings are as follows: $(\log C_0)^*$, $\log C_1, \ldots \log C_k$, where

$$(\log C_0)^* = \log C_1 - \frac{C_1 - C_0}{C_2 - C_1}[\log C_2 - \log C_1] \quad (2)$$

Note that although the log of 0 is undefined, the control should have a response which in most cases is not that much different than the lowest nonzero concentration. Thus, the log of 0 has been defined, $(\log C_0)^*$, so as to preserve the arithmetic spacing among the three lowest concentrations (*15, 16*).

The one-sided trend test is performed for each scaling, the corresponding one-sided P values are obtained, and the smallest P value is reported, i.e., the one that gives the best-fitting trend line. If the minimum observed P value is less than or equal to 0.05 (other levels of significance may be used depending on one's taste), the null hypothesis of no concentration effect is rejected in favor of the alternative of progressiveness of response with increasing concentration.

Stepwise Trend Test Procedure for Estimating the NOEC. For each parameter and stage the NOEC estimate is obtained by a stepwise application of the trend test (*16*). First, all of the appropriate concentrations are included. If a statistically significant (one-sided $P \leq 0.05$) negative trend is observed, the highest concentration is excluded and the trend test is applied to the data from the remaining concentrations. These steps are repeated until a concentration is reached at which no statistically significant negative trend is observed. The resulting highest concentration is then denoted as the *no statistical significance of trend* (NOSTASOT) concentration and serves as the NOEC estimate for a single parameter. Recall that in the second stage analysis of sublethal effects, only those concentrations at or below the NOSTASOT for lifetime are included in the first step of the sequential trend test.

The overall NOEC estimate for the test substance is then the smallest of all the individual parameter NOEC estimates. However, if, for all parameters, the minimum trend test P value is greater than 0.25 when all the concentrations are included, then a NOEC estimate is not reported for the test substance. Since the highest concentration is chosen to be toxic, obtaining such a result would indicate that the experiment is suspect because either (i) the concentrations are too small or (ii) there is excessive experimental variability. If none of the parameters exhibit a statistically significant trend but, for at least one parameter, a borderline result is obtained ($0.05 <$ minimum P value ≤ 0.25), then the NOEC estimate for the test substance is reported as greater than or equal to the highest tested concentration.

Results

Computational Aspects Illustrated by Example. The results of study 2 (*6*) are examined in detail to illustrate the computational techniques of the two-stage stepwise trend test procedure. The first stage of the analysis examines the observed lifetime of the test organisms. The lifetime data for each of the control and concentration groups are summarized in Table I. The 3.2 mg/L concentration obviously affected survival, with all organisms dead before 8 days. None of the other concentrations appeared to show evidence of lethality in relation to the controls. The stage I log rank analysis can be summarized as follows: The two control groups were not statistically significantly different (two-sided $P > 0.05$) so the groups were combined in the subsequent analysis. In this study the ordinal and the arithmetic–logarithmic scalings are equivalent. When all concentrations were included, the negative trend test log rank statistics for lifetime data were as follows:

scaling	log rank χ^2	one sided P value
arithmetic	97.88	<0.0001
ordinal	44.64	<0.0001

Table II. Summary of Second Stage Reproduction Results for Study 2

(A) Means Unadjusted for Reproductive Potential

group	$(BROOD)^{1/2}$	log (YOUNG + 1)	log YOUNG/ BROOD
methanol control	2.31	4.52	2.93
well water control	2.32	4.53	2.92
0.2 mg/L acridine	2.65	5.23	3.31
0.4 mg/L acridine	2.58	5.18	3.33
0.8 mg/L acridine	2.13	3.70	2.29[c]
1.6 mg/L acridine	0.56	0.09	0.12[c]
RMSE[a]	0.345	0.412	0.243

(B) Means Adjusted for Reproductive Potential

group	N^b	$(BROOD)^{1/2}$	log (YOUNG + 1)
methanol control	2	2.41	4.63
well water control	4	2.36	4.57
0.2 mg/L acridine	1	2.65	5.23
0.4 mg/L acridine	1	2.58	5.18
0.8 mg/L acridine	1	2.15[c]	3.72[c]
1.6 mg/L acridine	1	0.56[c]	0.09[c]
RMSE		0.312	0.327

[a] Root mean square error from one way ANOVA with concentration as a factor. [b] The number of organisms for which an adjustment was necessary. [c] For adjusted reproductive data and YOUNG/BROOD concentration was statistically significantly different from combined controls when a stepwise trend test procedure (*16*) at a one-sided 5% level of significance was used.

Both scalings gave highly statistically significant results. (Note that the log rank test statistic for the arithmetic scaling was twice that of the ordinal scaling. Such a result is often seen in situations where the highest of geometrically spaced concentrations gives a toxic result while the remaining concentrations are similar to the control. The arithmetic scaling gives more weight to the highest concentration than does the ordinal scaling.) The 3.2 mg/L group was excluded from further analysis, and the observed trend was then not in the direction of toxicity. Hence, the stage I NOSTASOT concentration was 1.6 mg/L.

The analysis of the sublethal effects only included concentrations ≤ 1.6 mg/L. Table II contains the means for the transformed reproductive parameters for each concentration (both unadjusted and adjusted for reproductive potential) as well as estimates of variability. In this study, there were a total of 10 organisms that died after the minimum age of onset of reproduction (min ATIME = 8 days) but before study completion. The adjustment for reproductive potential had little effect on the means of the transformed BROOD and YOUNG variables but did effect the variability. The root mean square error (RMSE) for the adjusted number of young was about 20% less than the unadjusted case, and the RMSE for the adjusted number of broods was about 10% less than the unadjusted case.

The trend test is illustrated on the log transformed adjusted number of YOUNG in Table III. The controls were combined since the methanol and well water controls were not statistically significant. A statistically significant negative trend was observed (i) when all concentrations ≤ 1.6 mg/L were included and (ii) when all the concentrations ≤ 0.8 mg/L were included but *not* (iii) when all concentrations ≤ 0.4 mg/L were included. Thus, 0.4 mg/L was the NOSTASOT concentration for YOUNG. Similarly, for BROOD and AYOUNG (Table II), 0.4 mg/L was the NOSTASOT concentration.

Table III. Illustration of Stepwise Trend Test for log Transformed Number of Young, Study 2 (*6*)

ANOVA for log (YOUNG + 1) Adjusted for Reproduction Potential

source	df	SS	MS	F	Prob > F
concn	5	377.40	75.48	706.4	<0.0001
error	11	11.86	0.107		
total	16	389.26	75.587		

scaling	estimate[a]	SE[b]	t^c	one-sided P value
Trend: All Concentrations ≤ 1.6 mg/L				
arithmetic	−108.38	2.00	−54.32	<0.0001
ordinal	−234.88	5.26	−44.69	<0.0001
Trend: Concentrations ≤ 0.8 mg/L				
arithmetic	−8.82	0.96	−9.13	<0.0001
ordinal	−25.10	3.75	−6.69	<0.0001
Trend: Concentrations ≤ 0.4 mg/L				
arithmetic	3.41	0.48	7.16	NC[d]

[a] Estimate of linear trend contrast among concentration means. [b] Standard error of contrast estimate. [c] t statistic. [d] Not computed since trend is positive, i.e., not in the direction of toxicity.

Table IV. Cumulative Proportion of Organisms That Reproduce According to Age of Onset of Reproduction for Study 2 (*6*) by Concentration

day	methanol[a]	well water	0.2	0.4	0.8	1.6[b]
0	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.70	0.55	0.45	0.32	0.00
10	0.82	1.00	1.00	1.00	0.95	0.00
12	1.00	1.00	1.00	1.00	1.00	0.00
14	1.00	1.00	1.00	1.00	1.00	0.05
17	1.00	1.00	1.00	1.00	1.00	0.20
21	1.00	1.00	1.00	1.00	1.00	0.40
22	1.00	1.00	1.00	1.00	1.00	0.45
24	1.00	1.00	1.00	1.00	1.00	0.70
26	1.00	1.00	1.00	1.00	1.00	0.85
28	1.00	1.00	1.00	1.00	1.00	0.85
N^c	18	19	20	20	20	20

[a] Methanol control group was statistically significantly different from the well control group when the log rank test (*17*) at a two-sided 5% level significance was used. [b] Concentration was statistically significantly different from the methanol control group when the log rank test (*17*) and the stepwise trend test (*16*) were used at a one-sided 5% level significance. [c] Number of organisms in each group.

Age of onset of reproduction data for each concentration is summarized in Table IV. The methanol control was statistically significantly different than the well water control. Thus, each concentration was compared with the methanol control by using the stepwise trend test. The resulting NOSTASOT concentration was 0.8 mg/L. The day of occurrence of the primiparous instar was not evaluated since, in this as in the other studies, it usually preceded age of onset of reproduction by 2 days. Thus, analysis of this variable would be redundant.

NOEC Estimates by Study and Methodological Technique. Table VA contains the NOEC estimates for the four studies using the two-stage NOSTASOT methodology, at a one-sided 5% level of significance. For purposes of comparison, the estimates obtained by using a two-stage Dunnett's multiple comparison approach also at a one-sided 5% level of significance are given in Table VB. Also, Parkhurst et al. (*6*) simultaneously evaluated the lethal and sublethal indexes (one-stage) using Duncan's multiple range test at a two-sided 5% level of significance. The resulting NOEC estimates are contained in Table VC.

Table V. NOEC Estimate by Study and Methodological Technique

variable	study 1	2	3	4
(A) NOSTASOT Concentration[a]				
survival	0.8	1.6	0.4	0.2
BROOD	0.8	0.4	0.4	0.2
YOUNG	0.8	0.4	0.4	0.2[d]
YOUNG/BROOD	0.8	0.4	0.4	0.2[d]
ATIME[d]	0.8	0.8	0.4	0.2
NOEC for acridine	0.8	0.4	0.4	0.2
(B) Dunnett's Test NOEC[b]				
SURVIVAL	1.6	1.6	0.4	0.8
BROOD	0.8	0.4	0.4	0.8
YOUNG	0.8	0.4	0.4	0.4[d]
YOUNG/BLOOD	0.8	0.4	0.4	0.4[d]
ATIME[d]	0.8	0.8	0.4	0.8
NOEC for acridine	0.8	0.4	0.4	0.4
(C) Parkhurst et al. (6) Estimates[c]				
SURVIVAL	0.8	1.6	0.4	0.8
BROOD	0.8	0.4	0.4	0.8
YOUNG	0.8	0.4	0.4	0.8
YOUNG/BROOD	0.8	0.4	0.4	0.4
ATIME	0.8	0.8	0.4	0.4
NOEC for acridine	0.8	0.4	0.4	0.4

[a] Estimates were obtained by employing a two-stage approach; i.e., survival was evaluated first, and then sublethal parameters were evaluated at concentrations that did not adversely affect survival. Concentrations compared with combined controls (except where noted) using one-sided stepwise linear trend test at 5% level of significance. [b] Estimate obtained with a two-stage approach. Concentrations compared with combined controls (except where noted) using one-sided Dunnett's test at 5% level of significance. [c] Estimates obtained using a one-stage approach. (cf. ref 5 and 6). Concentrations compared with each control group separately using a two-sided Duncan's multiple range test at overall 5% level of significance. A concentration was considered to be statistically significantly different from the control if it was statistically significantly different from both control groups. [d] Methanol control group was statistically significantly different (two-sided $P \leq 0.05$) than the well water control group. Concentrations compared with methanol control group only.

All three methods tended to give similar overall results; however, the NOSTASOT estimates were always less than or equal to both Dunnett's NOEC estimates and those obtained by Parkhurst et al. (*6*). This may indicate the greater sensitivity of the trend test in relation to Dunnett's test or Duncan's multiple range procedure. In study 4, there were differences among the techniques in the reported NOEC estimate for acridine (i.e., the minimum NOEC estimate over all parameters). Although there was nearly 50% mortality in the 0.8 and 0.4 mg/L groups (Table VI), the observed differences in survival relative to the combined controls were not statistically significant when either (i) the log rank test with Dunnett's adjustment or (ii) Duncan's multiple range test for mean survival time was used. (The unadjusted, for multiplicity of comparisons, one-sided P values for the individual 0.8 mg/L vs. control and 0.4 mg/L vs. control comparisons using the log rank test were less than 0.05; however, the adjusted P values after applying Dunnett's procedure were greater than 0.05.) However, when each concentration was compared with the combined controls by using the more sensitive stepwise trend test, the 0.8 and 0.4 mg/L groups were statistically significantly different than the controls. Further, since much of the mortality in the 0.4 and 0.8 mg/L groups occurred in the later stages of the study, there was no evidence of toxicity, in comparison with the control, (i) for the 0.4 and 0.8 mg/L groups in terms of maturation and (ii) for the 0.4 mg/L groups in terms of reproduction (the mean of each reproduction variable was greater than the control). Also, since the mortality was assumed not to be treatment related, the adjustment for reproductive potential would also help to dissipate any differences. Hence, the greater sensitivity of the trend test approach in detecting survival differences led to a lower reported NOEC for acridine (0.2 mg/L vs. 0.4 mg/L) than either of the two alternative analyses.

Table VI. Summary of Lifetime Data, Study 4 (*6*): Cumulative Proportion of Surviving Organisms by Day (When at Least One Death Occurred) and Concentration

day	combined controls	acridine, mg/L 0.2	0.4[a]	0.8[a]	1.6[a,b]	3.2[a,b]
0	1.0	1.0	1.0	1.0	1.0	1.00
3	1.0	1.0	1.0	1.0	1.0	0.35
6	1.0	1.0	1.0	1.0	0.95	0.10
9	0.90	1.0	0.95	0.85	0.85	0.00
13	0.90	0.95	0.95	0.85	0.85	0.00
16	0.88	0.95	0.85	0.85	0.80	0.00
20	0.88	0.95	0.85	0.85	0.30	0.00
22	0.88	0.95	0.85	0.85	0.25	0.00
23	0.85	0.90	0.55	0.75	0.00	0.00
25	0.85	0.80	0.50	0.60	0.00	0.00
27	0.80	0.80	0.50	0.55	0.00	0.00
28	0.80	0.80	0.50	0.55	0.00	0.00
N	20	20	20	20	20	20

[a] The concentration was statistically significantly different from the combined controls when the log rank test (*17*) and the stepwise trend test procedure (*16*) were used at a one-sided 5% level of significance. [b] Concentration was statistically significantly different from the combined controls when the log rank test (*17*) and Dunnett's test (*10*) were used at a one-sided 5% level of significance.

Sensitivity Considerations: An Example. The ability to obtain statistical significance is a function of the sample size, number of concentrations, within concentration variability, and the specific pattern under consideration. A statistical procedure that is capable of detecting smaller differences than another procedure is regarded as being more sensitive. As noted previously, procedures such as the trend test that take into account the dependence of response with concentration are generally more sensitive than procedures that do not (*11*, *14*, *21–23*). A simple example is provided to illustrate how the trend test compares with two other approaches: (i) the one-sided Dunnett's procedure for comparing each concentration with the control and (ii) Williams' test (*11*, *21*) for determining whether a monotonic concentration–response relationship exists. (Williams' test is recommended by Gelber et al. (*4*) for NOEC estimation.) The example, which simulates an analysis of the transformed reproductive parameters, considers a case where there are three equispaced concentrations (including a control) with 20 organisms per concentration group. In this situation, the three concentration scalings for the trend test give equivalent results. The one-sided trend test is recognized as a least significant difference t test (*20*) between the control group and the highest concentration group, i.e.

$$t = \frac{M_2 - M_0}{\mathrm{SE}(M_2 - M_0)} \tag{3}$$

where M_0 and M_2 are the observed means for the control and the highest concentration, respectively, and $\mathrm{SE}(M_2 - M_0)$ denotes the standard error of the difference. If we further assume that the means M_0, M_1, and M_2 are monotonically ordered, then the test statistic for Williams' test, Dunnett's test, and the trend test will be the same. The

Table VII. Comparison of Sensitivity of Three Test Procedures for NOEC Estimation in the Three Equispaced Concentration Case, One-Sided Level of Significance = 0.05 and Replication = 20/Concentration

test	critical value[a]	relative efficiency[b]	effective[c] sample size
trend	1.67	1.00	20
Williams	1.75	1.10	22
Dunnett	1.95	1.36	27

[a] One-sided 5% critical value for test statistic of eq 3. [b] Denotes how much the variance of the mean difference would need to be decreased in order to obtain the sensitivity of the trend test. [c] Approximate number of animals needed in each concentration group if the procedure were to have the same sensitivity as the trend test, i.e., trend test sample size × relative efficiency.

sensitivity of the three procedures can then be compared by examining the respective critical values for the test statistic t.

Table VII contains the one-sided 5% critical value for the three tests. As expected it is harder to reject the null hypothesis of no difference using Dunnett's test compared with Williams' test or the trend test. In this example, the trend test is also slightly more sensitive than Williams' test. The critical values reflect the fact that Dunnett's test was designed to answer more questions than either of the alternatives. Relative efficiency indicates how much the variance of the mean difference for each procedure would need to be decreased in order to obtain the sensitivity of the trend test. Alternatively, for a fixed level of variability, this would involve increasing the per group sample size. Thus, the effective sample size gives the approximate number of animals that would be needed in each concentration group for the procedure to have the same sensitivity as the trend test. As indicated in Table VII, in order for Dunnett's test to achieve the same sensitivity as the trend test, 27 organisms per group would be needed rather than 20.

For this same example, one can compute for the log transformed reproductive indexes (e.g., log [YOUNG + 1]) the probability of observing a statistically significant difference on the log scale between the highest concentration and the control (when the highest concentration actually provides a reduction from control), using each of the statistical tests. Table VIII contains these probabilities which were computed under the following conditions: number of observations per group, N = 20 or 40; pooled standard deviation of the log transformed data, SD = 0.25, 0.75, and 1.25 (these values are similar to those obtained for the four studies in the NOSTASOT analysis of log (YOUNG + 1)); actual percent reduction from back-transformed control mean = 10, 20, 35, and 50. The results indicate that for this example the trend test has greater sensitivity than Dunnett's test. For instance, if N = 20 and SD = 1.25, then there would be only a 54% chance of observing a statistically significant difference between the highest concentration and the control when the trend test is used if in fact the highest concentration provided a true 50% reduction from control. However, if Dunnett's test were employed, there would be an even smaller 43% chance of detecting such a reduction. These results also show the large effect that variability and sample size have on the ability to observe a statistically significant effect. In the case cited above, if an experiment was performed, it would be likely that no statistically significant difference between the control and highest concentration would be observed. In addition, the reported NOEC estimate would represent a concentration that would, on the average, produce one-half fewer young than the control group.

This simple example has illustrated some important points. Even with just two concentrations and a control group, the trend test and Williams' test provide slightly better sensitivity than Dunnett's comparison with control procedure. This difference in sensitivity would be more dramatic in situations involving more than three concentrations (*11*). With more concentrations, Williams' test and the trend test use all the information regarding the observed concentration–response relationship and would, therefore, be more sensitive than Dunnett's procedure in evaluating the question of interest. Under the restrictions of this example, the trend test is more sensitive than Williams' test, although this will not always be the case.

The dependency of the NOEC estimate on sample size and variability was also demonstrated. Environmentally important differences may not always be detectable. Thus, it is imperative that biologically important differences be defined and that experimental design requirements be based on a sensitivity analysis, i.e., specify the appropriate

Table VIII. Probability of Observing a Statistically Significant Difference[a] for the Following: (A) Three Tests,[b] i.e., Trend,[c] Williams, and Dunnett, (B) Number of Observations, N = 20 or 40, and (C) Pooled Standard Deviation, SD = 0.25, 0.75, or 1.25

actual % reduction from back-transformed control mean, %	N = 20 for test			N = 40 for test		
	trend	Williams	Dunnett	trend	Williams	Dunnett
			(A) SD = 0.25			
10	0.37	0.34	0.27	0.58	0.55	0.48
20	0.87	0.86	0.81	0.99	0.99	0.98
35	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99
50	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99
			(B) SD = 0.75			
10	0.11	0.10	0.07	0.15	0.13	0.10
20	0.24	0.21	0.16	0.37	0.34	0.27
35	0.56	0.53	0.45	0.81	0.79	0.73
50	0.89	0.88	0.83	0.99	0.99	0.98
			(C) SD = 1.25			
10	0.08	0.07	0.05	0.10	0.09	0.06
20	0.14	0.12	0.09	0.19	0.17	0.13
35	0.28	0.26	0.20	0.45	0.42	0.34
50	0.54	0.50	0.43	0.79	0.77	0.70

[a] Difference is on the log scale and is between the highest concentration and the control. [b] One-sided $P < 0.05$ test. [c] Scaling is 0, 1, and 2.

standard deviation and use a sample size necessary to detect environmentally meaningful differences with large probability (e.g., 80%). In addition, when the NOEC estimates are reported, the sensitivity of the study to detect important differences should also be provided.

Discussion

In a properly designed study, lack of statistical significance is viewed as evidence of no toxic effect, and thus, it is imperative to apply a sensitive procedure. The practice (*3, 5–7, 9*) of simultaneously evaluating lethal and sublethal indexes using a comparison with control procedure (that does not consider the underlying concentration–response pattern) seems inadequate. For the data sets considered, the NOEC estimates obtained with the one-stage method were generally consistent with either of the two-stage approaches; however, misleading results may occur if sublethal effects are examined when differential mortality is present. For instance, the higher concentration of a test substance could kill the least productive organisms, hence masking the true effect on reproduction. These results might be higher than those at low concentrations where there is little mortality. A biased measure of experimental variability may also result. In order to avoid such methodological and interpretative problems, the two-stage procedure (*4*) of first examining survival and then sublethal effects for those concentrations that do not adversely affect survival is recommended.

At each stage of the analysis, the trend test is advocated as a means for assessing evidence of an effect on the response of interest within the range of concentrations examined. The trend test addresses the specific question of interest in toxicity studies, i.e., progressiveness of response with increasing concentration, and will usually be more sensitive than standard methods for testing heterogeneity of response. The latter are concerned with a wide spectrum of concentration vs. control relationships rather than just the ordering of response to concentrations (*18*). The trend test should in the long run provide fewer false negatives than methods that rely on tests for heterogeneity, while maintaining an equivalent false positive rate (*18*).

Note that employing a trend test does not imply that the concentration–response relationships are considered linear. Three concentration scalings are examined, one of which is a linear trend while the remaining two are not, and the best-fitting trend is used. Since the scalings should span a wide range of biologically expected concentration–response patterns, the trend test is sensitive to most orderings of response with concentration in the direction of toxicity (*15, 16*). The trend test is expected to provide respectable power against a variety of response patterns, e.g., an initial threshold or an initial stimulatory response at low concentrations.

For purposes of NOEC estimation, stepwise multiple comparison procedures that, at each step, incorporate all the appropriate concentrations such as Williams' test (*11, 21*) and the stepwise trend test (*16*) should be utilized. They have been recommended for similar problems in other subject matter areas and are known to have equivalent or greater sensitivity than procedures such as Dunnett's (*10*) and Duncan's (*8*) which consider separately each concentration with the control (*21–23*). In addition, the step down procedures avoid the interpretative difficulties that one may encounter when employing the procedures which compare each concentration mean with the control such as finding a low concentration to be different from the control while a higher concentration is not. At each stage of the analysis, Gelber et al. (*4*) recommended that Williams' test (*11, 21*) be employed in order to estimate the NOEC. However, this procedure has a drawback, in that it assumes that there is a monotonic concentration trend. Although the concentration–response relationship will most often be monotonic, in certain cases the test substance might be hypothesized to slightly stimulate reproduction at very low concentrations before causing toxicity at moderate or high levels. The stepwise trend test seems preferable since it (i) does not assume monotonicity and (ii) does not require special tables.

As Mantel (*15*) noted in a different setting, "If it had not been for so long the custom to calculate and so carefully examine the comparisons of single doses with control, the best way to strengthen our experiments in this area would probably be to drop the single-dose comparisons from all our routine analyses. Today, however, it does not seem that such a recommendation would be acceptable". This statement also holds true for chronic aquatic toxicity tests. In addition to the stepwise trend test, if desired, it seems reasonable to also report the results of the separate concentration comparisons using a procedure such as Dunnett's (*10*) which take into account the fact that comparisons share a common control group (as was done in Table VB). This can be thought of as an option which may make the implementation of the stepwise trend test more palatable.

In each step of the recommended procedure, valid data analytic procedures must be used to evaluate trend. For the *Daphnia magna* toxicity data examined in this paper, the log rank test (*17*) was employed in order to examine the censored survival and maturation parameters. This test is recommended since (i) it is easy to apply and understand and (ii) it is efficient against reasonable biological alternatives (*18*). However, as two referees noted, if one has sufficient information to parametrically model both the contaminated and control curves, e.g., with the log normal or Weibull distributions, then even higher sensitivity could be achieved (*19*). Our experience has been that this information is rarely available and that one must guard against the loss of sensitivity that may ensue if the wrong model is mistakenly chosen.

The reproductive data were adjusted for a period of exposure. On the basis of the data evaluated, transformations were necessary. Further experience with many such experiments will determine a general policy on transformations. The transformed adjusted reproductive data were then evaluated by using appropriate linear contrasts. One-sided rather than two-sided statistical tests were more relevant in this setting.

Registry No. Acridine, 260-94-6.

Literature Cited

(1) "Standard Methods for the Examination of Water and Wastewater", 14th ed.; American Public Health Association, American Water Works Association, and Water Pollution Control Federation: Washington, DC, 1975; pp 762–766.
(2) Mount, D. I.; Stephan, C. E. *Trans. Am. Fish. Soc.* **1967**, *96*, 185–193.
(3) Maki, A. W. *J. Fish. Res. Board Can.* **1979**, *36*, 411–421.
(4) Gelber, R. D.; Lavin, P. T.; Mehta, C. R.; Schoenfield, D. A. Department of Biostatistics, Sidney Farber Cancer Institute, 1981, Technical Report 312.
(5) Parkhurst, B. R.; Bradshaw, A. S.; Forte, J. L.; Wright, G. P. *Environ. Pollut., Ser. A* **1981**, *24*, 21–30.
(6) Parkhurst, B. R.; Forte, J. L.; Wright, G. P. *Bull. Environ. Contam. Toxicol.* **1981**, *26*, 1–8.
(7) Winner, R. W.; Farrell, M. P. *J. Fish. Res. Board Can.* **1976**, *33*, 1685–1691.
(8) Duncan, D. B. *Biometrics* **1955**, *11*, 1–42.
(9) "Standard Practice for Conducting Toxicity Tests With Early Life Stages of Fish". American Society for Testing

and Materials, Philadelphia, PA, 1981, ASTM E-47.01 (draft).
(10) Dunnett, C. W. *Biometrics* **1964**, *20*, 482–491.
(11) Williams, D. A. *Biometrics* **1971**, *27*, 103–117.
(12) Duncan, D. O. *Technometrics* **1965**, *7*, 171–222.
(13) Dawkins, H. C. *Biometrics* **1983**, *39*, 789–790.
(14) Barlow, R. E.; Bartholomew, D. J.; Bremner, J. M.; Brunk D. "Statistical Inference under Order Restrictions"; Wiley: New York, 1972.
(15) Mantel, N. *Biometrics* **1981**, *36*, 381–401.
(16) Tukey, J. W.; Chiminera, J. L.; Heyes, J. F. *Biometrics*, in press.
(17) Mantel, N. *Cancer Chemother. Rep.* **1966**, *50*, 163–170.
(18) "Long-Term and Short-Term Screening Assays for Carcinogens: A Critical Appraisal; IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans", International Agency for Research on Cancer: Lyon, France, 1980; Suppl. 2, pp 311–426.
(19) Kalbfleisch, J. D.; Prentice, R. L. "The Statistical Analysis of Failure Time Data"; Wiley: New York, 1980; Chapters 1–3.
(20) Snedecor, G. W.; Cochran, W. G. "Statistical Methods"; Iowa State University Press: Ames, IA, 1980; pp 288–292.
(21) Williams, D. A. *Biometrics* **1972**, *28*, 519–531.
(22) Marcus, R.; Peritz, E.; Gabriel, K. R. *Biometrika* **1976**, *63*, 655–660.
(23) Abelson, R. P.; Tukey, J. W. *Ann. Math. Stat.* **1963**, *34*, 1347–1369.

Received for review September 12, 1983. Revised manuscript received June 14, 1984. Accepted August 16, 1984.

Chemical and Biological Characterization of Organic Material from Gasoline Exhaust Particles

Tomas Alsberg,* Ulf Stenberg, Roger Westerholm, and Michael Strandell

Department of Analytical Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

Ulf Rannug and Annica Sundvall

Department of Toxicology Genetics, Wallenberg Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

Lennart Romert, Vibeke Bernson, and Bertil Pettersson

Department of Cellular Toxicology, Wallenberg Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

Rune Toftgård, Bo Franzén, Marla Jansson, and Jan Åke Gustafsson

Department of Medical Nutrition, Huddinge University Hospital, S-146 86 Huddinge, Sweden

Karl Erik Egebäck and Gunnar Tejle

Vehicle Emission Research Laboratory, National Swedish Environment Protection Board, S-611 82 Nyköping, Sweden

■ Unfractionated gasoline exhaust particle extracts, silica gel fractions, and recombined fractions were analyzed with capillary gas chromatography–mass spectrometry and tested in three biological test systems based on different end points, namely, genotoxicity (Ames *Salmonella* mutagenicity test), aryl hydrocarbon hydroxylase (AHH) inducibility (2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) receptor affinity test), and cytotoxicity to pulmonary alveolar macrophages (PAM cytotoxicity test). Fraction I contained aliphatic hydrocarbons and showed no activity in the biological tests. Fraction II contained PAH and showed mutagenicity in the presence of a metabolizing system (S9), TCDD–receptor affinity, and PAM cytotoxicity. Fractions III and IV gave high effects in all bioassays. In these fractions polynuclear aromatic ketones are the most abundant species. Fraction V contained the most polar species, including nitrogen-containing compounds, and showed a much weaker activity in the bioassays as compared to fractions III and IV.

Introduction

Internal combustion engines emit a complex mixture of newly formed products and unburned fuel, which is both gaseous and particle associated. Due to regulations made during the 1960s and 1970s on-line measurement techniques have been developed for compounds such as NO/NO_2, CO, and HC (HC = unspecified gaseous hydrocarbons), but to analyze what nowadays usually is denoted "unregulated pollutants", other methods have to be used. This concept has often been synonymous to PAH/PAC (PAH, polycyclic aromatic hydrocarbons; PAC, polycyclic aromatic compounds), which is a component class to which much interest has been assigned. Motor traffic is a significant source of ambient PAH/PAC in urban areas (*1*) and the emission comes from both gasoline and diesel vehicles (*2*, *3*).

The improved fuel economy in light-duty vehicles equipped with diesel engines vs. those equipped with gasoline engines was expected to considerably increase the percentage of diesel vehicles on the market of new cars in the immediate future. The interest in adverse health effects of mobile source emissions has, therefore, been focused on diesel particles. Thus, most studies, including chemical and biological characterization, have been carried out on diesel particulate extracts, whereas relatively little has been reported for gasoline exhausts (*4*, *5*).

In this study, which is the first part of a more detailed investigation of gasoline exhaust particulate extracts, chemical analyses were combined with three bioassays. The bioassays chosen are based on different biological end points assumed to include critical events for the adverse effects of automobile exhausts.

Of much concern is the potential genotoxic effect. This type of effect was studied with Ames mutagenicity test on *Salmonella typhimurium* (*6*). This test has shown to be a useful tool in the screening of mutagens/carcinogens in complex chemical mixtures such as automobile exhaust (*7*, *8*). Also, fractionation in combination with chemical analysis and bacterial mutagenicity tests is an efficient

0013-936X/85/0919-0043$01.50/0

Table I. Average Emission per Kilometer of Particles, CO, CO_2, HC, and NO_x in the Two Series[a]

series	particles, mg	CO, g	CO_2, g	HC, g	NO_x g
B (n = 17)	11.7 ± 2.7	8.10 ± 0.98	231 ± 6.3	1.55 ± 0.11	2.18 ± 0.15
C (n = 19)	11.7 ± 3.2	9.99 ± 1.60	235 ± 3.5	1.69 ± 0.20	2.34 ± 0.11

[a] The tests were run according to the US 73 test procedure.

means for the characterization of the genotoxic species.

As previously mentioned PAH are abundant species in vehicle exhausts. These hydrophobic compounds require metabolic activation to exert a mutagenic/carcinogenic effect (*8–11*). The cytochrome P-450 isozymes of the mixed-function oxygenase enzyme systems which activate PAH are generally known as aryl hydrocarbon hydroxylase (AHH). The level, as well as the substrate specificity, of the cytochrome P-450 isozymes present in the target tissue is therefore of importance. An increased AHH activity, e.g., by induction, results in an increased activity of PAH which in turn may result in an increased toxicity. Thus, AHH-mediated PAH metabolism is suspected to play an important role in the etiology of cancer (*8–11*). The induction of AHH is believed to be mediated by the interaction of the inducer with a highly specific intracellular receptor protein (*12, 13*). The most potent inducer of AHH known, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), binds to this receptor with a very high specificity. Compounds competing with TCDD for receptor binding are screened with the second bioassay. Such compounds have previously been found in extracts of urban air samples (*14*). Furthermore, extracts of vehicle exhaust have been shown to induce AHH activity in a rat hepatoma cell line (B. Franzén, unpublished results).

The third bioassay is based on the primary role of pulmonary alveolar macrophages (PAM) in the defense of the lung against inhaled particles. By ingestion and degradation of particulate matter, they are scavenging the bronchioalveolar region of the respiratory tract (*15*). The key function of PAM in the pulmonary defense and the consequence of an impaired PAM function are a crucial part in the evaluation of the hazards of automobile exhausts or any other types of air pollution. In this study the phagocytic capacity and the oxidative metabolism of isolated PAM were used to investigate the cytotoxicity of the vehicle exhausts.

The aim of this study is to determine the possible presence of genotoxic, AHH-including, and PAM-cytotoxic components in gasoline exhaust particulate extracts and to identify the responsible components. The extract was divided into five different fractions according to polarity and tested in the three bioassays.

Materials and Methods

Test Vehicles. The test vehicle was a Saab 900 GL (noncatalyst) with a four-cylinder carburetor engine, swept volume 1985 cm^3. The vehicle was adjusted by the manufacturer to fulfill the Swedish emission regulation for vehicles of 1983 and later year models. The odometer reading of the vehicle was 4430 km at the start of the test series. The fuel was a leaded (0.15 g of Pb/L) 96 octane (RON) commercial gasoline. The average values for the emission per kilometer of particles, CO, CO_2, HC, and NO_x, are shown in Table I.

Sampling. All samples were collected in a dilution tunnel constructed according to the specifications given by U.S. Federal Register (*16*). The particle emission were collected on Teflon-coated filters (Pallflex T60A20) with a diameter of 240 mm. Prior to use, the filters were washed in 99% ethanol and heated to 200 °C for 1 h. The vehicle was driven according to the US-73 test procedure after at least 12 h standing at 22 °C. A total of 630 m^3 of diluted exhaust gas was collected, dilution ratio approximately 1:10. After sampling, the filters were stored in the dark in a desiccator with dry blue gel and weighed after 24 h. A new weighing was done after another 24-h period, and with a recorded difference less than 0.5% the sample was regarded to have constant weight.

In all, 36 samples were collected by using two series (B and C) run at different times.

Extraction. Four filters at a time were combined, folded, and put in a 500-mL Soxhlet extractor. Dichloromethane (DCM) was used as solvent, giving a siphoning rate of 30 min, and the extraction proceeded for 24 h. Following extraction the solvent was removed under reduced pressure at 30 °C. The final residues were combined and stored in a glass vial at −20 °C prior to fractionation and analysis.

Fractionation and Chemical Analysis. The crude extracts were fractionated on a gravity fed glass column (200 × 15 mm) packed with silica gel, 120 mm bed height (Merck Kieselgel 60, 0.063–0.200 mm particle diameter), which had been heated to 500 °C for 16 h prior to deactivation with 10% (w/w) distilled water. The column was conditioned with 100 mL of *n*-hexane before application.

The samples, dissolved in DCM, were adsorbed to approximately 1 mL of silica gel in hexane. The solvent was evaporated under dry nitrogen, yielding a dry powder, which was placed on top of the gel bed. Fractionated sample amounts were the equivalents of 20.5 (sample B) and 9.6 km of driving (sample C), corresponding to approximately 24 and 11 m^3 of raw exhausts, respectively. Half of the fractions of sample B was stored for further studies involving subfractioning with high-pressure liquid chromatography (HPLC).

Five fractions were collected from the preparative silica column as follows: fraction I, eluted with 22 mL of hexane; fraction II, 22–112 mL of hexane; fraction III, 0–112 mL of 25% DCM in hexane; fraction IV, 0–112 mL of DCM; fraction V, 0–112 mL of methanol (MeOH). Fractions were evaporated to dryness with a rotary evaporator. The residue was dissolved in acetone and transferred to 5-mL sample vials, which were stored at −20 °C.

The solvent strength progression was primarily based on literature data on the distribution of mutagenic activity in fractions of diesel exhaust particle extracts (*17, 18*). In addition, reference compounds were chromatographed on a silica column in order to establish the appropriate solvent strengths and cut points for the fractions (Table II). Chromatography of reference compounds was performed with on-line UV detection (Altex Analytical UV detector, Model 133) and/or capillary gas chromatography–flame ionization detection (GC–FID) or capillary gas chromatography–mass spectrometric detection (GC–MS) analysis.

All fractions as well as the crude extracts and recombined samples were analyzed with GC–FID and GC–MS. The GC–FID gas chromatograph was a PYE Unicam GCV with a split/splitless injector. The GC–MS gas chromatograph was a Carlo Erba Fractovap 2150 with a split/splitless injector and a fused silica column of 12.5 m length and 0.22 mm i.d. Stationary phase was SE-52. The tem-

Table II. Fractionation of Reference Substances on Silica Gel Column[a]

compound	fraction I, hexane, 0–22 mL	II, hexane, 22–112 mL	III, 25% DCM, 112 mL	IV, 100% DCM, 112 mL	V, MeOH, 112 mL
phenanthrene		X			
coronene		X			
xanthene		X			
9-chloroanthracene		X			
dichloroanthracene		X			
9-bromoanthracene		X			
9-cyanoanthracene			X		
1-nitronaphthalene			X		
1-nitropyrene			X		
10-nitrobenz[*a*]anthracene			X		
fluoren-9-one			X		
carbazole			X		
phenanthrene-9-carboxaldehyde			X[b]	x[c]	
1-methylanthracene-9-carboxaldehyde			x[c]	X[b]	
naphthalene-1-carboxaldehyde			X		
1-naphthol				X	
2,5-dinitrofluorene				X	
2,7-dinitrofluoren-9-one				X	
9-aminophenanthrene				X	
2-aminochrysene				X	
anthrone				X	
xanthone				X	
9,10-anthraquinone				X	
9,10-phenanthrenequinone				X	
3,4-dihydrobenz[*a*]anthracen-1(2*H*)-one				X	
benzanthrone				X	
9,10-dihydrobenzo[*a*]pyren-7(8*H*)-one				X	
acridine					X
dibenz[*a*,*j*]acridine					X

[a] Column dimensions 120 × 15 mm. [b] Major amount. [c] Minor amount.

perature program was the following: 70 °C for 1 min, increase 7 °C/min to 300 °C, isothermal for 10 min. The mass spectrometer was a Jeol D300 with electron impact ionization source (EI) and an Incos 2000 data system. MS parameters were the following: ion source temperature 200–230 °C; ionization voltage 70 eV; scan range 35–350 amu; scan time 1.0 s.

Thirteen PAH were quantified by GC–FID after cleanup by partitioning between dimethylformamide/water and cyclohexane as described elsewhere (*19*). Duplicate aliquots of the crude extracts and reconstituted samples were analyzed. The levels of the other PAH were estimated from the mass chromatograms of their respective molecular ion, as recorded from the full-scan GC–MS analysis of the PAH fraction (Table III).

Biological Testing. (1) Mutagenicity Tests. Mutagenicity tests were performed as described by Ames et al. (*6*) with *S. typhimurium* TA98 and TA100. The samples, dissolved in acetone, were tested on two occasions, each at a minimum of three doses with and without the addition of a metabolizing system, consisting of a liver S9 fraction from Aroclor-pretreated male Sprague-Dawley rats. The results are given as number of revertants per kilometer, determined by regression analysis from the linear part of the dose–response curve.

(2) TCDD–Receptor Affinity Tests. Before testing for TCDD–receptor affinity, the solvent was exchanged to dimethyl sulfoxide (Me_2SO) for all samples. The presence of compounds with affinity for the TCDD–receptor protein was determined from the extent of competition with [^{3}H]TCDD for binding to the receptor (*14*, *20*). Each experiment was carried out as described for urban air samples (*14*). The results are expressed as meter driving distance per milliliter that competes for 50% of the specific [^{3}H]TCDD binding (EC_{50}).

(3) PAM Cytotoxicity Tests. The pulmonary alveolar macrophages (PAM) were collected from the lungs of rabbits according to the procedure described earlier (*21*).

The acetone was replaced by Me_2SO, as a solvent for the extracts before testing on PAM for cytotoxicity.

The short time incubation (15 min) was performed according to the procedure previously described (*21*). The cellular oxygen consumption was continuously measured polarographically on 3×10^6 PAM suspended in 1 mL of Hank's balanced salt solution (HBSS). The fraction to be studied was added 5 min prior to the initiation of phagocytosis by addition of 30×10^6 heat-killed and opsonized yeast cells (*Saccharomyses cerevisiae*). Ten minutes later the experiment was stopped, and the phagocytic capacity was microscopically determined.

In the case of long time incubation (20 h) 0.5×10^6 PAMs, suspended in 1 mL of Ham's F10 medium complemented with 10% fetal calf serum, were allowed to adhere to each of the glass cover slips placed in tissue multiwell plates kept at 37 °C in an atmosphere of 5% CO_2 in air for 30 min. Nonadherent cells were washed off, and the cell culture medium was replaced by HBSS containing the fraction to be tested. Each fraction was added to a minimum of three wells in every experiment, and the cells were incubated for 20 h. Thereafter, the phagocytosis was initiated by the addition of 10×10^6 heat-killed and fluorescein isothiocyanate stained yeast particles to each well, and the PAMs were cultivated for an additional 30 min. The phagocytosis was stopped by decreasing the temperature to 0 °C. The cover slips were then immersed in a Trypan blue solution (2 mg/mL phosphate-buffered saline) to stain the noningested particles and thereby quench their fluorescence before the phagocytic capacity, in terms of particle uptake, was determined by epifluorescence microscopy.

Table III. Peak Assignments, PAH Levels, and Fractionation Yields of PAH

peak no.[a]	component[b]	emission,[c] μg/km		yield, %[d]	
		sample B	sample C	B	C
1	phenanthrene	2.6	2.9	107	86
2	anthracene	0.7	0.7		
3	methylphenanthrenes/anthracenes	17	11		
4	2-phenylnaphthalene	3.7	1.3		
5	dimethylphenanthrenes/anthracenes	30	17		
6	fluoranthene	20	14	85	93
7	pyrene	28	31	82	97
8	methylpyrenes/fluoranthenes + benzo[*a*]-, benzo[*b*]fluorene	26	28		
9	benzo[*ghi*]fluoranthene	5.6	12	93	92
10	cyclopenteno[*cd*]pyrene	2.9	12	79	80
11	benz[*a*]anthracene	5.7	5.9	79	78
12	chrysene	6.7	8.7	84	78
13	β,β′-binaphthyl[e]				
14	benzo[*b*]-, benzo[*k*]fluoranthene	3.9	7.0	72	81
15	benzo[*j*]fluoranthene	1.1	0.9		
16	benzo[*e*]pyrene	2.6	6.2	88	91
17	benzo[*a*]pyrene	1.9	4.5	89	94
18	perylene	0.3	0.5		
19	M_r 264 (methylenebenzopyrene[f])	0.4	1.2		
20	M_r 276 (indeno[1,2,3-*cd*]fluoranthene[f])	0.4	0.8		
21	indeno[1,2,3-*cd*]pyrene	1.7	3.6	94	92
22	benzo[*ghi*]perylene	5.9	13	92	107
23	anthanthrene	0.2	1.3		
24	coronene	6.5	12	85	100
		174[g]	196[g]	87[h]	89[h]

[a] Peak numbers refer to Figure 1. [b] Identification by means of retention time and mass spectrum of reference substances. [c] Compounds for which a yield is reported were quantified by GC–FID analysis, while the remaining compounds were quantified from the full-scan GC–MS analysis of fraction II. [d] Reconstituted samples compared with crude extracts. [e] Internal standard (i.s.). [f] See Grimmer et al. (*22*). [g] Sum. [h] Mean.

The results are expressed as particle-stimulated increase of cellular respiration, percent PAMs ingesting particles, and mean number of particles ingested per PAM. Statistical analysis was performed by χ^2 test of independent distribution of number of ingested particles per PAM.

Results and Discussion

Chemistry. Fractions II–IV from sample B were analyzed in more detail, as a consequence of the results from the biological tests. PAH levels in the crude extracts of samples B and C together with the yield of PAH from the silica gel column are given in Table III. The PAH were exclusively found in fraction II. Tables IV and V summarize the GC–MS analysis of fractions III and IV of sample B, and the total ion chromatograms of fractions I–V are shown in Figure 1.

Fraction BIII (Table IV) contains mainly oxygenated PAH, of which fluoren-9-one is the most abundant species. Reference compounds are lacking for many of the remaining constituents in this fraction, which makes the identification more or less tentative. However, by comparison with published mass spectra of mono- and dimethylfluorenones (*18*), these were also determined together with tri- and tetramethylfluorenones on the basis of mass spectra and gas chromatographic elution pattern. Other major ketones (characteristic fragments: M-28 or M-29) are cyclopenta[*def*]phenanthrenone and benzofluorenones. A group of compounds in this fraction shows mass spectra typical for diphenyl-substituted aliphatic ketones or diketones; i.e., they have a weak molecular ion and strong mass fragments at m/z 91, 105, and/or 119. Peaks 31, 34, 35, 37, 43, and 45 (Figure 1 and Table IV) represent compounds of this group. To our knowledge, such substances have not previously been detected in automobile exhausts. However, Grimmer et al. (*22*) reported the presence in gasoline exhausts of diphenylmethane, which is a likely precursor to benzophenone. It is interesting to note that benzophenone is not found in this investigation, which could mean that this compound is a precursor to fluoren-9-one and is converted to it during combustion. Polyaromatic aldehydes (characteristic fragments: M-1 and M-29) such as phenanthrenecarboxaldehyde, are also found in this fraction. In addition, a number of brominated and chlorinated derivatives of oxygenated PAH are present. These have been studied in more detail, the results of which are presented elsewhere (*23*).

Table V shows the contents of fraction BIV. This fraction also contains polynuclear ketones, although these are structurally different from those in fraction BIII. While most ketones in fraction BIII contain one five-membered ring as in fluorenone, those in fraction BIV, e.g., benzanthrone and benz[*cd*]pyrenone, are made up of six-membered rings only, with the exception of polyfunctional compounds and benzo[*b*]fluoren-9-one which is present in both fractions. The most abundant compound in fraction BIV is xanthone, an oxygen heterocyclic polynuclear ketone, and phenalen-1-one. Other species are polynuclear quinones (characteristic fragments: M-28 and M-56).

A comparison with literature data (*24–27*) shows that the dominant oxygenates in this gasoline exhaust extract are the same as for diesel exhausts and ambient air. Schuetzle et al. (*24*) also report fluoren-9-one and xanthone as the most abundant species in the two biologically most active fractions, which is in accordance with our results for fractions BIII and BIV.

Fraction BV contains polar oxygen and/or nitrogen-containing compounds of comparably low molecular weight. Structures proposed by the mass spectrometer spectrum library (National Bureau of Standards) are alkylated benzimidazoles, hydroxy- and ethoxybenzaldehyde, and indoledione.

Biology. (1) Mutagenicity Tests. Two samples, B and C, were tested for mutagenic activity. The results

Table IV. Peak Assignments and Mass Spectrometric Data for Fraction BIII

peak no.[a]	molecular ion, M^+ (intensity)	fragmentation pattern (rel intensity, %)[b]	proposed compound
25	170 (87)	−1 (125), −29 (40), −55(21)	methylnaphthalenecarboxaldehyde[c]
26	180 (2216)	−28 (23)	fluoren-9-one[c–g]
27	194 (482)	−29 (50)	methylfluoren-9-one[c,e–g]
28	210 (331)	−119 (263), −91 (39)	unknown[h]
29	196 (151)	−1 (105), −29 (36), −57 (23)	dibenzofurancarboxaldehyde
30	194 (1218)	−29 (55)	methylfluoren-9-one[c,e–g]
31	210 (197)	−119 (368)	unknown[h]
32	194 (1670)	−29 (50)	methylfluoren-9-one[c,e–g]
33	194 (1180)	−29 (68)	methylfluoren-9-one[c,e–g]
34	224 (277)	−119 (523), −105 (20)	unknown[h]
35	224 (145)	−119 (488), −145, (47), −105 (21)	unknown[h]
36	208 (744)	−43 (29), −30 (18), −15 (12)	C_2-alkylfluoren-9-one[e]
37	224 (51)	−119 (1190), −105 (164)	unknown[h]
38	208 (559)	−43 (34), −15 (29), −30 (16)	C_2-alkylfluoren-9-one[e]
39	208 (967)	−43 (27), −30 (14), −29 (11), −15 (2)	C_2-alkylfluoren-9-one[e]
40	208 (866)	−43 (30), −30 (13), −15 (10)	C_2-alkylfluoren-9-one[e]
41	238 (163)	−133 (415), −105 (45), −161 (45)	unknown[h]
42	260 (316)	−2 (95),[i] −109 (40), −30 (17), −28, (6)	bromofluoren-9-one[d]
43	238 (44)	−133 (377), −119 (238), −147 (80), 161 (41)	unknown[h]
44	204 (1836)	−28 (26), −54 (6)	cyclopenta[*def*]phenanthren-4-one[c–g]
45	252 (25)	−133 (922), −147 (261), −119 (50), −161 (61)	unknown[h]
46[a]	222 (212)	−43 (40), −44 (18), −15 (13), −29 (4)	C_3-alkylfluoren-9-one[e]
46[b]	206 (379)	−1 (63), −29 (54), −28 (27)	phenanthrene-9-carboxaldehyde[c–g]
47	206 (718)	−1 (43), −28 (42), −29 (39)	phenanthrene/anthracenecarboxaldehyde
48	206 (261)	−29 (36), −1 (33)	phenanthrene/anthracenecarboxaldehyde
49	276 (104)	−2 (83),[i] −137 (31), −81 (21)	bromoxanthone[d]
50	220 (87)	−1 (35), −29 (30), −31 (28)	methylphenanthrene/anthracenecarboxaldehyde[c,e]
51	220 (147)	−1 (43), −31 (35), −29 (29)	methylphenanthrene/anthracenecarboxaldehyde[c,e]
52	230 (2236)	−28 (16), −30 (30), −29 (9)	benzo[*a*]fluoren-9-one[d,e,f,g]
53a	230 (583)	−28 (39), −29 (19), −30 (19)	benzo[*c*]fluoren-9.-one[d,e,f,g]
53b	256 (175)	−28 (30), −30 (29), −29 (23)	unknown ketone[e]
54	230 (814)	−28 (15), −30 (9), −29 (7)	benzo[*b*]fluoren-9-one[d,e,f,g]
55	244 (141)	−29 (27)	methyl from a ketone with M_r 230[g]
56	244 (78)	−29 (34)	methyl from a ketone with M_r 230[g]
57	246 (115)	−57 (13), −28 (11)	coumarin from a PAH with M_r 228[g]
58	244 (156)	−29 (35)	methyl from a ketone with M_r 230
59	244 (66)		methyl from a ketone with M_r 230
60			phthalate (system peak)
61	256 (129)	−30 (19)	unknown ketone[e]
62	254 (4920)		β,β'-binaphthyl (i.s.)[j]
63	270 (79)	−28 (10)	unknown ketone
64	254 (152)	−28 (13)	unknown ketone
65	280 (89)	−30 (24)	ketone from PAH with M_r 266[f,g]

[a] Peak numbers refer to Figure 1. [b] Relative intensity = $[(M - R)^+/M^+] \times 100$, R = fragment loss given in table. [c] Same compound or compound with similar mass spectrum previously detected in diesel exhaust particle extract (*24*). [d] Retention time and fragmentation pattern in accordance with reference substance. [e] Same as for footnote *c* (*25*). [f] Same compound or compound with similar mass spectrum previously detected in extract of airborne particles (*27*). [g] Same as for footnote *f* (*26*). [h] Fragmentation pattern indicates diphenyl-substituted aliphatic ketone or diketone. [i] Fragment due to the isotopic distribution of bromine atoms. [j] The amount of internal standard (i.s.) corresponds to an emission of 8.6 μg/km.

summarized in Figure 2 show that the crude extract induced 9000–48 000 revertants/km, in accordance with our earlier results from gasoline-fueled vehicles (*7*, *28*). The highest effect was seen with sample C on strain TA100. For the crude extracts no major differences in mutagenicity with and without the addition of a metabolizing system could be noted.

All fractions, except fraction I, induced mutations in both tester strains. In the absence of S9, more than 60% of the total mutagenic activity seen with strain TA100 was related to fraction III; 30% of the mutagenicity was found in fraction IV, and the remaining mutagenicity was mainly found in fraction V. With strain, TA98, the mutagenicity was equally distributed among fractions III–V. The most potent compounds have not yet been determined. The major component of fraction III, fluoren-9-one, was tested on *Salmonella* but gave no mutagenic effects (data not shown), in accordance with the results of Florin et al. (*29*) and Leary et al. (*30*). Xanthone and phenalen-1-one, the major components of fraction IV, were also tested at doses corresponding to the levels found in this fraction as estimated from GC–MS analysis. No mutagenic effects were found on strain TA100 with or without S9 (data not shown). At much higher doses, however, phenalen-1-one induces mutations (8-azaguanine resistance) in *Salmonella typhimurium* (*30*). Fraction II gave mutagenic effects in the presence of S9. This fraction contains PAH, which are indirect mutagens and thus must be metabolically activated before mutations can be induced.

When the fractions were recombined, the mutagenicity was similar to that of the crude extract. With sample C, however, the addition of a metabolizing system resulted in an increased response in strain TA100, while the effect of the crude extract was not altered by the addition of S9.

In diesel particulate extracts, nitroarenes, especially nitropyrenes, may be significant contributors to the mutagenicity, although data are conflicting (*31–33*). In the case of gasoline particulate extracts nitroarenes, most likely, are of minor importance (*33*).

(2) TCDD–Receptor Affinity Test. The results show (Figure 3) the presence of compounds competing with TCDD for receptor binding in the crude extract, fractions

Table V. Peak Assignments and Mass Spectrometric Data from Fraction BIV

peak no.[a]	molecular ion, M^+ (intensity)	fragmentation pattern (rel intensity, %)[b]	proposed compound
66	146 (158)	−15 (86), −1 (85), −29 (73)	dimethylindenedione
67	148 (320)	−29 (195), −57 (83), −1 (36)	methylbenzofuranone
68	162 (460)	−29 (210), −57 (70), −1 (29)	dimethylbenzofuranone
69	170 (456)	−29 (122), −93 (110)	unknown
70	196 (3420)	−28 (25), −57 (18)	xanthone[c-g]
71	180 (2292)	−28 (61)	phenalen-1-one[d,f]
72			phthalate
73	210 (676)	−29 (30), −1 (25), −58 (7)	dihydroxyanthracene[c]
74	210 (551)	−29 (20), −1 (12), −58 (6)	dihydroxyanthracene[c]
75	208 (144)	−1 (70), −57 (39), −29 (27)	fluoren-9-onecarboxaldehyde
76	222 (388)	−57 (47), −28 (39), −15 (16)	methylanthraquinone[c,e,g]
77	236 (162)	−15 (20), −28 (17), −71 (19), −57 (13)	dimethylanthraquinone
78	220 (507)	−57 (20), −28 (12)	coumarin from PAH with M_r 202[g]
79	230 (1114)	−28 (14), −30 (12), −29 (8)	benzo[b]fluoren-9-one[d-g]
80	230 (777)	−28 (23), −30 (11), −29 (8)	benzanthrone[c-g]
81	246 (430)	−57 (14), −28 (12)	coumarine from PAH with M_r 228[c,g]
82			phthalate (system peak)
83	254 (4992)		β,β′-binaphthyl (i.s.)[h]
84	254 (629)	−28 (17), −30 (11), −29 (9)	benzo[cd]pyren-6-one[d-g]
85	282 (171)	−28 (36), −56 (7)	quinone from PAH with M_r 252[c,f]

[a] Same as for Table IV. [b] Same as for Table IV. [c] Sames as for Table IV. [d] Same as for Table IV. [e] Same as for Table IV. [f] Same as for Table IV. [g] Same as for Table IV. [h] Same as footnote *j* in Table IV.

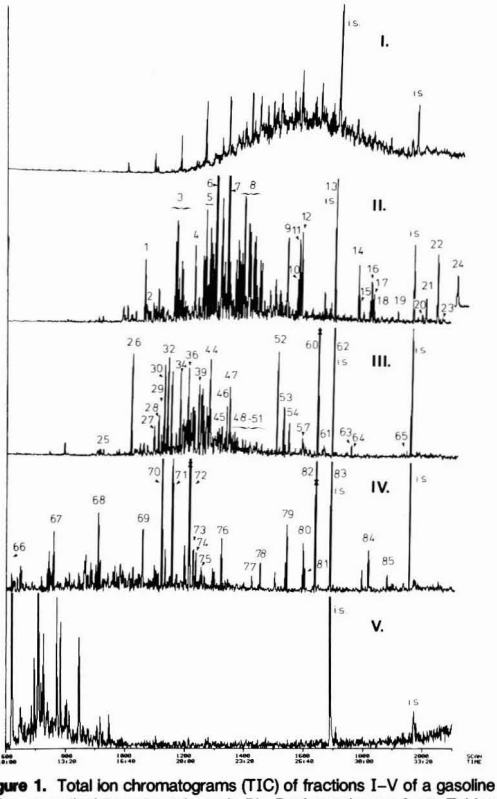


Figure 1. Total ion chromatograms (TIC) of fractions I–V of a gasoline exhaust particulate extract (sample B). Peak numbers refer to Tables III–V.

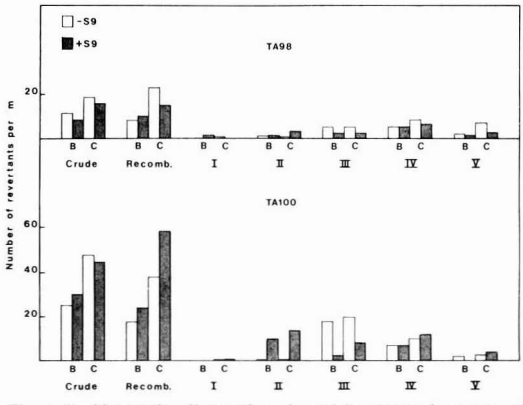


Figure 2. Mutagenic effects of crude and fractionated extracts of gasoline exhaust on *Salmonella typhimurium* TA98 and TA100. Two samples, B and C, were tested in the presence and absence of a metabolizing system (S9).

II–V, and recombined fractions. The highest concentration tested of fraction I, corresponding to 8.6 m of driving distance/mL of cytosol, did not show any competition with [^{3}H]TCDD for receptor binding. As expected from the high PAH content, fraction II was the most potent of all tested fractions. Under the assumption that the identified PAH with potential affinity for receptor binding in this fraction have the same binding characteristics as benzo[*a*]pyrene, most of the observed competition in this fraction can be accounted for. The relatively high potency of fraction III cannot be explained by the fluorenone content. Some fluorenone derivatives have been tested in pure form and show a low receptor affinity (R. Toftgård, unpublished results). More possible candidates are the halogenated derivatives of oxygenated PAH. According to the EC_{50} values of crude extract and recombined fractions, no significant amount of substances with TCDD–receptor affinity were lost during the fractionation procedure.

(3) PAM Cytotoxicity Tests. No difference in toxicity between the corresponding fractions from samples B and C could be detected tested on PAMs. Results obtained with sample B or C are therefore not separated in the further discussion concerning this test system.

By incubation of PAMs for a total of 15 min in 1 mL of HBSS-containing extract from vehicle exhausts in a dose corresponding to a 26-m distance of driving, it could be demonstrated that both the phagocytic capacity and the increased cellular respiration associated with phagocytosis were strongly inhibited Figure 4 (upper). An increased respiratory rate is a part of the metabolic burst taking place when unaffected macrophages are exposed to particles. The inhibition of this increase and the decreased

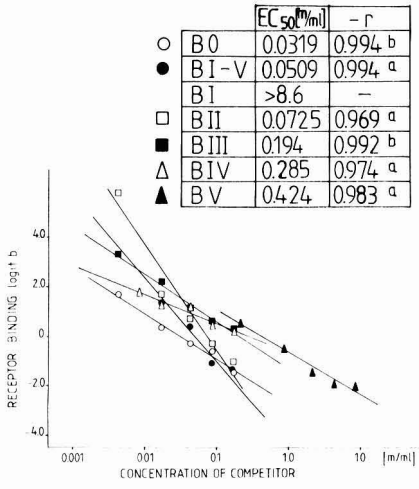


Figure 3. Log–logit plots of crude extract (BO), recombined fractions (BI–V), and fraction II–V (BII–BV). The relative affinity is expressed as the concentration of extracts that competes for 50% of the specific TCDD binding (EC_{50}, logit $b = 0$). The values given in the upper right corner are the results of the seven individual experiments shown in this figure. [a] Statistical analysis gives $p < 0.01$. [b] Statistical analysis gives $p < 0.001$.

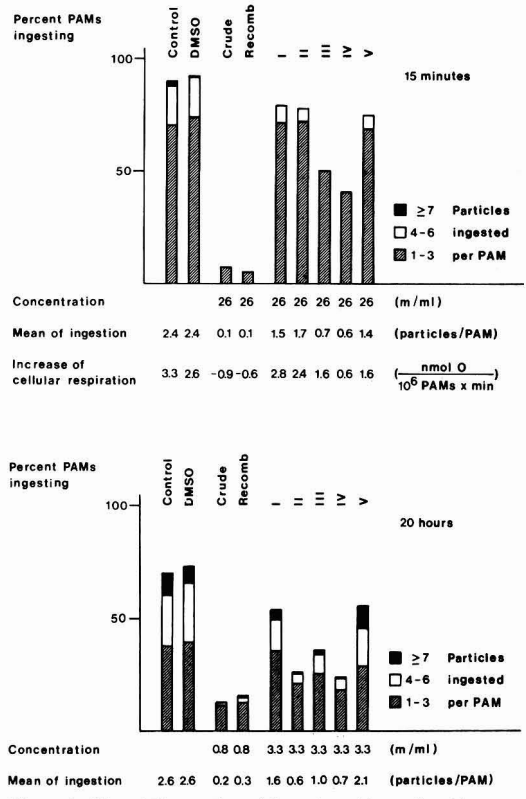


Figure 4. (Upper) Phagocytic activity and particle-mediated increase of cellular respiration of PAMs treated for 15 min with crude and fractionated extracts of car exhaust. Results shown are based on two experiments. Samples were added as Me_2SO solutions in a concentration corresponding to 26-m distance of driving/mL of HBSS. Mean number of cellular respiration before additions: 3.8 nmol of $O/10^6$ PAMs x min. Arrangement of the samples in increasing toxicity based on statistical analysis of the phagocytic activity gives the following order: control = Me_2SO; fraction I = fraction II = fraction V; fraction III = fraction IV; crude = recombinated. $p < 0.001$. (Lower) Phagocytic activity of PAMs treated for 20 h with crude and fractionated extracts of car exhaust. Results shown are based on two experiments. Crude and recombinated extracts were added as Me_2SO solutions in concentrations corresponding to 0.8-m distance of driving/mL of HBSS. The fractions were added as Me_2SO solutions at concentrations corresponding to 3.3-m distance of driving/mL of HBSS. Arrangement of the samples in increasing toxicity based on statistical analysis of the phagocytic activity gives the following order: control = Me_2SO; fraction V = fraction I = III; fraction II = fraction IV; crude = recombinated. $p < 0.001$.

phagocytic capacity are interpreted as a toxic influence of the added material. From Figure 4 (upper) it is also clear that toxic effects were found for all of the fractions when tested in the same dose as the crude extract. The strongest effect was found for fraction III and IV. When the five fractions were recombined, it was also possible to regain the same toxicity as in the crude extract.

Figure 4 (lower) shows the effect on the phagocytic capacity when PAMs were incubated for 20 h with crude extract and recombined extract at a concentration corresponding to 0.8-m distance of driving/mL of HBSS solution and fractions at a concentration corresponding to 3.3-m distance of driving/mL of HBSS solution. As in the short time incubations no significant effect of the solvent (Me_2SO) per se was detected when added in the same volume as used for the samples. The crude and the recombined extracts showed the same toxicity and were more toxic than the exhaust fractions. The distribution of the toxicity among the fractions was similar to that obtained with short time incubations, with one exception. The PAH-containing fraction II, which was among the least toxic fractions judged from the short time incubation, was shown to be one of the two most toxic fractions after the prolonged incubation time. This increase in toxicity, in relation to the other fractions, is possibly due to metabolic transformation by PAM of substances in this fraction to more toxic products. Rabbit PAM (*34*) and human PAM (*35*) have previously been reported to metabolize PAH. The elevation of the cytotoxic influence of fraction II as a function of time of incubation was also confirmed in experiments designed exactly as the 20-h incubation experiments but incubated for only 1 h in total.

Conclusions

In conclusion it can be noted that the crude particle extracts gave a response in all test systems, indicating the presence of genotoxic, AHH-inducing, and PAM-cytotoxic components in the gasoline exhausts. With regard to the fractions the following similarities and differences can be noted. Fraction I, mainly containing aliphatic hydrocarbons, gave no or low effects in all systems. In the TCDD–receptor affinity test fraction II, where the PAH exclusively were found, gave, as expected, the highest response. This fraction also gave significant effects in the other test systems, and the results indicated a metabolism-dependent effect, characteristic for PAH. Fractions III and IV giving the highest effect in *Salmonella* and in the PAM-cytoxicity test also gave significant effects in the TCDD–receptor affinity test. None of the major components of fraction III or IV, i.e., fluoren-9-one, phenalen-1-one, and xanthone, seems to be responsible for the observed mutagenicity. Fraction V, compared to fractions III and IV, showed lower effects in all test systems.

With regard to the question of which compounds are responsible for the effects, there are several possible candidates such as derivatives of fluoren-9-one and phenalen-1-one or other oxy-PAH in fractions III and IV. The

further work will therefore be focused on these fractions.

Acknowledgments

We thank Saab-Scania for supplying the test vehicle and Torben Nielsen at Risö National Laboratory, Denmark, for the gift of several reference substances.

Registry No. Phenanthrene, 85-01-8; anthracene, 120-12-7; methylphenanthrene, 31711-53-2; methylanthracene, 26914-18-1; 2-phenylnaphthalene, 612-94-2; dimethylphenanthrene, 29062-98-4; dimethylanthracene, 29063-00-1; fluoranthene, 206-44-0; pyrene, 129-00-0; methylpyrene, 27577-90-8; methylfluoranthene, 30997-39-8; benzo[*a*]fluorene, 238-82-4; benzo[*ghi*]fluoranthene, 203-12-3; cyclopenteno[*cd*]pyrene, 27208-37-3; benz[*a*]anthracene, 56-55-3; chrysene, 218-01-9; β,β'-binaphthyl, 612-78-2; benzo[*k*]fluoranthene, 207-08-9; benzo[*j*]fluoranthene, 205-82-3; benzo[*e*]pyrene, 192-97-2; benzo[*a*]pyrene, 50-32-8; perylene, 198-55-0; indeno[1,2,3-*cd*]pyrene, 193-39-5; benzo[*ghi*]perylene, 191-24-2; anthanthrene, 191-26-4; coronene, 191-07-1; benzo[*b*]fluorene, 14458-76-5; benzo[*b*]fluoranthene, 205-99-2.

Literature Cited

(1) Möller, M.; Alfheim, I.; Larsson, S.; Mikalsen, A. *Environ. Sci. Technol.* **1982**, *16*, 221.
(2) Grimmer, G.; Hildebrandt, A. *Zbl. Bakt. Hyg. I. Abt. Orig.* **1975**, *161*, 104.
(3) Alsberg, T.; Westerholm, R.; Stenberg, U.; Stillborn Elfver, L. In "Chemical and Biological Characterization of Exhaust Emissions from Vehicles Fueled with Gasoline, Alcohol, LPG and Diesel"; Egebäck, K. E.; Bertilsson, B. M., Eds.; National Swedish Environment Protection Board (Statens Naturvårdsverk): Solna, Sweden, 1983; SNV-PM 1635.
(4) Claxton, L. D. *Environ. Mutagen.* **1983**, *5*, 609.
(5) Zweidinger, R. B. In "Toxicological Effects of Emissions from Diesel Engines"; Lewtas, J., Ed.; Elsevier: Amsterdam, 1982; p 83.
(6) Ames, B. N.; McCann, J.; Yamasaki, E. *Mutat. Res.* **1975**, *31*, 347.
(7) Rannug, U. *Environ. Health Perspect.* **1983**, *47*, 161.
(8) Jeff, J. R.; Moses, H. L.; Branum, E. L.; Taylor, W. F.; Fontana, R. S. *Cancer* **1978**, *41*, 192.
(9) Kouri, R. E.; Rude, T. H.; Joglekar, R.; Dansette, P. M.; Jerina, D. M.; Atlas, S. A.; Owens, I. S.; Nebert, D. W. *Cancer Res.* **1978**, *38*, 2777.
(10) Kouri, R. E.; Billups, L. H.; Rude, T. H.; Whitmire, C. E.; Sass, B.; Henry, C. J. *Cancer Lett.* **1980**, *9*, 277.
(11) McLemore, T. L.; Warr, G. A.; Martin, R. R. *Cancer Lett.* **1977**, *2*, 161.
(12) Poland, A. P.; Glover, E.; Kende, A. S. *J. Biol. Chem.* **1976**, *251*, 4936.
(13) Nebert, D. W.; Jensen, N. M. *CRC Crit. Rev. Biochem.* **1979**, *6*, 401.
(14) Toftgård, R.; Löfroth, G.; Carlstedt-Duke, J. M. B.; Kurl, R.; Gustafsson, J.-Å. *Chem.-Biol. Interact.* **1983**, *46*, 335.
(15) Hunninghake, G. W.; Gadek, J. E.; Szapiel, S. V.; Strumpf, I. J.; Kawanami, O.; Ferrans, V. J.; Keogh, B. A.; Crystal, R. G. In "Methods in Cell Biology"; Harris, C.; Stoner, G., Eds.; Academic Press: New York, 1980; Vol. 21A, p 95.
(16) *Fed. Regist.* **1980**, 45.
(17) Schuetzle, D.; Riley, T. L.; Prater, T. J.; Salmeen, I. In "Proceedings of the 2nd International Congress on Analytical Techniques in Environmental Chemistry, Barcelona, Spain 11/23/81"; Pergamon Press, Oxford, England, 1981.
(18) Choudhury, D. R.; Doudney, C. O. In "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium"; Pepelko, W. E.; Danner, R. M.; Clarke, N. A., Eds.; U.S. Environmental Protection Agency: Cincinatti, OH, 1980; Vol. 1, EPA-600/9-80-057a, p 263.
(19) Stenberg, U.; Alsberg, T.; Bertilsson, B. M. SAE *Tech. Pap. Ser.* **1981**, No. 810441.
(20) Carlstedt-Duke, J. M. B.; Elfström, G.; Snochowski, M.; Högberg, B.; Gustafsson, J.-Å. *Toxicol. Lett.* **1978**, 2, 365.
(21) Romert, L.; Bernson, V.; Pettersson, B. *J. Toxicol. Environ. Health* **1983**, *12*, 417.
(22) Grimmer, G.; Böhnke, H.; Glaser, A. *Zbl. Bakt. Hyg. I. Abt. Orig. B* **1977**, *164*, 218.
(23) Alsberg, T.; Westerholm, R.; Stenberg, U.; Strandell, M.; Jansson, B. Eighth International Symposium on Polynuclear Aromatic Hydrocarbons, Columbus, OH 1983.
(24) Schuetzle, D.; Lee, F. S.-C.; Prater, T. J. *Int. J. Environ. Anal. Chem.* **1981**, *9*, 93.
(25) Choudhury, D. *Environ. Sci. Technol.* **1982**, *16*, 102.
(26) König, J.; Balfanz, E.; Funcke, W.; Romanowski, T. *Anal. Chem.* **1983**, *55*, 599.
(27) Ramdahl, T. *Environ. Sci. Technol.* **1983**, *17*, 666.
(28) Sundvall, A.; Rannug, U. National Swedish Environment Protection Board, 1983, Report SNV pm 1635, ISBN 91-7590-115-3.
(29) Florin, I.; Rutberg, L.; Curvall, M.; Enzell, C. R. *Toxicology* **1980**, *15*, 219.
(30) Leary, J. A.; Lafleur, A. L.; Liber, H. L.; Blemann, K. *Anal. Chem.* **1983**, *55*, 758.
(31) Gibson, T. L. *Atmos. Environ.* **1982**, *16*, 2073.
(32) Rosenkranz, H. S. *Mutat. Res.* **1982**, *101*, 1.
(33) Gibson, T. L. *Mutat. Res.* **1983**, *122*, 115.
(34) Romert, L.; Jenssen, D. *Mutat. Res.* **1983**, *111*, 245.
(35) Harris, C. C.; Hsu, I. C.; Stoner, G. D. *Nature (London)* **1978**, *272*, 633.

Received for review January 17, 1984. Revised manuscript received June 19, 1984. Accepted July 19, 1984. This research was sponsored by the National Swedish Environment Protection Board (Contract number 583 0032-2) in cooperation with Volvo and Saab-Scania.

Environ. Sci. Technol. 1985, 19, 51-56

Impact of Tubificid Oligochaetes on Pollutant Transport in Bottom Sediments

Samuel W. Karickhoff* and Kenneth R. Morris

U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, Georgia 30613

■ Pollutant transport in bottom sediments effected by tubificid oligochaetes was studied in laboratory microcosms. Tubificids burrow in surfacial sediments (typically 6–10 cm), ingest sediment fines (silt and clay particles), and egest them at the sediment/water interface as sand-sized fecal pellets. Sorbed pollutants are transported by default in this process irrespective of the relative pollutant fugacities in the system. For the compounds studied (hexachlorobenzene, pentachlorobenzene, and trifluralin), more than 90% of the chemicals contained in the biologically worked zone were transported to the sediment surface via this process during a 30–50-day period. Pollutant release into the water column was not comparably enhanced, which showed a 4–6-fold increase (over a 90-day period) in the presence of the worms. Pollutant release from intact fecal pellets was highly retarded by sorption. During a typical pellet residence time at the sediment surface (1–3 days), less than 20% of the pollutant contained in the pellet was released. Pollutant entrainment in fecal material further emphasizes the intimate link between benthic organisms and pollutant transport and fate in bottom sediments.

Introduction

Bottom sediments potentially constitute an important sink and/or source for aquatic pollutants. The direction of chemical movement across the sediment/water interface depends upon the relative fugacities of pollutant in the bottom sediment vs. the water column—with pollutant moving in the direction of lower fugacity (*1*, *2*). In this respect pollutant sorption to sediments is most important because sorption serves to lower pollutant fugacity and thereby increase the sink potential of bottom sediments for the more highly sorbed chemicals. It is not surprising, therefore, that bottom sediment is the eventual sink for many hydrophobic environmental contaminants.

The rate of chemical movement across the bottom sediment interface generally varies directly with the magnitude of pollutant fugacity difference between the sediment and water column, but the kinetic coefficient is highly dependent upon the actual exchange process(es) effecting the transport. In those aquatic systems where the bottom sediments are not subject to frequent resuspension, interfacial pollutant transport is generally rate controlled by chemical transport within the bottom, either in pore water or "piggybacked" on transported sediment particles. For hydrophobic pollutants, movement in pore water is highly retarded by sorption. For example, the kinetic coefficient for movement involving advection or dispersion is roughly reciprocally related to the sorption partition coefficient, K_p. If the partition coefficient is 10^4, the rate of chemical movement via pore water is attenuated 4 orders of magnitude, relative to a "nonsorbed" chemical species. Thus, for these types of chemicals, transport in the sorbed state (via sediment transport) may become quite significant.

Again, in those aquatic systems where the sediments are not subject to frequent resuspension, sediment reworking is largely the result of the activities of aquatic organisms (burrowing organisms and dimersal fishes). In freshwater systems, due to their trophic type, life habit, widespread distribution, and population densities, tubificid oligochaetes have the greatest potential for reworking surfacial sediments (*3*, *4*). In this study the transport of three hydrophobic pollutants (trifluralin, pentachlorobenzene, and hexachlorobenzene) in bottom sediments was investigated in small laboratory microcosms in the presence of tubificid oligochaetes. Behavior in these systems was contrasted with chemical transport in the absence of oligochaetes where molecular diffusion was the only operative transport process. Special emphasis was placed on elucidating the role of pollutant sorption in the transport process.

Experimental Section

Tubificid worms (>90% *Limnodrilus hoffmeisteri*, <10% *Tubifex tubifex*) were collected with their native sediment (largely fecal material) from Calls Creek, a small north Georgia stream draining predominantly undeveloped rural lands. In the laboratory the worms were placed in small open-air aquaria (sediment depth 5–10 cm) to allow the worms to recover and establish a fecal layer. Temperature and dissolved oxygen were maintained near field conditions, determined at the time of worm collection. Temperatures ranged from 15 to 20 °C at different collection times, with dissolved oxygen levels near saturation in each case. Larger quantities of whole sediment were collected from the same stream, air dried, and stored for brief periods (less than 30 days) prior to use. The "behavior" of processed sediments in these experiments was contrasted with fresh sediments (i.e., used immediately after collection) and found to be indistinguishable). These sediments were high in sand (50–80%) with organic carbon content varying from 0.5 to 1.5%. The fine fraction of the sediments (<50 μm) was fairly uniform in both organic carbon content (2.5–3.0%) and clay content (40–60%, largely kaolinite). Reagent-grade pentachlorobenzene and hexachlorobenzene (K and K Laboratories) and trifluralin (analytical reference standard, U.S. Environmental Protection Agency, Research Triangle Park, NC were used as received.

Sorption Equilibration of Test Chemicals. Pentachlorobenzene (pcb), hexachlorobenzene (hcb), and trifluoralin (tfn) were plated out of isooctane standard solutions (5–10 mg of each chemical) onto the walls of 40-L glass carboys. Air-dried whole sediment (6–10 kg) and distilled water (≈20 L) were added and the suspensions stirred until sorption equilibrium was achieved (at least 3 weeks).

Microcosm Setup. Portions of the whole sediment (≈1 kg dry weight), which had been previously equilibrated with the test chemicals, were transferred to 4-L flat-bottomed glass bottles (≈250-cm^2 cross-sectional area). Tubificid worms, which were removed from the incubation aquaria via air suction of small volumes of fecal material, were introduced into the microcosms and the containers slowly agitated to distribute the worms in the sediment. The containers were then placed on their sides, allowing the sediments to settle (≈5 min), and slowly righted to produce a fairly uniform sediment profile. The overlying water (and suspended solids) was siphoned off to within a centimeter of the sediment surface and replaced with distilled water. The microcosms were equipped with glass frits and resin traps (Figure 1) and air purge initiated. Air

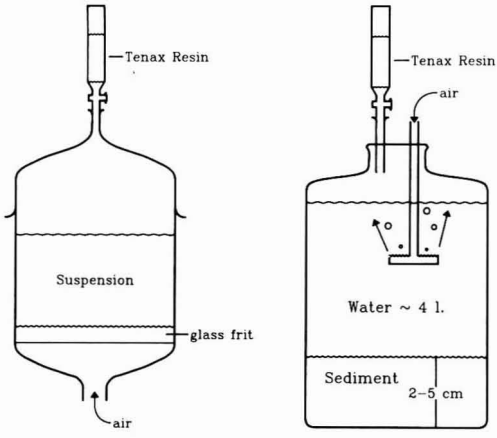


Figure 1. Microcosm and purge cell setup.

purge (≈0.8 L/min, directed upward so as not to resuspend bottom sediment) maintained the dissolved oxygen (d.o.) level in the aqueous phase near saturation (approximating field levels at the collection site). The collection and measurement of test chemicals sparged from the water column provided a direct quantification of the rates of chemical release from the bottom sediment. "Blank" microcosms were prepared in the same manner, excluding worms, to provide a contrast to worm-mediated pollutant transport.

Tubificid Activity. Although a conscious effort was made to maintain in the laboratory a sediment worm environment (i.e., d.o., temperature, sediment composition) similar to their native habitat, no comparison of organism activity in the field vs. the laboratory was made. Those aspects of worm activity deemed important to pollutant transport (i.e., sediment transport and restructuring) were monitored, and the resultant impact on pollutant movement was determined.

Tubificid Population. The worm density (i.e., worms per unit volume) in the fecal material used for microcosm spiking was determined by subsampling and enumeration (at least three samples). Target populations, which ranged from 100 to 5000 worms per microcosm, were then gauged by the volume of the spike. The coefficient of variation (standard deviation per mean) for multiple sampling of a given "spiking medium" was less than 0.3 in all cases. Upon termination of each experiment, the entire population was isolated (wet sieving, 60 mesh), counted, and characterized. For the higher populations, worm enumeration on small cores (≈1-cm^2 cross section) taken from the microcosm fecal layer were used to assess population stability and viability during the experiment. No counting statistics were determined on these cores. It was found that, for local sediments, "stable" tubificid populations could be maintained at population levels of 10^4–10^5 individuals/m^2 for a 90–100-day experimental period (with no added nutrients).

Sediment Transport. During fecal layer creation, sediment profile definition (i.e., depth of worm penetration and fecal layer depth) was monitored on the exterior wall of each microcosm. Depth measurements were taken at 2-day intervals on six equally spaced vertical transects of the sediment profile. Sediment transport during this period could be equated with fecal layer accumulation.

In addition, glass beads (125 μm, coated with fluorescent paints) were sprinkled on the sediment surface at roughly 30-day intervals. Black light was used to aid in bead observation, and variable color coding was used to differentiate times of introduction. Bead burial rates (from which sediment transport was inferred) were expressed in terms of burial depths on the exterior of the microcosms. The temporal and spatial frequency for bead depth measurements was the same as for strata formation described previously. At tubificid populations of 10^4–10^5 individuals/m^2, organism activity appeared uniform across the sediment surface. The coefficient of variation of depth measurements (fecal layer accumulation and/or bead burial) taken on different vertical transects was less than 0.4 in all cases, but generally less than 0.1. Also, bead disappearance from the surface appeared uniform across the microcosm. At populations $<10^4$ individuals/m^2, organism activity was nonuniform, with the worms tending to concentrate in small areas. At populations $>10^5$ individuals/m^2, stable populations could not be sustained during the experimental period without addition of nutrient.

Chemical Redistribution and Sediment Particle Restructuring. Sediment cores (10–20 g) were collected from each microcosm prior to the start of gaseous purge to establish initial chemical concentrations and whole sediment characteristics (i.e., volumetric water content, organic carbon content, etc.). In addition, sorption partition coefficients and desorptive chemical release kinetics were measured on the parent sediment. See Karickhoff et al. (*5*) for partition coefficient measurement and sediment characterization methods and Karickhoff (*6*) for kinetic release measurement.

Upon termination of each microcosm experiment, "discrete" zones (i.e., fecal layer, sand sublayer, biologically unworked sediment) were sampled individually and characterized with regard to the aforementioned chemical and sediment properties. Periodically, fecal pellets were removed during an experiment and the chemical content and chemical release kinetics determined. Chemical release from "intact" pellets was followed by dispersing fresh pellets (no worms) over a flat surface (not exceeding a single pellet thickness) and measuring the kinetics of release into the water under continuous sparging. The glass bottles and air purge/trap assemblies described previously for the microcosms (Figure 1) were used for these measurements. Chemical release from suspended pellets was followed by gaseous stripping of pellet suspensions in smaller purge cells (≈250 mL volume) with fine glass frit bottoms (Figure 1). Gas flow was routed upward through the cell bottom to keep the pellets suspended.

Chemical Recovery and Analysis. Test chemicals removed by air stripping were trapped on tenax resin columns. Collection columns were changed periodically—the sampling frequency was determined by the anticipated temporal properties of the process(es) in question. Highly purified laboratory air was used as the purge gas. The air was collected (oilless compressor), cleaned (brass frit, paper filter, charcoal, tenax resin), and flow regulated in three stages To avoid cummulative loss of water from purge vessels, purge air was saturated with water and/or makeup water was added periodically. Sparged chemicals were recovered from the resin columns by reverse flow solvent elution (1 pore volumen of acetone followed by 2 pore volumes of isooctane). Water was added to the eluate to remove the acetone, and the test chemicals were quantified in the isooctane phase by electron capture gas chromatography (Tracor 222 GC, SE-30 Gas Chrom Q column).

The test chemicals were extracted from sediment and fecal material with a mixed solvent system consisting of

distilled water, acetonitrile, and isooctane (6:1:1 v/v). Samples were sonicated for 8 h (Cole Palmer ultrasonic bath) with vortex mixing every 2 h. The test chemicals were quantified in the isooctane phase by GC as given above.

The test chemicals in aqueous phase samples were extracted with an acetonitrile/isooctane mixture (1:1), vortex mixed, sonicated for 2 h, and quantified as given above.

Results

Sorption of Hydrophobic Pollutants. Sorption equilibrium is typically characterized by an isotherm, which is a plot of pollutant concentration in the sorbed state (denoted S, expressed relative to dry mass sediment) vs. pollutant concentration in solution (C, expressed relative to aqueous phase volume). For hydrophobic pollutants at typical environmental concentrations (low ppm or less), sorption isotherms approximate linearity and can be described by ($S = K_pC$) where K_p is the equilibrium partition coefficient. By convention, concentration units are chosen with the volume element in solution equivalent in mass to the mass unit for sediment; typical units for K_p are therefore liters per kilogram or milliliters per gram. previous sorption studies with hydrophobic chemicals similar to those used in this study showed that sediment/water partitioning of a given compound on different sediments is highly correlated with the organic carbon content of the sediment (*7*). Partition coefficients normalized to organic carbon (denoted K_{oc}) were found to be fairly independent of sediment ($K_{oc} = K_p$/o.c.) where o.c. is the weight fraction of organic carbon in the sediment. K_{oc}'s for the test compounds on whole sediments were determined to be 80 000, 40 000, and 30 000 for hcb, pcb, and tfn, respectively.

Molecular Diffusion Attenuated by Sorption. Under the experimental conditions employed in the microcosms whereby the chemicals were initially distributed uniformly in a semiinfinite sediment column and subsequently stripped continuously from the overlying water, the pollutant flux out of the sediment, F (at time t), is given by

$$F(\text{mass/time}) = \frac{m}{l}\left(\frac{D}{t}\right)^{1/2} \quad (1)$$

where m is the total mass of the chemical initially incorporated in the sediment to depth l and D is the diffusion coefficient for the pollutant in bottom sediment (*8*). For nonconservative chemicals that degrade uniformly within the sediment zone contributing to interfacial transport (describable by a first-order rate constant, k_d), the flux into the water is given by

$$F(t) = \frac{m}{l}\left(\frac{D}{t}\right)^{1/2} \exp(-k_d t) \quad (2)$$

Pentachloro- and hexachlorobenzenes showed no measurable degradation over the 3–4-month purge experiments. Flux out of sediments with no oligochaetes was diffusion controlled and conformed to eq 1 (Figure 2) with effective diffusion coefficients of 10^{-4}–10^{-3} cm^2/day. Trifluralin, however, did degrade significantly in the sediment "blanks", and the flux out of the sediments conformed to eq 2. Degradation rate constants derived from purge data (0.005–0.008 day^{-1}) were generally a factor of 2–4 smaller than rate constants inferred from mass-balance deficit determined at the termination of the experiment. This suggests, perhaps, that tfn degraded more slowly in the near-surface zone contributing to purge release than at depth in the sediment. The diffusion coefficients for

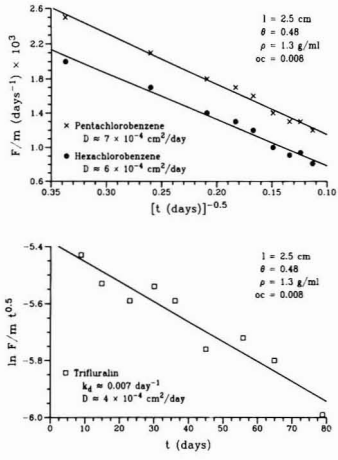


Figure 2. Diffusion-mediated chemical release from bottom sediment in absence of benthic organisms (microcosm 1, blank for microcosm 3).

tfn were in the same range as those observed for the chlorinated benzenes.

As expected, diffusive release of these chemicals is highly retarded by sorption. During a 90-day purge, only the top 1–3 mm of sediment was "stripped" of chemical. A characteristic time for diffusive movement of these chemicals is months per millimeter. Although it is doubtful molecular diffusion of highly sorbed chemicals will mediate transport directly at the sediment/water interface, this process may redistribute chemicals at depth in the sediments (in the absence of other transport mechanisms) and thus may control the long-term transport behavior.

If sorption equilibrium within the bottom sediment is assumed, apriori estimates of the pollutant diffusion coefficients can be made (*9*).

$$D = \frac{D_0\tau}{1 + K_p\rho/\theta} \quad (3)$$

θ is the volumetric water content, which varies from 0.3 to 0.8 but was 0.5 for the sediments used in this experiment; ρ is the sediment mass per unit total volume of sediment, which is 0.5–1.8 g/mL; D_0 is the bulk water diffusion coefficient of the pollutant, which is typically (5–10) × 10^{-6} cm^2/s for organic pollutants; τ is the sediment complexity number (in this case, largely tortuosity and porosity), which is close to unity for bottom sediments (*9*). As described previously, the sorption partition coefficient can be estimated from the organic carbon content of the sediment ($K_p \approx K_{oc}$ × o.c.). For the chemicals and whole sediments used in these experiments (with K_{oc}'s of 10^4–10^5 and organic carbon contents around 1%), one would expect D to be in the range observed, that is, 10^{-4}–10^{-3} cm^2/day.

The molecular diffusion experiments did not reveal nonequilibrium sorptive effects. This does not necessarily indicate the existence of true sorptive equilibrium during transport, but a lack of kinetic resolution to isolate such an effect.

Tubificid Oligochaetes: Impact on Pollutant Transport. Tubificids burrow in surfacial sediments (typically 2–10 cm), ingest sediment fines (silt and clay particles), and egest them at the sediment/water interface as sand-sized cylindrical fecal pellets (*10*). Sorbed pollutants are transported by default in this feeding process *irrespective of the relative pollutant fugacities in the*

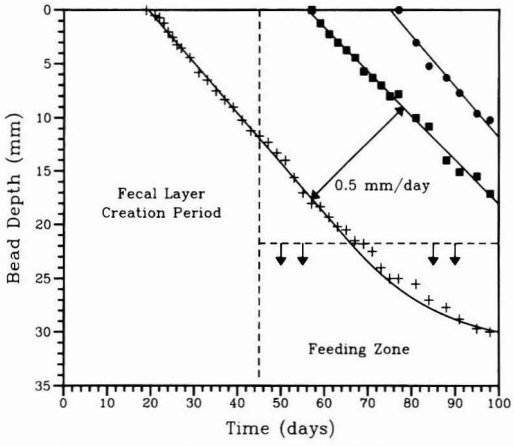


Figure 3. Measurement by glass bead burial of sediment transported by tubificids (microcosm 3, population 4 × 10^4 individuals/m^2).

system. If the flux of fecal pellets to the surface is denoted F_s, then the pollutant flux (via sediment movement) is given by the product $F_s S^f$, where S^f denotes the sorbed pollutant concentration on the *transported* sediment. The pollutant flux into the water, F_p, can be expressed as

$$F_p = F_s S^f R \tag{4}$$

where R describes the pollutant release from the fecal pellet during its residence time at the surface. In the absence of other benthic organisms and/or turbulence that produces stirring or scouring of surface pellets, the residence time (and thus R) is directly related to pellet flux, F_s.

Sediment Transport (F_s). In this study, sediment transport derives solely from tubificid activity and is assumed to be independent of the presence of pollutants. Worm activity is typically quantified in terms of fecal pellet depositional rate and maximum depth worked by the organisms. Recently, Fisher et al. (*11*) and Krezoski et al. (*9*) used an advective feeding model to describe oligochaete feeding; the approach used herein is patterned after these models.

When the worms were introduced into microcosms containing hcb, pcb, and tfn equilibrated with whole sediments, they began pumping the *sediment fines* (composited into fecal pellets) to the surface. The pumping of "virgin" sediment (previously unprocessed by the worms) continued for 30–50 days in different microcosms. During this period, the sediment, which was initially well mixed, became stratified—a surface zone of fecal material and a sublayer of sand (depleted of fines), highly perforated with worm burrows. Toward the end of this period, the microcosms approached a steady-state condition, in terms of sediment stratification, wherein some portion of the fecal material was reprocessed and "recycled". During this latter period, worm activity declined sharply below the fecal zone and was limited to the larger worms in the population.

The rate and depth dependence of worm feeding were followed by periodically introducing fluorescent glass beads onto the sediment surface and observing their subsequent burial on the edges of the microcosms (Figure 3). In systems where worm populations were stable throughout the experiment, bead burial rates showed no significant dependence upon time of introduction. Also, the beads descended at a fairly constant rate through at least half of the eventual depth of the fecal pellet zone, indicating that the major organism feeding was below this "near-surface" portion of the fecal layer (for example, below 2 cm for the microcosm described by Figure 3). This finding is significant because it suggests discrete "turnover" of the fecal layer. The turnover interval (τ_r) for fecal material was defined to be the time required for the glass beads to reach the midpoint of the *feeding* zone, which varied from 40 to 70 days in different microcosms.

Table I. Sediment Physical Properties: Effect of Tubificids–Microcosm 3 (Population 4 × 10^4 Individuals/m^2)

	whole sediment	fecal layer
volumetric water content (θ)	0.48	0.80[a]
solids concentration (ρ)	1.25	0.52[a]
organic carbon fraction (o.c.)	0.008	0.028[a]
hcb concentration, ppm	0.96	3.6[b]
pcb concentration, ppm	1.0	3.3[b]
tfn concentration, ppm	1.2	0.5[b]

[a] Determined at termination of run (day 90). [b] Determined on fresh fecal pellets collected on day 29.

Pollutant Concentration (S^f). During the period of fecal layer creation, the effect of particle sorting on pollutant concentration was clearly evidenced (Table I). Pollutant concentration in the fecal material relative to the whole sediment is given by

$$\frac{S^f}{\bar{S}} = \frac{K_p^f}{\bar{K}_p} \tag{5}$$

where the bar denotes whole sediment values and the superscript f denotes fecal material. For hydrophobic chemicals, this enhancement ratio can be expressed in terms of the relative organic carbon content of fecal material vs. the whole sediment.

$$\frac{S^f}{\bar{S}} = \frac{K_{oc}^f \text{o.c.}^f}{\bar{K}_{oc}\overline{\text{o.c.}}} \approx \frac{\text{o.c.}^f}{\overline{\text{o.c.}}} \tag{6}$$

For the conservative test chemicals (pcb and hcb), there was a 1:1 correspondence between organic carbon redistribution (i.e., $\text{o.c.}^f/\overline{\text{o.c.}}$), and chemical redistribution in all microcosms (correlation coefficient = 0.94, 12 microcosms). In every case, the vast majority (i.e., >90%) of the test chemicals in the biologically worked zone was incorporated into fecal material and transported to the sediment surface. A rough estimate of pollutant redistribution can be derived by assuming *all* the chemical in the "worked" zone ends up in fecal material or

$$S^f \approx \bar{s}/f_{\text{fecal}} \tag{7}$$

when f_{fecal} is the fractional mass of the whole sediment that is processed by the worms.

A characteristic rate for chemical movement (via sediment pumping) with worm populations around 5 × 10^4 individuals/m^2 was millimeters per day. For highly sorbed chemicals of the type used in these experiments, diffusive transport in fecal layer pore water (from eq 3, with o.c. ≈ 0.02–0.05, θ = 0.7–0.8, $\rho \approx 0.7$) is roughly 2 orders of magnitude less than sediment-mediated transport. Even given substantial enhancement of pore water movement effected by worm activity, sediment transport will still dominate chemical movement.

In addition to redistributing the test chemicals, the presence of the worms significantly altered the reactivity of tfn. In blank microcosms (no worms), tfn showed half-lives of 20–30 (subsurface) and 60–80 days for near-surface environments. With the introduction of worms,

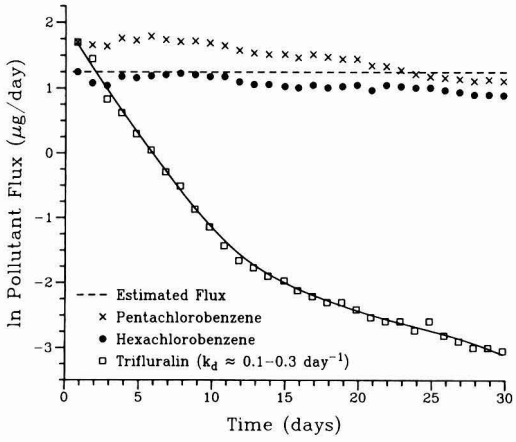


Figure 4. Oligochaete-mediated pollutant release–purge release from microcosm 3 (population 4 × 10^4 individuals/m^2).

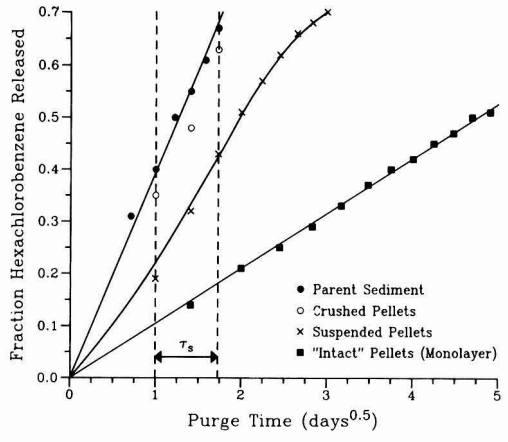


Figure 5. Release of chemicals from fecal pellets (pellets taken from microcosm 3 on day 29).

however, the tfn concentration in fresh fecal pellets decreased exponentially (half-life 2–4 days) during the period of fecal layer creation. Trifluralin flux into the water during this period reflected an equivalent decay (see Figure 4). Once the fecal layer was formed and "recycling" commenced, however, tfn showed very minimal decay (half-life >60 days). Willis et al. (*12*) observed similar behavior for tfn in bacterial suspensions, which they attributed to microbially mediated chemical reduction, where the organisms induced a drop in E_h sufficient to chemially reduce tfn.

Pollutant Release from Fecal Pellets (R). Pollutant release from intact fecal pellets is via molecular diffusion and, for hydrophobic chemicals, is highly retarded by sorptive interactions with the pellet material. Purge-induced release of the test chemicals from fresh pellets (distributed as a disperse monolayer) in the absence of worms showed that times of the order of a month were required for release of half the hcb and pcb contained in the pellets (see Figure 5). Suspending the pellets enhanced the release, primarily as a result of induced pellet fracturing. After extensive sonication (30 min in a sonicator), the pellet material appeared completely dispersed and release rates approached those characteristic of the unpelletized parent sediment. Since in the microcosms the pellet residence time at the surface (τ_s) is characteristically short compared to the time scale of chemical release from intact pellets, diffusive release can be treated in the "short time" limit, whereby the fraction of the chemical released from the pellet during the period τ_s (denoted $f(\tau_s)$) is given by

$$f(\tau_s) = k_t^f(\tau_s)^{1/2} \tag{8}$$

where k_t^f is the effective diffusive release coefficient for intact pellets that incorporates physical characteristics of the pellet and the diffusion coefficient for the pollutant in the pellet.

This equation assumes continuous purge of the released chemical from the water column and a uniform initial distribution of the chemical in that portion of the pellet contributing to release during this period. In addition, a planar source is assumed, which ignores any complex surface geometry of the pellet. These conditions seem plausible for fresh pellets during their brief period of surface exposure.

In the microcosms, these surface residence times were equated *operationally* with the mean "lifetime" of the fluorescent beads on the surface, which varied from 1 to 3 days. (This lifetime was dependent solely on the sediment flux and for different microcosms was approximately 0.5 mm/F_s with F_s expressed as millimeters per day.) Figure 5 shows that hcb release from intact pellets follows the prescribed temporal behavior (eq 8) for times well in excess of "measured" values of τ_s. For pellet residence times at the surface of 1–3 days, one would expect a 10–20% loss in the three test chemicals. Insufficient data (in terms of different chemicals and different sediment sources) were collected to evaluate the variability of k_t^f. It should be indicated, however, that one would expect an inverse relationship between k_t^f and the equilibrium partition coefficient, K_p. That is, any change in sediment properties that enhanced sorption (i.e., increased organic carbon content) would further retard chemical release. Likewise, more hydrophobic chemicals would be sorbed more strongly and, therefore, be released more slowly than those described herein. Also, it should be pointed out that fecal pellet stability (and therefore the chemical release coefficient, R) in a field situation will be impacted by the activities of other benthic organisms and dimersal fishes, which were not present in these microcosms. The diffusion component $f(\tau_s)$ should be considered a lower limit for the release coefficient, R.

Pollutant Flux from the Microcosms. Under continuous sparging of the water column, the pollutant flux into the water during the period of fecal layer creation was fairly constant for pcb and hcb, as given by

$$F_p = F_s S^f R \approx F_s \frac{\text{o.c.}^f}{\overline{\text{o.c.}}} \bar{s} f(\tau_s) \tag{9}$$

where again the bar denotes whole sediment values. In these experiments, F_s and τ_s were determined from bead burial, and o.c.^{f}, $\overline{\text{o.c.}}$, and $f(\tau_s)$ were measured independently on samples extracted from the microcosms; this enables an *independent* estimate of F_p and, thereby, provides a check on the process factoring of eq 9. For example, in microcosm 3 (see Table I and Figures 3–5)

F_s = 0.05 cm/day × 250 cm^2 × 0.52 g/cm^3 = 6.5 g/day

o.c.^{f} = 0.028 $\quad$ $\overline{\text{o.c.}}$ = 0.008

$\bar{s}$ = 1.0 ppm (pcb), 0.96 ppm (hcb), 1.2 ppm (tfn)

$\tau_s \approx$ 2 days; $f(\tau_s) \approx$ 0.15 for τ_s = 2 days

The estimated pollutant flux would be about 3.5 μg/day for each chemical, which corresponds closely with that observed for pcb and hcb during the fecal layer creation period and with the initial tfn flux (Figure 4).

For nonconservative chemicals, in this case tfn, the flux is given by

$$F_p = F_s \frac{\text{o.c.}^f}{\text{o.c.}} \bar{s} f(\tau_s) \exp(-k_d t) \quad (10)$$

where k_d describes the degradation, assumed to be first order and uniform in the zone contributing to sediment flux. As indicated previously, this degradation rate constant was 0.2–0.4 day^{-1} in the presence of worms.

Subsequent to fecal layer creation, pollutant flux out of the microcosms decreased fairly continuously (for the duration of the experiments, ~90 days), and fitted reasonably well to a first-order decay function with rate constants of 0.01–0.03 day^{-1} for the three test chemicals. If one assumes repetitive cycling of fecal material with some chemical fraction, $f(\tau_s)$, lost to the water during each successive turnover (period τ_r)

$$F_p \approx F^0 \exp[-f(\tau_s)t/\tau_r] \quad (11)$$

where F^0 is the flux during fecal layer creation. If $0.1 < f(\tau_s) < 0.2$ for each turnover, and τ_r = 30–60 days, the estimated decay constant is approximately 3×10^{-3} day^{-1}, which is somewhat smaller (3–10-fold) than observed. The independent measurements of sediment turnover (bead burial) and pollutant release (pellet monolayer purge) did not indicate dramatic changes during this recycle period, so the cause for the enhanced decay in chemical flux was not elucidated.

An obvious change in chemical behavior during fecal layer recycle was the abated tfn decay, which now approximated decay in the surface zone of the blank microcosms ($k_d \approx 5 \times 10^{-3}$ day^{-1}) with no worm effect.

Conclusions

The transport of hydrophobic pollutants in bottom sediments in the presence of oligochaete worms was contrasted with transport via molecular diffusion. In microcosms containing stable worm populations ($10^4 - 10^5$ individuals/m^2) pollutant transport within the biologically worked surfacial sediment was entirely worm mediated. Sorbed pollutants were redistributed and/or cycled within the fecal zone in accordance with the life habit of the organism(s), irrespective of pollutant fugacity. Sediment was pumped in a generally vertical direction at rates of 0.3–1.0 mm/day, dependent primarily upon worm population and size. This compared to maximal pollutant diffusion rates of a millimeter per month for highly sorbed chemicals of the type used in these experiments.

The discharge of pollutants out of the sediment into the water (under continuous stripping) was enhanced substantially by the presence of the worms (4–6-fold increase over a 90-day period), but not orders of magnitude as might be expected from the enhanced chemical transport within the sediment profile. The mitigating process appeared to be sediment pelletization by the worms, which "entraps" the sorbed chemical and significantly retards chemical release into the aqueous phase. For the hydrophobic chemicals studied, less than 20% of the chemical contained in an intact pellet was released during a characteristic pellet lifetime at the surface (which was 1–3 days). Chemical release was enhanced by pellet fracturing, but in these microcosms, appreciable pellet fracturing occurred only at depth in the sediment and did not appear to contribute to chemical release into the water.

This study substantiated the intimate link between oligochaete activity and pollutant movement in bottom sediments. Although no effort was made to integrate (with worm activity) other pollutant transport processes operative in a field situation, several inferences can be drawn regarding oligochaete impact on pollutant transport.

Over short time periods (days to weeks) the presence of oligochaetes can expand considerably the depth of sediment "interacting" with the water column. Pollutants can be transported to (or from) depths of up to 10 cm in this time period. Redistribution of highly sorbed chemicals within the biologically active layer is via sediment movement, which in large part derives from benthic organism activity. An important facet of this behavior is that the time required for hydrophobic pollutant mixing in this zone is relatively independent of pollutant physical properties and pollutant fugacity. Hexachlorobenzene, pentachlorobenzene, and trifluralin are "mixed" at the same rate. Pollutant exchange at the water/sediment interface is "driven" by the fugacity differential between the surface sediment and overlying water. The kinetic exchange coefficient is the sediment turnover rate modified by a release factor (such as R in eq 4) that compensates for chemical release or uptake retardation due to sediment pelletization.

Pollutant transport below the fecal zone is typically controlled by molecular diffusion. For "long-lived" pollutants, this process may rate limit chemical uptake and release over long periods of time (months to years).

Acknowledgments

We gratefully acknowledge the advice and assistance of Donald Schultz of the EPA Region IV Environmental Services Division, Athens, GA, and Jarl Hiltunen of U.S. Fish and Wildlife Services' Great Lakes Fishery Lab, Ann Arbor, MI, in the collection, identification, and handling of the macrobenthic invertebrates used in these studies.

Registry No. hcb, 118-74-1; pcb, 608-93-5; tfn, 1582-09-8.

Literature Cited

(1) Mackay, D. *Environ. Sci. Technol.* **1979**, *13*, 1218.
(2) Mackay, D. *Environ. Sci. Technol.* **1982**, *16*, 654A.
(3) Fisher, J. B. Ph.D. Dissertation, Case Western Reserve University, Cleveland, OH, 1979.
(4) Robbins, J. A. *Hydrobiologia* **1982**, *92*, 611–622.
(5) Karickhoff, S. W.; Brown, D. S.; Scott, T. A. *Water Res.* **1979**, *13*, 241.
(6) Karickhoff, S. W. In "Contaminants and Sediments"; Baker, R. A., Ed.; Ann Arbor Science: Ann Arbor, MI, 1980; Vol. II, p 193.
(7) Karickhoff, S. W. *Chemosphere* **1981**, *10*, 833.
(8) Crank, J. "The Mathematics of Diffusion", 2nd ed.; Clarendon: Oxford, 1975.
(9) Krezoski, U. R.; Robbins, J. A.; White, D. S., submitted for publication in *J. Geophys. Res.*
(10) McCall, P. L.; Fisher, J. B. In "Aquatic Oligochaete Biology"; Brinkhurst, R. O.; Cook, D. G., Eds.; Plenum Press: New York, 1980; pp 253–318.
(11) Fisher, J. B.; Lick, W. J.; McCall, P. L.; Robbins, J. A. *J. Geophys. Res.* **1980**, *85*, 3997–4006.
(12) Willis, G. H.; Wander, R. C.; Southwick, L. M. *J. Environ. Qual.* **1974**, *3* (3), 262–265.

Received for review January 30, 1984. Accepted August 15, 1984.

Environ. Sci. Technol. 1985, 19, 57-62

Partition Coefficients of Organic Compounds in Lipid–Water Systems and Correlations with Fish Bioconcentration Factors

Cary T. Chiou

U.S. Geological Survey, Denver Federal Center, Denver, Colorado 80225

■ Triolein–water partition coefficients (K_{tw}) have been determined for 38 slightly water-soluble organic compounds, and their magnitudes have been compared with the corresponding octanol–water partition coefficients (K_{ow}). In the absence of major solvent–solute interaction effects in the organic solvent phase, the conventional treatment (based on Raoult's law) predicts sharply lower partition coefficients for most of the solutes in triolein because of its considerably higher molecular weight, whereas the Flory–Huggins treatment predicts higher partition coefficients with triolein. The data are in much better agreement with the Flory–Huggins model. As expected from the similarity in the partition coefficients, the water solubility (which was previously found to be the major determinant of the K_{ow}) is also the major determinant for the K_{tw}. When the published BCF values (bioconcentration factors) of organic compounds in fish are based on the lipid content rather than on total mass, they are approximately equal to the K_{tw}, which suggests at least near equilibrium for solute partitioning between water and fish lipid. The close correlation between K_{tw} and K_{ow} suggests that K_{ow} is also a good predictor for lipid–water partition coefficients and bioconcentration factors.

Introduction

A major problem in environmental contamination by pollutants is the extent that these pollutants may concentrate from water into aquatic organisms such as fish (*1–12*). The extent of such concentration is expressed as the bioconcentration factor (BCF), i.e., by the ratio of the steady-state concentration of a chemical in the organism (or a part of it) to that in water. It has been generally assumed that the mechanism leading to the uptake of organic pollutants by organisms is analogous to the partitioning between an organic phase and water. Hence, log (BCF) values of organic compounds from both laboratory bioconcentration testings and natural water systems have been correlated either with the corresponding octanol–water partition coefficients (log K_{ow}) (*1, 2, 4–9, 11–13*) or with their water solubilities (log S) (*2, 3, 5, 6, 9, 11*), to which the log K_{ow} are strongly correlated (*3, 14*).

Reinert (*15*), Anderson and Fenderson (*16*), Roberts et al. (*17*), and Sugiura et al. (*18*) found that concentrations of various organic compounds accumulated in fish are determined largely by fish lipid content. Hansen et al. (*19*) showed that residual PCB contents vary considerably in different tissues of fish, presumably because of variations in lipid content. BCF values of various chlorinated compounds in laboratory bioconcentration studies of Könemann and van Leeuwen (*8*) with guppies, and of Oliver and Nimi (*12*) with rainbow trout, show about the same magnitudes for the same compounds, when BCF values are expressed in terms of fish lipid content. These results indicate that the lipid fraction of aquatic organisms (fish) is an important controlling factor in the bioconcentration of relatively insoluble organic compounds. The importance of lipids in bioconcentration has also been speculated in correlation analysis (*8, 9, 11, 12, 18*).

To better understand the effect of lipids in bioconcentration, it is of both theoretical and practical interest to examine the partition characteristics of organic compounds in lipid-water systems. This information may serve as a basis for validating the use of empirical parameters (such as log K_{ow} and log S) in the assessment of bioconcentration and for characterizing the lipophilicity of organic compounds in biochemical studies. Apart from the study by Platford (*20*) on the partition coefficients of three compounds (hexane, benzene, and carbon tetrachloride) in lipid (triolein)–water systems, an in-depth analysis of the subject has not been reported. The primary objectives of this study are therefore to identify the thermodynamic factors affecting the lipid–water partition coefficient (K_{tw}) and to evaluate the relationship between K_{tw}, K_{ow}, and BCF. Triolein (glyceryl trioleate) is selected in this study as the model lipid because of its abundance and its structural similarity to other triglycerides in organisms and because it is a liquid at room temperature.

Raoult's law based equation describing the partitioning of slightly water-soluble organic solutes in a solvent–water mixture, in which the solvent has small solubility in water, is given by (*14*)

$$\log K = -\log S - \log \bar{V}_o^* - \log \gamma_o^* - \log (\gamma_w/\gamma_w^*) \quad (1)$$

where K is the solute partition coefficient, S is the molar water solubility of the liquid or supercooled liquid solute (mol/L), $\bar{V}_o^*$ is the molar volume of water-saturated organic phase (L/mol), γ_o^* is the solute activity coefficient (Raoult's law convention) in water-saturated solvent phase, γ_w is the solute activity coefficient in water, and γ_w^* is the solute activity coefficient in solvent-saturated water. Equation 1 has been used to analyze the relative significance of log S, log γ_o^*, and log (γ_w/γ_w^*) on log K of the solute in solvent–water systems (e.g., octanol–water and heptane–water) (*14, 21*), where the size difference between solute and solvent is generally small. We know, however, from the work of Flory and Huggins (*22–24*) that eq 1 is inappropriate for describing the partitioning of ordinary solutes into a macromolecular phase, because it does not take into account any size disparities between solute and solvent. The Flory–Huggins treatment (*25*) leads to the expression for the partition coefficients of dilute solutes between a macromolecular phase and water as

$$\log K = -\log S\bar{V} - (1 - \bar{V}/\bar{V}_o^*)/2.303 - \chi/2.303 - \log (\gamma_w/\gamma_w^*) \quad (2)$$

where $\bar{V}$ is the molar volume of the solute, $\bar{V}_o^*$ is the molar volume of water-saturated polymer phase, and χ is the Flory–Huggins interaction parameter, a sum of excess enthalpic (χ_H) and excess entropic (χ_S) contributions to the solute–polymer interaction. The magnitude of χ_S depends presumably on the characteristics of the polymer network that affect the orientation of the chain segment (*26, 27*). For triolein in liquid state at room temperature, χ_S should approximate zero because of flexibility of the hydrocarbon chain to assume various orientations. The $\chi_H/2.303$ term in eq 2 is equivalent to the log γ_o^* term in

eq 1. In the absence of size disparity (i.e., $\bar{V}_o^* = \bar{V}$), eq 2 is thus equivalent to eq 1.

The fundamental difference between the predictions of eq 1 and 2 may be illustrated for systems in which one postulates no excess enthalpic or entropic interactions between solute and solvent (i.e., $\chi = 0$) and where, for simplicity, there is effectively zero water miscibility of the solvent (i.e., where log (γ_w/γ_w^*) is zero). Let us consider what happens to the partition coefficient of a solute as we increase the molecular weight of the solvent to macromolecular magnitudes. Equation 2 predicts an asymptotic approach of log K to some constant value. To the extent that this constancy is followed, it can be accounted for on the basis of eq 1 only by making activity coefficient (γ_o^*) inversely proportional to an increasingly large solvent molar volume. For such systems, therefore, Raoult's law model could be reconciled with the data only by assuming a vanishingly small fractional activity coefficient that is characteristic of large specific interactions for which there is no apparent physical justification.

Although triolein is not polymeric in the ordinary sense, it is an interesting solvent (in addition to its obvious biochemical significance) because of its high molar volume relative to a common solvent (e.g., octanol), which is sufficient to distinguish between the predictions of the alternative models. In this study, partition experiments were carried out for a large group of organic compounds in the triolein–water systems to determine whether or not the effects of size disparity between solute and triolein may be safely ignored and how they are best handled if they exist. We may anticipate that the data are better fit by the Flory–Huggins model in view of its recognized success for macromolecular solvent systems; therefore, the reduction in partition coefficient that one would expect from eq 1 on going from octanol to triolein (which corresponds to about a 7-fold increase in molar volume) should not materialize.

Experimental Section

Lipid triolein ($C_{57}H_{104}O_6$, also known as glyceryl trioleate) was purchased from Nu-Check Prep Inc., Elysian, MN and used without further purification. The lipid had been analyzed previously by the supplier using gas–liquid chromatography (GLC) and thin-layer chromatography (TLC) techniques; it was claimed to have a purity greater than 99%. At room temperature, the lipid is a liquid having a density of 0.915 g/mL at 15 °C. Test compounds were reagent grades or analytical standards from Aldrich, Analabs, Mallinckrodt, and Chem Service; they were used as received. The water used was distilled and filtered through an XAD-2 column to remove residual organic contaminants.

The method used for determining the triolein–water partition coefficient is virtually the same as described for the determination of octanol–water partition coefficients (*3*, *14*). In the present study, 0.25–0.50 mL triolein containing 1000–2000 mg/L of test compounds were added to 20 mL of water. Samples were equilibrated at 20–23 °C in 30-mL screw-capped Corex centrifuged tubes (equipped with Teflon-lined lids) for 8–16 h on a reciprocating shaker. Control experiments indicated that samples of all test compounds reached equilibrium in less than 8 h with mechanical shaking, as the observed solute partition coefficients were practically time independent after this period of equilibration.

Generally, four replicate samples were prepared for each test compound. Samples were subsequently centrifuged at 20 °C at ~3500 rpm (1560*g*) for 1 h to attain phase separation. Aliquots of the triolein phase were removed and stored in 2-mL microvials for subsequent dilution and analysis. Remaining triolein was then removed from the sample tubes by using suction pipets, and glass walls previously in contact with the upper triolein phase were carefully cleaned by wiping with tissue paper. To assist in complete removal of excess triolein, the tubes were recentrifuged and the cleaning procedure repeated, as it was observed that residual triolein has a tendency to slide down the glass wall and form droplets on newly cleaned water surfaces. Following this procedure, aliquots of aqueous samples (normally, 10 mL) were removed with pipets and concentrated, when necessary, by extraction with hexane or heptane for subsequent analysis. Partition coefficients of test compounds were calculated as ratios of the concentrations in the triolein phase to those in the aqueous phase. For each compound, log K_{tw} values of the four replicate samples were averaged. Standard deviations were generally less than 0.05 for compounds with average log $K_{tw} < 3.00$, less than 0.10 with $3.00 < \log K_{tw} \leq 5.50$, and less than 0.14 for a few compounds with log $K_{tw} > 5.50$. For DDT and some PCBs, which have high log K_{tw} values, experiments had been carried out with more than four replicate samples to enhance accuracies; in many instances, experiments were repeated until nearly constant log K_{tw} values were obtained.

Determinations of the concentrations of most simple benzene derivatives lacking electron-capture (EC) sensitivity (most of compounds 1–15 in Table I) were done by UV. The triolein phase was diluted with heptane; the aqueous phase was analyzed either directly, or as concentrated heptane extracts (as for benzene and other relatively nonpolar compounds). Interference in UV absorption by the residual triolein in diluted samples was corrected through a blank containing equal amounts of triolein in heptane. In some cases, when concentrations of the aqueous phase had insufficient sensitivity, analyses were done by high-pressure liquid chromatography (HPLC) using a C-18 column, with methanol–water mixture as the mobile phase. Concentration analysis for compounds containing multiple chlorine atoms was carried out by GC with (^{63}Ni) EC detectors, which eliminated the triolein background problem. Whenever possible, the same technique was used to analyze concentrations in both phases. Finally, the solubility of water in triolein was determined by the standard Karl–Fisher titration method.

Results and Discussion

Determined triolein–water partition coefficients (K_{tw}), octanol–water partition coefficients (K_{ow}), solubilities in water (S), and molar volumes ($\bar{V}$) of 38 organic compounds at room temperatures (20–25 °C) are listed in Table I. The S values for solid solutes are values of the corresponding supercooled liquids, calculated from solid solubilities, heats of fusion ($\Delta\bar{H}_f$), and melting points (T_m) according to the method described earlier (*14*). For 1,2,3-trichlorobenzene, 1,3,5-trichlorobenzene, 1,2,3,4-tetrachlorobenzene, 1,2,3,5-tetrachlorobenzene, and pentachlorobenzene, which have low melting points (<370 K), calculations were made with the assumption of $\Delta\bar{H}_f = 13.5T_m$, along with solid solubilities of 16.3, 10.6, 7.18, 3.23 and 0.385 mg/L, respectively, at 23 °C. Similarly, the molar volumes are those for the solutes in the liquid state. Densities of 1,2,3-trichlorobenzene, 1,2,3,4-tetrachlorobenzene, pentachlorobenzene, hexachlorobenzene, and DDT at their melting points were determined and used to calculate their $\bar{V}$ values. Liquid molar volumes of 1,3,5-trichlorobenzene and 1,2,3,5-tetrachlorobenzene were assumed to be the same as those of 1,2,3-trichlorobenzene and 1,2,3,4-tetrachlorobenzene, respectively. Liquid molar volumes of PCBs were

Table I. Water Solubilities (S), Liquid Molar Volumes ($\bar{V}$), and Partition Coefficients (K) of Selected Organic Compounds in Triolein–Water and Octanol–Water Systems: $K_{ow} = K$(Octanol–Water) and $K_{tw} = K$(Triolein–Water)

	compound	$\bar{V}$, L/mol[a]	log S, mol/L[b]	log $S\bar{V}$	log K_{ow}[c]	log K_{tw}[d]
1	aniline	0.0911	−0.405	−1.45	0.90	0.91
2	*o*-toluidine	0.107	−0.817	−1.79	1.29	1.24
3	benzaldehyde	0.102	−1.51	−2.50	1.48	1.58
4	acetophenone	0.117	−1.31	−2.24	1.58	1.61
5	anisole	0.109	−1.85	−2.82	2.11	2.31
6	benzene	0.0894	−1.64	−2.69	2.13	2.25
7	toluene	0.106	−2.25	−3.22	2.69	2.77
8	nitrobenzene	0.102	−1.78	−2.77	1.85	2.15
9	ethylbenzene	0.123	−2.84	−3.75	3.15	3.27
10	*n*-propylbenzene	0.139	−3.30	−4.16	3.68	3.77
11	1,3,5-trimethylbenzene	0.139	−3.09	−3.95	3.42	3.56
12	fluorobenzene	0.0938	−1.80	−2.83	2.27	2.33
13	chlorobenzene	0.102	−2.36	−3.35	2.84	2.97
14	bromobenzene	0.105	−2.55	−3.53	2.99	3.12
15	iodobenzene	0.112	−2.78	−3.73	3.25	3.42
16	1,2-dichlorobenzene	0.113	−2.98	−3.98	3.38	3.51
17	1,3-dichlorobenzene	0.114	−3.04	−3.98	3.38	3.63
18	1,4-dichlorobenzene	0.118	(−3.03)	−3.96	3.39	3.55
19	hexachloroethane				4.14	4.21
20	1,2,3-trichlorobenzene	0.125	(−3.74)	−4.64	4.14	4.19
21	1,2,4-trichlorobenzene	0.125	−3.72	−4.62	4.02	4.12
22	1,3,5-trichlorobenzene	0.125	(−3.82)	−4.72	4.31	4.36
23	1,2,3,4-tetrachlorobenzene	0.142	(−4.24)	−5.09	4.60	4.68
24	1,2,3,5-tetrachlorobenzene	0.142	(−4.53)	−5.38	4.59	4.69
25	1,2,4,5-tetrachlorobenzene	0.142			4.70	4.70
26	hexachlorobutadiene	0.158	−5.01	−5.81	4.90	5.04
27	pentachlorobenzene	0.166	(−5.18)	−5.96	5.20	5.27
28	hexachlorobenzene	0.186	(−5.57)	−6.30	5.50	5.50
29	biphenyl	0.155	(−3.88)	−4.69	4.09	4.37
30	2-PCB	0.174	(−4.57)	−5.33	4.51	4.77
31	2,2′-PCB	0.189	(−5.08)	−5.57	4.80	5.05
32	2,4′-PCB	0.189	(−5.28)	−5.97	5.10	5.30
33	4,4′-PCB				5.58	5.48
34	2,4,4′-PCB	0.204	(−5.98)	−6.67	5.62	5.52
35	2,5,2′,5′-PCB				5.81	5.62
36	2,4,5,2′,5′-PCB				6.11	5.81
37	2,4,5,2′,4′,5′-PCB				6.72	6.23
38	*p,p*′-DDT	0.250	(−6.74)	−7.34	6.36	5.90

[a] Calculated from molecular weights and liquid densities. [b] The 20–25 °C values cited in ref 14 and 25 for all compounds, except compounds 20–27 from this work. Numbers in parentheses are for the supercooled liquids. [c] Values cited in ref 14 and 28 for all compounds, except compounds 19–27 from this work. [d] Values from this work.

approximated by using the densities of liquid Aroclor mixtures that have approximately the same chlorine numbers as the individual PCBs.

In analyzing the partition data in triolein–water systems, the molar volume of the triolein phase was calculated based on the solubility of water in triolein and on the assumption of volume additivity of the liquids (*14*). The water content in triolein at 25 °C was found to be 0.11% by weight (or 5.1% by mole fraction), which is in reasonable agreement with the reported value of 0.20% by weight at 37 °C (*20*). This gives $\bar{V}_o^* = 0.919$ L/mol, or log $\bar{V}_o^* = -0.037$, for water-saturated triolein at room temperature. To simplify the analysis further, the term log (γ_w/γ_w^*) (≥ 0) describing the enhancement of solute solubility in water by dissolved triolein is assumed to be zero. With small mutual solubility of triglyceride and water (*28*), neglect of this term will not be serious for solutes with relatively large log S.

As shown in Table I, determined log K_{tw} values are generally somewhat greater than corresponding log K_{ow} values for most relatively small nonpolar organic compounds that would not be expected to form stable associations with triolein. Platford (*20*) reported log $K_{tw} = 2.5 \pm 0.2$ for benzene at 20–37 °C, in reasonable agreement with the value from this study. With increasing molecular size of the solute, e.g., for larger PCBs, hexachlorobenzene, and DDT, log K_{tw} and log K_{ow} values become more comparable. The large log K_{tw} values for small solutes can be explained by eq 1 only by assuming γ_o^* to be from 0.27 to 0.42, i.e., by an ad hoc assumption of negative deviations from Raoult's law that has no physical justification and that we may at least suspect to be an artifact of the model. In a similar study (*20*), less-than-one γ_o^* values (0.20–0.50) were also found for hexane, benzene, and carbon tetrachloride in triolein at dilution, based on the Raoult's law theory.

The apparent negative deviations from Raoult's law of small solutes in triolein reflect presumably the result that activities of small molecules in a polymer (or a macromolecular phase) are lower than can be expected on the mole fraction basis because of the size-disparity effect (*22–25*), which makes eq 1 ineffective. This occurs to practically all substituted benzenes whose molecular volumes are only small fractions of the triolein molecule (i.e., $\bar{V}_o^*/\bar{V} > 6$). In support of this view, magnitude of the size effect on γ_o^* becomes progressively reduced (i.e., γ_o^* increases) as solute size increases. For instance, the calculated log γ_o^* values by eq 1 for DDT, hexachlorobenzene, and some PCBs, which have only moderate size disparity with triolein (i.e., $\bar{V}_o^*/\bar{V} = 3.7–5.0$), are positive (i.e., $\gamma_o^* > 1$), as is normally the case for a solute in solution. It should be noted, however, that since log (γ_w/γ_w^*) can be significant for larger solutes (which is omitted in our calculations), the actual γ_o^* values may still be <1 for some of these compounds.

According to the Flory–Huggins model, as incorporated into eq 2, activities of the two components differing in size and shape in a binary solution will always deviate from those defined by Raoult's law theory. Although this has been well recognized in those systems where components show a large size disparity, as for small molecules in triolein, it has been difficult to verify the superiority of the Flory–Huggins model to Raoult's law model when the size disparity is only moderate. Hildebrand and Sweny (*29*) found that component activities in the hexadecane–hexane system ($\bar{V}_2/\bar{V}_1 = 2.25$) are very close to predictions of Raoult's law. With moderate size disparity, however, the difference between the two theories is relatively small. For instance, when $\bar{V}_0^*/\bar{V}$ is 4, the log γ_0^* calculated by using eq 1 differs from $\chi/2.303$ calculated by using eq 2 by only about 0.3; with $\bar{V}_0^*/\bar{V} \leq 2$, as for common solutes in octanol, the difference is reduced to less than 0.09. We therefore made no attempts to resolve the relative merits of the two theories in this case. With $\bar{V}_0^*/\bar{V} > 6$, Raoult's law model (eq 1) obviously fails to account for the solute activity in triolein.

Since eq 2 is able to accommodate the experimental log K_{tw} values of all solutes in Table I, it is therefore used as the basis for interpreting the solute behavior. By eq 2, when $\bar{V}/\bar{V}_0^*$ is neither constant nor approaching zero, no single "ideal line" relating log K_{tw} vs. log S or log K_{tw} vs. log $S\bar{V}$ (*14*, *25*) can be established to describe solute incompatibilities with triolein ($\chi/2.303$). The magnitude of $\chi/2.303$ is therefore determined individually for each solute according to eq 2, with log (γ_w/γ_w^*) assumed to be zero.

The values of $\chi/2.303$ thus calculated are generally quite small but positive (<0.25) for small benzene derivatives that have relatively large log S values; respective values are somewhat greater for larger, less-soluble solutes such as 2,4′-PCB (0.32), 2,4,4′-PCB (0.81), hexachlorobenzene (0.45), and DDT (1.1). True $\chi/2.303$ values for larger solutes are likely smaller than calculated because of the neglect of log (γ_w/γ_w^*), which can be significantly greater than zero. However, since the sum of $\chi/2.303$ and log (γ_w/γ_w^*) is nevertheless small compared to $-\log S$ for all solutes and the variation of $\bar{V}$ is also relatively small, it is evident that log S is the major factor affecting the value of log K_{tw}, similar to the effect of log S on the octanol–water partition coefficient (log K_{ow}) (*14*). The log K_{tw} values are therefore quite comparable with respective log K_{ow} values. Data in Table I show that difference between the two is generally less than 0.2 (or, a factor of 2 between K_{tw} and K_{ow}) for all compounds except 2,4,5,2′,4′,5′-PCB and DDT (where the log K_{tw} could be less accurate because of experimental difficulties). Small differences between log K_{tw} and log K_{ow} appear to be related to size differences of the solutes. All simple benzene derivatives that are relatively nonpolar tend to have larger values of log K_{tw} than of log K_{ow} by about 0.10–0.20. In contrast, log K_{tw} values of larger solutes (PCBs, hexachlorobenzene, and DDT) are more comparable with (and in some cases slightly smaller than) respective log K_{ow} values, a result to be further clarified with more intensive experimental work.

Because log S is the major factor affecting both log K_{tw} and log K_{ow}, log K_{tw} can therefore be estimated in terms of log S (or log $S\bar{V}$) and log K_{ow}. A plot of log K_{tw} vs. log $S\bar{V}$ is given in Figure 1 and a plot of log K_{tw} vs. log K_{ow} in Figure 2. Results show that although log K_{tw} is closely related to log $S\bar{V}$ and to log K_{ow}, the correlations are noticeably curvilinear, rather than linear, when extended over the upper range of log K_{tw}. Curvature is noted more visibly at log $K_{tw} = \sim 5$ in both plots, about the point that separates most benzene derivatives from relatively larger PCBs and DDT. This nonlinearity appears to result partly from the uneven size effect and partly from the difference in χ and log (γ_w/γ_w^*) on log K_{tw} for small and large solutes. According to eq 2, the size effect favors the log K_{tw} values of small molecues; consequently, a systematic increase in χ and/or log (γ_w/γ_w^*) with solutes of increasing size (of decreasing log S) would result in deviations from linearity between log K_{tw} and log $S\bar{V}$. It is possible that part of the deviation from linearity could also result from the inaccuracy of large log K_{tw} values.

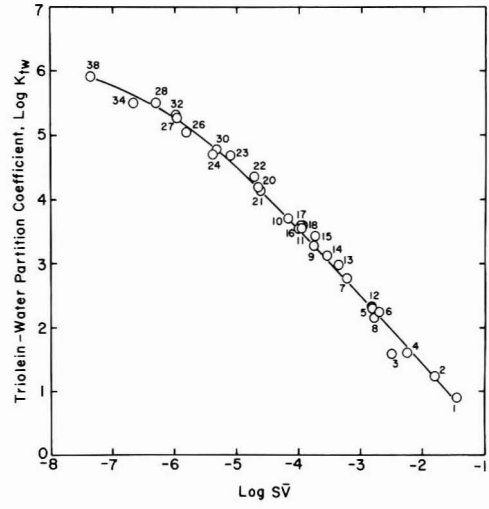


Figure 1. Plot of log K_{tw} values vs. log $S\bar{V}$ for the selected compounds. See Table I for compound numbers.

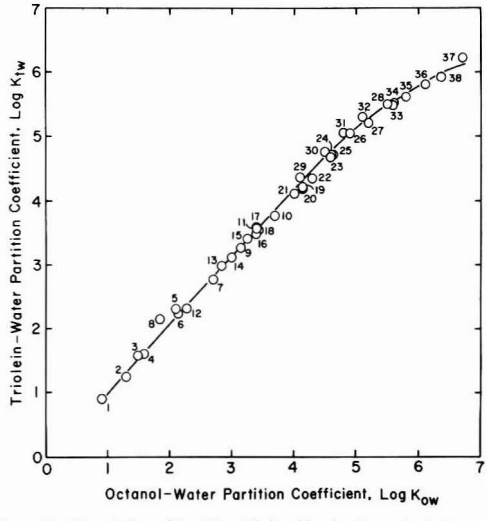


Figure 2. Correlation of log K_{tw} with log K_{ow} for the selected organic compounds. See Table I for compound numbers.

Barring some unknown effects that may also contribute to the curvature in Figures 1 and 2 at high log K_{tw}, the values of log K_{tw} for most substituted benzenes that have comparable molar volumes can be satisfactorily correlated in linear form with respective values of log $S\bar{V}$ and log K_{ow}. For compounds **1–25**, where log $K_{tw} < 5$, the linear regression between log K_{tw} and log K_{ow} gives

$$\log K_{tw} = 1.00 \log K_{ow} + 0.105 \quad (3)$$

Table II. Comparison of Lipid-Based Bioconcentration Factor (BCF) and Triolein–Water Partition Coefficients (K_{tw}) of Selected Organic Compounds

compound	log K_{tw}	lipid-based log (BCF) guppies[a]	lipid-based log (BCF) rainbow trout[b]
1,2-dichlorobenzene	3.51		3.51–3.80
1,3-dichlorobenzene	3.63		3.70–4.02
1,4-dichlorobenzene	3.55	3.26	3.64–3.96
hexachloroethane	4.21		3.79–4.13
1,2,3-trichlorobenzene	4.19	4.11	4.15–4.47
1,2,4-trichlorobenzene	4.12		4.19–4.56
1,3,5-trichlorobenzene	4.36	4.15	4.34–4.67
1,2,3,4-tetrachlorobenzene	4.68		4.80–5.13
1,2,3,5-tetrachlorobenzene	4.69	4.86	
1,2,4,5-tetrachlorobenzene	4.70		4.80–5.17
hexachlorobutadiene	5.04		4.84–5.29
pentachlorobenzene	5.27	5.42	5.19–5.36
hexachlorobenzene	5.50	5.46	5.16–5.37

[a] Data from Könemann and van Leeuwen (*8*). [b] Data from Oliver and Nimi (*12*) with BCFs obtained at two exposure levels of the test compounds.

with $r^2 = 0.995$ and $n = 25$. The linear regression between log K_{tw} and log $S\bar{V}$ for the same set (excluding compounds 19 and 25) gives

$$\log K_{tw} = -1.05 \log S\bar{V} - 0.646 \quad (4)$$

with $r^2 = 0.987$ and $n = 23$. If log S is used to replace log $S\bar{V}$ in eq 4 the correlation gives

$$\log K_{tw} = -0.960 \log S + 0.537 \quad (5)$$

with $r^2 = 0.959$ and $n = 23$.

It is worth noting that the small curvilinearity in the plot of log K_{tw} vs. log K_{ow} at large values of log K_{ow} resembles the one noticed in the plot of log (BCF) vs. log K_{ow} in the studies of Sugiura et al. (*7*), Könemann and van Leeuwen (*8*), and Watarai et al. (*13*). While the nonlinearity in log (BCF) – log K_{ow} plots appears to result partly from the use of inaccurately estimated log K_{ow} values in some studies, it has also been suggested that the curvature at large values of log K_{ow} reflects the transport resistance of large molecules across tissues and membranes in organisms (*7*, *13*) before the system reaches a steady-state condition. In the present study of the correlation of log K_{tw} with log K_{ow}, the observed nonlinearity is recognized as an equilibrium effect rather than as a kinetic effect of the solutes in triolein and octanol phases. A critical investigation of the linearity in plots of log (BCF) vs. log K_{ow} and log K_{tw} vs. log K_{ow} would require a large set of accurate data on these properties.

We now examine the relationship between log K_{tw} and log (BCF). A comparison of log K_{tw} values with corresponding log (BCF) values reported by Könemann and van Leeuwen (*8*) with guppies, and by Oliver and Nimi (*12*) with rainbow trout, expressed on the basis of the fish lipid content, is shown in Table II. Results show a close agreement between K_{tw} and BCF values for the compounds studied. In most cases, the agreement appears to be within a factor of 2, which is about as good as can be expected since the combined error associated with K_{tw} and BCF determinations can often be just as large. The log (BCF) values on the lipid basis do not appear to show systematic differences between the two fish species, when one considers the uncertainty associated with BCF values and the possible dependence of BCF on solute concentration as indicated in the study of Oliver and Nimi (*12*).

The proximity of log K_{tw} to log (BCF) on the lipid basis is consistent with the suggestion that the lipid content of the fish is the principal component for achieving concentrations of (nonionic) organic compounds. The correspondence between log K_{tw} and log K_{ow} therefore supports the use of log K_{ow} in estimating log (BCF) values. A practically linear correlation is found by plotting log (BCF) vs. log K_{tw} (Figure 3) by using the BCF values of guppies from Könemann and van Leeuwen (*8*) and of rainbow trout from Oliver and Nimi (*12*). The correlation gives

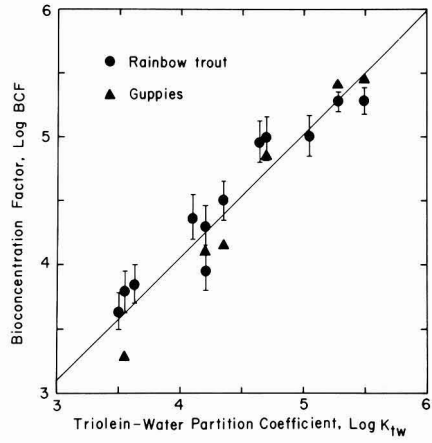


Figure 3. Correlation of log (BCF) on the basis of fish lipid content with log K_{tw} for the compounds in Table II. The BCF values for rainbow trout are the average values given in Table II. The solid line is the correlation line given by eq 6.

$$\log (\text{BCF}) = 0.957 \log K_{tw} + 0.245 \quad (6)$$

with $n = 18$ and $r^2 = 0.915$. The fact that the slope is nearly 1 and the intercept is small further supports the postulate that lipid content mainly is responsible for the uptake of organic compounds. The correlation given by eq 6 is not statistically different from log (BCF) = log K_{tw} at the 95% confidence level for the compounds in Table II. When log BCF values of the compounds are correlated with corresponding log K_{ow} values, the result gives

$$\log (\text{BCF}) = 0.893 \log K_{ow} + 0.607 \quad (7)$$

with $n = 18$ and $r^2 = 0.904$. Although eq 7 is statistically different from log (BCF) = log K_{ow} at the 95% level for the range of BCF values in Table II, the difference between eq 6 and 7 is relatively small, and we cannot be sure that the correlation of log (BCF) with log K_{ow} will be statistically different from that of log (BCF) with log K_{tw} for wider or different range of data set. On the basis of the relationship between log K_{tw} and log K_{ow}, and correlations of log (BCF) with log K_{tw} and log K_{ow}, octanol appears to be a sufficiently good model for biological lipids in the study of bioconcentration of nonionic organic compounds, since the uncertainty of log (BCF) can be as high as 0.30 (as shown in Table II), more than the difference between log K_{tw} and log K_{ow} for most compounds.

There are several factors that can lead to discrepancies between BCF and K_{tw} (or K_{ow}). Compounds that are unstable in water or that are readily metabolized by organisms (to the extent that the rate of degradation is greater than the rate of equilibration) will give anomalous BCF values because of the inability of the system to reach true equilibrium state. Similarly, BCF values obtained with short exposure times before steady-state concentrations in both biotic and water phases are reached may be expected to differ greatly from equilibrium values. For compounds with very large log S (or very small log K_{ow}),

it is also possible that the solute concentration in nonlipid phases (e.g., protein or membrane phase) of the organism may be significant (*11*) and consequently may affect the correlation between log (BCF) and log K_{tw} (or K_{ow}). Certain macromolecular organic materials (e.g., humic substances) in natural waters also could significantly enhance the solubility of relatively insoluble solutes (*30*, *31*), thus decreasing apparent BCF values. Thus, BCF values from laboratory experiments must be considered to be somewhat "idealized". Nonetheless, they provide useful references in fate assessment, particularly in situations where experimental BCF values are not available. Estimation of BCF values in fish for relatively stable compounds (such as chlorinated hydrocarbons) on the basis of K_{tw} or K_{ow} and fish lipid content would seem to be a convenient and economical approach. To substantiate the effect of lipids and the use of K_{tw} and K_{ow} in estimating bioconcentration, the relationship between K_{tw} (or K_{ow}) and (lipid-based) BCF needs to be further evaluated with more compounds in the range of $5 < \log (BCF) < 3$.

Acknowledgments

I thank D. W. Schmedding, Z. D. Hill, and T. I. Brinton for technical assistance and L. C. Friedman and L. J. Schroder for statistical consultation.

Literature Cited

(1) Neely, W. B.; Brnason, D. R.; Blau, G. E. *Environ. Sci. Technol.* **1974**, *8*, 1113.
(2) Metcalf, R. L.; Sanborn, J. R.; Lu, P. Y.; Nye, D. *Arch. Environ. Contam.* **1975**, *3*, 151.
(3) Chiou, C. T.; Freed, V. H.; Schmedding, D. W.; Kohnert, R. L. *Environ. Sci. Technol.* **1977**, *11*, 475.
(4) Southworth, G. R.; Beauchamp, J. J.; Schmieder, P. K. *Environ. Sci. Technol.* **1978**, *12*, 1062.
(5) Veith, G. D.; Macek, J. R.; Petrocelli, S. R.; Caroll, J. In "Aquatic Toxicology"; Eaton, J. D.; Parrish, P. R.; Hendricks, A. C., Eds.; American Society for Testing and Materials: Philadelphia, 1980; pp 116–129.
(6) Kenega, E. E.; Goring, C. A. I. In "Aquatic Toxicology"; Eaton, J. R.; Parrish, P. R.; Hendricks, A. C., Eds.; American Society for Testing and Materials: Philadelphia, 1980; pp 78–115.
(7) Sugiura, K.; Ito, N.; Matsumoto, N.; Michara, Y.; Murata, K.; Tsukakoshi, Y.; Goto, M. *Chemosphere* **1978**, *7*, 731.
(8) Könemann, H.; van Leeuwen, K. *Chemosphere* **1980**, *9*, 3.
(9) Chiou, C. T. In "Hazard Assessment of Chemicals: Current Developments"; Saxena, J.; Fisher, F., Eds.; Academic Press: New York, 1981; Vol. I, pp 117–153.
(10) Trujillo, D. A.; Ray, L. E.; Murray, H. E.; Giam, C. S. *Chemosphere* **1982**, *11*, 25.
(11) Mackay, D *Environ. Sci. Technol.* **1982**, *16*, 274.
(12) Oliver, B. G.; Nimi, A. J. *Environ. Sci. Technol.* **1983**, *17*, 287.
(13) Watarai, H.; Tanaka, M.; Suzuki, N. *Anal. Chem.* **1982**, *54*, 702.
(14) Chiou, C. T.; Schmedding, D. W.; Manes, M. *Environ. Sci. Technol.* **1982**, *16*, 4.
(15) Reinert, R. E. *Pestic. Monit. J.* **1970**, *3*, 233.
(16) Anderson, R. B.; Fenderson, O. C. *J. Fish. Res. Board Can.* **1970**, *27*, 1.
(17) Roberts, J. R.; DeFrietas, A. S. W.; Gidney, M. A. J. *J. Fish. Res. Board Can.* **1977**, *34*, 89.
(18) Sugiura, K.; Washino, T.; Hattori, M.; Sata, E.; Goto, M. *Chemosphere* **1979**, *8*, 359.
(19) Hansen, D. J.; Parrish, P. R.; Lowe, J. I.; Wilson, A. J., Jr.; Wilson, P. D. *Bull. Environ. Contam. Toxicol.* **1971**, *6*, 113.
(20) Platford, R. F. *Bull. Environ. Contam. Toxicol.* **1979**, *21*, 68.
(21) Chiou, C. T.; Schmedding, D. W.; Block, J. H.; Manes, M. J. *Pharm. Sci.* **1982**, *71*, 1307.
(22) Flory, P. J. *J. Chem. Phys.* **1942**, *10*, 51.
(23) Huggins, M. L. *Ann. N.Y. Acad. Sci.* **1942**, *43*, 1.
(24) Flory, P. J. "Principles of Polymer Chemistry"; Cornell University Press: Ithaca, NY, 1953; pp 495–520.
(25) Chiou, C. T.; Porter, P. E.; Schmedding, D. W. *Environ. Sci. Technol.* **1983**, *17*, 227.
(26) Scott, R. L. *J. Chem. Phys.* **1949**, *17*, 268.
(27) Hildebrand, J. H.; Prausnitz, J. M.; Scott, R. L. "Regular and Related Solutions"; Van Nostrand-Reinhold: New York, 1970.
(28) Leo, A.; Hansch, C.; Elkins, D. *Chem. Rev.* **1971**, *71*, 525.
(29) Hildebrand, J. H.; Sweny, J. W. *J. Phys. Chem.* **1939**, *43*, 297.
(30) Carter, C. W.; Suffet, I. H. *Environ. Sci. Technol.* **1982**, *16*, 735.
(31) Chiou, C. T.; Porter, P. E.; Shoup, T. D. *Environ. Sci. Technol.* **1984**, *18*, 295.

Received for review February 13, 1984. Revised manuscript received August 6, 1984. Accepted August 23, 1984. The work was supported in part by NIH Grant ES-02400 and by EPA Cooperative Research Grant CR-808046 from the EPA Environmental Research Laboratory at Corvallis, OR. Any use of commercial names is for descriptive purposes only and does not constitute endorsement by the U.S. Geological Survey.

Environ. Sci. Technol. 1985, 19, 63–69

Mutagenic Transformations of Dilute Wood Smoke Systems in the Presence of Ozone and Nitrogen Dioxide. Analysis of Selected High-Pressure Liquid Chromatography Fractions from Wood Smoke Particle Extracts

Richard Kamens,* Douglas Bell, Andrea Dietrich, Jean Perry, and Randall Goodman

Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, North Carolina 27514

Larry Claxton and Sylvestre Tejada

Environmental Protection Agency, Research Triangle Park, North Carolina 27711

■ Dilute wood smoke from a residential wood stove was added to 25-m^3 outdoor Teflon chambers and then reacted in the dark with sub-ppm levels of O_3 and NO_2. Chemical fractionation of unreacted and O_3 and NO_2 reacted wood smoke indicated that most of the extracted mass as well as the mutagenicity was contained within the most polar fractions. The PAH fraction contributed 12–17% of the total TA98+S9 mutagenicity of unreacted wood smoke. After reaction with O_3 and NO_2, the mutagenic contribution of the PAH fraction declined substantially. One of the moderately polar fractions that contained compounds with the polarity of aromatic ketones contributed 4% to the total direct-acting mutagenicity in unreacted samples. After reaction with O_3 + NO_2 wood soot extract in this fraction made up 16–30% of the total direct-acting mutagenicity. Analysis of this reacted fraction tentatively indicated the formation of odd nitrogen organic compounds and other oxygenated species.

Introduction

Laboratory studies have suggested (*1–3*) the potential for atmospheric reactions of particle-bound polycyclic organic compounds, but actual documentation of the extent to which these reactions and subsequent mutagenic changes take place is much more limited. Recently, workers (*4–6*) at the University of North Carolina outdoor chamber facility have shown that dilute wood smoke systems can react with sub-ppm levels of O_3 and NO_2 and cause a manyfold increase in the direct-acting TA98 mutagenicity of wood smoke particle extracts.

Given the large mutagenic increases that O_3 and NO_2 mixtures impart to wood smoke, a chamber study was undertaken to generate unreacted and O_3 + NO_2 reacted wood smoke for chemical fractionation. The purpose was to determine the fractions in which mutagenic changes were occurring and to isolate associated chemical transformations which gave rise to some of these changes in mutagenicity. The preliminary results of that study are presented in this paper.

Experimental Section

Sample Generation and Preparation. Wood smoke was added to two 25-m^3 outdoor Teflon film chambers directly from the chimney of a residential woodstove (free standing intermediate size Buck woodstove, Smoky Mountain Enterprises, Asheville, NC). After the addition of wood smoke to the chambers, ~30–60 mg of wood smoke particles was collected on 13.34-cm Teflon-impregnated glass fiber filters (Pallflex). This left, depending on the experiment, 2000–2500 $\mu g/m^3$ smoke particles in the chambers. UV-generated O_3 and NO_2 (in nitrogen) from a commercially prepared high concentration cylinder were then added and immediately diluted into the chambers. The dilute wood smoke and NO_2 + O_3 mixture were permitted to react in the dark for periods of up to 4 h at which time another 13.34-cm filter sample was taken. A more detailed description of the chambers, injection of wood smoke, associated instrumentation, sampling apparatus, and aerosol behavior is given in previous papers published elsewhere (*4–6*).

Table I. Step Gradient Program for Fractionation of Wood Smoke[a]

time, min	% hexane	% $MeCl_2$	% ACN
0–12	100	0	0
12–17	100–95	0–5	0
17–25	95	5	0
25–26	95–80	5–20	0
26–35	80	20	0
35–36	80–40	20–60	0
36–48	40	60	0
48–49	40–0	60–100	0
49–70	0	100	0
70–72	0	100–0	0–100
72–90	0	0	100
90–92	0	0–100	100–0
92–102	0	100	0
102–104	0–100	100–0	0
104–120	100	0	0

[a] Fractions collected at the following times to optimize separation: A fraction, 0–12 min; B fraction, 12–28.5 min; C fraction, 28.5–37.5 min; D fraction, 37.5–50.5 min; E fraction, 50.5–72.5 min; F fraction, 72.5–93.0 min. Column flow rate 2.0 mL/min.

Wood smoke particulate filter samples were immediately Soxhlet extracted with 100 mL of methylene chloride ($MeCl_2$) for 16 h and concentrated to ~10 mL by rotary evaporation. Gravimetric determinations on the extracted mass were made by evaporating 100-μL aliquots of the extract on preweighed 47-mm Teflon-impregnated glass fiber filters (Pallflex). Extracts were concentrated with a dry nitrogen stream to 50–200 μL. Most of the extract was then fractionated for subsequent chemical and bioassay analysis on the basis of polarity, with a normal-phase liquid chromatographic technique (*7*).

Fractionation was conducted with a ternary Spectra-Physics Model 8700 high-pressure liquid chromatographic (HPLC) pump and a 5 mm × 30 cm Bio-sil A, 20–44 μm, (Bio-Rad Laboratories, Richmond, California) column using a multistep gradient program (as shown in Table I). Five milligrams of concentrated extract was injected onto the column, and six fractions were collected at the detector outlet according to the time windows shown in Table I. The fractionation was monitored with a Varian Fluorichrom (340–380-nm excitation and 470–530-nm emission) fluorescence detector. A chromatographic trace of a typical fractionation is shown in Figure 1. Characterization of this system has shown the recoveries of selected aliphatic, aromatic, nitroaromatic, and aromatic carbonyl compounds to be greater than 80% (*3*).

Individual fractions were then concentrated to 1 mL, and gravimetric determinations of the mass were made in the

0013-936X/85/0919-0063$01.50/0

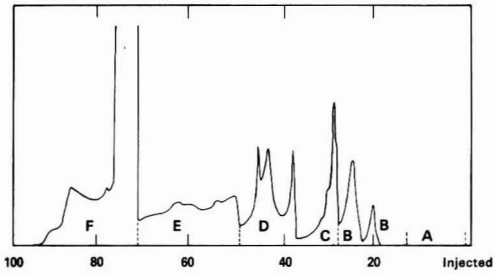


Figure 1. Fluorescence chromatogram of normal phase fractionation of O_3 + NO_2 chamber reacted dilute wood smoke extract. Excitation wavelength 340–380 nm and emission wavelength 470–530. 5 mm × 30 cm Bio-Sil A, 20–44 μm column, 2 mL/min; mobile phase and fraction windows given in Table I.

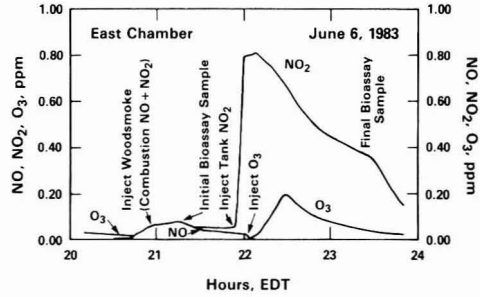


Figure 2. NO_x and O_3 time vs. concentration profiles for dilute wood smoke experiment on June 6, 1983.

same manner described above. Chemical analysis was routinely conducted on a Carlo Erba Model 4130 gas chromatograph with a 30-m DB-5 fused silica column or a Spectra Physics 8700 HPLC with UV or fluorescence detectors and a reverse-phase C18 column. Selected fractions were analyzed on a VG-Micromass 7070F (double-focusing, magnetic sector) mass spectrometer interfaced to a Hewlett-Packard 5710A capillary gas chromatograph. Analysis conditions are presented elsewhere (*8*). These fractions were also analyzed for the presence of selected nitroaromatic compounds by using the reverse-phase HPLC technique developed by Tejada and coworkers (*9*). The method takes advantage of the high sensitivity at which aminoarenes can be detected by fluorescence and the fact that nitro-PAH generally are difficult to measure with fluorescence at sub-ppb levels. Conversion of nitro-PAH to their complementary amino-PAH compounds was achieved catalytically within the HPLC system.

Mutagenicity Assay. The *Salmonella typhimurium* plate incorporation assay was performed as described by Ames et al. (*10*) with minor modifications (*11*). Tester strain TA98 was used with and without rat liver microsomal activation (S9) at five to six doses using triplicate plates. When sample mass was low, duplicate or single plates were used. To conserve sample, the dose range was compressed to assure a maximum number of points in the linear range of the dose/response curve.

Slope values from mutagenicity dose/response curves were reported as revertants per microgram of extract. All data points were used unless toxicity to the bacteria was observed. The positive controls were 2-nitrofluorene and 2-aminoanthracene (+S9), and tester strain monitoring and maintenance procedures were followed to assure reproducibility (*11*). Samples from an experiment were assayed on the same day by the same worker at the Health Effects Research Laboratory, U.S. EPA, Research Triangle Park, NC.

Results and Discussion

Three wood smoke O_3 + NO_2 experiments were conducted in the dark to generate both unreacted and reacted wood smoke particulate extracts for fractionation followed by chemical and bioassay analysis. The experiments on June 6 and April 24, 1983, will be described in detail, and additional information from the other experiment (Dec 4, 1982) will be used to address similarities and variations that occurred in these experiments.

The June 6, 1983, experiment started with ~2500 μg/m^3 wood smoke particles, 0.79 ppm of NO_2, and 0.20 ppm of O_3. The April 24, 1983, experiment began with ~2000 μg/m^3 wood smoke particles, 0.76 ppm of NO_2, and 0.31 ppm of O_3. Both experiments exhibited similar O_3 and NO_2 behavior, and for illustrative purposes the NO_x and O_3 time–concentration profiles for the June 6 experiment are shown in Figure 2. Note the appearance of combustion NO and NO_2 as wood smoke was added to the chambers and the reaction of both O_3 and NO_2 soon after they were injected into the chambers.

Table II. Mass Contribution of Each Fraction to the Unfractionated $MeCl_2$ Wood Soot Particle Extract

fraction	June 6, 1983[a] unreacted, %	June 6, 1983[a] reacted, %	April 24, 1983[b] unreacted, %	April 24, 1983[b] reacted, %
A	0.89	0.23	1.60	0.34
B	0.62	0.11	0.94	0.29
C	0.39	0.16	0.56	0.29
D	2.82	1.35	2.69	2.80
E	6.03	4.06	8.31	3.27
F	80.51	90.43	83.86	86.28
A–F	91.26	96.34	97.96	93.27
wood smoke particle mass, mg	80.39	90.53	38.71	58.13
unfractionated extracted mass, mg	61.02	56.13	31.90	38.19

[a] Red oak (6 cm × 0.5 m logs) used as the fuel and stove operated at a constant burn rate of 4.3 kg/h. [b] Same fuel with stove burn rate of 4.8 kg/h.

The percent mass determined in each fraction from both experiments is shown in Table II. Ninety-one to ninety-eight percent of the original wood soot extract that was fractionated was recovered through this system. Only 11–14% of the unreacted filter mass extract appeared in the first five neutral or semipolar fractions, and 81–84% eluted in the sixth or very polar fraction. This agrees with the original findings of Hubble et al. (*12*), who reported that most of the particle-bound organics in wood smoke were very polar in nature. After reaction with O_3 + NO_2, the particle extract became more polar. Only 6–7% of the mass appeared in the first five fractions, and more than 86–90% appeared in the final most polar fraction.

The TA98±S9 dose/response curves for selected fractions from the June 6, 1983, experiment, both before and after reaction, are shown in Figure 3. As can be seen, large increases in the mutagenic response for the C–E fractions were observed when unreacted and reacted samples were compared. These curves usually had linear regression correlation coefficients greater than or equal to 0.9 for fractions having mutagenicities greater than 0.3 revertant/μg. At the doses tested (1–100 μg), slope values for nonmutagenic and marginally mutagenic fractions (i.e.,

Table III. Estimation of the Mutagenic Contribution of Each Fraction to the Total Sample for June 6, 1983, Dilute Wood Smoke Experiment: Initial Wood Smoke Particle Concentration = 2500 $\mu g/m^3$, Ozone = 0.20, and NO_x = 0.79 ppm

	TA98–S9						TA98+S9					
	unreacted			reacted			unreacted			reacted		
	slope,[a] rev/μg	rev/mg[b]	%[c]	slope, rev/μg	rev/mg[b]	%	slope, rev/μg	rev/mg[b]	%	slope, rev/μg	rev/mg[b]	%
unfractionated sample	0.22	167.0		2.08	1289.6	100.0	0.37	280.8	100.0	0.99	613.8	100.0
fraction												
A	−0.03	0.0	0	−0.11	0.0	0.0	1.05	7.1	2.5	−0.05	0.0	0.0
B	0.91	4.3	2.6	NT[d]			7.27	34.2	12.2	1.58	1.1	0.2
C	0.40	1.2	0.7	9.67	9.6	0.7	2.22	6.6	2.4	NT		
D	0.31	6.6	4.0	25.07	209.8	16.3	1.47	31.5	11.2	13.40	112.2	18.3
E	0.28	12.8	7.7	6.09	153.3	11.9	0.71	32.5	11.6	5.56	140.0	22.8
F	0.09	55.0	32.9	1.04	583.1	45.2	0.30	183.3	65.3	0.56	314.0	51.2
∑A–F		79.9	47.9		955.8	74.1		295.2	105.2		567.3	92.5

[a] Mutagenic slope from linear portion of dose/response curves in revertants per microgram (rev/μg) of extract. [b] rev/mg of wood smoke particles = slope[a] in rev/mg × mg of extract in fraction ÷ mg of particles, i.e., 80.39 mg for unreacted sample and 90.53 mg for reacted sample. [c] Percent mutagenic contribution to unfractionated sample. [d] Not tested.

Table IV. Estimation of the Mutagenic Contribution of Each Fraction to the Total Sample for April 24, 1983, Dilue Wood smoke Experiment: Initial Wood Smoke Particle Concentration = 1985 $\mu g/m^3$, Ozone = 0.31 ppm, and NO_2 = 0.76 ppm

	TA98–S9						TA98+S9					
	unreacted			reacted			unreacted			reacted		
	slope,[a] rev/μg	rev/mg[b]	%[c]	slope, rev/μg	rev/mg[b]	%	slope, rev/μg	rev/mg[b]	%	slope, rev/μg	rev/mg[b]	%
unfractionated sample	0.20	164.8	100	1.16	762.1	100	0.42	346.1	100	0.96	630.7	100.0
fraction												
A	0.20	2.6	1.6	−0.28	0.0	0.0	1.77	23.3	6.7	NT[d]		
B	0.07	0.5	0.3	−0.38	0.0	0.0	7.75	60.0	17.3	1.29	2.5	0.4
C	0.09	0.4	0.2	5.28	10.1	1.3	1.50	6.9	2.0	NT		
D	0.29	6.4	3.9	12.86	236.6	31.0	1.98	43.9	12.7	11.93	219.5	34.8
E	0.88	60.3	36.6	5.26	113.0	14.8	1.23	84.2	24.3	4.78	102.7	16.3
F	0.10	69.1	41.9	0.43	243.7	32.0	0.08	55.3	16.0	0.44	249.4	39.5
∑A–F		139.3	84.5		603.4	79.1		273.6	79.0		574.1	91.0
reconstituted ∑A–F	0.16	131.9	80.0	0.85	525.6	68.4						

[a] Estimated slope from linear portion of dose/response curve in revertants per microgram (rev/μg) of extract. [b] rev/mg of wood smoke particles = slope[a] in rev/mg × mg of extract in fraction ÷ mg of particles, i.e., 38.71 mg of wood soot particles for unreacted sample and 58.13 mg for reacted sample. [c] Percent mutagenic contribution to unfractionated sample. [d] Not tested.

<0.1 revertants/μg) had correlation coefficients equal to or less than 0.7. This lower correlation was primarily a function of plate count scatter at or near the spontaneous revertant level.

In order to qualitatively assess each fraction's mutagenic contribution to the whole sample, a weighted estimate was calculated by taking the product of the mass in a given fraction and the mutagenic slope value determined from that fraction's dose/response curve and dividing this by the product of the mass and the slope of the unfractionated sample. Results of these calculations along with the number of revertants per milligram of wood smoke particles which appears in each fraction are displayed in Tables III and IV. In addition, in the April 24 experiment aliquots of the appropriate mass portion from the −S9 fractions were recombined (reconstituted sample), bioassayed, and compared to the unfractionated sample. Approximately 80% of the mutagenicity was accounted for by reconstituting the unreacted fractions and 68% by reconstituting the reacted fractions.

Nonpolar Fractions. The most nonpolar fraction, A, was eluted with 100% hexane and contributed approximately 1% to unreacted, extracted particle mass. Gas chromatography/mass spectrometry (GC/MS) analysis indicated that this fraction was initially dominated by a mixture of alkanes and alkenes, primarily in the C_{16}–C_{30} range. After reaction with O_3 + NO_2, the percentage of mass in this fraction declined substantially. Analysis of the O_3 + NO_2 reacted A fraction did not show detectable levels of olefinic compounds. Presumably these alkenes had reacted with O_3 to form carbonyl and acid derivatives. These product compounds would appear in the more polar fractions of the final or reacted extract. All of our experimental data have shown that the aliphatic fraction did not contribute substantially to the direct-acting mutagenicity before or after reaction with O_3 + NO_2. In the April 24 experiment we did observe ~7% of the indirect-acting mutagenicity in the unreacted fraction. On this day, however, the fractionating column became slightly deactivated after the fifth pass through the system, and some two- and three-ring PAH did elute into the A fraction.

The PAH fraction, fraction B, was eluted by using a mobile phase of 95% hexane and 5% $MeCl_2$. It was found that this fraction contributed less than 1% of the mass to the total unfractionated extract. After reaction with O_3 + NO_2 the mass in this fraction decreased by 70–90%. The PAH compounds that have been identified by GC/MS and gas chromatography/flame ionization detection (GC/FID) retention time data were similar to those previously reported by Ramdahl et al. (*13*, *14*). The distribution of PAH compounds that were typically observed in unreacted

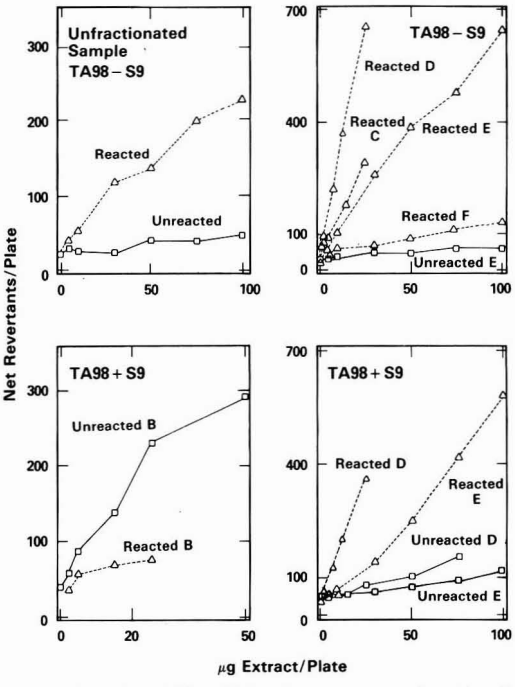


Figure 3. Selected TA98 ± S9 dose/response curves from June 6, 1983, dilute wood smoke + O_3 + NO_2 experiment.

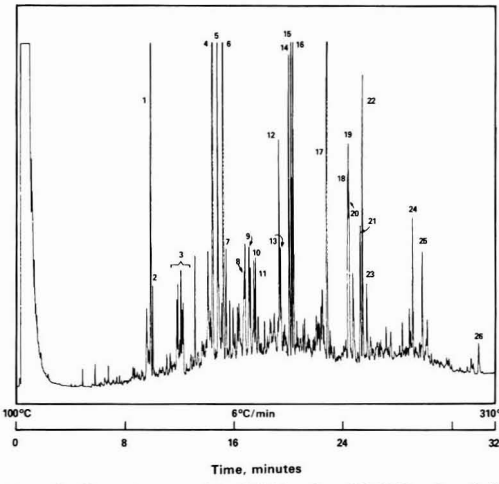


Figure 4. Chromatogram of the PAH fraction. DB-5 30 m fused silica column, H2 carrier. Identifications based on retention time of knowns (denoted by superscript a), comparison to GC/MS reconstructed ion chromatograms and MS reference spectra (denoted by superscript b), and comparison to Ramdahl chromatograms (10, 11) (denoted by superscript c): (1) phenanthrene[a,b], (2) anthracene[a,b], (3) order of elution according to Ramdahl[c,b], 3-methylphenanthrene[b], 2-methylphenanthrene[b], 4,5-methylenephenanthrene[b], 4- and/or 9-methylphenanthrene[b], 1-methylphenanthrene[b], (4) fluoranthene[a,b], (5) acephenanthrylene[b,c], (6) pyrene[a,b], (7) ethylmethylenephenanthrene[b,c], (8) benzo[*a*]fluorene[b,c], (9) benzo[*b*]fluorene/4-methylpyrene[b,c], (10) 2-methylpyrene and/or methylfluoranthene[b,c], (11) 1-methylpyrene[b,c], (12) benzo[*ghi*]fluoranthene[b,c], (13) benzo[*c*]phenanthrene[b,c], (14) cyclopenta(*cd*]pyrene[a,b], (15) benzo[*a*]anthracene[a,b], (16) chrysene and triphenylene[a,b,c], (17) internal standard β,β'-binaphthyl, (18) benzo[*b*]fluoranthene[a,b], (19) benzo[*j*]fluoranthene[b,c], (20) benzo[*k*]-fluoranthene[a,b], (21) benzo[*e*]pyrene[a,b], (22) benzo[*a*]pyrene[a,b], (23) perylene[a,b], (24) indeno[1,2,3-*cd*]pyrene[a,b], (25) benzo[*ghi*]perylene[a,b], and (26) coronene[b,c].

chamber wood smoke samples is shown in Figure 4.

In certain experiments, the concentrations of selected PAH were monitored over the course of the O_3 + NO_2 reaction. This was done by analyzing the extracts from 47-mm filter samples which were taken hourly during the experiment. As shown in Figure 5, PAH disappeared with time. By comparison (Figure 5), wood smoke aged in the dark in the presence of 45 ppb of combustion NO_x showed comparative stability of particle-bound PAH.

Mutagenicity testing of the PAH fractions showed a small (<3%) contribution to the direct-acting mutagenicity of unreacted samples and no apparent contribution to reacted samples. Given, however, that PAH require metabolic activation for full expression in Ames bacterial assays, it was not surprising that the PAH fraction contributed 12–17% to the total unreacted indirect-acting mutagenicity. After reaction with O_3 + NO_2, almost no indirect-acting mutagenicity was observed in this fraction. This loss of indirect-acting mutagenicity between unreacted and reacted samples was also consistent with the above-mentioned decay of PAH concentrations over time (Figure 5) and the dramatic loss of mass in this fraction.

Semipolar and Moderately Polar Fractions. A 20% $MeCl_2$ and 80% hexane mobile phase was used to elute the first of the semipolar and moderately polar fractions. This fraction, fraction C, generally comprised less than 0.6% of the unreacted filter extract. After reaction the percent mass of this fraction declined by 50%.

Analysis of the unreacted C fraction for nitroarenes by conversion to amino-PAH did not suggest the significant presence of nitroaromatic compounds. After reaction with O_3 + NO_2, however, the possible formation of some unidentified nitroarenes was indicated.

The initial TA98–S9 slope of the dose/response curve for the C fraction was in the 0.3–0.4 revertant/μg range. After reaction with O_3 + NO_2 the slope of the direct-acting dose/response curve increased to 5–10 revertants/μg, depending on the experiment. Although this increase in slope was rather significant, the final mass in the fraction was comparatively small. Thus, when compared to the net increase in the final unfractionated sample, the increase in the C fraction only made up a small percentage of the total direct-acting mutagenicity.

The D fraction was eluted with a 60% $MeCl_2$ and 40% hexane solvent mixture and was characterized by compounds with the polarity of aromatic ketones. Four-ring nitroaromatics also eluted in this fraction. As will be seen, the D fraction was the most interesting fraction and thus had the greatest amount of analysis directed toward it.

This fraction consistently showed the largest direct-acting mutagenicity slope increase between unreacted and reacted samples. The unreacted slope (~0.3 revertant/μg) when combined with the initial mass in the fraction accounted for 4% of the initial direct-acting mutagenicity. After reaction, slope values increased to 12–25 revertants/μg. Thus, even though less than 3% of the reacted mass remained in this fraction, it contributed ~16–30% of the total direct-acting mutagenicity (Tables III and IV). Similar changes in indirect-acting mutagenicity (+S9) were also observed.

The most prominent aromatic carbonyl compounds in this fraction which were confirmed by GC/MS with authentic standards and retention time included fluoren-9-one, 9,10-anthraquinone, and 7*H*-benz[*de*]anthracen-7-one (benzanthrone). These compounds along with many other aromatic ketones have been tentatively identified in wood smoke by Ramdahl (*15*). Very little is known about the mutagenic or carcinogenic qualities of aromatic carbonyl

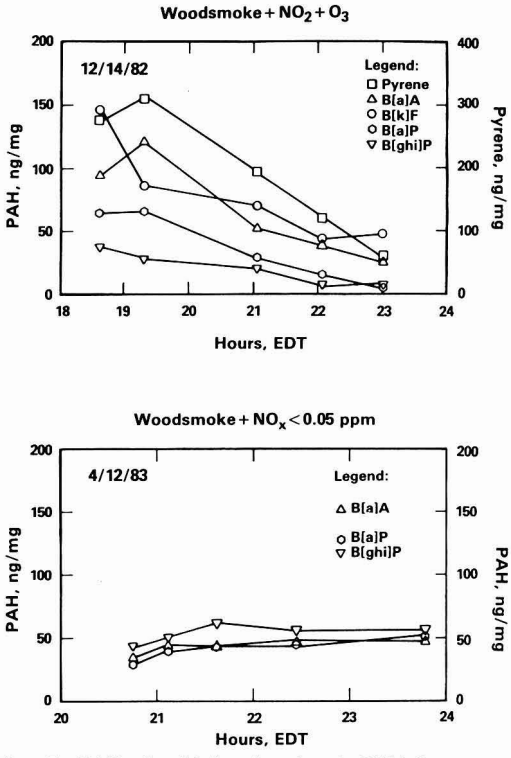


Figure 5. Stability of particle-bound wood smoke PAH in the presence of (1) 0.63 ppm of NO_2 + 0.43 ppm of O_3 (particle concentration = 3850 $\mu g/m^3$) and (2) NO_x below 0.05 ppm + 0.00 O_3 (particle concentration = 3702 $\mu g/m^3$).

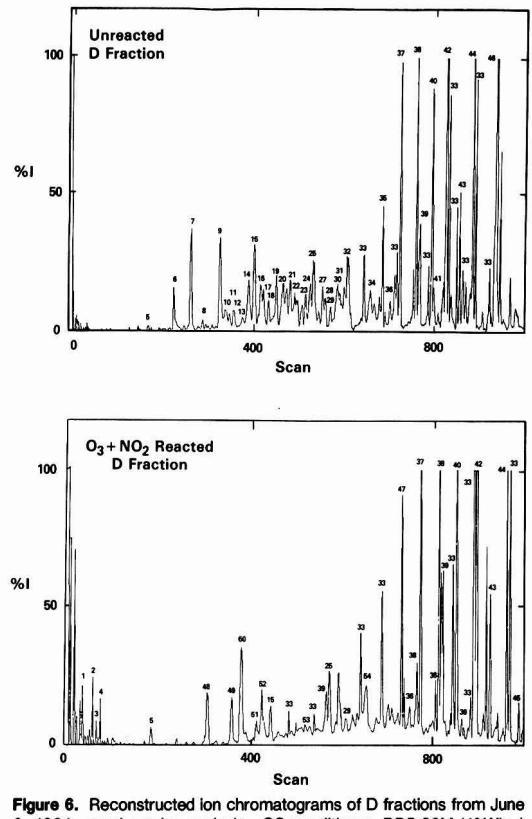


Figure 6. Reconstructed ion chromatograms of D fractions from June 6, 1984, wood smoke analysis. GC conditions: DB5-30M (J&W); 1 μL on column at room temperature, programmed 100–300°C at 8 °C/min. Italicized compounds have been identified on the basis of the comparison of mass spectral and retention time data to those of an authentic standard. Other compounds have been tentatively identified on the basis of mass spectral interpretation and library matching. The presence of xanthone (M_r 196), anthrone (M_r 194) and naphthaquinone (M_r 158) have been ruled out on the basis of retention times of authentic samples. (1) M_r 120 acetophenone, (2) M_r 122 hydroxybenzaldehyde, (3) M_r 120 methylbenzaldehyde, (4) M_r 136 ethylbenzaldehyde, (5) M_r 148, (6) M_r 164 methoxypropenylphenol, (7) M_r 144 1-hydroxynaphthalene, (8) M_r 176 alkyl-substituted benzophenone, (9) M_r 158 hydroxymethyl-1-hydroxynaphthalene, (10) M_r 158 hydroxymethylnaphthalene, (11) M_r 190 dihydroxymethoxynaphthalene, (12) M_r 174 hydroxymethoxynaphthalene, (13) M_r 204, (14) M_r 172 dimethylhydroxynaphthalene, (15) M_r 180 *9-fluorenone*, (16) M_r 184 hydroxydibenzofuran or dibenzo-*p*-dioxane, (17) M_r 188 hydroxymethoxymethylnaphthalene, (18) M_r 184, (19) M_r 200 methoxymethylnaphthaldehyde, (20) M_r 184, (21) M_r 198, (22) M_r 182 phenylbenzaldehyde, (23) M_r 214, (24) M_r 198, (25) M_r 208 *9,10 anthraquinone*, (26) M_r 212, (27) M_r 196 methoxyfluorene, (28) M_r 194, (29) M_r 204 cyclopenta[*def*]phenanthrone, (30) M_r 270 chlorinated compound, (31) M_r 224, (32) M_r 194 hydroxyanthracene or phenanthrane, (33) alkyl derivative, (34) M_r 208 isomers, (35) M_r 236 alkyl derivative, (36) M_r 230 *benzanthrone*, (37) M_r 340 alkyl derivative, (38) M_r 354 alkyl derivative, (39) phthalate ester, (40) M_r 368 alkyl derivative, (41) M_r 218, (42) M_r 382 alkyl derivative, (43) M_r 396 alkyl derivative, (44) M_r 410 alkyl derivative, (45) M_r 424 alkyl derivative, (46) M_r 438 alkyl derivative, (47) M_r 326 alkyl derivative, (48) M_r 183 C_2-oxynitrophenol, (49) M_r 197 C_3-oxynitrophenol, (50) M_r 167 C_2-oxynitrophenol, (51) M_r 211, (52) M_r 181 C_3-nitrophenol, (53) M_r 194, and (54) M_r 245.

compounds. A few compounds like fluoren-9-one have been tested and found to be nonmutagenic or toxic in Ames type assays. Leary et al. (*16*) using the forward mutation 8-azaguanine assay obtained a very strong mutagenic response from 1*H*-phenalen-1-one and almost no response from fluoren-9-one. 1*H*-Phenalen-1-one has been tentatively identified by Ramdahl (*15*) in urban air samples. The mutagenicity of larger molecular weight aromatic ketones with a phenalen-1-one molecular sub-unit is unknown.

Compounds that have been confirmed and tentatively identified in the D fractions are listed in Figure 6. Both fractions contained the aromatic ketones 9-fluorenone, 9,10-anthraquinone, and benzanthrone. Additionally, there was a homologous series of high molecular weight alkyl compounds which was common to the reacted and unreacted fraction. Through interpretation of EI spectra and the use of library search techniques, a series of substituted naphthalenes and hydroxyaromatics have tentatively been identified in the unreacted D fraction. On the basis of the interpretation of molecular weight data, there was no evidence of odd nitrogen-containing compounds. In contrast, on the basis of molecular weight there were six odd N-containing compounds in the O_3 + NO_2 reacted D fraction; four of these have been tentatively identified as substituted nitrophenols and nitrobenzene compounds. The formation of nitrophenol possibly resulted from an NO_3/NO_2 type oxidation. This mechanism has been proposed (*2*, *3*) for the oxidation of hydroxyaromatic compounds to hydroxynitro-PAH. Presumably in this scheme, NO_3 produced from the reaction of O_3 + NO_2 abstracts a phenolic hydrogen. NO_2 then adds to one of the intermediate species, and the OH group is regenerated.

As in fraction C, we selectively looked for certain nitro-PAH in both the unreacted and reacted D fraction using the method of Tejada et al. (*9*). No appreciable amino-PAH enhancement was observed when the un-

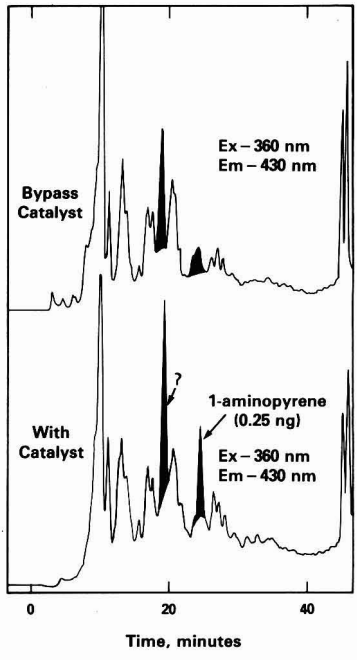


Figure 7. Fluorescence detection of 1-aminopyrene using Tejada et al. (*9*) HPLC technique. 15 cm × 5 mm Zorbax ODS column, mobile phase 25:75 H_2O/MeOH for 30 min, 15:85 For 30–40 min, flow = 1 mL/min, platinum–rhodium catalyst at 70 °C, 10-μL sample.

reacted D fraction was analyzed. As shown in Figure 7, however, the reacted D fraction had two peaks which are significantly enhanced after passage through the platinum–rhodium catalyst. The latter eluting peak was tentatively identified as 1-nitropyrene. This was done by optimizing the sensitivity of the fluorescence detector to the 360- and 430-nm excitation and emission maxima for 1-aminopyrene. When another C_{18} reverse-phase column was placed after the catalyst, additional chromatographic confirmation of 1-aminopyrene was made. The first peak noted in Figure 7 had an elution time that did not exactly correspond to that of 1,6-dinitropyrene. When samples were rerun with optimum excitation and emission maxima of 369 and 442 nm for 1,6-diaminopyrene, a decrease as opposed to an increase in response was observed. Hence, a positive identification of 1,6-dinitropyrene could not be made. No enhancement was observed in the region where 1,8-dinitropyrene elutes.

The observed concentration of the 1-nitropyrene peak was used to estimate the total amount of 1-nitropyrene in the reacted fraction. For the April 24 experiment this translated into 0.7 ng/mg of reacted wood soot particles. In this same system, a comparison between unreacted and reacted filter samples suggested that 84 ng of pyrene/mg of soot had reacted. This did not include pyrene, which potentially existed and reacted in the vapor phase, and/or pyrene lost during filter sampling. It may also be presumed that more 1-nitropyrene mass actually proceeded through this reaction but that some of the formed nitropyrene was additionally reacted with chamber O_3. In either event, it appeared that only a small portion of the reacted pyrene was ultimately observed in the form of 1-nitropyrene.

The mutagenic contribution of the observed 1-nitropyrene to the direct-acting mutagenicity in the reacted D fraction in the April 24 experiment was computed from its observed mass and its reported direct-acting mutagenicity of 2000 revertants/μg (*17*). This was then compared to the number of revertants in this fraction (237 revertants/mg, Table IV) and indicated that 1-nitropyrene contributed ~0.6% to the total direct-acting mutagenicity in the final D fraction and thus less than 0.1% to the total mutagenicity of the sample. Although these calculations are qualitative in nature, they suggest that the formation of 1-nitropyrene had little impact on the observed mutagenic increases resulting from the reaction of $O_3 + NO_2$ with dilute wood smoke. If 1,6- and 1,8-dinitropyrene formed, however, at trace levels just below our detection limits (i.e., 0.03 and 0.10 ng), then these compounds, given their large individual mutagenicity, would substantially contribute to the observed mutagenicity in this fraction. It is also possible that nitro derivatives of other PAH like fluoranthene, benz[*a*]anthracene, and benz[*a*]pyrene formed. Since the detection of these amino analogues is also not as sensitive as for 1-aminopyrene, these may have been present but below our detection limit as well.

The next fraction of higher polarity, fraction E, was eluted with 100% $MeCl_2$. It initially comprised 6–8% of the total fractionated mass, and after reaction it was reduced to 3–4%. Compounds that were tentatively identified in the unreacted E fraction by GC/MS (by comparison to library spectra) included substituted hydroxyaromatics such as a phenolic specie at M_r 154, a vanillic acid at M_r 168, napthols (M_r 144), and substituted dibenzofurans at M_r 182 and 196. In addition, a homologous series of C_{16}–C_{20} aliphatic alcohols appeared to be present. As in the D fraction, the reacted E fraction contained nitrogen-substituted compounds. Compounds that have been tentatively identified are *p*-nitrophenol (M_r 139) and *p*-nitroanisole (M_r 153). Benzaldehyde (M_r 106), hydroxybenzaldehyde (M_r 122), and naphthoic anhydride (M_r 198) were also identified.

The E fraction underwent large changes in mutagenicity after reaction with $NO_2 + O_3$. Its direct-acting slope showed an 8–20-fold increase and indirect mutagenicity increased from 4- to 8-fold. The change in percent mutagenic contribution of this fraction was not directionally consistent between experiments.

Polar Fraction (F). The most polar fraction, fraction F, was eluted with 100% ACN. As indicated before more than 80% of the mass appeared in this fraction both before and after reaction. The very polar nature of this fraction has proved to be a major obstacle for analytical work. This fraction has not been particularly amenable to mutagenicity analysis either, due to occasional toxicity to the tester bacteria. Because of the large percentage of mass appearing in this fraction, it contributes proportionally large percentages to the mutagenicity of wood smoke particles. In unreacted samples, although the slope values were relatively low compared to those of some of the moderately polar fractions, this fraction made the largest contribution to the direct-acting mutagenicity of the sample. After reaction with $NO_2 + O_3$, a large proportion (30–70%) of the direct- and indirect-acting mutagenicity was still observed in this fraction.

Summary and Conclusions

Chemical fractionation from unreacted and $O_3 + NO_2$ reacted wood smoke indicated that most of the extracted mass as well as the mutagenicity was contained within the most polar fractions. The PAH fraction generally made up less than 1% of extracted wood sample mass and contributed 12–17% of the total indirect-acting TA98 mutagenicity to fresh or unreacted chamber wood smoke. After reaction with $O_3 + NO_2$, the mutagenic contribution of the

PAH fraction declined substantially, and this was consistent with the observed loss in particle-bound PAH which were monitored over the course of the reaction. One of the moderately polar fractions, which contained compounds with the polarity of aromatic ketones contributed ~4% to the total direct-acting mutagenicity in unreacted samples. After reaction with $O_3 + NO_2$, this fraction, which only contained 2–3% of the extracted mass, made up 16–30% of the total direct-acting mutagenicity. GC/MS analysis of this reacted fraction tentatively indicated the formation of odd nitrogen organic hydroxy compounds and other oxygenated species. 1-Nitropyrene was found to contribute less than ~0.1% to the overall reacted mutagenicity.

It may be assumed that nitro-PAH analogues of all of the reacted PAH originally present in wood smoke could form to some degree in the $O_3 + NO_2$ system. Since many of these compounds were below the detection limit of our analytical systems, the mutagenic contribution of this class of compounds could not be evaluated.

Acknowledgments

We acknowledge the help of Joellen Lewtas for the useful discussions during the course of this project. The data processing efforts of Jeff Hoffner, the laboratory assistance of Maurice Jackson and Ed Edmondson, and the document preparation of Donna Simmons were appreciated.

Literature Cited

(1) Pitts, J. N., Jr.; VanCauwenberghe, K. A.; Gorsjean, D.; Schmid, J. P.; Fitz, D. R. *Science (Washington, D.C.)* **1978**, *202*, 515–519.
(2) Pitts, J. N., Jr. *Environ. Health Perspect.* **1983**, *47*, 115–140.
(3) Torben, N.; Ramdahl, T.; Bjorseth, A. *Environ. Health Perspect.* **1983**, *47*, 103–114.
(4) Kamens, R. M.; Rives, G. D.; Perry, J. M.; Bell, D. A.; Paylor, R. E., Jr.; Goodman, R. G.; Claxton, L. D. *Environ. Sci. Technol.* **1984**, *18*, 523–530.
(5) Bell, D. A.; Rives, G. D.; Kamens, R. M.; Perry, J. M.; Saucy, D. A.; Claxton, L. D. Proceedings of the 8th International Battelle Symposium on Polynuclear Aromatic Hydrocarbons, Columbus, OH, 1984.
(6) Kamens, R. M.; Rives, G. D.; Perry, J. M.; Goodman, R. G.; Bell, D. A.; Saucy, D. A. *APCA Proc.* **1983**, *83-54*.12.
(7) Schuetzle, D.; Perez, J. M. *APCA Proc.* **1983**, *81-56*.4.
(8) Kamens, R. M.; Bell, D. A.; Dietrich, A. M.; Perry, J. M.; Goodman, R. G.; Claxton, L. D.; Tejada, S. B. Proceedings of the 8th International Batelle Symposium on Polynuclear Aromatic Hydrocarbons, Columbus, OH, 1984.
(9) Tejada, S. B.; Zweidinger, R. B.; Sigsby, J. E. *SAE Tech. Pap. Ser.* **1982**, *No. 820775*.
(10) Ames, B. N.; McCann, J.; Yamasaki, E. *Mutat. Res.* **1975**, *31*, 347–364.
(11) Claxton, L. D.; Kohan, M.; Austin, A.; Evans, C. *U.S. Environ. Prot. Agency* **1982**, *EPA-HERL-0323*.
(12) Hubble, B. R.; Stetter, J. R.; Gebert, E.; Harkness, J. B. L.; Flotard, R. O.; Cooper, J. A.; Malek, D., Eds. In "Residental Solid Fuels"; Oregon Graduate Center: Beaverton, OR, 1981; p 349.
(13) Ramdahl, T.; Alfeheim, I.; Rustad, S.; Olsen, T. *Chemosphere* **1982**, *11*, 601–611.
(14) Ramdahl, T.; Moller, M. *Chemosphere* **1983**, *12*, 23–34.
(15) Ramdahl, T. *Environ. Sci. Technol.* **1983**, *17*, 666–670.
(16) Leary, J. A.; Lafleura, L.; Liber, H. L.; Blemenann, K. *Anal. Chem.* **1983**, *55*, 758–761.
(17) Schuetzle, D. *Environ. Health Perspect.* **1983**, *47*, 65–80.

Received for review February 13, 1984. Revised manuscript received August 6, 1984. Accepted August 20, 1984. The contents of this paper do not necessarily represent the views of the U.S. Environmental Protection Agency. This work was supported by Grants R-808112 and R-810773 from the U.S. Environmental Protection Agency and Contract 57 from the North Carolina Energy Institute.

Environ. Sci. Technol. 1985, 19, 70–74

2,4-Dinitrophenylhydrazine-Coated Florisil Sampling Cartridges for the Determination of Formaldehyde in Air

Frank Liparl*† and Stephen J. Swarin‡

Environmental Science Department and Analytical Chemistry Department, General Motors Research Laboratories, Warren, Michigan 48090

■ A new ambient formaldehyde (HCHO) method was developed on the basis of quantitative collection of HCHO in a cartridge containing Florisil coated with 2,4-dinitrophenylhydrazine (DNPH). The formaldehyde–DNPH derivative is eluted from the cartridge and analyzed by high-performance liquid chromatography. This novel approach utilizes commercially available Thermosorb/F air sampling cartridges packed with Florisil as the sorbent. The cartridges are readily coated with DNPH and are easy to use. The formaldehyde collection efficiency of the method in a single cartridge is 97.3 ± 1.4%. The detection limit of the method is about 1.0 ppb of HCHO for a 100-L air sample. Interferences from NO_2, SO_2, and humidity were investigated and found to have no effect on the method. The method was used to measure HCHO in ambient air, workplace air, and automobile exhaust emissions. The cartridges provide for a low-cost, simple, and sensitive method for ambient HCHO analysis with many potential environmental applications.

Introduction

Formaldehyde is a well-known irritant of the eyes, skin, and nasopharyngeal membranes and recently was labeled as a suspected carcinogen by the American Conference of Government Industrial Hygienists (*1*). Formaldehyde is also important in atmospheric photochemistry and plays a central role in the radical-initiating reactions that lead to photochemical smog formation (*2*, *3*). Many of the current atmospheric models developed to simulate oxidant formation require formaldehyde and other aldehyde data as inputs (*4*). In many cases, such data are lacking because there are no convenient analytical methods that have the sensitivity to measure the low levels of aldehydes (0.6–2 ppb) commonly found in clean environments (*2*).

The methods usually used for ambient aldehyde analyses are either impinger or spectroscopic methods. Most of the liquid impinger methods employed for formaldehyde utilize bisulfite (*5*), chromotropic acid (*6*), 3-methyl-2-benzothiazolone (MBTH) (*6*), pararosaniline (*7*), or 2,4-dinitrophenylhydrazine (DNPH) (*8–11*) absorbing solutions. Liquid impingers are not ideally suited for routine field use. Impinger sampling is inconvenient and cumbersome and requires a considerable amount of operator time to dispense, transport, and store both the reagents and samples in the field. Spectroscopic techniques such as Fourier transform infrared spectroscopy (FTIR) (*12*) for formaldehyde are not readily portable and involve a considerable expense in operating and maintaining these instruments in the field.

Solid sorbent methods could eliminate many of these problems. They utilize simple, inexpensive devices that require little operator care. They are easily transported and stored and are ideal for field use. Generally, the solid sorbent (*13–17*) or passive dosimeter (*18–20*) methods for formaldehyde were originally developed for industrial hygiene purposes to operate at sampling rates of 50–100 cm^3/min. Because of the low sampling rates, such devices lack the required sensitivity for ambient formaldehyde monitoring. In addition, many of the solid sampling devices have problems with tedious cartridge preparation (*10*, *14*) and low collection efficiencies (*10*, *16*) for formaldehyde. Furthermore, many of these methods are also temperature and humidity dependent.

This paper describes a method for determining formaldehyde in which air-containing formaldehyde is sampled by a cartridge containing Florisil coated with 2,4-dinitrophenylhydrazine (DNPH). The formaldehyde–DNPH derivative formed during sampling is subsequently eluted with acetonitrile and analyzed by high-performance liquid chromatography (HPLC). These cartridges can operate at air sampling rates up to 4.0 L/min and are able to collect larger air volumes than is possible with many other methods. This higher air sampling capacity results in the lower detection limits required for ambient formaldehyde monitoring. This approach provides for a low-cost, simple, and sensitive method for ambient formaldehyde analysis. During the course of this work a similar method, based on Waters Sep-PAK C_{18} cartridges coated with DNPH and phosphoric acid, was reported (*21*). Unfortunately, little method validation and interference data were presented in their report. In our work, we utilize a commercially available Thermosorb/F air sampling cartridge packed with Florisil as the substrate for the deposition of DNPH. Our report presents validation and interference data and shows the application of DNPH-coated Florisil samplers to measuring formaldehyde in ambient air, industrial environments, and automotive exhaust emissions. Furthermore, an independent method was used to validate the method in the above applications.

Experimental Section

Experimental Apparatus. The HPLC system used consisted of a Varian Model 5060 liquid chromatograph with a Vista 401 data station (Varian Associates, Palo Alto, CA), a Perkin-Elmer Model LC-85 (Perkin-Elmer Corp., Norwalk, CT) variable wavelength UV–visible absorbance detector operated at 365 nm, and a Valco (Valco Instruments, Houston, TX) air actuated injection valve with a 25-μL sample loop. The analytical column used was a 4.6 mm × 25 cm Zorbax-ODS (Du Pont Instruments, Wilmington, DE) reverse-phase column with a Rainin (Rainin Instrument Co., Woburn, MA) 0.5-μm prefilter.

Air sampling was performed either with Bendix BDX-55-HD super sampler pumps (Bendix Corp., Lewisburg, WV) or with Gilian Model HFS 113 UT (Gilian Instruments Corp., Wayne, NJ) portable constant flow pumps. For field studies, air sampling was performed with VWR Model 4K Dynapumps (VWR Scientific, Detroit, MI), and the total gas volumes were measured with a Precision Scientific Model 63125 (VWR Scientific) wet-test meter.

Gas flows for the calibration and dilution gases were measured and controlled with Tylan mass flow controllers (Tylan, Carson, CA). Nitrogen dioxide and sulfur dioxide used in the interference study were purchased from Scott Specialty Gases (Troy, MI). Relative humidities and temperatures were measured with an EG&G Model 880

†Environmental Science Department.
‡Analytical Chemistry Department.

0013-936X/85/0919-0070$01.50/0

dew point hygrometer (Princeton Applied Research, Princeton, NJ) and a Keithley Model 871 Digital thermometer (Kiethley Instruments, Cleveland, OH), respectively.

Reagents. The mobile phase was prepared, and the cartridges were eluted with HPLC-grade solvents obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ) or Fisher Scientific (Pittsburgh, PA). The 2,4-dinitrophenylhydrazine was obtained from Aldrich Chemical Co. (Milwaukee, WI) and recrystallized twice from HPLC-grade acetonitrile before use. All other chemicals were reagent grade.

Cartridge Preparation. Thermosorb/F air sampling cartridges packed with high purity 60/80-mesh Florisil (magnesium silicate, Floridian Co.) were used in this study. These cartridges were purchased from Thermo Electron Corporation (Waltham, MA) and are similar to the Thermosorb/N air sampling cartridges used for airborne nitrosamine sampling (*22*). The cartridges are constructed of polyethylene and are about 1.5 cm o.d. × 2.0 cm long. A 100-mesh stainless steel screen at the inlet and a glass wool plug at the outlet holds the sorbent in the cartridge. Standard Luer-lok fittings at both the inlet and outlet ports are also present, which facilitate sorbent coating and elution. The cartridges contain about 1.2 g of dry sorbent.

The sorbent is coated with DNPH by filling the cartridge with about 2 mL of a 2 mg/mL DNPH solution in CH_2Cl_2. The cartridges are capped at the bottom (air inlet) and placed in a vacuum chamber at 37–50 cmHg for 1 h to volatilize the CH_2Cl_2 solvent. The cartridges are then capped and are ready for use.

Generation of Formaldehyde Atmospheres and Standards. A Metronics (VICI Metronics, Santa Clara, CA) certified permeation device along with a Metronics Model 350 Dynacalibrator permeation system was used to generate known atmospheres of formaldehyde. The permeation rate of the device was determined by bubbling the formaldehyde vapors produced by the permeation system through an acetonitrile solution of the DNPH reagent and analyzing the corresponding formaldehyde–DNPH derivative by HPLC (*8*). The permeation rates were determined at 90 and 78 °C and found to be 379 and 110 ng/min, respectively. These permeation rates are equivalent to 300 and 90 ppb/L formaldehyde, respectively.

Sample Elution. The cartridges are back-flushed with acetonitrile to elute the formaldehyde–DNPH derivative. Generally, 2–3 mL of acetonitrile was required to quantitatively elute the formaldehyde derivative. However, in standard practice, 5 mL of the acetonitrile eluant was collected to ensure that all the formaldehyde–DNPH derivative was eluted from the cartridge. In standard practice, the sampling cartridges are eluted with 5 mL of acetonitrile into graduated test tubes and, after addition of 100 μL of 1 N $HClO_4$ to the collected eluant, are made up to 10.0 mL with deionized water. In this way, the sample matrix approximates the composition of the mobile phase and allows large sample volume injections (~25 μL) to be made on the column without peak distortion.

Results and Discussion

The development of a sampling cartridge for the collection and determination of formaldehyde, as reported here, is an extension of previous work on an improved DNPH-impinger technique for aldehyde determinations (*8*) and the sampling cartridge developed for ambient NO_2 determinations (*23*). This report gives validation data for formaldehyde. Although other aldehydes can be collected on the cartridge, our discussion is limited to formaldehyde analyses.

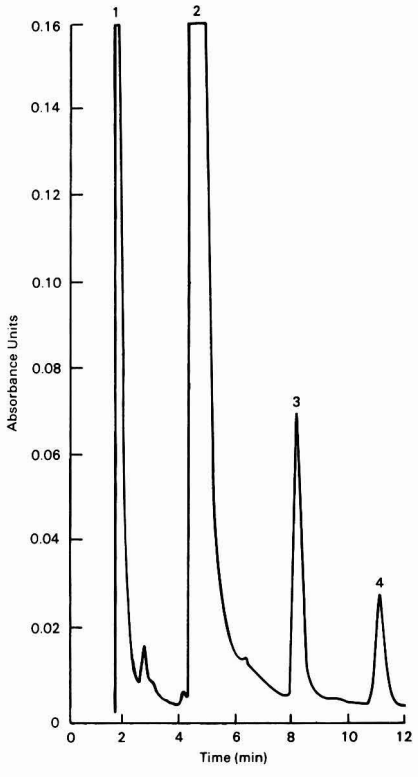


Figure 1. Liquid chromatogram of the eluant from a cartridge after sampling ambient air containing 3.8 ppb of HCHO at 1.0 L/min for 120 min. Conditions given under Experimental Section. Peak identities: 1, solvent front; 2, DNPH reagent; 3, DNPH–formaldehyde derivative; 4, DNPH–acetaldehyde derivative.

Liquid Chromatography. Figure 1 shows a typical liquid chromatogram of the eluant from a sampling cartridge after sampling ambient air containing 3.8 ppb of formaldehyde. The separation of DNPH derivatives of carbonyl compounds by liquid chromatography was discussed in a previous publication (*8*). For the separation of the DNPH reagent and formaldehyde derivative, we found that a 55/45 acetonitrile/water mobile phase at 1.0 mL/min gives the best separation in a reasonable time period. Acetaldehyde was also present in this air sample but was not quantitated because of lack of validation data for this species by the method.

Collection Efficiency. The collection efficiency of the sampling cartridge was determined by passage of known amounts of formaldehyde vapor through two sampling cartridges connected in series. The concentration of formaldehyde was varied from 308 to 77 ppb by varying the dilution gas flow rate from 1.0 to 4.0 L/min. Higher flow rates were not investigated because of the limitation of our sampling system. The total gas volume sampled during a 30-min period ranged from 30 to 120 L for the highest and lowest concentrations of formaldehyde, respectively. The results, which are summarized in Table I, showed that formaldehyde is quantitatively collected at rates up to 4.0 L/min in a single sampling cartridge. The overall formaldehyde recovery from the cartridges ranged from 95 to 99% with an average of 97.3 ± 1.4% for two determinations at each concentration level.

To assess the precision of the method, the permeation device was adjusted to give 92 ppb of formaldehyde in air

Table I. Sampling Rate Effects on HCHO Recovery[a]

ppb of HCHO generated	sampling rate, L/min	volume sampled, L	ppb of HCHO[b-d] found	% recovery[c]
308.0	1.0	30	304.0	98.7
154.0	2.0	60	151.2	98.2
103.0	3.0	90	98.5	95.6
77.0	4.0	120	74.4	96.6
				97.3 ± 1.4[e]

[a] HCHO was generated from permeation system. [b] Recovery from a single cartridge. [c] Average of two determinations at each concentration level. [d] Back-up cartridges contained less than 1.5% of total HCHO and were not included. [e] Average.

Table II. NO_2 Effects on Formaldehyde Recovery

ppb of NO_2/ ppb of HCHO[a]	NO_2/HCHO ratio[b]	% recovery[c,d]
550/77	7.1	98.5
275/77	3.6	94.7
137/77	1.8	102.1
		98.4 ± 3.7[e]

[a] Thirty liters of the NO_2/HCHO gas mixture was collected. [b] Ratio of concentration of NO_2/HCHO mixture. [c] Recovery from a single cartridge. [d] Average of two determinations at each concentration level. [e] Average.

at 1.0 L/min, and 20 sampling cartridges were used to collect 20-L volumes of this air stream. These samples showed an average formaldehyde recovery of 99.7 ± 2.3% for 20 determinations which corresponded to a relative standard deviation of less than 2.5%.

Capacity and Sensitivity. With the permeation device set to deliver 92 ppb of formaldehyde at 1.0 L/min, the capacity of the cartridges was determined. Sets of two sampling cartridges in series were used to collect the output of the permeation device for 6, 12, 18, 24, and 48 h. Analyses of the eluted cartridges showed that over 95% of the formaldehyde is collected on the first cartridge for 6, 12, and 18 h of sampling, after which breakthrough occurred. However, over 90% of the formaldehyde is collected on the two cartridges for 24 and 48 h of sampling. This means that the capacity of a single cartridge is about 1.6 ppm·h HCHO, thereby, making these devices suitable for both long-term low level ambient air monitoring and short-term high level personal exposure monitoring.

The amount of DNPH loaded onto a single sampling cartridge was about 3 mg. Even though the DNPH used in this study was recrystallized twice from acetonitrile, it still contained a trace amount of the formaldehyde–DNPH derivative. This amount corresponds to about 2 ng of formaldehyde/mg of DNPH on the cartridge. This leads to a cartridge blank of 0.5 ppb of formaldehyde in 100 L of air and a detection limit (a response at twice the blank level) of 1 ppb of formaldehyde in 100 L of air.

Potential Interferences. Potential interferences from NO_2, SO_2, and humidity were also investigated in this study. Ordinarily, NO_2 does not interfere with ambient formaldehyde methods. However, since NO_2 is a commonly found atmospheric species, we decided to investigate its effect on our sampling cartridges. Table II summarizes the results of our NO_2 interference study. Nitrogen dioxide from a calibration gas cylinder was blended with formaldehyde from the permeation system to produce gas mixtures containing 77 ppb of formaldehyde and from 137 to 550 ppb of NO_2 in air. The data show that NO_2 had no effect on the formaldehyde recovery even at NO_2/HCHO ratios of 7 to 1.

Table III. SO_2 Effect on Formaldehyde Recovery[a]

		% HCHO recovered		
test	ppb of SO_2/ ppb of HCHO	front cartridge[b]	back-up cartridge[b]	total[b]
1	1000/92	95.1	3.2	98.3
2	1000/92	97.6	0.0	97.6
3	1000/92	98.5	0.7	99.2
4	1000/92	103.5	0.2	103.7
5	1000/92	92.5	1.3	93.8
				98.5 ± 3.6[c]

[a] Twenty liters of the SO_2/HCHO gas mixture was collected. [b] Average of two determinations. [c] Average.

Table IV. Humidity Effects on Formaldehyde Recovery

RH, %	ppb of HCHO generated	sample flow rate, L/min	sample time, min	% recovery[a]
30	308	1.0	30	96.6
60	215	1.0	45	96.0
70	154	1.0	60	102.7
80	154	1.0	60	101.2

[a] Average of two determinations.

Sulfur dioxide was another atmospheric species that was investigated because of the possibility of forming a formaldehyde–bisulfite addition compound which could lower formaldehyde recovery. In this case, SO_2 and formaldehyde were blended to produce a mixture that contained 1000 ppb of SO_2 and 92 ppb of formaldehyde in air which corresponded to a SO_2/HCHO ratio of about 10/1. Table III summarizes the results of five 20-L samples of this air mixture which were collected on dual cartridges. The results showed an average HCHO recovery of 98.5 ± 3.6% which implied that SO_2 had no effect on the method.

Relative humidity (RH) effects on the sampling and recovery efficiency of the method were also investigated. A nearly saturated (~95% RH) air stream was produced by passage of clean air through two water impingers with gas dispersing tips. The humid air stream was then mixed with formaldehyde from the permeation system and passed through a cartridge at 1.0 L/min. Various mixtures of air containing 154–308 ppb of HCHO at humidities ranging from 30 to 80% were generated by varying both the humid air and formaldehyde permeation flow rates.

These results, which are summarized in Table IV, showed that relative humidity had no effect on the formaldehyde sampling or recovery efficiency of the method. Unlike other formaldehyde sorbent (*11*, *13*) or passive dosimeter (*24*) methods, this method was not affected by humidity.

Storage Stability. Since unavoidable delays may occur between collection and analysis, the stability of formaldehyde samples collected on cartridges was investigated. For this investigation four parallel overnight samples of ambient air (~1000 L each) were collected at the same time by using four sampling cartridges. One of the cartridges was eluted and analyzed immediately, while the others were stored at room temperature. One sample was eluted and analyzed each week for the next 3 weeks. The four samples had an average value of 3.80 ± 0.23 ppb of HCHO which corresponded to a relative standard deviation of ±6.1%. Thus, the sampling cartridges are stable after sample collection for at least 3 weeks when stored at 21 °C. Studies conducted in which cartridge blanks were stored at 21 °C for periods up to 4 weeks before use showed no adverse effects as long as the inlet and outlet ends of the cartridges were capped.

Table V. Parallel Ambient Formaldehyde Measurements Using the Cartridge and Impinger Methods

date	time (EDST)[a]	HCHO, ppb cartridge[b]	HCHO, ppb impinger[b,c]	cartridge/ impinger[d]
7/6	11:00–13:00	1.28	1.31	0.98
7/6	13:00–15:00	1.83	1.40	1.31
7/6–7/7	15:00–8:00	1.96	ND[d]	ND
7/7	8:00–10:00	3.73	4.21	0.89
7/7	11:00–13:00	2.84	3.14	0.90
7/7	13:00–15:00	3.56	3.19	1.12
7/7–7/8	15:00–8:00	2.98	ND	ND
7/8	8:00–10:00	4.09	4.46	0.92
7/8	13:00–15:00	5.01	4.26	1.18
7/11	13:00–15:00	6.10	6.29	0.97
7/11–7/12	15:00–8:00	3.04	ND	ND
7/12	8:00–10:00	6.46	5.88	1.10
7/12	13:00–15:00	4.56	5.03	0.91
7/12–7/13	15:00–8:00	4.22	ND	ND
				1.02 ± 0.14

[a] Time is eastern daylight savings time for the sampling period. [b] Cartridge and impinger sampling rate is about 1.0 L/min. [c] Impingers contain 18 mL of DNPH-absorbing solution. [d] Ratio of cartridge to impinger results. [e] ND means not determined.

Table VI. Parallel Formaldehyde Measurements in Foundry Workplace Air Using the Cartridge and Impinger Methods

test[a]	HCHO, ppm cartridge[b]	HCHO, ppm impinger[c]	cartridge/ impinger[d]
1	0.645	0.580	1.11
2	0.435	0.458	0.95
3	0.442	0.423	1.04
4	1.172	1.105	1.06
5	0.969	0.994	0.98
			1.03 ± 0.07[e]

[a] The sampling rate and time were 1.0 L/min and 20 min, respectively, for both cartridge and impinger methods. [b] Back-up cartridges contained less than 1.5% of total HCHO and were not included. [c] Impingers contained 10 mL of DNPH-absorbing solution. [d] Ratio of cartridge to impinger results. [e] Average.

Field Evaluation. The performance of these cartridges was evaluated for monitoring formaldehyde levels in ambient air, workplace air, and automotive exhaust emissions. The accuracy of the method was assessed with parallel samples obtained with an independent DNPH-impinger method (*8*).

Table V summarizes the results obtained for parallel formaldehyde measurements made in Warren MI, by using the cartridge and impinger methods. The data represent 2-h parallel samples obtained at 1.0 L/min for both methods. Long-term or overnight parallel samples were not obtained because of loss of the DNPH-absorbing solution from the impinger during extended sampling periods. The results for the 2-h samples showed the excellent agreement obtained between the cartridge and impinger methods. The average ratio of the cartridge to impinger results was 1.02 for the 10 samples obtained. The overnight cartridge results are also presented in Table V to illustrate the usefulness of these devices in long-term formaldehyde monitoring. These results indicate that the cartridges are capable of monitoring low-level ambient formaldehyde levels and are suitable for such field use.

Formaldehyde levels were also monitored in a foundry where sand coated with phenol–formaldehyde resin was cured. These results, which are summarized in Table VI, again show the excellent agreement obtained between the cartridge and impinger methods. The average ratio of the cartridge to impinger results was 1.03 for five determinations. In all cases, the formaldehyde levels in the workplace air were below the OSHA threshold limit value of 3 ppm. These results illustrate the potential of these cartridges for workplace air monitoring.

Table VII. Determination of Formaldehyde in Automobile Exhaust Emissions Using the Cartridge and Impinger Methods

test	vehicle	HCHO,[c] ppm cartridge	HCHO,[c] ppm impinger	cartridge/ impinger
1	diesel[a]	0.491	0.422	1.16
2	diesel	0.434	0.450	0.96
3	diesel	0.401	0.411	0.98
4	methanol[b]	0.155	0.191	0.81
5	methanol	0.153	0.141	1.09
6	methanol	0.167	0.149	1.12
				1.02 ± 0.13[d]

[a] Light-duty diesel vehicle operating on diesel fuel. [b] Experimental vehicle operating on neat-methanol fuel and equipped with a catalytic converter. [c] Sample volumes collected ranged from 10 to 20 L of CVS diluted exhaust. [d] Average.

Finally, the performance of these cartridges were evaluated for automotive aldehyde emission sampling. Formaldehyde emissions were measured from various vehicles as they were driven on a chassis dynamometer at a constant 50 mph speed. Parallel cartridge and impinger samples were obtained from the vehicle's diluted exhaust by using a constant volume sampler (CVS) system.

Table VII summarizes the results for three tests on a light-duty diesel automobile and three tests on a neat methanol-fueled vehicle. The results show the excellent agreement of the two methods for formaldehyde emissions. Other aldehydes were also detected in the diesel tests for both the cartridge and impinger samples, but these are not included in the table for lack of validation data for these species by the cartridge method.

Acknowledgments

We acknowledge the assistance of William Scruggs and Daniel Blossfeld in all aspects of the experimental work. The authors would also like to thank Kelly Thurston of Oldsmobile Division and Fay Colden of General Motors Advanced Product Manufacturing and Engineering Staff for providing the automobiles used in the emission tests.

Registry No. DNPH, 119-26-6; Florisil, 1343-88-0; formaldehyde, 50-00-0.

Literature Cited

(1) ACGIH "Threshold Limit Values for Chemical Substances and Physical Agents in the Work Environment". American Conference of Government Industrial Hygienists, Cincinnati, OH, 1982.
(2) Committee, on Aldehydes "Formaldehyde and Other Aldehydes"; National Research Council, National Academy Press: Washington, DC, 1981.
(3) Bailey, R. A.; Clark, H. M.; Ferris, J. P.; Krause, S.; Strong, R. L. "Chemistry of the Environment"; Academic Press: New York, 1978.
(4) Carter, W. P. L.; Winer, A. M.; Pitts, J. N., Jr. *Atoms. Environ.* **1982**, *16*, 113.
(5) Levaggi, D. A.; Feldstein, M. *J. Air Pollut. Control Assoc.* **1970**, *20*, 312.
(6) Katz, M., Ed. "Methods of Air Sampling and Analysis", 2nd ed.; American Public Health Association Intersociety Committee: Washington, DC, 1977.
(7) Miksch, R. A.; Anthon, D. W.; Fanning, L. Z.; Hollowell, C. D.; Revzan, K.; Kenneth, J. G. *Anal. Chem.* **1981**, *53*, 2118.
(8) Lipari, F.; Swarin, S. J. *J. Chromatogr.* **1982**, *247*, 297.
(9) Grosjean, D. *Environ. Sci. Technol.* **1982**, *16*, 254.

(10) Grosjean, D.; Fung, K. *Anal. Chem.* **1982**, *54*, 1221.
(11) Kuwata, K.; Urebori, M.; Yamasaki, Y. *J. Chromatogr. Sci.* **1979**, *17*, 264.
(12) Tuazon, E. C.; Graham, R. A.; Winer, A. M.; Easton, R. R.; Pitts, J. N., Jr.; Hanst, P. L. *Atoms. Environ.* **1979**, *12*, 865.
(13) Matthews, T. G.; Howell, T. C. *Anal. Chem.* **1982**, *54*, 1495.
(14) Beasley, R. K.; Hoffmann, C. E.; Rueppel, M. L.; Worby, J. W. *Anal. Chem.* **1980**, *52*, 1110.
(15) Kim, W. S.; Geraci, C. L.; Kupel, R. E. *Am. Ind. Hyg. Assoc. J.* **1980**, *41*, 334.
(16) Anderson, G.; Anderson, K.; Nilsson, C. A.; Levin, J. O.; *Chemosphere* **1979**, *10*, 823.
(17) Kennedy, E. R.; Hill, R. H., Jr. *Anal. Chem.* **1982**, *54*, 1739.
(18) Du Pont "Pro-Tek Colorimetric Air Monitoring Badge System Laboratory Validation Report, Formaldehyde Badge, Series II, Type C-60", E. I. du Pont de Nemours & Co.: Wilmington, DE, 1981.
(19) Air Quality Research Inc. "Product Literature for PF-1 Passive Formaldehyde Monitor"; Air Quality Research, Inc.: Berkeley, CA, 1982.
(20) Rodriguez, S. T.; Olson, P. B.; Lund, V. R. "Colorimetric Analysis of Formaldehyde Collected on a Diffusion Monitor"; 3M Co.: St. Paul, MN, 1981.
(21) Kuwata, K.; Urebori, M.; Yamasaki, H.; Kuge, Y.; Kiso, Y. *Anal. Chem.* **1983**, *55*, 2013.
(22) Thermo Electron Corp. "Product Literature for Thermosorb/N Air Sampling Cartridges"; Thermo Electron Corp.: Waltham, MA, 1983.
(23) Lipari, F. *Anal. Chem.* **1984**, *56*, 1820.
(24) Hodgson, A. T.; Geisling, K. L.; Girman, J. R.; Remijn, B. "Validation of a Passive Sampler for Determining Formaldehyde in Residential Air". paper presented at the Third Annual EPA National Symposium on Recent Advances in Pollutant Monitoring of Ambient Air and Stationary Sources, Raleigh, NC, May 3–6, 1983.

Received for review March 12, 1984. Revised manuscript received August 2, 1984. Accepted August 21, 1984. This paper was presented at the 187th National Meeting of the American Chemical Society, St. Louis, MO, April 8–13, 1984.

Photosensitized Transformations Involving Electronic Energy Transfer in Natural Waters: Role of Humic Substances

Richard G. Zepp,* Patricia F. Schlotzhauer, and R. Merritt Sink

Environmental Research Laboratory, U.S. Environmental Protection Agency, Athens, Georgia 30613

■ Studies are reported of the kinetics of photosensitized reactions involving the transfer of absorbed light energy from humic substances to various trace organic chemicals in water. The photoisomerization of 1,3-pentadiene and the photooxygenation of 2,5-dimethylfuran were used to probe the nature and concentrations of the excited species that mediate humus-sensitized photoreactions of aquatic pollutants. Evidence is presented that the photosensitized reactions of pentadiene and dimethylfuran do not involve binding of the chemicals by the humic substances. Kinetic results indicate that the key steps in both photoreactions involve the transfer of electronic energy from triplet states of the humic substances. Up to half of these triplets are estimated to have energies of at least 250 kJ/mol, sufficiently high to transfer energy to polycyclic aromatic hydrocarbons, nitroaromatic compounds, conjugated dienes, and other types of chemicals. Steady-state concentrations of the humus-derived triplets are estimated to typically fall in the 10^{-15}–10^{-13} M range in the upper layers of sunlight-irradiated natural waters. The concentrations are proportional to UV absorption coefficients of the water. Action spectra for the photosensitized reactions indicate that solar ultraviolet radiation is most important in inducing the reactions. Results of the study are used to estimate maximum rates of reactions photosensitized by humic substances.

Introduction

As sunlight penetrates down into freshwater and coastal marine waters, the great bulk of the radiation is absorbed by natural dissolved or particulate substances. Initial studies of the influence of these natural substances on rates of photochemical and photobiological processes in aquatic environments have focused on light attenuation effects (*1*, *2*). Results of these studies have been used to predict rates of pollutant photoreactions that are initiated through direct absorption of sunlight by the pollutant (*1*).

A number of recent investigations have shown that the influence of natural substances on photoreactions in freshwaters and seawater is not limited to light attenuation (3–13). Sunlight-induced reactions involving free radicals may be initiated through photolysis of natural inorganic constituents such as nitrite (*3*) and hydrogen peroxide (*4*, *5*). Moreover, a significant portion of the solar radiation absorbed by freshwater humic substances results in formation of electronically excited molecules that are capable of participating in a variety of reactions with aquatic pollutants (*8–15*). These photosensitized reactions can greatly accelerate the light-induced transformation of trace chemicals in natural waters, in some cases resulting in rapid photoreaction of compounds that are stable to sunlight in distilled water (*6–13*).

Humic substances have been shown to sensitize oxygenations and other photoreactions of organic chemicals involving electronic energy transfer (*9*), but there is a dearth of information concerning factors that influence the rates of these processes in aquatic environments. In this paper, humus-sensitized photoreactions of two organic chemicals, 2,5-dimethylfuran and 1,3-pentadiene, are used to explore the nature and concentrations of the excited species that are involved in the rate-determining steps of the energy-transfer processes. Results of this study are used to define types of pollutants that may be susceptible to photosensitized reactions involving energy transfer and to estimate maximum rates of these photoreactions in natural waters.

As used in this paper, the terms "humic substances" or "humus" refer to the colored organic matter that is dissolved in natural waters or extractable from soil by base. No attempts were made to elucidate the structures of the photoactive chromophores.

Experimental Section

Materials. *cis*-1,3-Pentadiene (99%) (cP) and *trans*-1,3-pentadiene (99%) (tP) were obtained from Chemical Samples Co. and used as received. 2,5-Dimethylfuran (DMF) was obtained from Chemical Samples Co. and distilled prior to use. 2,4-Hexadien-1-ol (99%), mixed isomers, was obtained from Chemical Samples Co. and distilled prior to use. Valerophenone from Eastman was distilled before use, and *p*-nitroanisole from Aldrich

Chemical Co. was recrystallized from 95% ethanol. Reagent-grade acetophenone from Fisher Scientific was used as received, as was a sample of *p*-(trimethylammonio)benzophenone chloride that was obtained as a gift from Dr. Richard Hautala, University of Georgia, Athens, GA. Aldrich 2,6-naphthalenedisulfonic acid disodium salt was purified by recrystallization from aqueous sodium chloride solution. The natural water samples were obtained from the top meter of water bodies and refrigerated at 5 °C prior to use. Commercial humic acids were obtained from Aldrich Chemical Co. and Fluka AG, and a commercial fulvic acid was obtained from Contech E.T.C. Ltd. A sample of humic substances isolated from Wylde Lake (Ontario) was contributed by Dr. John Carey of the Canada Center for Inland Waters. Dr. Paul Ringhand, USEPA, Cincinnati, OH, provided a sample of Ohio River fulvic acid. Spectrograde acetonitrile from Burdick & Jackson was used as received. Other chemicals used were reagent grade obtained from various commercial sources.

Preparation of Solutions for Irradiation. Procedures used for preparing solutions of the commercial humic acids and fulvic acid are described elsewhere in detail (*9*). These solutions and the natural water samples were centrifuged at 15 000 rpm for 1 h to remove particulates. For all studies in sunlight the solutions were optically matched by adjusting their absorbances to the same value at 365 nm through addition of distilled water. Aqueous stock solutions of valerophenone, *p*-nitroanisole, and the pure photosensitizers were prepared by adding to distilled water an amount of chemical that exceeded its aqueous solubility by about 10%. The resulting mixture was stirred for 24 h and then centrifuged as described above to remove undissolved chemical. The resulting stock solutions were diluted by addition of distilled water to obtain concentrations required for the irradiations.

With the exception of the irradiations of DMF solutions in the monochromator, the following procedure was used to prepare solutions for the irradiations. Aqueous solutions containing the photosensitizers and other additives were prepared in volumetric flasks. Then 8.50-mL portions of these solutions were added to 100 × 13 mm Pyrex test tubes equipped with gas-tight Mininert caps. Finally, 40 μL of a 2 mM solution of substrate (DMF, cP, or tP) was injected into the solution in each test tube, resulting in a substrate concentration of about 10 μM.

Irradiation of Samples. Three procedures were used to irradiate samples in this study: by exposure to sunlight, by exposure to monochromatic light in a "merry-go-round" apparatus, and by exposure to monochromatic light derived from a monochromator.

The procedures used in the exposures to sunlight have been described elsewhere (*9*). Dilute solutions of valerophenone (10 μM) were used as outdoor actinometers in these experiments (*16*).

The irradiations in the merry-go-round apparatus (*17*) were performed as previously described (*10*). The merry-go-round apparatus ensures that the same intensity of irradiation impinges on each sample of a set of simultaneously irradiated samples. Dilute aqueous solutions of *p*-nitroanisole were used as chemical actinometers in the experiments with 313- and 366-nm light, and ferrioxalate actinometers (*18*) were used for experiments with 405- and 436-nm light.

Wavelength studies of the humus-sensitized photoreaction of DMF were conducted using the Schoeffel Reaction Chemistry System. This apparatus is basically an optical bench that consists of a 1000-W xenon lamp, high intensity monochromator, and sample compartment. The light intensity impinging on the samples was determined on a YSI Model 65A radiometer that was calibrated vs. ferrioxalate actinometers (*18*).

For the purpose of determining the ratios of *cis*- to *trans*-pentadiene at a photostationary state, aqueous solutions containing the photosensitizers and cP or tP were irradiated for about 10 half-lives and then analyzed. The samples were irradiated for another half-life and reanalyzed to ensure that the ratio was no longer changing. The standard error observed in triplicate experiments was typically ±2% of the mean.

Equipment. Electronic absorption spectra were obtained on a Perkin-Elmer Model 356 spectrophotometer. *cis*- and *trans*-1,3-pentadiene were analyzed as previously described (*9*) on a Tracor MT-222 gas chromatograph equipped with flame detector and a 6 m long by 2 mm i.d. gas–liquid chromatographic (GLC) column packed with 20% 1,2,3-tris(2-cyanoethoxy)propane on Gas-Chrom RA. A Micromeritics high-pressure liquid chromatograph equipped with a UV detector was used to analyze the reaction mixtures containing 2,5-dimethylfuran, valerophenone, or *p*-nitroanisole. Acetonitrile–water mixtures were used as the mobile phase in a column packed with ODS-2. Dioxygen concentrations were measured by using a YSI Model 54 oxygen meter.

Procedures for Kinetic Studies. Both DMF and the pentadienes are volatile compounds in water. Therefore, all kinetic studies were conducted by using gas-tight reaction cells that were completely filled with air-saturated aqueous solutions. After introduction of the substrate to the filled test tube or cuvette, the solutions were exposed to light as described above. In the outdoor experiments three replicates of each solution and two sunlight actinometers were simultaneously irradiated, typically for a period less than 20 min. Rate constants were computed assuming that the photoreactions were described by first-order rate expressions. In the kinetic runs using the merry-go-round, a series of samples and actinometers were simultaneously irradiated with periodic removal of samples for analysis. With the monochromator, three replicates were irradiated separately, and first-order rate constants were computed from the relative change in concentration of the DMF for each replicate. The radiometer, which was monitored throughout the exposure, generally indicated no change in incident light intensity during the irradiations. Standard errors in the computed rate constants were typically ≤ ±15% of the mean value.

The kinetic studies of the influence of dioxygen and 2,4-hexadien-1-ol on the photosensitized isomerization of cP were conducted in sunlight with the humic substances and in the merry-go-round with acetophenone as sensitizer. Aqueous solutions containing the same concentrations of sensitizer but varying concentrations of quencher (including no added quencher) were prepared, and portions of each solution were added to two equivalent test tubes. After the test tubes were capped, the same amount of cP was then injected into each test tube. The test tubes were irradiated for the same length of time. Then the reaction mixtures were analyzed for cP and tP.

In all the experiments dark controls showed no loss of substrate. Also, cP, tP, and DMF did not react when irradiated as described above in air-saturated distilled water.

Action Spectra. Spectral response functions, $X_{s,\lambda}$, were computed from the kinetic data obtained at various wavelengths by using the following equation:

$$X_{s,\lambda} = k_{s,\lambda}/(I_\lambda l S_\lambda) \quad (1)$$

where $k_{s,\lambda}$ is the first-order rate constant for the reaction

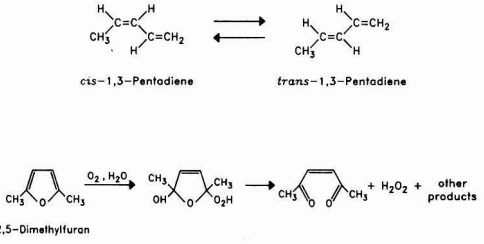


Figure 1. Photosensitized reactions of 1,3-pentadiene and 2,5-dimethylfuran in water.

of DMF, cP, or tP, I_λ is the light intensity term (number of photons that enter the reaction cell per unit time and volume), S_λ is the "light screening factor", and l is the light path length. The values of I_λ were computed from kinetic data obtained from irradiation of chemical actinometers in the same way as the solutions of DMF or the dienes. With *p*-nitroanisole (2 μM; no pyridine) as the actinometer at 313 or 366 nm, the following simple equation can be used to compute I_λ (in einsteins/(L h)):

$$I_\lambda = C_\lambda k_p / l \qquad (2)$$

where k_p is the first-order rate constant for photolysis of the nitroanisole (in h^{-1}) and C_λ is a constant equal to 0.15 einstein/L for 313-nm light and 0.77 einstein/L for 366-nm light. The value of S_λ was computed by using

$$S_\lambda = (1 - 10^{-\alpha_\lambda l})/(2.303\alpha_\lambda l) \qquad (3)$$

where α_λ is the decadic absorption coefficient of the solution. The mean value of the light path length l for the experiments in the merry-go-round was estimated by exposing a series of solutions containing valerophenone (10 μM) and varying concentrations of Fluka humic acid. The light screening factor S_λ was computed by assuming that the observed rate constant in the humic acid solution equalled $S_\lambda k_p$(dw) where k_p(dw) was the rate constant in distilled water. This experimental value of S_λ was used along with the absorption coefficients and eq 3 to compute the mean light path length. This procedure was validated by conducting it in the monochromator (310 nm) using a reaction cell with a path length known to be 1.00 cm.

Results and Discussion

A wealth of investigations have established that both photosensitized oxygenations (*19*) and sensitized photoisomerizations of alkenes (*20*) involve electronic energy transfer from triplet states of photosensitizers. For this study we selected the oxygenation of 2,5-dimethylfuran (DMF) and the cis–trans isomerization of 1,3-pentadiene (Figure 1) as convenient chemical processes to investigate transfer of light energy in natural waters.

2,5-Dimethylfuran and the pentadiene isomers were selected as substrates for several reasons. Experimental considerations include their relatively high water solubilities (>0.005 mol/L), their transparency to solar UV radiation and extremely low direct photolysis rates, and their great sensitivity to photosensitized reactions. In addition, convenient methods had been developed to directly analyze for DMF, cP, and tP at trace concentrations in water (*9*). Finally, both substrates have previously been studied extensively with pure photosensitizers in organic solvents, so a great deal of background information was already available concerning the kinetics of their reactions.

Rate Expressions. Young and co-workers (*21*) have previously shown that photosensitized oxygenations are first order with respect to substrate when substrate concentrations are very low. Under these conditions, which

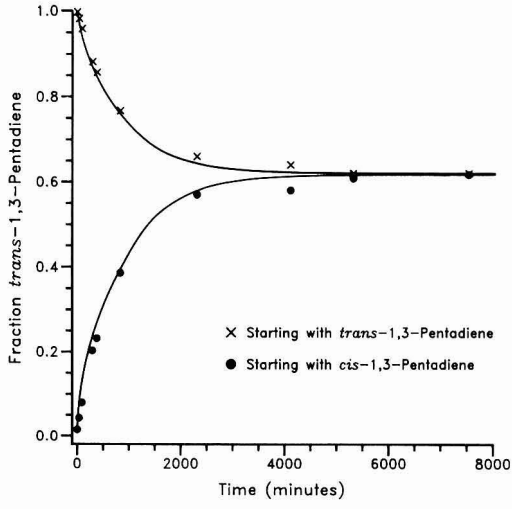


Figure 2. Kinetic data for the photosensitized isomerization of *cis*- and *trans*-1,3-pentadiene (10 μM) in a water sample from the Aucilla River.

are usually found in aquatic environments, the substrate has practically no effect on the lifetime of singlet molecular oxygen, the reactive intermediate in oxygenations (see below). In the present study, as in earlier studies (*8–10*), we found that the photooxygenation of DMF (10 μM), measured as the disappearance of substrate in irradiated solutions, was cleanly first order in all the aqueous systems that were studied. The kinetic data were generally treated by computing first-order rate constants from the concentration vs. time data; that is, $k_s = \ln (C_0/C)/t$ where C_0 represents initial DMF concentration and C represents DMF concentration after light exposure for time t.

Previous studies in organic solvents using a variety of photosensitizers have shown that the photoisomerization of cP or tP is a reversible reaction (*22*). With prolonged irradiation in the presence of a photosensitizer, isomerization proceeds until the ratio of cis to trans diene no longer changes. When this "photostationary state" is reached, the rate of cis to trans isomerization equals the rate of the reverse reaction. A typical plot of the kinetic data obtained for the photoisomerizations in natural waters is shown in Figure 2. The same photostationary state was reached starting with either cP or tP. At the concentration of diene used in this study (10 μM), the initial rate of photoisomerization was first-order with respect to diene in all the experiments. The integrated rate expression for the photoisomerization is shown in eq 4, where t_∞, t_0, and

$$\ln \frac{(t_\infty - t_0)}{(t_\infty - t_t)} = k_i t \qquad (4)$$

t_t represent the fraction of the diene that was trans at the photostationary state, at time zero, and at time t. The rate constant, k_i, for the isomerization equals the sum of the rate constants for the cis–trans conversion and for the trans–cis conversion (*23*).

For the studies in sunlight, outdoor actinometers consisting of dilute valerophenone in distilled water were simultaneously irradiated. To correct for the variability in sunlight intensity, the rate constants were ratioed to the first-order rate constant for the actinometer, k_{act}, to give a relative rate constant, k_{rel}.

$$k_{rel} = k_s/k_{act} \qquad (5)$$

At latitude 40°N, the value of k_{act} averaged over a full year is 0.88 h^{-1}.

Table III. Kinetic Data for Photosensitized Reactions of 2,5-Dimethylfuran (DMF) and *cis*-1,3-Pentadiene (cP) in Optically Matched Natural Water Samples and Aqueous Solutions of Humic Substances[a]

reaction medium	k_{rel}[b] DMF	k_{rel}[b] cP	$10^{13}\times$ [$^1O_2$], M[c,d]	$10^{13}\times$ [$^3H^*$] + [$^3S^*$], M[c,e]	$10^{13}\times$ [$^3H^*$], M[c,f]
Aucilla River water sample, Lamont, FL	1.4	0.22	4.4	2.2	1.0
Suwannee River water sample, Suwannee Springs, FL	1.3	0.18	4.1	2.0	0.71
Wylde Lake (Ontario) humus, in distilled water	0.81	0.092	2.5	1.3	0.37[g]
Ohio River (Cincinnati) fulvic acid, in distilled water	3.0	0.43	9.4	4.7	1.8[g]
Fluka AG humic acid, in distilled water	1.3	0.14	4.1	2.0	0.31
Aldrich humic acid, in distilled water	1.2	0.13	3.8	1.9	1.0
Contech fulvic acid, in distilled water	1.25	0.10	3.8	1.9	0.61
mean values			4.6	2.3	0.84

[a] Absorbance at 365 nm equals 0.25 cm^{-1} in a 1.00-cm cell. [b] Ratio of k_s for DMF reaction or k_i for cP isomerization to photolysis rate constant for valerophenone actinometer. [c] Computed using sunlight photolysis rate constants that were estimated from k_{rel} assuming that k_{act} equals 0.88 h^{-1} (eq 5); the mean value for the valerophenone actinometer at latitude 40° N. [d] Typical near-surface concentration of singlet oxygen (eq 21). [e] Typical near-surface concentration of triplets that transfer energy to dioxygen (eq 22). [f] Typical near-surface concentration of triplets with energy 240–250 kJ/mol (eq 23). [g] Computed assuming that $k_q = 6 \times 10^8$ M^{-1} s^{-1}.

considerably, with soil-derived humic acids much more effective than dissolved organic substances in natural waters. The humic acids used in this study, however, were not found to be significantly more efficient sensitizers than the dissolved organics in the natural water samples. Finally, the UV absorption spectra of DMF and pentadiene in water were found to be unaffected by the presence of humic substances, whereas spectral changes would be likely if the humus bound these substrates.

Although static sensitization does not account for the results of this study, it may come into play with substrates that are much more hydrophobic than DMF or the pentadienes. The substrates used in this research are over one million times more water soluble than DDT, the solute used by Carter and Suffet (*24*) in their binding studies.

Scheme for Energy Transfer. On the basis of the above considerations as well as a large number of other results from previous studies of photosensitized reactions in solution (*19*, *20*, *22*, *25*), it appears most likely that the kinetic results of this study can be described by the following scheme. This scheme depicts the key energy-transfer steps as occurring when excited sensitizer, denoted by the asterisk, and energy acceptor (substrate or dioxygen) diffuse together.

$$H \xrightarrow{\text{light}} {}^1H^* \rightarrow {}^3H^* \quad (8)$$

$${}^3H^* \xrightarrow{k_d} H + \text{heat} \quad (9)$$

$${}^3H^* + O_2 \xrightarrow{k_{ox}} H + {}^1O_2 \quad (10)$$

$${}^3H^* + Q \xrightarrow{k_q} H \quad (11)$$

$${}^3H^* + cP \xrightarrow{k_c} T + H \quad (12)$$

$${}^3H^* + tP \xrightarrow{k_t} T + H \quad (13)$$

$$T \rightarrow cP \quad (14)$$

$$T \rightarrow tP \quad (15)$$

$$S \xrightarrow{\text{light}} {}^1S^* \rightarrow {}^3S^* \quad (16)$$

$${}^3S^* \rightarrow S + \text{heat} \quad (17)$$

$${}^3S^* + O_2 \xrightarrow{k_{ox}} {}^1O_2 + S \quad (18)$$

$${}^1O_2 \xrightarrow{1/\tau_0} O_2 \quad (19)$$

$${}^1O_2 + A \xrightarrow{k_A} AO_2 \quad (20)$$

Because humic substances are known to be mixtures, we subdivided the sensitizers in humus solutions into two main categories: the symbol H indicates components that can transfer energy to 1,3-pentadiene as well as dioxygen, O_2, and the symbol S denotes substances that can transfer energy only to dioxygen. A brief discussion of this scheme follows. Light absorption promotes a photosensitizer molecule to its first excited singlet state $^1H^*$ or $^1S^*$. Singlet excited states, with lifetimes usually only on the order of 10 ns or less, are far too short lived to significantly interact with organic substrates or dioxygen present at the very low concentrations used in this study. Molecules in singlet states, however, decay in part by undergoing intersystem crossing to excited triplet states, $^3H^*$ or $^3S^*$, which are considerably longer lived. A variety of pathways may deactivate such triplets (eq 9–13, 17, and 18). These include decay to ground state (eq 9 and 17), energy transfer to dioxygen (eq 10 and 18) or some other interaction that quenches $^3H^*$ (eq 11), and energy transfer to the substrate cP or tP, in the natural water (eq 12 and 13), forming its triplet state T. The diene triplet decays back to a mixture of ground-state cP and tP (*22*).

Singlet molecular oxygen, $^1O_2$, the product of energy transfer to dioxygen (eq 10 and 18), is an excited form of oxygen that is a more powerful oxidant than dioxygen (*19*). It reacts rapidly with certain "acceptors", A (eq 20), often forming peroxidic products. Reactions of singlet oxygen with various substrates must compete with its decay back to ground state (eq 19). DMF is one of the most reactive of known singlet oxygen acceptors. Its photooxidation in air-saturated natural waters (*8*, *12*) or in solutions of humic substances (*9*) involves the intermediacy of $^1O_2$.

The rates of the various energy-transfer processes depicted in the above scheme depend upon the concentration of the energy acceptor as well as the rate constants for the energy-transfer processes. Energy transfer occurs rapidly only when the triplet state energy of the sensitizer equals or exceeds that of the energy acceptor (*22*). The energy required to excite dioxygen to its first singlet state is only 94 kJ/mol. Thus, most triplet sensitizers that absorb only visible or UV light transfer energy to dioxygen rapidly ($k_{ox} = 2 \times 10^9$ L/(mol s) in water) (*26*). On the other hand, the triplet state energy of dienes is about 250 kJ/mol, well above the energy required to excite dioxygen, and certainly more typical of the triplet energies of various organic pollutants such as polycyclic aromatic hydrocarbons and nitroaromatic compounds. Triplets capable of transferring energy to dienes also are able to rapidly transfer energy to dioxygen, but the reverse is not necessarily true.

In the following sections, we apply the concepts and the scheme discussed here to estimate the concentrations and energies of triplets in highly colored natural waters and

humus solutions exposed to light.

Near-Surface Triplet Concentrations in Natural Waters. In the photic zone of water bodies, the water is typically close to air saturated and the concentration of dioxygen (0.2–0.3 mM) is considerably higher than that of natural organic chemicals that are triplet energy acceptors. This factor, coupled with the great magnitude of the energy-transfer rate to dioxygen, indicates that most of the electronic energy that is transferred is likely to result in formation of singlet oxygen. This condition is also applicable to almost all of the experiments with DMF or the pentadiene that are discussed here. In all cases, the dioxygen concentration was at least an order of magnitude larger than the substrate concentration. With exposure of natural waters to sunlight, concentrations of triplets, $[^3H^*]$ and $[^3S^*]$, and of singlet oxygen, $[^1O_2]$, rapidly build to a steady state. It has previously been shown (*8*) that steady-state concentrations of $^1O_2$ can be computed by using eq 21 where k_s is the rate constant for the DMF

$$[^1O_2] = k_s/k_A \tag{21}$$

photooxygenation and k_A is the bimolecular rate constant for reaction of DMF with singlet oxygen. We estimate that k_A is 7.8×10^8 L/(mol s) on the basis of data in the chemical literature (*27*, *28*). It can readily be shown that, at steady state, the total concentration of triplets available for photosensitized reactions, $[^3H^*] + [^3S^*]$, is related to $[^1O_2]$ by eq 22. At 25 °C, k_{ox} is 2×10^9 L/(mol s), τ_0, the

$$[^3H^*] + [^3S^*] = \frac{[^1O_2]}{k_{ox}\tau_0[O_2]} \tag{22}$$

lifetime of $^1O_2$, is 4 μs (*27*), and $[O_2]$ is 2.5×10^{-4} mol/L in air-saturated water. The maximum concentration of triplets is thus equal approximately to $0.5[^1O_2]$.

The steady-state concentration of triplets capable of transferring energy to cP and tP can be computed by using eq 23 where k_i is the rate constant for the photoisomer-

$$[^3H^*] = k_i/k_q \tag{23}$$

ization (eq 4) and k_q is the effective rate constant for energy transfer from $^3H^*$ to diene. It is assumed that k_c and k_t (eq 12 and 13) both equal k_q, although, as discussed below, energy transfer to the trans isomer is slightly slower than to the cis isomer.

To estimate k_q, the quenching effect of another water-soluble diene, 2,4-hexadien-1-ol, on the photoisomerization of cP was quantitated. Both pentadiene and 2,4-hexadienol have the same published triplet state energies (*22*, *29*), so it is reasonable to assume that the triplets in natural waters transfer energy to both dienes at about the same rate. The well-known Stern–Volmer equation (*25*) was found to describe the quenching data with quencher concentrations in the 0.5–5.0 mM range (eq 24). In the

$$k_i(0)/k_i(Q) = 1 + k_q\tau[Q] \tag{24}$$

equation, $k_i(0)$ and $k_i(Q)$ are the rate constants in sunlight without and with the hexadienol present, respectively, τ is the effective lifetime of the various triplets that mediate the photoisomerization, and [Q] is the molar concentration of the hexadienol. Results of quenching studies in various air-saturated, aqueous solutions of dilute cP are shown in Table I. Acetophenone, a known high-energy triplet sensitizer, was included in these studies for comparison. The $k_q\tau$ values for acetophenone were larger than those observed in the humus solutions, indicating that either the lifetimes of $^3H^*$ and/or the k_q values for energy transfer from $^3H^*$ to dienol are lower than those for triplet acetophenone.

To estimate the lifetime(s) of $^3H^*$, kinetic data were obtained concerning the effects of dioxygen concentration on k_s for DMF and k_i for cP in sunlight. With no quencher present, the lifetime of a triplet τ equals $1/(k_d + k_{ox}[O_2])$, and the efficiency for formation of singlet oxygen from triplets equals $k_{ox}\tau[O_2]$. The maximum triplet lifetime with O_2 present equals $1/k_{ox}[O_2]$, or 2 μs in air-saturated water. Under these conditions, because τ is inversely proportional to $[O_2]$, the photooxygenation rate of DMF is predicted to be independent of dioxygen concentration, and the photoisomerization rate of cP is predicted to be inversely proportional to dioxygen concentration. Thus, as shown in Table II, the pronounced reduction in k_i and lack of change in k_s for the DMF reaction in going from air-saturated to oxygen-saturated solutions strongly indicates that the lifetimes of most of the triplets are about 2 μs.

Assuming that τ equals 2 μs, we computed k_q values for the systems in Table I. A k_q value of 2.8×10^9 L/(mol s) was computed for quenching of triplet acetophenone by hexadienol, in excellent agreement with the literature value (*29*). This result indicates that acetophenone transfers energy to dienes at the maximum possible rate in water, a result that is expected (*22*) when the energy of the sensitizer (305 kJ/mol for acetophenone) exceeds that of the diene (251 kJ/mol). Triplet energy transfer from the humic substances to dienes was also very rapid (Table I), although it was not quite as fast as with acetophenone as sensitizer. This result suggests that, on the average, triplet energy transfer from humic substances to the dienes is slightly endothermic (*22*), in agreement with the results in the following section.

The various kinetic parameters and equations that are discussed above were used to estimate typical near-surface concentrations of singlet oxygen and triplet states in the sunlight-exposed solutions (Table III). These calculations indicate that up to half of the triplets capable of transferring energy to dioxygen can also transfer it to the much higher energy dienes. On the average $[^3H^*]/([^3S^*] + [^3H^*])$ is 0.35. The rate constants for these photosensitized reactions are proportional to the UV absorption coefficients of the colored natural waters and humus solutions. For these waters the mean ratios of steady-state concentrations to the absorption coefficient at 365 nm equals 8×10^{-15} mol/(L m) for $[^1O_2]$, and 1.5×10^{-15} for $[^3H^*]$. The absorption coefficient used for these calculations was defined as 2.303 times the absorbance at 365 nm/M. Using known absorption coefficients of natural waters (*2*, *8*), we estimate that triplet concentrations in the photic zone range from about 10^{-15} (clear lakes) to 10^{-13} M (highly colored water bodies, e.g., swamps). Singlet oxygen concentrations are 5–6 times higher.

The steady-state concentrations in Table III (average for a full year) are estimated (*1*) for latitude 40°N (mid-United States) during the day near the surface of a water body. Action spectra for the reactions (see below) were used in conjunction with a previously described computer program (*1*) to make these estimates (*10*). Variations with latitude, season, and depth should be similar to the changes in photolysis rate constants of UV-absorbing pollutants or of solar UV irradiance that are discussed elsewhere (*1*, *2*).

Triplet Energies of Photosensitizers. Studies by Hammond et al. (*22*) of the photoisomerization of alkenes indicated that the photostationary state (PSS) depends on the triplet state energy of the photosensitizer. The data in Table IV illustrate this effect in the case of 1,3-pentadiene with several pure photosensitizers in distilled water.

Table IV. Photostationary States Reached in Sensitized Isomerization of 1,3-Pentadiene Using Pure Photosensitizers in Distilled Water

sensitizer	t_∞[a]	triplet state energy, kJ/mol
acetophenone	0.56	305
p-(trimethylammonio)-benzophenone chloride	0.56	293
naphthalene	0.60	254
naphthalene-2,6-disulfonic acid sodium salt	0.60	251
2-acetonaphthone	0.66	248
1-acetonaphthone	0.71	236

[a] Fraction *trans*-1,3-pentadiene at photostationary state using 313-nm light.

Table V. Fraction *trans*-1,3-Pentadiene at Photostationary State for Isomerization of 1,3-Pentadiene in River Water Samples and in Aqueous Solutions of Humic Substances

	sunlight	313 nm	366 nm
Aucilla River, FL	0.638	0.592	0.620
Suwannee River, FL	0.645	0.588	0.616
humus isolated from Wylde Lake, Ontario	0.635	*a*	*a*
Fluka humic acid	0.647	0.59	0.628
Aldrich humic acid	0.644	0.587	0.630
Contech fulvic acid	0.634	0.611	0.620
Ohio River fulvic acid	0.625	*a*	*a*

[a] Not determined.

These results are consistent with past studies showing that, with sensitizers having triplet energies in excess of 265 kJ/mol, the same fraction trans at PSS (t_∞) is obtained regardless of the triplet energy of the sensitizer. We find that t_∞ for pentadiene in water with high energy sensitizers (0.56) is about the same as that observed in organic solvents (*22*). When the triplet energy drops to about 250 kJ/mol, energy transfer to *trans*-1,3-pentadiene becomes slower than to the cis isomer and the PSS becomes more trans rich. The fraction trans at PSS increases sharply as the triplet energy of the photosensitizer drops from 254 to 236 kJ/mol. As discussed earlier, photostationary states were also obtained in the natural water samples and solutions of humic substances (Table V). Comparisons of the results in Table V with those in Table IV suggest that the predominant triplets in sunlight-irradiated humus solutions have energies of around 250 kJ/mol. The PSS (and thus available triplet energies) are slightly wavelength dependent, with the fraction trans lower with 313-nm light than with 366-nm light or sunlight, but are remarkably similar in all the natural water samples and solutions of humic substances. Because humic substances are mixtures, we cannot exclude the possibility that some of the triplets have energies higher than 250 kJ/mol.

Wavelength Dependence of the Photosensitized Reactions. The penetration of sunlight into natural waters depends strongly on wavelength in the UV and blue spectral region (*2*). To estimate the depth dependence of rate constants for photoreactions of pollutants in natural waters, it is necessary to have data concerning the wavelength dependence of the reactions (*1*). As a part of this study we have determined action spectra, i.e., the dependence of response functions on wavelength, for both the DMF and diene reactions. Typical results of these studies are shown in Figure 4 for Aucilla River water and a solution of a commercial, soil-derived humic acid. These action spectra are compared to the absorption spectra of the waters. Ultraviolet light (300–400 nm) is clearly most

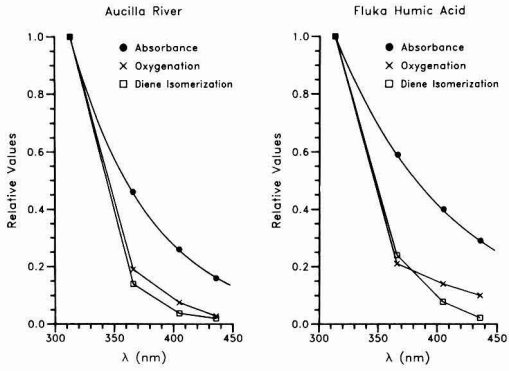


Figure 4. Comparison of action spectra for photosensitized reactions to ultraviolet–visible absorption spectra for Aucilla River water and solution of Fluka humic acid.

effective at inducing the photoreactions. There is little difference in the relative action spectra in the UV region, but in the visible region the DMF photooxygenation becomes much more efficient than the diene isomerization. This result can be explained in terms of energy-transfer considerations. The DMF reaction is mediated by energy transfer to dioxygen, whereas the diene reaction involved energy transfer to diene as the key step. As discussed above, dioxygen can accept energy from a wider variety of sensitizers, including some that absorb lower energy visible light.

It can be shown that the quantum yield for production of singlet oxygen, $Y_{s,\lambda}$, can be computed from the response function by using eq 25, where α_λ is the decadic absorption

$$Y_{s,\lambda} = \frac{X_{s,\lambda}}{2.303\alpha_\lambda k_A \tau_0} \qquad (25)$$

coefficient at wavelength λ. For DMF this reduces to $Y_{s,\lambda} = (1.4 \times 10^{-4})(X_{s,\lambda}/\alpha_\lambda)$. In the near-UV region (365 nm) quantum yields in the 0.004–0.016 range were computed for the air-saturated humus solutions (see supplementary material; see paragraph at end of paper regarding supplementary material). Similar results have been reported recently by Haag and co-workers (*13*) and Baxter and Carey (*12*) with natural waters from Switzerland and Canada. The results in Table II indicate that the Y_s values closely correspond to quantum yields for the formation of triplet states.

Conclusions

The results of this study indicate that absorption of sunlight by humic substances in natural waters can lead to rapid photosensitized reactions of certain pollutants via energy transfer from molecules in their triplet states. Pollutants with triplet state energies less than 250 kJ/mol should be able to participate in these photoreactions. These chemicals include most aromatic compounds with two or more fused aromatic rings, nitroaromatic compounds, and chemicals with conjugated double bonds such as diketones and polyenes. Rates of the humus-sensitized reactions depend strongly on dioxygen concentration, indicating that the major fate of a humus triplet involves energy transfer to dioxygen to form singlet molecular oxygen. As shown in earlier studies (*9*, *19*), singlet oxygen can in turn rapidly oxidize certain substrates, including furans, sulfides, and electron-rich olefins.

Kinetic studies of photosensitized reactions in colored natural water samples and in solutions of humic substances

isolated from water bodies or soils indicated that (1) with substrates used in this study the reactions do not involve binding of the substrate by the humus (however, such binding may be significant with chemicals that strongly sorb on humus), (2) a substantial fraction (about one-third) of the humus triplets that transfer energy to dioxygen have triplet energies greater than 240 kJ/mol, (3) UV radiation is mainly responsible for inducing the photosensitized reactions, and relative action spectra for the oxygenation of DMF and the isomerization of cP were very similar in the UV region, and (4) quantum efficiencies for the production of triplets in the near-UV region fall in the 0.4–1.5% range. These conclusions provide further supporting evidence that certain commercial humic substances can be used as sensitizers that mimic the behavior of the humic substances in fresh waters.

In the upper layers of sunlit water bodies, it is estimated that typical concentrations of humus triplets with energies greater than 240 kJ/mol range from about 10^{-15} (clear lakes) to 10^{-13} M (swamps and other highly colored natural water bodies). The concentrations of singlet oxygen are estimated to be about 5–6 times higher. The rate constant of a photosensitized reaction is related to the triplet concentration by eq 26. In this equation, k_{et} is the rate

$$\text{rate constant} = k_{et}\phi_I[^3H^*] \qquad (26)$$

constant for energy transfer to substrate and ϕ_I is the probability that the product of such transfer, the substrate in its triplet state, goes on to products. The maximum values of k_{et} and ϕ_I in water are 3×10^9 L/(mol s) and unity, respectively. Therefore, average near-surface rate constants for humus-sensitized photoreactions involving triplet energy transfer in natural waters may theoretically range up to 10^{-4}–10^{-6} s^{-1} (half-lives of a few hours to several days of average sunlight).

In previous studies, it has been shown that aquatic humic substances can photosensitize reactions involving hydrogen atom transfer in addition to reactions involving energy transfer. These other photoreactions also are likely to involve triplet state intermediates, so the results of these studies can be applied to estimate environmental rate constants for these reactions as well.

These estimates, however, are based on only a few colored freshwater samples and soil-derived humus solutions. Further photochemical and flash spectroscopic research is required to determine the generality of the results reported here. In particular, recent results of Harvey et al. (*30*) indicate that the spectral properties, and thus probably the photochemical properties, of marine humus differ significantly from those of freshwater humus.

Supplementary Material Available

Derivations of the equations and a table of quantum yields for singlet oxygen production in natural water samples (5 pages) will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper or microfiche (105 × 148 mm, 24× reduction, negatives) may be obtained from Microforms Office, American Chemical Society, 1155 16th St., N.W., Washington, DC 20036. Full bibliographic citation (journal, title of article, author, page number) and prepayment, check or money order for $9.00 for photocopy ($11.00 foreign) or $6.00 for microfiche ($7.00 foreign), are required.

Registry No. cP, 1574-41-0; DMF, 625-86-5.

Literature Cited

(1) Zepp, R. G.; Cline, D. M. *Environ. Sci. Technol.* **1977**, *11*, 359.
(2) Baker, K. S.; Smith, R. C. *Limnol. Oceanogr.* **1982**, *27*, 500–509.
(3) Zafiriou, O. C. In "Chemical Oceanography"; Riley, J. P., Ed.; Academic Press: London, 1983; Vol. 8, Chapter 48.
(4) Zika, R. G.; Cooper, W. J. *Science (Washington, D.C.)* **1983**, *220*, 711.
(5) Draper, W.; Crosby, D. G. *Arch. Environ. Toxicol. Chem.* **1983**, *12*, 121.
(6) Joussot-Dubien, J.; Kadiri, A. *Nature (London)* **1970**, *227*, 700.
(7) Ross, R.; Crosby, D. G. *J. Agri. Food Chem.* **1973**, *21*, 335.
(8) Zepp, R. G.; Wolfe, N. L.; Baughman, G. L.; Hollis, R. C. *Nature (London)* **1977**, *267*, 421.
(9) Zepp, R. G.; Baughman, G. L.; Schlotzhauer, P. F. *Chemosphere* **1981**, *10*, 109.
(10) Zepp, R. G.; Baughman, G. L.; Schlotzhauer, P. F. *Chemosphere* **1981**, *10*, 119.
(11) Wolff, C. J. M.; Halmans, M. T. H.; van der Heijde, H. B. *Chemosphere* **1981**, *10*, 59.
(12) Baxter, R. M.; Carey, J. H. *Freshwater Biol.* **1982**, *12*, 285.
(13) Haag, W. R.; Hoigne, J.; Gassman, E.; Braun, A. M. *Chemosphere*, in press.
(14) Slawinski, J.; Puzyna, W.; Slawinska, D. *Photochem. Photobiol.* **1978**, *28*, 75.
(15) Slawinski, J.; Puzyna, W.; Slawinska, D. *Photochem. Photobiol.* **1978**, *28*, 459.
(16) Dulin, D.; Mill, T. *Environ. Sci. Technol.* **1982**, *16*, 815.
(17) Moses, F. G.; Liu, R. S. H.; Monroe, B. M. *Mol. Photochem.* **1969**, *1*, 245–249.
(18) Hatchard, C. G.; Parker, C. A. *Proc. R. Soc. London, Ser. A* **1956**, *235*, 518.
(19) Wilkinson, F.; Brummer, J. G. *J. Phys. Chem. Eng. Data, Suppl.* **1981**, *10*, 809.
(20) Saltiel, J.; D'Agostino, J.; Megarity, E. D.; Metts, L.; Neuberger, K. R.; Wrighton, M.; Zafiriou, O. C. *Org. Photochem.* **1972**, *3*, 1.
(21) Young, R. H.; Wehrly, K.; Martin, R. L. *J. Am. Chem. Soc.* **1971**, *93*, 5774.
(22) Hammond, G. S.; Saltiel, J.; Lamola, A. A.; Turro, N. J.; Bradshaw, J. S.; Cowan, D. O.; Counsell, R. C.; Vogt, V.; Dalton, C. *J. Am. Chem. Soc.* **1964**, *86*, 3197–3217.
(23) Frost, A. A.; Pearson, R. G. "Kinetics and Mechanisms"; Wiley: New York, 1961; p 186.
(24) Carter, C. W.; Suffet, I. H. *Environ. Sci. Technol.* **1982**, *16*, 735.
(25) Wagner, P. J. In "Creation and Detection of the Excited State"; Vol. 1, pt. A, Lamola, A. A., Ed.; Marcel Dekker: New York, 1971; Vol. 1, Part A, Chapter 4.
(26) Foote, C. S. In "Free Radicals in Biology"; Pryor, W., Ed.; Academic Press: New York, 1976; Vol. II, p 85.
(27) Rodgers, M. A. J.; Snowden, P. J. *J. Am. Chem. Soc.* **1982**, *104*, 5541.
(28) Usui, Y.; Tsukada, M.; Nakamura, H. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 379.
(29) Yip, R. W.; Riddell, W. D.; Szabo, A. G. *Can. J. Chem.* **1970**, *48*, 987.
(30) Harvey, G. R.; Boran, D. A.; Chesal, L. A.; Tokar, J. M. *Mar. Chem.* **1983**, *12*, 119.

Received for review April 6, 1984. Accepted August 13, 1984.

Environ. Sci. Technol. 1985, 19, 82–86

Surface Area and Porosity of Coal Fly Ash

Mark R. Schure,* Pat A. Soltys,† David F. S. Natusch,‡ and Thad Mauney§

Department of Chemistry, Colorado State University, Ft. Collins, Colorado 80523

■ Results of surface area measurements and morphology studies on two size-fractionated samples of coal fly ash reveal that small ash particles are predominately nonporous spheres with irregular surface morphology. The surface area is dependent on particle size for both samples. For the sample taken from a particle collector it is shown that large surface areas are attributable to carbonaceous particles of highly porous character. The surface area of particles in a coal-fired power plant is suggested to be dependent on the point of collection.

Introduction

The surface of particulate matter emitted from any combustion process is important since the amount of surface, the morphology, and its chemical nature may determine the ability of these particles to act as gaseous pollutant sinks.

Many mechanisms have been proposed to explain the enhanced biological effects observed when certain combinations of vapors and particles are inhaled. One mechanism to explain this behavior involves the adsorption of vapor by the particle presenting a high local concentration of adsorbed compounds at the site of impact in the lung (*1*).

Although the surface area controls the total adsorption capacity of particles, the rate of release of adsorbed material into the lung may be influenced by the morphology of the surface. If porosity is present, then release can be retarded by the slowness of diffusion out of the pores. Additionally, the porosity may influence the eventual chemical state of the adsorbed vapor, for example, by shielding material contained in pores from photochemical degradation. Therefore, determination of surface area and porosity are both essential to understanding of the environmental properties of particulate emissions.

There are currently few data and limited understanding of the surface area and porosity of airborne particulate matter. Studies by Corn et al. (*2*) reveal that some environmental particles are composed of polycyclic aromatic hydrocarbons. The measured surface area of these particles almost doubled upon outgassing, suggesting that a porous matrix is coated with adsorbed matter.

In a study of the surface area of coal fly ash, Kaakinen and co-workers (*3*) using the BET technique (*4*) showed that the measured surface area varies according to the location in the power plant from which the ash was taken. These authors attempt to explain these differences in terms of a correlation between surface area and trace metal content.

In a study investigating the adsorption–desorption isotherm behavior of argon on coal fly ash, Ditl and Coughlin (*5*) concluded from the presence of hysteresis that fly ash is porous. The carbonaceous components of the sample were purposely burned off by heating the fly ash in air at 800 °C for 12 h. In studies of trace metal vaporization of coal fly ash (*6*), it was noted that carbonate decomposition can occur at temperatures below 800 °C. This implies that the outgassing procedure of Ditl and Coughlin may be too rigorous to preserve the sample integrity.

The adsorption–desorption behavior of water vapor on coal fly ash, studied by Rothenberg (*7*), revealed large hysteresis loops which Rothenberg tentatively attributed to porosity. However, the rates of desorption measured at several temperatures suggest (*8*) that water forms chemical complexes with fly ash. Because of strong chemical interactions between water and ash, a pore structure cannot be implied from these adsorption–desorption isotherms.

Using nitrogen adsorption methods, Ondov (*9*) examined the surface area of a number of size-fractionated coal fly ash samples. This work also focused on the contribution of carbonaceous particles to the measured surface area. The carbonaceous components were suggested to be responsible for the discrepancy between measured and calculated surface area.

It is the purpose of this paper to report on studies of the surface area of size-fractionated coal fly ash under carefully controlled conditions. The surface morphology of a number of ash samples is examined and in conjunction with mathematical models is used to explain the relationship between measured and calculated surface area. In addition, one size-fractionated sample was analyzed in detail for pore structure by using gas adsorption methods.

Experimental Section

Samples. Two types of coal fly ash were used in this study. Coal fly ash was collected from the stack breeching after the electrostatic precipitator of a coal-fired electric power plant burning low-sulfur, high-ash, high-moisture coal and aerodynamically fractionated during collection into four size fractions. The reader is referred to Fisher's papers (*10*, *11*) for further details of collection and morphological characterization. These samples are referred to here as western ash.

Coal fly ash was also collected from the baghouse of the Corrette power plant in Billings, MT, burning low-sulfur, subbituminous, Montana Rosebud coal. The Corrette ash was fractionated according to particle size by using precision sieves (ATM Corp., Greendale, WI) driven by a sonic sifter (ATM Corp.). The sieve sizes used here had openings of 5, 10, 45, 75, and 125 μm. Each sieve has a cutoff of ±2 μm rated size. No other physical or morphological fractionations were performed.

Scanning Electron Microscopy. The four samples of western ash and the seven samples of Corrette ash were examined by scanning electron microscopy (SEM) using a Hitachi HHS-2R electron microscope equipped with an energy dispersive X-ray spectrometer (Model 5000A, Kevex Corp.). Particles were mounted on adhesive copper tape affixed to aluminum stubs. The samples were coated by sputtering a Au/Pd (60:40) alloy for 2-min, forming a deposited layer of approximately 120 Å.

Surface Area Measurement. Specific surface area measurements were performed by the BET method using

*Address correspondence to this author at Digital Equipment Corp., Marlboro, MA 01752.

†Present address: Elars Bioresearch Laboratory, Ft. Collins, Colorado 80523.

‡Present address: Liquid Fuels Trust Board, Wellington 1, New Zealand.

§Present address: Department of Chemistry, University of Antwerp (UIA), B2610 Wilrijk, Belgium.

0013-936X/85/0919-0082$01.50/0

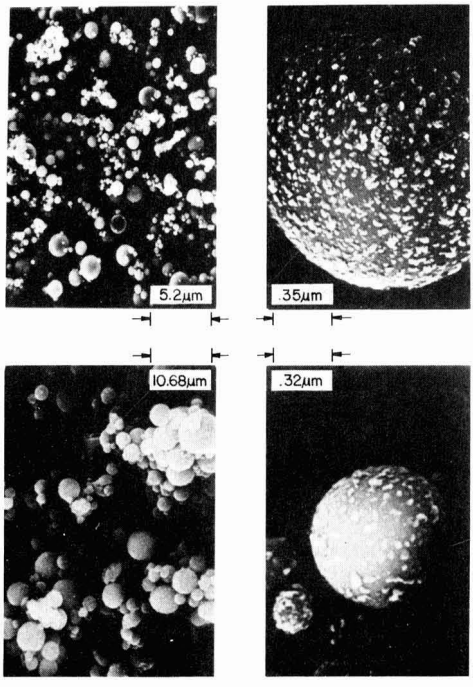


Figure 1. Two smallest size fractions of Corrette ash and corresponding surface views. Top left: <5-μm ash; Top right: <5-μm ash. Bottom left: 5–10-μm ash. Bottom right: 5–10-μm ash.

nitrogen as the adsorbate at three partial pressures. All measurements were made by using a Quantasorb Sorption System (Quantachrome Corp., Syosset, NY). Premixed nitrogen–helium mixtures of 0.1005, 0.207, and 0.309 mole fraction of nitrogen (UHP quality) were supplied by Scientific Gas Products (Denver, CO). Other mole fractions of nitrogen were obtained by blending UHP quality nitrogen and helium by using an electronic flow controller (5841; Brooks Instrument Co., Hatfield, PA).

Density Determination. Density determinations were performed by using the classical pycnometer technique with mesitylene (1,2,5-trimethylbenzene) as the displaced fluid.

Particle Size Distributions. The four samples of western ash were found by Fisher and co-workers (*11*) to fit the log-normal size density function. The size distribution histogram of the smallest Corrette ash sample was obtained by manually measuring the particle diameters from SEM photographs. The integrated log-normal distribution was least squares fitted to the cumulative histogram yielding the log-normal parameter estimates.

Results

The SEM micrographs of the four western ash fractions have been previously published by Fisher et al. (*11*). As noted in their study, a variety of very small particles and crystalline formations appear to be attached to the surface of the predominately small particles. This behavior is also found in the case of the Corrette ash. Figure 1 shows this effect where low- and high-resolution micrographs of the two smallest Corrette fractions are shown. Although low-resolution photographs show that the particles are spherical, high-resolution photographs demonstrate the true character of the surface.

Despite exhaustive sieving, electron microscopy reveals that the 5–10-μm size fraction is heavily contaminated by particles with diameters around 3 μm. This may be due to the formation of electrostatically bound agglomerates, preventing proper fall through in sieving.

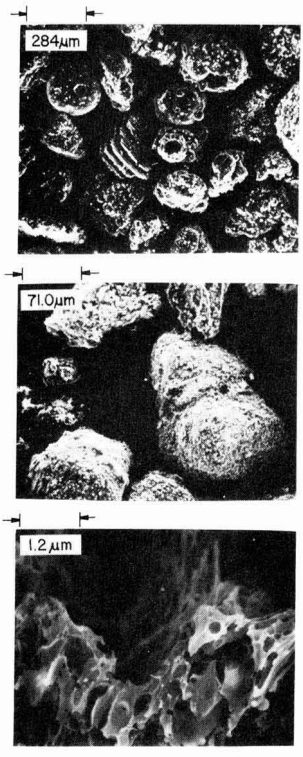


Figure 2. Largest size (>125 μm) fraction of Corrette ash. The middle and bottom views are of carbonaceous particles.

Table I. Results of Surface Area and Density Determinations

size, μm	S_m, m^2/g	ρ, g/cm^3	roughness	S_v, m^2/cm^3
	Corrette Fly Ash[a]			
5 (<7)	1.27	1.91	1.88	2.42
5–10 (3–12)	0.90	1.72	*d*	1.5
10–20 (8–22)	0.63	2.24	*d*	1.4
20–45 (18–47)	0.45	1.97	*d*	0.89
45–75 (43–77)	0.52	1.93	*d*	1.0
75–125 (73–127)	1.10	1.97	*d*	2.17
>125 (>123)	9.44	1.75	*d*	16.5
	Western Fly Ash			
0.92,[b] 1.20[c]	2.89	2.45	1.69	7.09
1.14,[b] 1.79[c]	1.72	2.36	1.63	4.05
2.58,[b] 4.60[c]	0.85	2.19	*d*	1.8
2.73,[b] 6.94[c]	0.58	1.85	*d*	1.2

[a] Sizes in parentheses denote sieve cutoff size(s). [b] Count median diameter from SEM determination. [c] Calculated diameter of average volume. [d] Not applicable due to presence of irregular particles.

As the particle size increases, the shape deviates from an ideal spherical shape. At the higher size range very irregular porous "spongelike" particles appear. These are shown in Figure 2 at various levels of magnification for the greater than 125-μm fraction. These particles are predominantly carbonaceous in origin, as determined from studies utilizing combustion techniques (*12*) and the solid NMR technique (*12*), and are most likely the result of incomplete combustion.

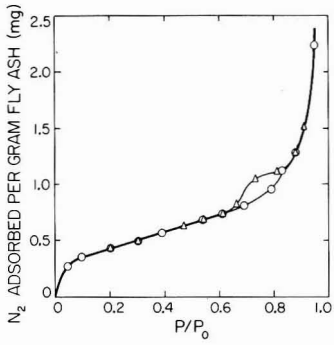


Figure 3. Adsorption–desorption isotherm of the <5-μm Corrette ash fraction. Circles are the adsorption leg; triangles are the desorption leg.

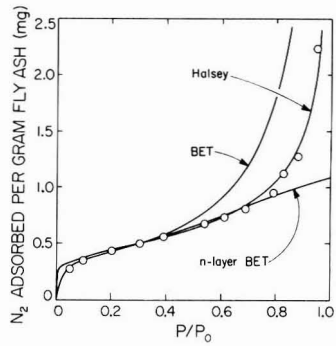


Figure 4. Fit of the BET model, Halsey model (n = 2.25), and n-layer BET model (n = 4) to the <5-μm Corrette ash adsorption isotherm.

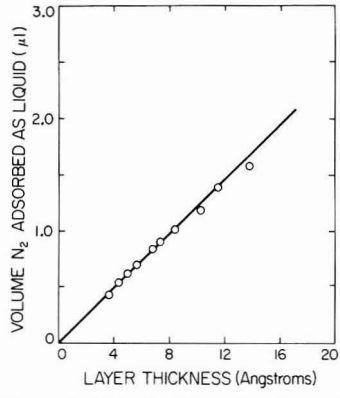


Figure 5. "t-curve" plot for the <5-μm Corrette ash fraction.

Surface areas and densities of the ash fraction are listed in Table I. Volume specific surface area (S_v) is calculated as the product of mass specific surface area (S_m) and particle density; this measure permits comparison between surface areas of solids of different densities. For both ash samples surface area decreases as particle size increases over the range from 0 to 75 μm. The two largest size fractions of Corrette ash, diameters from 75 to 125 μm and diameters greater than 125 μm, have higher surface areas than the other fractions, undoubtedly due to the presence of the highly porous carbonaceous particles already mentioned.

The calculation of surface roughness, defined as the ratio of the surface area measured by gas adsorption to the surface area obtained by particle size measurement, is discussed in the Appendix and given in Table I for the smallest ashes which approximate spheres. These ashes have surface areas that are almost twice that of surface area obtained by geometric consideration. We shall discuss this aspect in more detail.

The adsorption–desorption isotherm of the smallest size fraction of Corrette ash is shown in Figure 3. A hysteresis loop is noted which opens and closes between P/P_0 values of 0.62 and 0.83. Capillary condensation of adsorbate between particles of a fine powder has been demonstrated to occur in a number of cases (*14–17*). In an attempt to see if the hysteresis is caused by capillary condensation of nitrogen in the region where particles touch each other, the sample cell of the gas adsorption instrument was vibrated with an engraving tool for the duration (3 h) of adsorption equilibrium. The adsorption and desorption values upon shaking the sample appear to trace the original desorption leg although reproducibility was poor in this experiment. This result suggests a minor contribution of interparticle effects in the hysteresis region. However, the disappearance of the hysteresis loop does not strictly disprove the presence of porosity; certain porous powders have been found to be devoid of hysteresis (*18*).

The adsorption isotherm was fitted by a least-squares method to three theoretical adsorption models which include the BET model (*19*), the n-layer BET model (*19*), and the Halsey model (*19*). This is shown in Figure 4. Of these, the best fit is achieved by the Halsey equation with n = 2.25, n being an empirical parameter common to this model (*19*). The Halsey model is known to fit adsorption data of nonporous solids above P/P_0 of 0.1 (*19*) and is commonly used as a model reference isotherm for nitrogen adsorption on nonporous solids.

A powerful method of analyzing an experimental isotherm is the "t-curve" method devised by deBoer and co-workers (*20*), in which the volume of the adsorbed gas, assuming that the adsorbed gas density is that of the liquid, is plotted vs. film thickness calculated for a reference solid. The slope of the plotted line yields the effective surface area. Deviation from this line is indicative of porosity: points above the line indicate additional volume held by capillary condensation and points below the line indicate blockage of pore throats, occluding the interior surface area. With the exception of small deviations at three points, the t curve of the less than 5-μm fraction of Corrette ash, plotted in Figure 5, is linear; the slope of the line indicates a surface area of 1.23 m^2/g, in excellent agreement with the BET surface area of 1.27 m^2/g. The linearity of the t curve suggests that this ash fraction is nonporous.

Discussion

The results presented above indicate that the Corrette fly ash fraction of less than 5 μm is predominately nonporous. This hypothesis is contradicted by the calculated surface roughness value of 1.88 which is higher than the surface roughness value of unity expected for a nonporous solid.

The high magnification micrograph of this fraction, shown in Figure 1, demonstrates that small particles, on the order of tens of nanometers in diameter, are embedded in the micrometer-sized particles. The effect on surface roughness of these small particles can be calculated by introducing two idealized models: spheres with small embedded hemispheres and spheres with small embedded spheres. For these models the surface roughness is equal to $1 + f$ and to $1 + 4f$ (*21*) where f is the fraction of surface

covered by hemispheres and spheres, respectively.

These models demonstrate the importance of the small embedded particles upon surface roughness and surface area. For a sphere half covered by embedded hemispheres a surface roughness of 1.5 would be expected. Although the surface roughness of the smallest Corrette fraction and that of the two smallest western ashes are 1.88, 1.63, and 1.69, respectively, the excess surface area can be qualitatively justified by the presence of the embedded particles.

The origin of the embedded particles may be due to the existence of what many workers call the "condensation" fraction of coal ash fly (*22*, *23*). These very small submicrometer particles could conceivably adhere to the bigger particles through chemical means in storage; however, it seems more likely that the agglomeration process is due to collisions which take place in the formation stage. Another possibility is that the embedded particles are formed by crystallite growth during storage; however, the appearance of the embedded particles suggests a similar origin to the main sphere. Chemical analysis of crystal growth on fly ash (*24*) has shown the composition of crystalline material associated with the particle surface to be of different composition than that of the bulk ash; additional study is needed to ascertain with certainty the origin of the embedded particles. Although the smallest size particles are the most important, from an inhalation toxicology standpoint, we now discuss the largest particles found in the Corrette ash.

The presence of the largest particles of carbonaceous origin in the Corrette ash tends to bias a surface area measurement of the bulk ash when examined without size fractionation. In a separate study (*12*) it was found that the bulk surface area of the Corrette ash is 1.19 m^2/g, and carbonaceous particles isolated by a float–sink density separation had a surface area of 97.9 m^2/g. These results show that there are two controlling aspects of the surface area of Corrette ash: (1) the size distribution of the particles and (2) the amount of porous carbonaceous particles in the ash.

It should be noted, however, that minute amounts of carbon in samples containing primarily small particles may have some effect on the surface area and consequently the calculated surface roughness. For the western ash (*25*) it was found that the two smallest size fractions contained 0.18 and 0.23% of carbonaceous carbon. This bias in the surface roughness lends further creditability to the hypothesis that small particles are essentially nonporous spheres with irregular surfaces; the excess roughness can be explained by the existence of porous carbonaceous particles.

The carbonaceous particles are found to exist primarily in the largest fraction of the Corrette ash and are the result of incomplete combustion of improperly ground coal. The Corrette samples were collected from a baghouse in bulk form and do not represent particles found in the atmosphere. The western ash collected after the particle precipitator, however, is a realistic sample for atmospheric studies.

The measurments performed on the western ash and the smaller Corrette ash fraction in conjunction with the interpreting models indicate that ash released to the environment has a minimal porosity. Any vapor deposited on the ash is likely to be available for direct interaction with a biological interface although photochemical degradation may alter the initial character of the deposited vapor.

One may speculate that the presence of carbonaceous particles in the power plant may be beneficial in trapping vapors; however, the adsorption of vapors such as polycyclic aromatic hydrocarbons has been suggested to be highly temperature dependent (*21*, *26*). The trapping effect will therefore be dependent on conditions unique to the design of the power plant under study. For coal-fired power plants operating without particle collection devices, the trapping effect may occur to some extent in the atmosphere, but due to the size of the carbonaceous particles, this effect would happen local to the power plant; the setting velocity of particles with diameters in excess of 100 μm is very fast.

Acknowledgments

The help of Jennifer Askew in the preparation of the manuscript is gratefully acknowledged. We also thank Gerald L. Fisher of the Battelle Columbus Laboratories for the donation of the four samples of western ash.

Appendix

Surface Roughness for the log-Normal Density Function. An estimate of the specific surface area of a powder can be derived from geometrical considerations based on microscopic examination of samples of known density (*13*). If the particles are spherical the specific surface area S_G of a single particle is given by

$$S_G = \frac{A}{m} = \frac{\pi d^2}{(\rho/6)\pi d^3} = \frac{6}{\rho d} \tag{1}$$

where A is the particle surface area, m is the mass, ρ is the density, and d is the particle diameter. The specific surface area of a collection of spherical particles is

$$S_G = \frac{\sum_{i=1}^{n} A_i}{\sum_{i=1}^{n} m_i} = \frac{6\sum_{i=1}^{n} d_i^2}{\rho\sum_{i=1}^{n} d_i^3} \tag{2}$$

where n represents the total number of particles. If the particles are uniformly shaped but not sperhical, a shape factor k replaces the value of 6 (*13*). If the size distribution of the particles is known and the population is large enough to neglect the discreteness of individual particles, the summations are replaced by integrals:

$$S_G = \frac{6}{\rho} \frac{\int_0^\infty f(d)\ d^2\ \mathrm{d}(d)}{\int_0^\infty f(d)\ d^3\ \mathrm{d}(d)} \tag{3}$$

where $f(d)$ is the size density function.

The quantity r, surface roughness, is defined to be the ratio of the specific surface area, S_N, as measured by nitrogen adsoption, to the specific surface area, S_G, as measured from geometrical considerations:

$$r = S_N/S_G \tag{4}$$

The log-normal density function can be written as (*27*)

$$f(d) = \frac{1}{d\sqrt{2\pi}\ \ln \sigma_g} \exp\left[\frac{-(\ln d - \ln d_0)^2}{2(\ln \sigma_g)^2}\right] \tag{5}$$

where σ_g is the geometric standard deviation and d_0 is the count median diameter. By substitution of eq 5 into eq 3 and by use of the identity (*27*)

$$\int_0^\infty d^q f(d)\ \mathrm{d}(d) = (d_0)^q \exp\left[\frac{q^2(\ln \sigma_g)^2}{2}\right] \tag{6}$$

the surface roughness of spheres obeying the log-normal density function is given as

$$r = \frac{S_N \rho}{6} d_0 \exp[2.5(\ln \sigma_g)^2] \quad (7)$$

For the case where particles obey the log-normal density function up to some truncation point (as in the sieving fractionation of the less than 5-μm Corrette fraction), the above equations are combined, and a finite upper integration limit, d_{max}, is substituted to give

$$r = \frac{S_N \rho}{6} \frac{\int_0^{d_{max}} f(d)\, d^3\, \mathrm{d}(d)}{\int_0^{d_{max}} f(d)\, d^2\, \mathrm{d}(d)} \quad (8)$$

The integrals in the above equation can be evaluated by computer using Simpson's rule of integration.

Literature Cited

(1) Goetz, A. *Int. J. Air Water Pollut.* **1961**, *4*, 168.
(2) Corn, M.; Montgomery, T. L.; Esmen, N. A. *Environ. Sci. Technol.* **1971**, *5*, 155.
(3) Kaakinen, J. E.; Jordan, R. M.; Lawasani, M. H.; West, R. E. *Environ. Sci. Technol.* **1975**, *9*, 862.
(4) Brunauer, S.; Emmett, P. H.; Teller, E. *J. Am. Chem. Soc.* **1938**, *60*, 309.
(5) Ditl, P.; Coughlin, R. W. *Environ. Sci. Technol.* **1977**, *11*, 701.
(6) Bauer, C. F. Ph.D. Dissertation, Colorado State University, Ft. Collins, CO, 1979.
(7) Rothenberg, S. J. *Atmos. Environ.* **1980**, *14*, 445.
(8) Rothenberg, S. J.; Cheng, Y. S. *J. Phys. Chem.* **1980**, *84*, 1644.
(9) Ondov, J. "Status of Research on Physical Chemical and Biological Characterization of Particulate and Organic Emissions from Conventional and Fluidized Bed Combustion of Coal: 1976 to the Present"; National Technical Information Service, U.S. Department of Commerce: Springfield, VA, 1983; DOE/ER-0162.
(10) McFarland, A. R.; Bertsch, R. W.; Fisher, G. L.; Prentice, B. A. *Environ. Sci. Technol.* **1977**, *11*, 781.
(11) Fisher, G. L.; Prentice, B. A.; Silberman, D.; Ondov, J. M.; Biermann, A. H.; Ragaini, R. C.; McFarland, A. R. *Environ. Sci. Technol.* **1978**, *12*, 447.
(12) Soltys, P. A. Masters Thesis, Colorado State University, Ft. Collins, CO, 1980.
(13) Gregg, S. J.; Sing, K. S. W. "Adsorption, Surface Area, and Porosity"; Academic Press: New York, 1967.
(14) Wade, W. H. *J. Phys. Chem.* **1964**, *68*, 1029.
(15) Wade, W. H. *J. Phys. Chem.* **1965**, *69*, 322.
(16) Pierce, C. *J. Phys. Chem.* **1959**, *63*, 1076.
(17) Kanellopoulos, N. K.; Petrou, J. K.; Petropoulos, J. H. *J. Colloid Interface Sci.* **1983**, *96*, 90.
(18) Bruanuer, S.; Mikhail, R. S.; Bodor, E. E. *J. Colloid Interface Sci.* **1968**, *26*, 45.
(19) Adamson, A. W. "Physical Chemistry of Surfaces"; Wiley: New York, 1976.
(20) deBoer, J. H.; Lippens, B. C.; Linsen, B. G.; Broekhoff, J. C. P.; van den Heuvel, A.; Osinga, Th. J. *J. Colloid Interface Sci.* **1966**, *21*, 405.
(21) Schure, M. R. Ph.D. Dissertation, Colorado State University, Ft. Collins, CO, 1981.
(22) Smith, R. D.; Campbell, J. A.; Nielson, K. K. *Atmos. Environ.* **1979**, *13*, 607.
(23) Ondov, J. M.; Ragaini, R. C.; Biermann, A. H. *Atmos. Environ.* **1978**, *12*, 1175.
(24) Fisher, J. L.; Chang, D. P. Y.; Brummer, M. *Science (Washington, D.C.)* **1976**, *192*, 553.
(25) Fisher, J. L.; Silberman, D. *Anal. Chim. Acta* **1979**, *106*, 299.
(26) Miguel, A. H.; Korfmacher, W. A.; Wehry, E. L.; Mamantov, G.; Natusch, D. F. S. *Environ. Sci. Technol.* **1979**, *13*, 1229.
(27) Raabe, O. G. *Aerosol Sci.* **1971**, *2*, 289.

Received for review April 17, 1984. Accepted July 30, 1984. This work was supported by the Department of Energy under Grant DE-AC02-78EV04960.A003.

Environ. Sci. Technol. 1985, 19, 87–90

Rate Constants for the Gas-Phase Reactions of NO_3 Radicals with Furan, Thiophene, and Pyrrole at 295 ± 1 K and Atmospheric Pressure

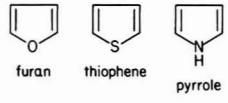
Roger Atkinson,* Sara M. Aschmann, Arthur M. Winer, and William P. L. Carter

Statewide Air Pollution Research Center, University of California, Riverside, California 92521

■ By use of a relative rate technique, rate constants have been determined for the gas-phase reactions of NO_3 radicals with furan, thiophene, and pyrrole, possible heterocyclic impurity emissions from shale oil derived fuels. These rate constant measurements were relative to rate constants for the reaction of NO_3 radicals with propene, *trans*-2-butene, and 2-methyl-2-butene, which in turn are based on the equilibrium constant for the $NO_2 + NO_3 \rightleftarrows N_2O_5$ reactions. On the basis of an equilibrium constant for these reactions of $2.4 \times 10^{-27} (T/300)^{0.32} e^{11080/T}$ cm^3 molecule^{-1}, the NO_3 radical reaction rate constants obtained at 295 ± 1 K and atmospheric pressure were the following (in cm^3 molecule^{-1} s^{-1} units): furan, $(1.4 \pm 0.2) \times 10^{-12}$; thiophene, $(3.2 \pm 0.7) \times 10^{-14}$; pyrrole, $(4.9 \pm 1.1) \times 10^{-11}$. From these data and our previous kinetic data for the reactions of OH radicals and O_3 with these heterocycles, it is evident that nighttime reaction with the NO_3 radical, a common constituent of continental nighttime atmospheres at levels of ~10–100 parts per trillion, can be an important, and even dominant, loss process for these organics.

Introduction

Furan, thiophene, and pyrrole are five-member heterocycles that are anticipated to be emitted in association with alternate energy production processes such as coal gasification and shale- and coal-based oil production (*1*). In order to assess the atmospheric lifetimes and fates of these heteroatom-containing organics, the kinetics and mechanisms of their reactions with OH and NO_3 radicals and with O_3 need to be determined (*2*, *3*).



Since reaction with the NO_3 radical has been shown to be an important atmospheric loss process for several classes of organics (*3–7*), we have determined the rate constants for the gas-phase reaction of NO_3 radicals with furan, thiophene, and pyrrole at 295 ± 1 K as part of an experimental and chemical kinetic computer modeling study of the atmospheric chemistry of these and other constituents of shale oil derived aviation fuels (*8*). Kinetic data for the reactions of OH radicals and O_3 with these three heterocycles have been reported previously (*9*, *10*).

Experimental Section

The experimental technique used for the determination of NO_3 radical reaction rate constants was a relative rate method which has been described in detail previously (*3–6*). This technique is based upon monitoring the relative decay rates of a series of organics, including at least one organic whose NO_3 radical reaction rate constant is reliably known, in presence of NO_3 radicals. NO_3 radicals were generated by the thermal decomposition of N_2O_5 (*11*) in air:

$$N_2O_5 \xrightarrow{M} NO_2 + NO_3 \quad (1)$$

$$NO_2 + NO_3 \xrightarrow{M} N_2O_5 \quad (2)$$

Providing that furan, thiophene, and pyrrole and the reference organics reacted only with NO_3 radicals (see below)

$$NO_3 + \text{heterocycle} \rightarrow \text{products} \quad (3)$$

$$NO_3 + \text{reference organic} \rightarrow \text{products} \quad (4)$$

then (*3–6*)

$$\ln\left(\frac{[\text{heterocycle}]_{t_0}}{[\text{heterocycle}]_t}\right) - D_t = \frac{k_3}{k_4}\left[\ln\left(\frac{[\text{reference organic}]_{t_0}}{[\text{reference organic}]_t}\right) - D_t\right] \quad (I)$$

where $[\text{heterocycle}]_{t_0}$ and $[\text{reference organic}]_{t_0}$ are the concentrations of the heterocycle and the reference organic, respectively, at time t_0, $[\text{heterocycle}]_t$ and $[\text{reference organic}]_t$ are the corresponding concentrations at time t, k_3 and k_4 are the rate constants for reactions 3 and 4, respectively, and D_t is the dilution factor at time t, due to the small amounts of dilution occurring from the incremental additions of N_2O_5 to the reactant mixtures. [During these experiments, the dilution factor D_t was typically 0.0015 (i.e., ~0.15%) per N_2O_5 addition.] Hence, plots of $[\ln([\text{heterocycle}]_{t_0}/[\text{heterocycle}]_t) - D_t]$ vs. $[\ln([\text{reference organic}]_{t_0}/[\text{reference organic}]_t) - D_t]$ should yield straight lines of slope k_3/k_4 and zero intercepts.

With this experimental technique, the intitial concentrations of the heterocycles and the reference organics were ~1–4 ppm (1 ppm = 2.41×10^{13} molecule cm^{-3} at 295 K and 735 torr total pressure), and up to five incremental amounts of N_2O_5 [~(0.1–3) ppm per addition] were added to the chamber during an experiment. In order to extend the reaction times beyond the mixing time, 2–10 ppm of NO_2 was also included in the reaction mixtures to drive the equilibrium between NO_3 radicals, NO_2, and N_2O_5 toward N_2O_5.

The reference organics propene, *trans*-2-butene, and 2-methyl-2-butene and the heterocycles were quantitatively monitored during these experiments by gas chromatography with flame ionization detection (GC–FID). Propene was analyzed on a 34 ft × 0.125 in. stainless steel (SS) column of 10% 2,4-dimethylsulolane on C-22 Firebrick (60/80 mesh), operated at 273 K. *trans*-2-Butene, 2-methyl-2-butene, furan, and thiophene were analyzed on a 20 ft × 0.125 in. stainless steel (SS) column packed with 5% 50/50 mixture of DC703/C20M on 100/120 mesh AW, DMCS Chromosorb G, operated at 333 K. For pyrrole, as described previously (*10*), gas samples of 100 cm^3 volume were drawn through 3.5 in. × 0.25 in. glass traps packed with Tenax GC (60/80 mesh) adsorbent. These samples were then transferred by the carrier gas at ~523 K from these traps to the head of a 6 ft × 0.25 in. glass column packed with 4% Carbowax 20M + 0.8% KOH on Super Pak II, which was initially at 373 K. The column was then temperature programmed to 433 K at 10 K min^{-1}.

All rate constant determinations were carried out at 295 ± 1 K and atmospheric pressure (~735 torr) in a ~4000-L all-Teflon chamber, with dry purified matrix air (*12*) as the diluent gas. As described previously (*3–6*), N_2O_5 was

0013-936X/85/0919-0087$01.50/0

Table I. Relative Rate Constant Ratios k_3/k_4 and Rate Constants k_3 for the Reaction of NO_3 Radicals with Furan, Thiophene, and Pyrrole at 295 ± 1 K and Atmospheric Pressure

heterocycle	k_3/k_4[a] relative to: propene	*trans*-2-butene	2-methyl-2-butene	k_3, cm^3 molecule^{-1} s^{-1} [b]	
furan		3.72 ± 0.05		$(1.4 \pm 0.2) \times 10^{-12}$	
thiophene	4.14 ± 0.09	0.092 ± 0.023		$(3.1 \pm 0.7) \times 10^{-14}$,[c] $(3.5 \pm 1.0) \times 10^{-14}$[d]	$(3.2 \pm 0.7) \times 10^{-14}$[e]
pyrrole			4.92 ± 0.09	$(4.9 \pm 1.1) \times 10^{-11}$	

[a] Indicated erros are two least-squares standard deviations derived from the slopes of the plots shown in Figures 1–3. [b] Based upon an equilibrium constant for the reaction $NO_2 + NO_3 \rightleftarrows N_2O_5$ of $(2.4 \times 10^{-27})(T/300)^{0.32}e^{11080/T}$ cm^3 molecule^{-1} (*11*, *15*). The indicated errors are two least-squares standard deviations and take into account the errors in the rate constants k_4 of $(7.6 \pm 1.6) \times 10^{-15}$ cm^3 molecule^{-1} s^{-1} for propene, $(3.80 \pm 0.43) \times 10^{-13}$ cm^3 molecule^{-1} s^{-1} for *trans*-2-butene, and $(9.9 \pm 2.2) \times 10^{-12}$ cm^3 molecule^{-1} s^{-1} for 2-methyl-2-butene (*3*, *4*, *11*, *15*). [c] Relative to propene. [d] Relative to *trans*-2-butene. [e] Weighted average.

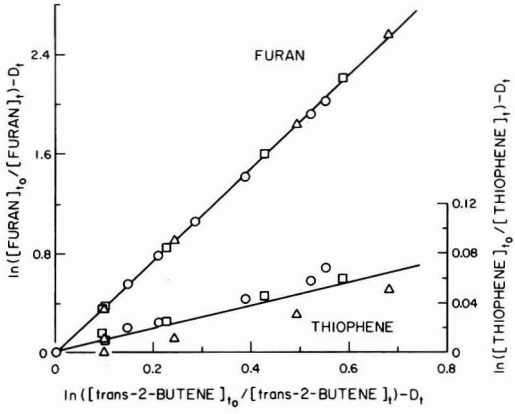


Figure 1. Plots of eq I for furan and thiophene, with *trans*-2-butene as the reference organic [initial NO_2 concentrations: (O) 5, (Δ) 2, and (□) 8 ppm].

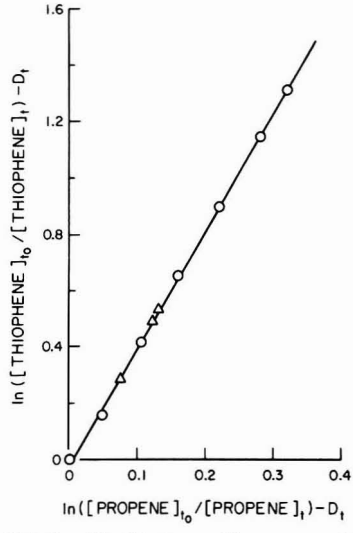


Figure 2. Plot of eq I for thiophene, with propene as the reference organic [initial NO_2 concentrations: (O) 4 and (Δ) 2 ppm].

prepared by the method of Schott and Davidson (*13*). Known pressures of N_2O_5 (as measured by an MKS Baratron capacitance manometer) in 1.0-L Pyrex bulbs were flushed into the chamber for 3 min by a 2 L min^{-1} flow of N_2 (≥99.995% purity level), with simultaneous rapid stirring by a fan rated at 300 L s^{-1}.

Furan, thiophene, and pyrrole were obtained from Aldrich Chemical Co., with stated purity levels of ≥99%, ≥99%, and 98%, respectively. No impurities were observed by GC–FID analyses.

Results

By use of this relative rate technique, rate constants were determined for the three heterocycles, with propene, *trans*-2-butene, or 2-methyl-2-butene as the reference organics. Duplicate experiments were carried out in all cases with differing initial NO_2 concentrations, and the data obtained are plotted in accordance with eq I in Figures 1–3. For thiophene initial experiments were carried out with *trans*-2-butene as the reference organic. However, because of the small amounts of thiophene consumed by reaction during these experiments (<7%), a further set of experiments was carried out with propene as the reference organic.

The rate constant ratios k_3/k_4 obtained by least-squares analyses of the slopes of the plots shown in Figures 1–3 are given in Table I. In all cases the least-squares intercepts of these plots were within three standard deviations of zero.

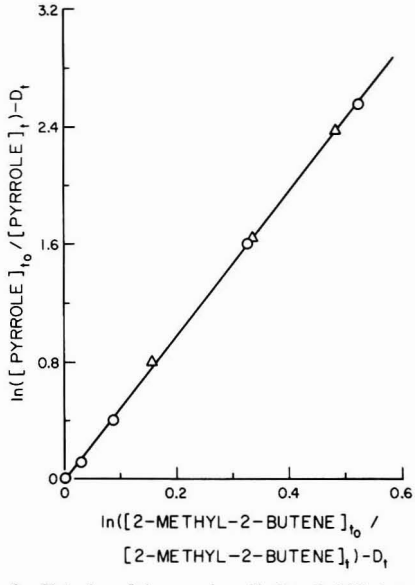


Figure 3. Plot of eq I for pyrrole, with 2-methyl-2-butene as the reference organic [initial NO_2 concentrations: (O) 5 and (Δ) 10 ppm].

Discussion

Apart from the plot of eq I for thiophene with *trans*-2-butene as the reference organic, the data shown in Figures 1–3 are in excellent accord with eq I. In previous studies the reference organics propene, *trans*-2-butene, and 2-

Table II. Rate Constants and Lifetimes for Furan, Thiophene, and Pyrrole due to Reaction with O_3 and OH and NO_3 Radicals at Room Temperature

heterocycle	rate constant, cm^3 molecule^{-1} s^{-1}			lifetime[a]		
	O_3	OH	NO_3[b]	O_3 (24 h)	OH (daytime)	NO_3 (nighttime)
furan	2.4×10^{-18} [c]	4.0×10^{-11} [c]	1.4×10^{-12}	6.7 day	6.9 h	50 min
thiophene	$<6 \times 10^{-20}$ [c]	9.6×10^{-12} [c]	3.2×10^{-14}	>270 day	29 h	36 h
pyrrole	1.6×10^{-17} [d]	1.2×10^{-10} [d]	4.9×10^{-11}	24 h	2.3 h	1.4 min

[a] Calculated for assumed clean tropospheric concentrations of 7.2×10^{11} molecule cm^{-3} (30 ppb) of O_3 (*22*), 1×10^6 cm^{-3} (0.04 ppt) of OH radicals (during daylight hours) (*23*), and 2.4×10^8 cm^{-3} (10 ppt) of NO_3 radicals (during nighttime hours) (*20*). [b] This work. [c] From ref 9. [d] From ref 10.

methyl-2-butene have been shown to react with NO_3 radicals, and not with N_2O_5 (*4*, *14*). Since the $[N_2O_5]/[NO_2]$, and hence the $[N_2O_5]/[NO_3]$, ratios were varied by factors of 2–4 in the present study, the excellent straight line plots in Figures 1–3 show that furan, thiophene, and pyrrole also react with NO_3 radicals, and not with N_2O_5. Furthermore, no observable decays of these heterocycles ($<5 \times 10^{-4}$ min^{-1}) were observed in the presence of 2–10 ppm of NO_2, showing that no significant reaction of NO_2 with these organics occurs. This was also confirmed by the excellent agreement of the sets of data obtained at differing initial NO_2 concentrations (Figures 1–3).

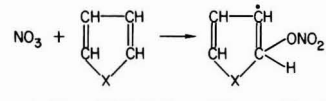
The rate constant ratios k_3/k_4 given in Table I can be placed on an "absolute" basis (but still linearly dependent on the value of the equilibrium constant used for the reactions $NO_2 + NO_3 \rightleftarrows N_2O_5$) by using the rate constants, k_4, for propene, *trans*-2-butene, and 2-methyl-2-butene (*3*, *4*). While in our earlier NO_3 radical rate constant studies (*3–6*) we used the $NO_2 + NO_3 \rightleftarrows N_2O_5$ equilibrium constant given by Malko and Troe (*11*), in this work we use our recently determined equilibrium constant of 3.4×10^{-11} cm^3 $molecule^{-1}$ at 298 K (*15*), which is a factor of 1.8 higher than that given by Malko and Troe (*11*). The rate constants k_3 so obtained are also given in Table I.

These rate constants show that NO_3 radicals react rapidly with furan and pyrrole, and substantially less rapidly with thiophene. This trend in reactivity toward the NO_3 radical of pyrrole > furan > thiophene mirrors those for their reactions with both OH radicals (*9*, *10*) and O_3 (*9*, *10*). The atmospheric importance of these NO_3 radical reactions can now be assessed by using the ambient concentrations of O_3 and of OH and NO_3 radicals.

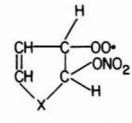
By use of long path-length differential optical absorption spectroscopy, NO_3 radical concentrations have been determined at a variety of locations in the United States and Europe (*16–21*). Measured NO_3 radical concentrations have ranged from the detection limit of the technique [$\sim$1 parts per trillion (ppt)] up to $\sim$350 ppt at a downwind receptor site in the Los Angles Air Basin (*16*), with levels of $\sim$10–100 ppt being routinely observed in relatively clean air masses in southern California (*20*).

Table II gives the room temperature rate constants for the reactions of these three heterocycles with OH and NO_3 radicals and O_3 and their corresponding atmospheric lifetimes under "clean" tropospheric conditions. In these lifetime calculations, O_3, daytime OH radical, and nighttime NO_3 radical concentrations of 7.2×10^{11} molecule cm^{-3} (*22*), 1×10^6 cm^{-3} (*23*), and 2.4×10^8 cm^{-3} (*20*), respectively, were used. These data show that nighttime reactions with the NO_3 radical can be an important, if not dominant, loss process for these heterocycles. While for thiophene (under the conditions employed for the calculations in Table II) the nighttime NO_3 radical reaction is of similar importance as a loss process as is the daytime OH radical reaction, for furan and pyrrole the NO_3 radical reactions lead to significantly shorter lifetimes than do the corresponding daytime OH radical reactions. Thus, as in the case of the more reactive alkenes (including the monoterpenes) (*3*, *4*, *7*, *14*, *24*), dimethyl sulfide (*5*, *7*), and the hydroxy-substituted aromatics (*6*, *25*), the NO_3 radical reactions with thiophene, furan, and pyrrole must be considered in assessing the atmospheric lifetimes and fates of these heteroatom-containing organics.

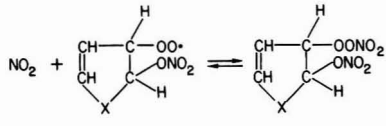
Finally, while we have now determined the kinetics of these reactions, the products formed under atmospheric conditions are not yet known. On the basis of the analogy with the alkenes and dialkenes (*3*, *4*, *14*, *26*), it is expected that these NO_3 radical reactions with furan, thiophene, and pyrrole will proceed via initial addition of the NO_3 radical to the olefinic double bonds:



where X ≡ O, S, or NH, followed by rapid addition of O_2 to yield the peroxy radical



While reaction with NO_2 can then yield the thermally unstable peroxynitrates



the ultimate fates of these peroxy radicals under nighttime conditions are not known, though it is likely that ring cleavage will ultimately occur, which leads to species such as CHOCH=CHXCHO. Obviously, product studies are necessary before such reaction pathways can be completely elucidated.

Literature Cited

(1) Sickles, J. E., II; Eaton, W. C.; Ripperton, L. A.; Wright, R. S. "Literature Survey of Emissions Associated with Emerging Energy Technologies". 1977, EPA-600/7-77-104.
(2) Atkinson, R.; Darnall, K. R.; Lloyd, A. C.; Winer, A. M.; Pitts, J. N., Jr. *Adv. Photochem.* **1979**, *11*, 375–488.
(3) Atkinson, R.; Aschmann, S. M.; Winer, A. M.; Pitts, J. N., Jr. *Environ. Sci. Technol.* **1984**, *18*, 370–375.
(4) Atkinson, R.; Plum, C. N.; Carter, W. P. L.; Winer, A. M.; Pitts, J. N., Jr. *J. Phys. Chem.* **1984**, *88*, 1210–1215.
(5) Atkinson, R.; Pitts, J. N., Jr.; Aschmann, S. M. *J. Phys. Chem.* **1984**, *88*, 1584–1587.
(6) Atkinson, R.; Carter, W. P. L.; Plum, C. N.; Winer, A. M.; Pitts, J. N., Jr. *Int. J. Chem. Kinet.* **1984**, *16*, 887–898.
(7) Winer, A. M.; Atkinson, R.; Pitts, J. N., Jr. *Science (Washington, D.C.)* **1984**, *224*, 156–159.

(8) Carter, W. P. L.; Winer, A. M.; Atkinson, R.; Dodd, M. C.; Long, W. D.; Aschmann, S. M. "Atmospheric Photochemical Modeling of Turbine Engine Fuels. Phase I: Environmental Chamber Studies". Final report to Engineering and Services Laboratory, Air Force Engineering and Services Center, Tyndall Air Force Base, FL, 1984.
(9) Atkinson, R.; Aschmann, S. M.; Carter, W. P. L. *Int. J. Chem. Kinet.* **1983**, *15*, 51-61.
(10) Atkinson, R.; Aschmann, S. M.; Winer, A. M.; Carter, W. P. L. *Atmos. Environ.* **1984**, *18*, 2085-2087.
(11) Malko, M. W.; Troe, J. *Int. J. Chem. Kinet.* **1982**, *14*, 399-416.
(12) Doyle, G. J.; Bekowies, P. J.; Winer, A. M.; Pitts, J. N., Jr. *Environ. Sci. Technol.* **1977**, *11*, 45-51.
(13) Schott, G.; Davidson, N. *J. Am. Chem. Soc.* **1958**, *80*, 1841-1853.
(14) Japar, S. M.; Niki, H. *J. Phys. Chem.* **1975**, *79*, 1629-1632.
(15) Tuazon, E. C.; Sanhueza, E.; Atkinson, R.; Carter, W. P. L.; Winer, A. M.; Pitts, J. N., Jr. *J. Phys. Chem.* **1984**, *18*, 3095-3098.
(16) Platt, U.; Perner, D.; Winer, A. M.; Harris, G. W.; Pitts, J. N., Jr. *Geophys. Res. Lett.* **1980**, *7*, 89-92.
(17) Noxon, J. F.; Norton, R. B.; Marovich, E. *Geophys. Res. Lett.* **1980**, *7*, 125-128.
(18) Platt, U.; Perner, D.; Schroder, J.; Kessler, C.; Toennissen, A. *J. Geophys. Res.* **1981**, *86*, 11965-11970.
(19) Platt, U.; Perner, D.; Kessler, C. Proceedings of the Second Symposium on Composition of the Non-Urban Troposphere, Williamsburg, VA, May 25-28, 1982.
(20) Platt, U. F.; Winer, A. M.; Biermann, H. W.; Atkinson, R.; Pitts, J. N. Jr. *Environ. Sci. Technol.* **1984**, *18*, 365-369.
(21) Pitts, J. N., Jr.; Biermann, H. W.; Atkinson, R.; Winer, A. M. *Geophys. Res. Lett.* **1984**, *11*, 557-560.
(22) Singh, H. B.; Ludwig, F. L.; Johnson, W. B. *Atmos. Environ.* **1978**, *12*, 2185-2196.
(23) Crutzen, P. J. In "Atmospheric Chemistry"; Goldberg, E. D., Ed.; Springer-Verlag: Berlin, 1982; pp 313-328.
(24) Atkinson, R.; Aschmann, S. M.; Winer, A. M.; Pitts, J. N., Jr. *Environ. Sci. Technol.*, in press.
(25) Carter, W. P. L.; Winer, A. M.; Pitts, J. N., Jr. *Environ. Sci. Technol.* **1981**, *15*, 829-831.
(26) Bandow, H.; Okuda, M.; Akimoto, H. *J. Phys. Chem.* **1980**, *84*, 3604-3608.

Received for review June 21, 1984. Accepted August 30, 1984. This work was supported by U.S. Air Force Contract FO8635-83-C-0278 (Project Officer, Daniel F. Stone).

On the Constancy of Sediment–Water Partition Coefficients of Hydrophobic Organic Pollutants

Philip M. Gschwend* and Shian-chee Wu

Ralph M. Parsons Laboratory, Department of Civil Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

■ If precautions are taken to eliminate or account for nonsettling (or nonfilterable) microparticles or organic macromolecules which remain in the aqueous phase during laboratory sorption tests, the observed partition coefficients (K_p or K_{oc}) for a group of model hydrophobic organic compounds (PCBs) are found to remain constant over a wide range of solid-to-solution ratios. Further, the partition coefficients for either sorptive uptake or desorptive release are indistinguishable and confirm the reversible nature of hydrophobic sorption. It is proposed that descriptions of the "speciation" of hydrophobic compounds in natural waters should include not only dissolved and sorbed-to-suspended-sediment fractions but also a component sorbed to nonsettling microparticles or organic macromolecules.

Introduction

One of the fundamental processes controlling the fate of hydrophobic organic compounds in aquatic environments is the exchange of these chemicals between dissolved and sorbed states. Many studies have shown that it is essential to distinguish dissolved from sorbed concentrations to describe either equilibrium conditions or kinetic rates. The tendency of hydrophobic organic compounds to associate with natural particles has been modeled by various workers (*1–3*) using an equilibrium expression for sorption: S (mass of compound/g of solid) = K_pC (mass of compound/mL of solution). Further, the equilibrium constant in this relation generally can be predicted as a product, $K_p = f_{oc}K_{oc}$, where f_{oc} is the organic carbon weight precent in the solids of interest and K_{oc} quantifies the hydrophobic partitioning tendency of the compound of interest to "generic" natural organic matter. K_{oc}'s are typically found to be constant within about a factor of 2 for sorption of a given hydrophobic compound to a variety of soils and sediments (e.g., ref 2). This simple partitioning model implies that sorption is reversible and that isotherms are linear. On the basis of our present "physical picture" of this process of sorption (*4*, *5*) as a phase equilibration in which only weak attractive forces (no covalent bonds or ion exchange) between organic solutes and natural organic matter are involved, these qualities (i.e., linearity and reversibility) are expected.

However, two sets of observations have been made that challenge this view of sorption. The first involves the results of several studies showing that the equilibrium partition coefficient (K_p or K_{oc}) decreases as the ratio of solids-to-water increases (e.g., see ref 6, and references therein, and 7). The second set of results suggests that sorption is not a reversible process; that is, some organic molecules sorbed to natural particles are not free to be released when the exterior water concentrations decline (*8–10*). Both of these results conflict with the view described above of hydrophobic organic sorption as a partitioning process between two immiscible "solvents", and both suggest that using the associated linear isotherm approach for predicting the fate of organic pollutants will be inaccurate.

Since it is difficult to envision mechanistic explanations for these complex sorptive phenomena, we initiated this study to determine if they resulted from artifacts associated with the common phase separation techniques employed in batch equilibration sorption experiments. If the phase separations in these experiments were incomplete, noncentrifugable or nonfilterable microparticles or organic macromolecules released from the solids remained in the aqueous phase. Carter and Suffet (*11*) and Voice et al. (*7*)

 0013-936X/85/0919-0090$01.50/0

have recently suggested the possibility that dissolved organic materials may cause the decline in partition constants with higher suspended solid loading, but they provided no direct evidence to support this assertion. In this report, we demonstrate that these nonsettling organic materials have a major impact on observed partition constants. Our results strongly suggest that this analytical consideration can fully explain the "solid concentration effect" and irreversible sorption phenomena observed in laboratory batch equilibration experiments. Moreover, we suggest that similar concepts must be employed in any discussion of truly dissolved hydrophobic pollutants in the "real world".

Materials and Methods

Materials. Several polychlorinated biphenyl isomers (Analabs Inc., North Haven, CT) were used as model nonpolar organic compounds for sorption studies. Isomers, containing two to seven chlorines (2Cl-PCB to 7Cl-PCB) to provide a range of hydrophobic tendencies (log K_{ow} of 5–7), were utilized as received. Lake Superior bottom sediment solids, obtained from Dr. Steven Eisenreich (box core S78-11 Bx, ref 12), had a 2.5% organic carbon content and were generally clayey in nature. The Missouri River sediments were supplied by Dr. Samuel Karickhoff (EPA-6, ref 2), exhibited an f_{oc} of 0.72%, and were of a silty-clay composition. Milli-Q water (Millipore, Bedford, MA) was used for aqueous preparations.

General Batch Equilibration Protocol. Several microliters of our stock PCB solution (at 2–90 ng of individual PCB isomers/μL of acetone) were spiked into 40 mL of water in 45-mL glass graduated centrifuge tubes with Teflon-lined screw caps. A preweighed portion of solids was then added to each tube to yield various, but known, solids-to-water ratios (ρ_s). The tightly capped tubes were then agitated with a wrist-action shaker for 48 h to allow sorption equilibration. Subsequently, the tubes were centrifuged at either 760*g* for 20 min or 1700*g* for 60 min (corresponding to complete settling of particles having a density of 1.2 g/cm^3 and a 1.0- or 0.4-μm diameter, respectively, based on Stokes' settling velocities) to separate the solid and aqueous phases. If desorption experiments followed, the supernatant was carefully withdrawn, replaced with clean water, agitated again, and finally recentrifuged. After centrifugation, the supernatant was transferred with a Pasteur pipet to a clean centrifuge tube; the pellet was resuspended in a minimum of Milli-Q water and transferred by pipet to a second centrifuge tube. The original cap and tube used for equilibration, the tube containing the water, and the tube with the sediments were each spiked with an internal standard (4-Cl PCB) and then were individually extracted overnight by shaking with 2 mL of 1:1 pentane–acetonitrile. The organic phase was collected from each sample, and the three fractions were each shaken briefly 2 additional times with 1 mL of pentane which was combined with the appropriate first extracts. The resultant organic extracts were spiked with a second internal standard (6-Cl PCB) and reduced to approximately 0.2 mL by evaporating under a N_2 gas stream.

The PCB contents of these extracts were ultimately determined by glass capillary gas chromatography with electron capture detection. This GC approach allows quantitation of several isomers simultaneously and provides direct evidence that biodegradation of sorbates had not occurred even over prolonged equilibrations. The instrument was a Carlo Erba Fractovap, Model 2100, equipped with a ^{63}Ni electron capture detector. The 15 m long by 0.32 mm i.d. fused silica capillary column coated with SE-54 (Analabs, Inc., North Haven, CT) was operated with H_2 carrier at 0.5 m/s linear velocity and was held isothermally at 70 °C for 2 min and then programmed from 70 to 250 °C at 6 °C/min; splitless injections were made. The detector was operated at 300 °C with 5% CH_4–Ar in the constant current mode with 0.5-μs pulse intervals, a differential electrode potential of 50 V, and a standing current of about 250 nA.

PCB peak heights were used for quantitation, and results were not corrected for losses as both standards were consistently recovered with about 95% efficiency. Some losses of 2-Cl and 3-Cl compounds were commonly observed (80% and 85% average recoveries, respectively, while more highly chlorinated PCB's were always recovered at >90%), and these were traced to losses during evaporation under N_2. Losses of PCB's to the glass walls of the equilibration tubes plus Teflon liners of the screw caps proved to be less than 4% of the dissolved PCB load in all cases. Analytical reproducibility for concentration determinations in each phase from replicate equilibration experiments was ±10%, which indicates a precision on our K_p determinations of better than ±15% due to propagation of errors. Since f_{oc} could be measured to within ±5%, calculated K_{oc} errors were ±15%.

In some cases, the sediments used for equilibrations were "prewashed" to remove nonsettling microparticles and macromolecules. This washing was done by suspending the sediments in 40 mL of water in the 45-mL graduated centrifuge tubes, shaking for 48 h, centrifuging at 760*g* for 20 min, and discarding the supernatant. After several (approximately five) washes, the solids were used in the batch experiments.

In order to assess the impact of prewashing sediments, the quantities of solids lost with the discarded supernatants were determined in three ways. Small aliquots of the supernatant were carefully evaporated on small preweighed aluminum pans, and the dry weight was determined with a Cahn microbalance. Additionally, turbidity of the supernatant was monitored by measuring the absorbance of the solution in a 10-cm cell at 500 nm in a spectrophotometer. Finally, dissolved organic carbon (DOC) measurements were made on the supernatants by persulfate wet oxidation of acidified CO_2-free aliquots (*13*) and subsequent CO_2 determination with a Shimadzu GC 8A (Columbia, MD) equipped with a thermal conductivity detector. The organic carbon content of the noncentrifugable microparticles and macromolecules is the ratio of the "DOC" to the dry weights.

Results and Discussion

Nonsettling Microparticles or Macroparticles in the Supernatant. All three measures of "dissolved" sorbing phase (weight of dissolved solids, turbidity, and dissolved organic carbon) demonstrated the increased loading of nonsettling microparticles or macromolecules (NSPs) in the supernatants of batch equilibrium experiments as the solids-to-water (ρ_s) ratio increased (Figure 1). The NSPs were seen to increase in a fixed proportion to the total solids. For the Missouri River sediment this fraction (weight of NSP/weight of solids) was 5.6%, while the Lake Superior solids maintained a 4.9% NSP fraction under the conditions of our phase separations. At suspended solids concentrations less than 500 mg/L, we were unable to measure NSPs by dissolved organic carbon methods due to blank problems. Voice et al. (*7*) also attempted to assess NSPs by turbidity and DOC measurements of supernatants recovered from equilibrations with 10–200 mg of solids/L and had poor success, concluding that the methods were insufficient. Nonetheless, it is clear

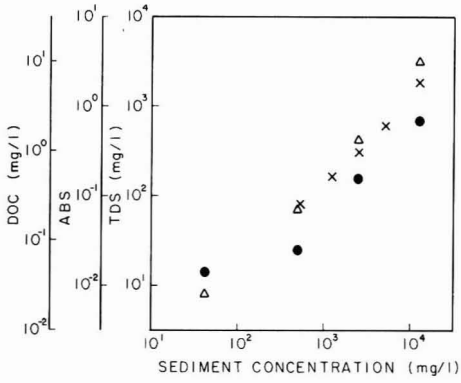


Figure 1. Nonsettling particles in the first wash supernatants vs. varying Missouri River solid concentrations as determined by DOC (crosses), light absorption (triangles), and total dissolved solids (solid circles).

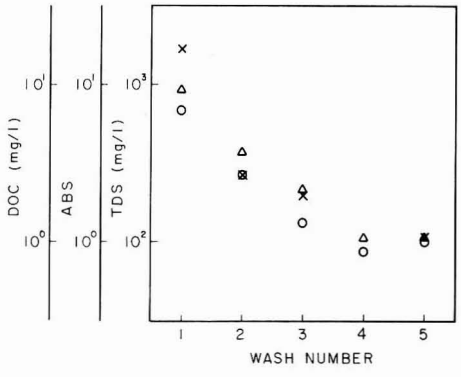


Figure 2. Nonsettling particles in successive washes of Lake Superior sediment (12 000 mg/L) as determined by DOC (crosses), light absorption (triangles), and total dissolved solids (circles).

from our data that NSPs vary regularly with suspended solid concentration.

That this occurs should not be particularly surprising. First, particle size distributions of natural sediments and soils are undoubtably continuous and do not drop to zero abundance in the region of typical centrifugation or filtration separation capabilities. Additionally, there is some evidence to indicate that the DOC and POC (particulate organic carbon) in natural waters are in dynamic equilibrium, causing new particles or new dissolved molecules to be formed when others are removed (*14*, *15*). Finally, experiments with soil columns have shown that natural soils can release large quantities of DOC into percolating fluids (*16*). Consequently, since there are NSPs remaining in the aqueous phase, we should take their effects into account.

One approach to diminish the NSPs effect is to try to reduce their abundance, for example, by washing the sediments. Figure 2 shows the decrease in NSP content of supernatants after successive washes of 12 000 mg/L Lake Superior sediment. The NSP content dropped by about an order of magnitude, yet remained at an amazingly high level of 100 mg/L even after five washes. As a result, if K_p for readily settled particles is desired, we recommend the strategy of centrifuging washes relatively weakly (e.g., 760*g* for 20 min) and discarding the supernatant containing microparticles and macromolecules that may be incompletely settled during subsequent phase separation operations (e.g., 1700*g* for 60 min).

Using the quotient of the observed DOC and NSP weight results, we calculated the f_{oc} values for the NSPs in our experiments. Lake Superior NSPs contained 2.5% organic carbon, and Missouri River NSPs had 0.83% organic carbon. Both results are indistinguishable from the f_{oc} values of the parent solids, and as a result we did not find that washing significantly affected the sediment f_{oc}. Thus, at least for these laboratory-derived microparticles and macromolecules, it may be reasonable to assign to them a similar affinity for hydrophobic compounds as that exhibited by the larger mass of solids (i.e., $K_{NSP} = K_p$ or $K_{oc\text{-}NSP} = K_{oc}$). This assumption is reasonable if the organic matter of the NSPs is of similar composition as that in the settling solids and if sorption is truly a partitioning process.

Other recent studies have provided mixed insights to this last assumption. Means and Wijayaratne (*17*) found that atrazine and linuron sorbed 10–35 times more strongly on natural colloids than on soils and sediments, suggesting that small particles or macromolecules should be treated with enhanced K_{oc}'s. If sorption entails an *ad*sorption process to the "surface" exposed by organic macromolecules, then K_{oc}'s associated with colloids could reasonably be expected to be greater than those for soil and sediment organic matter since colloidal organic matter has a greater surface area per mass of organic carbon. However, it is known that nitrogen-containing organic compounds may "sorb" by ion exchange (*18*, *19*) or by condensation reactions (*18*, *20–22*) in addition to hydrophobic interactions. Since the sorption constants observed for atrazine and linuron varied with pH (*17*), these nonhydrophobic sorption mechanisms were likely operating. Thus, these enhanced K_{oc}'s may simply reflect the greater reactive nature of colloidal organic matter than that in sediments and soils. In contrast, Carter and Suffet (*11*) have focused on DDT (nonionizable and unable to participate in condensation reactions) sorption to natural humic acids and found carbon normalized partition constants ($\log K_{oc} = 4.8$–5.7) which were similar to values previously reported for soils [$\log K_{oc} = 5.3$ (*23*)]. Finally, at least for some components of the DOC, one could reasonably expect the phase equilibrium partition coefficients to be less than those of the organic matter on particles since macromolecules in solution must be relatively hydrophilic in nature to maintain favorable aqueous interactions. This view is supported by the reports describing heteroatom compositional differences between fulvic and humic acids recovered from natural waters. The smaller, more water-soluble fulvic acids have higher oxygen-to-carbon ratios compared to the larger humic compounds (*24*). Presumably the sequence of "defunctionalization" continues into condensed phases (*25*). Thus, smaller, more water-soluble macromolecules may be expected to be more polar sorbents (i.e., exhibit relatively lower K_{oc}'s) than related larger macromolecules and particulate organic matter. Clearly more work is needed to understand these possibilities.

Effect of Suspended Solids on Observed Partition Coefficients. The observed sorption partition coefficients (determined with no precautions against NSP effects) were found to diminish with suspended solid loadings as reported previously by many workers (Figure 3). This effect was strongest for the sediment with the greatest organic carbon content (i.e., Lake Superior) and for hydrophobic compounds with the strongest tendencies to sorb (i.e., 7-Cl > 5-Cl). This result is precisely as predicted if the observed ratio is viewed:

$$K_P^{obsd} = \frac{P/\text{mass particles}}{(D + N)/\text{volume of water}} \qquad (1)$$

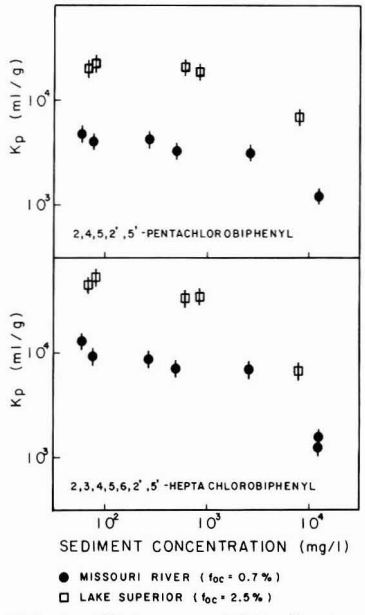


Figure 3. K_p for two PCB isomers vs. initial sediment concentration when no precautions are taken for NSPs. Missouri River (solid circles) and Lake Superior (squares).

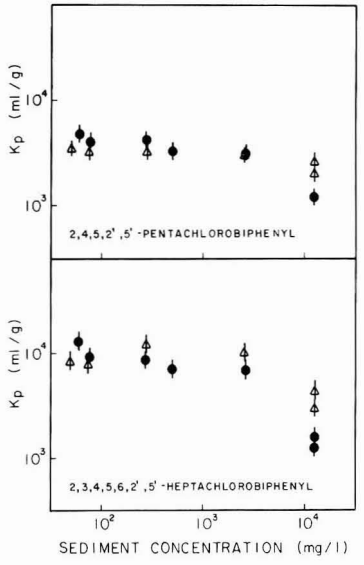


Figure 4. K_p for two PCB isomers vs. initial sediment (Missouri River) concentration when different centrifugation conditions are used: 760*g* for 20 min (solid circles) and 1700*g* for 60 min (triangles).

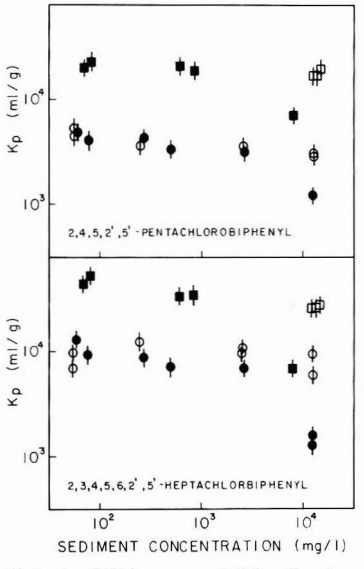


Figure 5. K_p for two PCB isomers vs. initial sediment concentration with (open symbols) and without (closed symbols) prewashing to remove NSPs. Missouri River (circles) and Lake Superior (squares).

where P is the mass of compound sorbed to settleable particles, D is the mass of compound dissolved, and N is the mass of compound sorbed to NSPs. At sufficiently low suspended solid loadings, the volume of water to weight of NSPs is great enough that the $D \gg N$; under these conditions the K_P^{obsd} could be considered the "true" value for the compound of interest. This is the value which should be used to quantify sorption to settling particles irrespective of the suspended solid concentration. However, as the suspended solid loadings are increased and the NSPs increase concomitantly and/or as the hydrophobicity of the compounds of interest increases, then D will no longer greatly exceed N and the K_P^{obsd} will decline accordingly. This observed partitioning constant has no real phase equilibration meaning.

By utilization of the simple approach of centrifuging with greater settling forces for longer periods to reduce the abundance of NSPs, it can be readily seen that the K_P^{obsd} remains unchanged at low ρ_s and increases somewhat in equilibrations with high ρ_s especially with the more hydrophobic 7-Cl PCB (Figure 4). Nonetheless, as indicated by these results and those of other workers (*7*), more stringent centrifugal conditions still do not eliminate NSPs completely, and the observed partition constants are inaccurate.

In order to greatly reduce the effect of the NSPs, a succession of prewashing treatments is necessary. This was particularly true for Lake Superior and Missouri River solids since they contained a significant proportion of fine clay grains, and the vigorous mixing provided by our wrist-action shaker undoubtedly continuously released these particles from larger aggregates. When prewashed sediments were used for batch equilibration experiments, the observed K_p remained virtually constant over the range of suspended solids tested (Figure 5). This is most dramatically shown for the partitioning of the most hydrophobic compound, 7-Cl PCB, and the difference in K_P^{obsd} with and without prewashing clearly reflects the great sensitivity of very strongly sorbed compounds to small NSP loadings in the aqueous phase.

Utilizing the measured NSP loadings in batch equilibrations with no prewashings, we can predict the K_P^{obsd} (or K_{oc}^{obsd}) vs. ρ_s. By definition

$$K_p^{true} = \frac{P/\text{mass settleable particles}}{D/\text{volume of water}} \quad (2)$$

By incorporating the hypothesis that some sorption to NSPs occurs in the supernatant [as suggested by this work and previous studies (*7*, *11*)], we have

$$K_P^{obsd} = \frac{P/\text{mass settleable particles}}{(D + N)/\text{volume of water}} \quad (3)$$

Combining (2) and (3)

$$K_p^{obsd} = K_p^{true}\left(1 + \frac{N}{D}\right)^{-1} \quad (4)$$

Now defining

$$K_{NSP}^{true} = \frac{N/\text{mass of NSPs}}{D/\text{volume of water}} \quad (5)$$

then

$$K_p^{obsd} = K_p^{true}\left(1 + K_{NSP}^{true}\frac{\text{mass of NSPs}}{\text{volume of water}}\right)^{-1} \quad (6)$$

Recalling $K_p = f_{oc}K_{oc}$ (and correspondingly $K_{NSP} = f_{oc\text{-}NSP}K_{oc\text{-}NSP}$), then we may write

$$K_{oc\text{-}p}^{obsd} = K_{oc\text{-}p}^{true}\left[1 + K_{oc\text{-}NSP}^{true}\frac{f_{oc\text{-}NSP}(\text{mass of NSPs})}{\text{volume of water}}\right]^{-1} \quad (7)$$

Finally, since the $f_{oc\text{-}NSP}$ was determined in our experiments by utilizing DOC measurements, then

$$\text{DOC} = \frac{f_{oc\text{-}NSP}(\text{mass of NSPs})}{\text{volume of water}} \quad (8)$$

and

$$K_{oc\text{-}p}^{obsd} = K_{oc\text{-}p}^{true}(1 + K_{oc\text{-}NSP}^{true}\,\text{DOC})^{-1} \quad (9)$$

Expressions 6 and 9 allow us to predict the observed change in partition coefficients due to varying suspended solid concentrations, if the values of K_{NSP} or $K_{oc\text{-}NSP}$ can be estimated. For example, using our measured fixed proportions of NSP mass to suspended solids (for Lake Superior, 4.9%, and for Missouri River, 5.6%), recalling that the organic carbon contents of the settleable and nonsettleable particles were identical, allowing K_p^{true} to be just high enough to fit the data at the lowest solids loads, and assuming $K_p^{true} = K_{NSP}^{true}$, we have predicted the observed K_p for the batch equilibrations with untreated suspended solids (Figure 6). The fit for the Missouri River solids for the entire range of sediment concentrations tested is excellent. Although the model shows the correct trend for experiments using Lake Superior solids, it overpredicts the decline in K_p^{obsd} by about a factor of 2 at the highest ρ_s. This may indicate that the $K_{oc\text{-}NSP}$ is lower than the $K_{oc\text{-}p}$ for these solids from Lake Superior. Despite this minor misfit, we conclude that the NSPs were the primary cause of declining observed K_p's and therefore that K_{oc} remains constant irrespective of the proportion of solids-to-solution. Interestingly, this result predicts that at high NSP masses the K_p's for all the PCBs collapse to the same values which are simply the volume of water/mass of NSPs.

Effect of Suspended Solids on Observed Sorption Reversibility. It has been suggested (*8*) that hydrophobic organic compound sorption to natural sediment particles may be irreversible to some degree. Unless either some unusual chemical bond between these nonreactive compounds (e.g., PCB's or PAH) and natural organic matter or mineral surfaces is forming or some deformation of the natural organic matter around the sorbates to form a "cagelike" structure is occurring, we do not understand how such an apparently irreversible uptake could take place (especially on the time scale of hours to days). Consequently, we hypothesized that irreversible sorption behavior could also be due to washing out the NSPs during sequential sorption and desorption experiments.

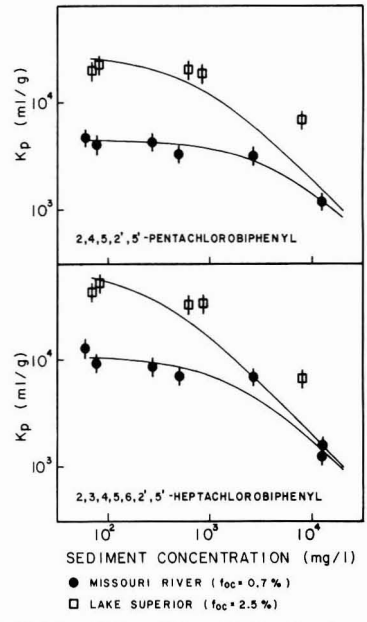


Figure 6. Model predictions of K_p vs. initial sediment concentrations assuming weight of NSPs/weight of solids is 5% and $K_{NSP} = K_p$.

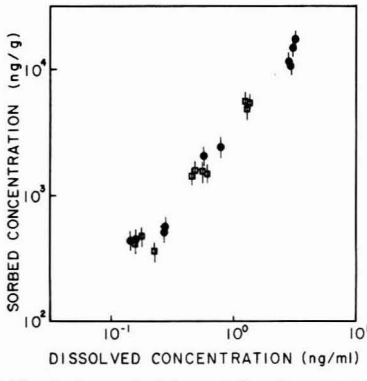


Figure 7. Dissolved vs. sorbed concentrations for pentachlorobiphenyl in sorption (solid circles) and desorption (squares) tests using prewashed Lake Superior solids.

In typical desorption protocols, a sorptive batch equilibration is performed by first establishing a sorption equilibrium between the solid and aqueous phases. In preparing to establish desorption equilibrium, the initial aqueous layer is discarded and with it the first wash load of NSPs, and clean water is added to take its place. After shaking and allowing hydrophobic compounds in the solids to exchange back into the aqueous phase, the solid and aqueous phases are separated again and concentrations determined in each. However, unlike the initial sorptive uptake experiment, the NSPs in this inadvertently prewashed condition are reduced in quantity, and the resultant aqueous load contains proportionately less NSP-sorbed material. Hence the *observed* $K_{oc}^{desorption}$ is greater than the previous $K_{oc}^{sorption}$. Further successive desorption tests will continue to be effected by NSPs less and less.

When we performed sorption and desorption experiments with prewashed sediments, our uptake and release isotherms were indistinguishable (Figure 7). This result was more easily obtained with less hydrophobic com-

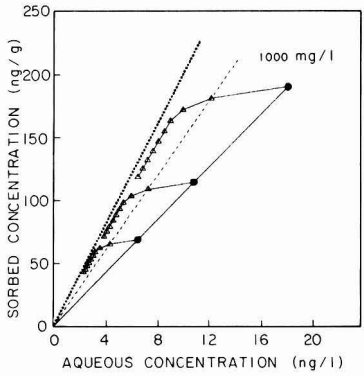


Figure 8. Model predictions for successive desorption equilibrations (triangles) after initial uptake experiments using 1000 mg of solids/L of solution (solid circles). Solid line through uptake data indicates observed uptake isotherm, while dashed line through first desorption points demonstrates associated desorption isotherm. Dotted line reflects uptake isotherm when NSPs have been eliminated or are negligible.

pounds, with solids containing lower organic contents, and with lower initial solid-to-water ratios as in these cases the resulting NSP-sorbed contents in equilibrations were more readily diminished relative to the dissolved concentrations.

Given our data on the NSP abundance in successive wash solutions of Lake Superior and Missouri River solids (NSPs sequentially declined to the following percentages of the solids: 5%, 2%, 1%, and ≈0.6% each wash thereafter), we can estimate the impact of these nonsettling sorbents in a typical succession of sorption-then-desorption equilibrations with ρ_s = 1000 mg/L. For example, DiToro and Horzempa (*8*) have studied the desorptive behavior of a hexachlorobiphenyl from Saginaw Bay sediments (f_{oc} ≈ 2.3%). Using eq 7 and their observed K_p and assuming an NSP loading of 5% in the first equilibration, we can calculate a "true" $K_p = 2.0 \times 10^4$ mL/g. By combining this result and our observed decline in NSPs with successive washes and by maintaining a mass balance for the hexachlorobiphenyl (i.e., system load of equilibration n equals system load of equilibration $n - 1$ minus the amount discarded with the aqueous phase), we can predict the desorptive isotherm points shown in Figure 8. These results are remarkably similar to those observed experimentally by DiToro and Horzempa, as well as in other studies (*9*, *10*) particularly when large solid-to-solution ratios were used. The isotherms predicted by using the first desorption equilibration data indicates a higher $K_{oc}^{desorption}$ than $K_{oc}^{sorption}$. These results would lead to the erroneous conclusions that irreversible binding was occurring and that there was a hysteretic effect in the extent of irreversibly bound material as a function of the initial dissolved concentrations to which the solids were exposed. Notably, previous studies showed successive desorption data eventually to trend toward the origin. We believe these data asymptotically coincided with the true isotherms as shown in Figure 8 for our model calculations using a relatively low ρ_s (and hence low NSPs concentration). Clearly the effects of nonsettling microparticles and macromolecules can account for these apparent nonideal sorption observations.

Implications. It is clear that precautions must be taken to avoid laboratory artifacts derived from incomplete phase separations. These precautions may consist of either elimination of the nonsettling (or nonfilterable) sorbents by prewashing the solids or by actually measuring the abundance of the NSPs in the otherwise presumed "purely" aqueous phase. Simply centrifuging at greater forces will be helpful but will not necessarily remove all the sorbents from suspension (especially neutrally buoyant organic macromolecules).

More importantly, our results support the idea that modeling sorption in the "real world" must include binding to two types of materials, one with and one without appreciable settling velocities. This second sorbing material includes a poorly characterized mixture of "microparticles" and dissolved macromolecules. This view of three exchanging environmental compartments is necessary not only to predict the actual transport properties of hydrophobic pollutants (i.e., their tendency to be removed from solution by settling to bottom sediments) but also to assess their water column chemical activities. It is likely that nonpolar compounds sorbed to nonsettling particles (be they humic or fulvic acids, colloidal aggregates, or other neutrally buoyant phases) behave much differently in various environmental processes such as bioconcentration and photo- or biodegradation than do the truly dissolved molecules. Since much of the DOC in natural waters is polymeric humic substances, measurements of DOC may allow us to roughly quantify nonsettling organic sorbents. Using this information in addition to knowledge of the suspended sediment load, we may more accurately estimate the hydrophobic compound partitioning between the three water column compartments. That is, we may evaluate the "speciation" of hydrophobic organic compounds with the following:

$$\text{dissolved fraction} = \frac{1}{1 + \text{DOC}\ K_{\text{oc-NSP}} + \text{POC}\ K_{\text{oc-p}}}$$

$$\text{NSP-sorbed fraction} = \frac{\text{DOC}\ K_{\text{oc-NSP}}}{1 + \text{DOC}\ K_{\text{oc-NSP}} + \text{POC}\ K_{\text{oc-p}}}$$

$$\text{fraction sorbed to settling particles} = \frac{\text{POC}\ K_{\text{oc-p}}}{1 + \text{DOC}\ K_{\text{oc-NSP}} + \text{POC}\ K_{\text{oc-p}}}$$

Summary

Our results support the view of Chiou and others that sorption of hydrophobic organic compounds to natural sediments and soils can be viewed as a phase partitioning process, quantifiable with a single K_{oc} equilibrium constant. This constant is the same for uptake or desorption and does not vary as the "volumes" of the solid and aqueous phases change with respect to one another. Previous experiments suggesting "complex" sorptive behaviors were probably subject to analytical artifacts caused by incomplete phase separations. Our work, taken in the context of recent studies showing sorption to natural microparticles or organic macromolecules, suggests that equilibrium environmental speciation of hydrophobic organic compounds should include three "phases": dissolved, sorbed to nonsettling particles or macromolecules, and sorbed to settling solids. With further work on partition constants appropriate for natural nonsettling particles or macromolecules, this three-phase equilibration process, possibly using site-specific DOC and POC measurements, can be used to greatly improve our predictive capabilities concerning the fate of hydrophobic organic pollutants.

Acknowledgments

We thank Robert Ambrose and Sam Karickhoff for fruitful discussions during this work. Oliver Zafiriou kindly made his gas chromatograph available for our use. We are

also grateful to Dave Dzombak, Bruce Brownawell, and Debera Backhus for comments on the manuscript.

Literature Cited

(1) Karickhoff, S. W.; Brown, D. S.; Scott, T. A. *Water Res.* **1979**, *13*, 241–248.
(2) Means, J. C.; Wood, S. G.; Hassett, J. J.; Banwart, W. L. *Environ. Sci. Technol.* **1980**, *14*, 1524–1528.
(3) Schwarzenbach, R. P.; Westall, J. *Environ. Sci. Technol.* **1981**, *15*, 1360–1367.
(4) Chiou, C. T.; Peters, L. J.; Freed, V. H. *Science (Washington, D.C.)* **1979**, *206*, 831–832.
(5) Chiou, C. T.; Porter, P. E.; Schmedding, D. W. *Environ. Sci. Technol.* **1983**, *17*, 227–231.
(6) O'Connor, D. J.; Connolly, J. P. *Water Res.* **1980**, *14*, 1517–1523.
(7) Voice, T. C.; Rice, C. P.; Weber, W. J., Jr. *Environ. Sci. Technol.* **1983**, *17*, 513–518.
(8) DiToro, D. M.; Horzempa, L. M. *Environ. Sci. Technol.* **1982**, *16*, 594–602.
(9) Swanson, R. A.; Dutt, G. R. *Soil Sci. Soc. Am. Proc.* **1973**, *37*, 872–876.
(10) Peck, D. E.; Corwin, D. L.; Farmer, W. J. *J. Environ. Qual.* **1980**, *9*, 101–106.
(11) Carter, C. W.; Suffet, I. H. *Environ. Sci. Technol.* **1982**, *16*, 735–740.
(12) Johnson, T. C.; Evans, J. E.; Eisenreich, S. J. *Limnol. Oceanogr.* **1982**, *27*, 481–491.
(13) Menzel, D. W.; Vacarro, R. F. *Limnol. Oceanogr.* **1964**, *9*, 138–142.
(14) Riley, G. A. *Adv. Mar. Biol.* **1970**, *8*, 1–118.
(15) Sheldon, R. W.; Sutcliffe, W. H.; Prakash, A. *Limnol. Oceanogr.* **1973**, *18*, 719–733.
(16) Leenheer, J. A.; Stuber, H. A. *Environ. Sci. Technol.* **1981**, *15*, 1467–1475.
(17) Means, J. C.; Wijayaratne, R. *Science (Washington, D.C.)* **1982**, *215*, 968–970.
(18) Stevenson, F. J. *ACS Symp. Ser.* **1976**, *No. 29*, 180–207.
(19) Karickhoff, S. W. *J. Hydraul. Div., Am. Soc. Civ. Eng.* **1984**, *110*, 707–735.
(20) Hsu, T.; Bartha, R. *Soil Sci.* **1974**, *116*, 444–452.
(21) Hsu, T.; Bartha, R. *J. Agric. Food Chem.* **1976**, *24*, 118–122.
(22) Appleton, H. T.; Banerjee, S.; Sikka, H. C. In "Dynamics, Exposure, and Hazard Assessment of Toxic Chemicals"; Haque, R., Ed.; Ann Arbor Science: Ann Arbor, MI, 1980; pp 251–272.
(23) Kenaga, E. E.; Goring, C. A. I. In "Aquatic Toxicology"; Eaton, J. G.; Parrish, P. R.; Hendricks, A. C., Eds.; American Society for Testing and Materials: Philadelphia, PA, 1980.
(24) Thurman, E. M.; Malcolm, R. L. *Environ. Sci. Technol.* **1981**, *15*, 463–466.
(25) Stuermer, D. H.; Peters, K. E.; Kaplan, I. R. *Geochim. Cosmochim. Acta* **1978**, *42*, 989–997.

Received for review March 19, 1984. Accepted September 4, 1984. This work was supported by U.S. EPA Contract CR 810472-01-0.

COPYRIGHT STATUS FORM

Name of American Chemical Society Publication

Author(s)

Ms No.

Ms Title

Received

This manuscript will be considered with the understanding you have submitted it on an exclusive basis. You will be notified of a decision as soon as possible.

Print or Type Author's Name and Address

[THIS FORM MAY BE REPRODUCED]

COPYRIGHT TRANSFER

The undersigned, with the consent of all authors, hereby transfers, to the extent that there is copyright to be transferred, the exclusive copyright interest in the above cited manuscript (subsequently referred to as the "work") to the **American Chemical Society** subject to the following (Note: if the manuscript is not accepted by ACS or if it is withdrawn prior to acceptance by ACS, this transfer will be null and void and the form will be returned.):

A. The undersigned author and all coauthors retain the right to revise, adapt, prepare derivative works, present orally, or distribute the work provided that all such use is for the personal noncommercial benefit of the author(s) and is consistent with any prior contractual agreement between the undersigned and/or coauthors and their employer(s).

B. In all instances where the work is prepared as a "work made for hire" for an employer, the employer(s) of the author(s) retain(s) the right to revise, adapt, prepare derivative works, publish, reprint, reproduce, and distribute the work provided that all such use is for the promotion of its business enterprise and does not imply the endorsement of the American Chemical Society.

C. Whenever the American Chemical Society is approached by third parties for individual permission to use, reprint, or republish specified articles (except for classroom use, library reserve, or to reprint in a collective work) the undersigned author's or employer's permission will also be required.

D. No proprietary right other than copyright is claimed by the American Chemical Society.

E. For works prepared under U.S. Government contract or by employees of a foreign government or its instrumentalities, the American Chemical Society recognizes that government's prior nonexclusive, royalty-free license to publish, translate, reproduce, use, or dispose of the published form of the work, or allow others to do so for noncommercial government purposes. State contract number: _____.

SIGN HERE FOR COPYRIGHT TRANSFER [Individual Author or Employer's Authorized Agent (work made for hire)]

Print Author's Name

Print Agent's Name and Title

Original Signature of Author on Behalf of All Authors (in Ink)

Date

Original Signature of Agent (in Ink)

CERTIFICATION AS A WORK OF THE U.S. GOVERNMENT

This is to certify that **ALL** authors are or were bona fide officers or employees of the U.S. Government at the time the paper was prepared, and that the work is a "work of the U.S. Government" (prepared by an officer or employee of the U.S. Government as a part of official duties), and, therefore, it is not subject to U.S. copyright. (This section should NOT be signed if the work was prepared under a government contract or coauthored by a non-U.S. Government employee.)

INDIVIDUAL AUTHOR **OR** AGENCY REPRESENTATIVE

Print Author's Name

Print Agency Representative's Name and Title

Original Signature of Author (in Ink)

Date

Original Signature of Agency Representative (in Ink)

FOREIGN COPYRIGHT RESERVED (NOTE: If your government permits copyright to be transferred, refer to section E and sign this form in the top section.)

☐ If **ALL** authors are employees of a foreign government that reserves its own copyright as mandated by national law, **DO NOT SIGN THIS FORM.** Please check this box as your request for the FOREIGN GOVERNMENT COPYRIGHT FORM (Blue Form) which you will be required to sign. **If you check this box**, mail this form to: Copyright Administrator, Books and Journals Division, American Chemical Society, 1155 Sixteenth Street, N.W., Washington, D.C. 20036, U.S.A.