U.S. Geological Survey
Toxic Substances Hydrology Program—

Proceedings of the Technical Meeting, Charleston, South Carolina, March 8–12, 1999

Water-Resources Investigations Report 99-4018B
Volume 2 of 3 - Contamination of Hydrologic Systems and Related Ecosystems

USGS
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Toxic Substances Hydrology Program— Proceedings of the Technical Meeting, Charleston, South Carolina, March 8-12, 1999

David W. Morganwalp and Herbert T. Buxton, Editors

U.S. GEOLOGICAL SURVEY

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U.S. DEPARTMENT OF THE INTERIOR

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PREFACE

The U.S. Geological Survey (USGS) Toxic Substances Hydrology (Toxics) Program was initiated in 1982. The goal of the Program is to provide earth science information on the behavior of toxic substances in the Nation's hydrologic environments. Contamination of surface water, ground water, soil, sediment, and the atmosphere by toxic substances is among the most significant issues facing the Nation. Contaminants such as excessive nutrients, organic chemicals, metals, and pathogens enter the environment, often inadvertently, via industrial, agricultural, mining, or other human activities. The extent of their migration and their persistence often are difficult to ascertain. Estimates of the costs and time frames for cleanup of contamination and protection of human and environmental health can best be described as astounding, despite continual efforts by governments and industries worldwide to improve environmental technologies.

Contaminant sources and environmental occurrence have a wide range of scales. Some contaminants are released from point sources, such as leaks or discharges from industrial facilities. Some are released from multiple, closely spaced releases, such as domestic septic systems. Still others are released relatively uniformly over broad areas with similar land-use practices, such as agricultural and residential land uses. Contaminants are detected at high concentrations locally in the immediate vicinity of a release, at varied concentrations where multiple releases disperse within watersheds or regional hydrologic systems, and at relatively low (but still potentially toxic) concentrations where they enter systems from broad uniform sources. Common to contamination at all these scales is the need to:

- Measure the contaminants and their transformation products in environmental samples;
- Characterize the physical processes and properties that affect their propagation in the environment;
- Define the chemical and microbial processes that transform or degrade the contaminants;
- Describe contaminant-biota interactions that control their effects on ecosystems, the food chain, and human health;
- Understand the ultimate fate of contaminants with the potential long-term implications for human and environmental health; and
- Develop simulation models that enable prediction of potential exposure and effective design of waste disposal facilities, monitoring networks, and remediation alternatives.

To meet these needs, the Toxics Program provides information and technology to Federal and State resource-management agencies and industry. The Toxics Program: (1) conducts intensive field investigations of representative cases of subsurface contamination at local releases; (2) conducts watershed- and regional-scale investigations of contamination affecting aquatic ecosystems from nonpoint and distributed point sources; and (3) develops methods and models — methods to detect, identify, and measure emerging environmental contaminants; and models to interpret the persistence and fate of contamination and to design waste-disposal and remediation strategies.

Intensive field investigations are established at sites contaminated with predominant types of environmental contamination, in commonly occurring geohydrologic and geochemical settings. Contamination types currently under investigation include chlorinated solvents, sewage effluent, toxic metals, radionuclides, and petroleum products, including fuel oxygenates. These long-term research projects are conducted by interdisciplinary research teams that comprehensively identify and characterize the physical, chemical, and biological processes that affect contaminant transport, transformation, and fate at the site. Through extensive characterization and field experimentation, the sites provide field-laboratory conditions that enhance research opportunities. Results from the sites are generalized by focused field and laboratory experiments at other sites that describe the range of field conditions for the controlling processes. Knowledge and methods produced at these representative sites improve the effectiveness and reduce the cost of characterization and remediation at similar sites across the Nation.

A unifying theme of these investigations is characterization of the natural response of hydrologic systems to contamination. This, when combined with comprehensive assessment of the processes that affect contaminant transport and fate, make assessing the potential of natural attenuation and remediation-performance monitoring undertakings in which the Toxics Program can excel. The long-term nature of the research provides a unique opportunity to evaluate the potential and limitations of natural-attenuation remediation alternatives.

Watershed- and regional-scale investigations are developed to address contamination problems typical of specific land uses or human activities that may pose a threat to human and environmental health throughout significant parts of the Nation. Current watershed- and regional-scale investigations address contamination from agricultural chemicals in the Midwest corn belt; cotton agriculture across the southern U.S.; human activities in estuarine ecosystems; historic hard-rock mining in watersheds in mountain terrain and southwestern alluvial basins; and mercury emissions on aquatic ecosystems.

In some cases, these investigations involve characterizing contaminant sources and their mechanisms for affecting aquatic ecosystems. This is the case in ongoing investigations of watersheds that may be affected by hundreds of abandoned mine sites. In some cases, watershed- and regional-scale investigations involve widespread detection of mixtures of contaminants or contaminant transformation products at levels near or below existing water-quality standards or advisories. This is the case in investigations of agricultural land uses which have documented that mixtures of pesticides and their metabolites accumulate to significantly higher levels than the individual parent compounds. In still other cases, these investigations identify chemicals in environmental samples for which standards have not yet been developed. In these cases, the Program provides information to resource managers and regulators that is useful for developing new water-quality standards or registering use of new chemicals, such as new pesticides or industrial chemicals.

These investigations complement the National Water-Quality Assessment (NAWQA) Program, which has the goal of assessing the status and trends of the quality of the Nation's ground- and surface-water resources. The Toxics Program watershed- and regional-scale investigations focus rapidly on new issues, emerging contaminants, and understanding the processes that affect whether a chemical may be of widespread concern. This information is used for planning future NAWQA Program activities.

New scientific models and methods are developed as part of both intensive field investigations and watershed- and regional-scale investigations. Simulation models provide tools to predict environmental occurrence and estimate exposure risks, as well as design remediation and monitoring strategies. A strength of models developed by the Toxics Program is that they are developed and applied to explain the complex field conditions at Program field sites. This makes them particularly well suited for application to real problems. New and improved water-quality analytical methods enable (1) detection of new chemicals in environmental samples, such as new pesticides and fuel oxygenates; (2) detection of chemicals at lower levels, which enables our understanding of the processes that control the environmental and human health effects of chemicals, such as mercury; and (3) identification of persistent transformation products of contaminants, such as pesticide metabolites. These methods and models are transferred to public and private practitioners for widespread use across the Nation.

Most scientists involved with Toxics Program activities are from the USGS National Research Program and District (state) Offices. However, as interdisciplinary approaches to solving contamination problems have become more successful, more ecologists, geologists, chemists, hydrologists, geochemists, and digital data-collection experts from across the USGS have become involved in Program activities. In addition, many scientists from universities, other Federal agencies, and industry are taking advantage of research opportunities afforded by the Program and its field sites, and are active members of the research teams.

Each project is steered by a core group of scientists from the research team. This core group, led by the project coordinator(s), guides the development of a research plan that integrates the multi-disciplinary activities at the site. They facilitate opportunities that become available for a wide range of related research. Although not an emphasis of the Toxics Program, many innovative, engineered-remediation technologies have been tested at the Program field sites because their extensive characterization provides a basis for effective evaluation of technology design and performance. Long-term data sets from the sites have been used by other Federal agencies to test decision-support software for site characterization or to test new hydrologic simulation models. Research plans for each project undergo periodic review by a panel of USGS and non-USGS scientists to improve the research approach and identify opportunities to enhance the research team. Field support for research projects is provided by experienced specialists located in the local USGS District Office.

The Toxics Program is coordinated with the U.S. Environmental Protection Agency, the U.S. Department of Agriculture, the Department of Defense, the Department of Energy, the Nuclear Regulatory Commission, and other U.S. Department of the Interior agencies to ensure that current and future research priorities are being addressed.

The long-term cooperation and assistance offered by the Federal, State, and local agencies, and by private entities that administer or own the Program's research sites has been essential to the success of the Toxics Program. Their continued support is greatly appreciated.

Herb Buxton
Coordinator, Toxic Substances
Hydrology Program

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INTRODUCTION

This report contains papers presented at the seventh Technical Meeting of the U.S. Geological Survey (USGS), Toxic Substances Hydrology (Toxics) Program. The meeting was held March 8-12, 1999, in Charleston, South Carolina. Toxics Program Technical Meetings are held periodically to provide a forum for presentation and discussion of results of recent research activities.

The objectives of these meetings are to:

- Present recent research results to essential stakeholders,
- Encourage synthesis and integrated interpretations among scientists with different expertise who are working on a contamination issue, and
- Promote exchange of ideas among scientists working on different projects and issues within the Toxics Program.

The Proceedings is published in three volumes. Volume 1 contains papers that report on results of research on contamination from hard-rock mining. Results include research on contamination from hard rock mining in arid southwest alluvial basins, research on hard rock mining in mountainous terrain, and progress from the USGS Abandoned Mine Lands Initiative. This Initiative is designed to develop a watershed-based approach to characterize and remediate contamination from abandoned mine lands and transfer technologies to Federal land management agencies and stakeholders.

Volume 2 contains papers on contamination of hydrologic systems and related ecosystems. The papers discuss research on the response of estuarine ecosystems to contamination from human activities. They include research on San Francisco Bay; mercury contamination of aquatic ecosystems; and investigation of the occurrence, distribution, and fate of agricultural chemicals in the Mississippi River Basin. This volume also contains results on development and reconnaissance testing of new methods to detect emerging contaminants in environmental samples.

Volume 3 contains papers on subsurface contamination from point sources. The papers discuss research on: hydrocarbons and fuel oxygenates at gasoline release sites; ground-water contamination by crude oil; complex contaminant mixtures from treated wastewater discharges; waste disposal and subsurface transport of contaminants in arid environments; ground water and surface water affected by municipal landfill leachate; natural attenuation of chlorinated solvents; and characterizing flow and transport in fractured rock aquifers.

In all, the more than 175 papers contained in this proceedings reflect the contributions of more than 350 scientists who are co-authors. These scientists are from across the USGS, as well as from universities, other Federal and State agencies, and industry.

More information on the Toxic Substances Hydrology Program, including a searchable bibliography of publications and selected on-line publications, is available on the World Wide Web at: **http://toxics.usgs.gov/toxics/**

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The editors acknowledge with great appreciation the assistance provided by Kim Crutchfield, Judy Salvo, Nana Snow, Judy Griffin, Patti Greene, and Ray Douglas. Their efforts in the planning, organization, and arrangements made for this seventh Technical Meeting of the Toxics Program made it both successful and enjoyable. Sincere thanks to Bill Ellis for assisting authors with preparation of papers for publication and for preparation and layout of this manuscript. Chet Zenone, Larry Slack, Anthony Buono, and John Flager assisted through review and improvement of the papers in this proceedings. Thanks are extended to the South Carolina District for their warm hospitality. Thanks also to the many scientists whose contributions and accomplishments are reflected in this proceedings; their continued efforts ensure continued success for the USGS Toxic Substances Hydrology Program.

KEYNOTE PAPER

Emerging Contaminant Issues from an Ecological Perspective

By Samuel N. Luoma

ABSTRACT

An ultimate goal of toxic substance hydrology is to understand the ecological, biological and human health implications of the toxic substances released by human activities. It is known that contaminants can be toxic, but it can be difficult to unambiguously identify their effects in specific circumstances. Thus controversy and contentious debate can occur when contaminants are suspected of causing ecological damage. Our knowledge of how contaminants exert their effects on ecosystems has advanced in important ways in recent years, and will change even more rapidly in the years ahead. These changes have the potential to reduce some of the ambiguities in evaluations of the implications of contamination. The new knowledge could create a demand for changes in the basic approaches for contaminant management and changes in the tools employed in those approaches. Changing approaches and tools will be a challenge for existing institutions, including regulatory agencies and the USGS. This paper attempts to summarize some of the broad "emerging contaminant issues" that could result in improved management approaches. These issues are relevant to both the newly discovered potential agents for ecological damage (Thurman, 1999) and some of the traditional contaminants.

INTRODUCTION

Managing the contamination problems of the future will require a strong partnership between experimental scientists, field scientists and managers. Never has there been a greater need for application of sophisticated contaminant-relevant principles from geochemistry, biology, ecology and hydrology. There are several reasons for this:

1. Many of the instances where it is easiest to identify the ecological effects of chemical contamination have been at least partly resolved by traditional management approaches. Waste treatment improved after passage of the Clean Water Act in 1970, and some chemicals were banned because of obvious adverse ecological effects (organochlorine pesticides, polychlorinated biphenols (PCBs), and tributyl tin). Some inputs of severe industrial pollution were eliminated as heavy industries moved away from North America. Overt instances of chemical effects (fish kills, instances where fauna were completely eliminated by toxins) are now less common than they once were. Complete elimination of fauna around outfalls is rare. Populations of large piscivorous birds are recovering after the ban of organochlorine pesticides and PCBs.

2. Discoveries in animal populations of chemically induced problems such as endocrine disruption (Colburn and Clement, 1992), teratogenicity (Skorupa and others, 1998), and chemical interference with reproduction at very low level exposures (Hook and Fisher, 1998) illustrate that at least some contaminant effects are manifested in ways not detected by traditional approaches. The reduced frequency of obvious contaminant effects does not mean that contaminants no longer have significant

influences on ecosystems. It is more likely that the remaining effects are manifested in ways that are difficult to detect with traditional tools. Therefore, toxicological mechanisms may not yet be appreciated or even recognized for some types of modern chemical contamination problems.

3. Even where chemical concentrations of contaminants are relatively high by most standards (abandoned mine lands; selenium and methyl-mercury contamination in some specific instances), controversy exists about ecological implications.

MANAGEMENT UNCERTAINTIES

Uncertainties about the ecological implications of contamination are an important detriment to effective environmental management. Costs of management, mitigation or remediation of contamination will grow as the most overt problems are resolved and the more widespread or complex problems are addressed. Optimal solutions to contamination problems can be facilitated by better understanding ecological implications. For example, in restoring damage to rivers from mining operations, is it necessary to completely remove all contaminated floodplain soils, or is it adequate to stabilize banks and let some contamination remain in the stream? Unambiguous knowledge of the metal concentrations and toxicant pathways that affect the status of fish resources in the river could greatly aid such decisions. Perhaps most importantly, we may be quickly approaching a state where intuitive management of environmental problems generates unacceptable risks. If a proposed solution to an environmental problem is single-minded, it may create problems just as bad or worse than the one being resolved. For example, methyl tert-butyl ether (MTBE) was prescribed as an additive to oxygenate gasoline and thereby reduce air pollution. However, its solubility created an unanticipated water pollution problem that we are only beginning to quantify. Furthermore, restoring marshlands or attaining zero net loss of wetlands by trading development of one marsh for restoration of another may be ostensibly desireable for reducing or reversing effects of past mistakes in land use. But biological availability of mercury through methylation processes is enhanced by the area of wetland in a watershed, and mercury contamination of soils and sediments may be widespread because of atmospheric inputs (Wiener, 1999). What are the tradeoffs (for example, in San Francisco Bay) when wetland restoration projects could make mercury contamination of fish and piscivorous birds increasingly problematic?

Today's water-quality criteria served an important legal purpose, and have been effective in reducing some forms of environmental pollution. But their ambiguities in terms of scientific accuracy have become a serious limitation to future applications. Management of contaminants in natural waters is presently accomplished by directly comparing the concentration of contaminant in water or sediment with a concentration predicted to cause adverse ecological effects. Most recent advances in regulatory science have emphasized improving the geochemical basis for incorporating environmental pollutant concentrations into water quality guidelines (DiToro and others, 1990). The new geochemical approaches include the switch from total metals in water to dissolved metals; incorporation of site-specific "water effects ratios" to correct for metal speciation; and use of equilibrium partitioning theory to predict pore water concentrations of some non-ionic organic compounds. They also include proposals to employ comparison of extractable metal and acid volatile sulfides (AVS-SEM) in sediments to model biologically available metal from pore waters.

Even if the geochemical approach is highly accurate, the environmental concentration must be compared to toxicity predicted by traditional toxicity testing methodologies. When dissolved concentrations of metals in nature, for example, are compared to the toxicities predicted by traditional bioassays **environmental concentrations are well below LC50 values**. Does this mean that there are no adverse effects from contaminants remaining in nature? Or is the toxicity value in these comparisons somehow under-estimating the contaminant concentrations that induce ecological implications? Suggestions of the latter lie in a growing new body of biological knowledge of contaminant effects.

The basic paradigm for toxicity testing was developed in the 1970's and remains unchanged. Recently, however, new knowledge and new tools provide alternatives to a number of the long-recognized uncertainties about the ecological implications of contaminants. These are best understood via comparison with traditional approaches.

Exposure Pathways: All existing bioassay methodologies (including sediment bioassays) predict toxic concentrations of contaminants from a dose delivered only via the dissolved route. It is known that animals inhabiting a contaminated environment ingest contaminated food. It is known that contaminant uptake from food by aquatic animals can occur. The contribution of the dietary pathway to dose and toxicity has remained controversial, however, at least partly because we could not quantitatively state how much contaminant was taken up from food. New protocols and models now clearly show that dietary exposure to contaminants is quantitatively important for some contaminants (although it may vary somewhat with contaminant and circumstance) (Luoma, 1996; Thomann and others, 1995; Wang and others, 1996). It is also clear that dietary exposure and dissolved exposures are additive. Therefore experiments employing dissolved exposures alone underestimate the dose an animal receives in nature from a combination of dissolved and diet exposures. Combined with the abbreviated exposures (24 – 96 hours) typical of most toxicity tests, these limitations result in underestimations of contaminant concentrations that cause adverse effects in nature (as much as 100 fold for Se; Luoma, 1996).

New toxicity testing protocols that include exposures to contaminated diet show surprising results. Trout fed food from a metal contaminated river show adverse effects not observed in simple dissolved replications of the concentrations found in the contaminated system (Woodward and others, 1995). Although an excess of sulfide over metal in a sediment may reduce pore water metal concentrations, dietary uptake of sulfide-bound metals has been demonstrated (B. G. Lee, USGS, written communication). Therefore dietary uptake of metal and toxicity can result in sediments predicted to be innocuous based upon metal-sulfide differences (B. G. Lee, USGS, written communication). Exposure of zooplankton to dissolved Ag concentrations in the range that occurs in contaminated natural waters (0.001 - 0.100 µg/L) has no effect on the animals. Hook and Fisher (1998) exposed phytoplankton to those silver concentrations then fed the phytoplankton to zooplankton. Both egg production by females and egg hatching in the zooplankton were affected by silver concentrations as low as 0.05 µg/L. Multiple pathway bioassays are more complicated to employ than the traditional dissolved exposures, so it will be challenging to incorporate such tests into management-related protocols. Until multi-pathway tests are refined, field scientists should be aware that at least some species are more sensitive than predicted from toxicities based upon dissolved exposures alone.

Effects on reproduction: The recent debate about effects of endocrine disruption illustrate the extreme sensitivity of reproductive processes in animals to chemical disruption. Mechanisms for disruption of reproduction include hormone disruption (pharmaceuticals; organochlorines), other modes of chemical interference with gamete production or embryo development (disruption of tertiary protein structure by Se substitution for sulfur) or even by disruption of energetics. Recruitment is as important as mortality for a population; so the relevance of reproductive toxicity cannot be disputed. Nevertheless, testing approaches are not routinely available to evaluate reproductive disruption; although such tests could be relevant for both "emerging contaminants" (Thurman, 1999) and many traditional contaminants (e.g. cadmium, silver, methyl mercury, selenium, PAHs, modern pesticides). The traditional toxicity tests employ toxicity to adult animals. These results are then corrected by a value derived from what are termed "chronic" exposures. Chronic tests usually consider a single life stage in isolation and can be abbreviated compared to the generation time of the life stage. Life cycle tests or specific tests of reproductive function are necessary to evaluate chemical interference with reproduction. These experiments are more difficult than traditional toxicity tests. Long term studies supported by the USGS Toxic Substances Hydrology program have shown, however, that reproductive status is more feasible to evaluate in the field than is toxicity. A demand for field evaluations might arise as awareness of reproductive sensitivities increases. It is not unreasonable to incorporate this type of effect into water quality evaluations of monitoring and field data (M. H. Hornberger and others, USGS, written communication). A combination of dietary exposure evaluations, focus on reproductive disruption, and field studies that evaluate reproductive activity and effects on fecundity, offer immediate possibilities for expanding the sophistication of knowledge about important contaminant effects.

Species-specific responses: Traditional bioassays evaluate toxicity for relatively few organisms. It is assumed that the response of these surrogate species can be extrapolated to the plethora of species that occupy communities in nature. Aside from the obvious limitations to choosing "the most sensitive species" for bioassays (Cairns, 1986), this approach is not consistent with how contaminants exert their effects in nature. Defaunation of ecosystems is not the mode of action of modern contamination. Contamination first eliminates the most sensitive species at a site. And as contamination increases, progressively less sensitive species are eliminated. We know little about which species are the most sensitive to contamination (and thus most likely to be eliminated). Only a few authors have directly evaluated sensitivities in toxicity tests (Clements, 1991). Where these studies have been done they have linked laboratory outcomes with field expectations in unprecedented ways. For example, it is recognized that some species of mayflies are among the first to disappear from metal contaminated streams. Paucity of mayflies (or mayfly species) are, therefore, a relatively sensitive indication that metals are an important variable structuring stream communities. Such knowledge offers the ecologist a basis for developing hypotheses to separate metal effects from other influences on community structure. Mechanistic studies may be another powerful way to compare sensitivities among species. New mechanstic approaches to studying bioaccumulation allow unambiguous comparisons of exposure differences among species. Bioaccumulation models, conceptual understanding of detoxification and mechanistic understanding of the most sensitive aspects of toxicology together will aid species-specific and, thus, site-specific evaluation of toxicity from monitoring data in the years ahead. The implicit, practical and traditional view that toxicity is biologically generic will yield in the future to the view that species-specific attributes determine susceptibility to contaminants.

Unambiguous linkage of field and experiment or mechanistic study: The traditional approaches to regulatory science lack unambiguous protocols for linking field observations with laboratory evaluations of toxicity. New protocols and bioaccumulation models might allow some solutions to this problem. Bioaccumulation is a direct indicator of the dose of a bioaccumulative chemical experienced by an organism. As described above, bioaccumulation can be modeled from field geochemical concentrations and species-specific physiological information (Luoma, 1996). Bioaccumulation can also be monitored in the field; tissue concentrations of bioaccumulative chemicals can thereby be used for model verification. Translating multi-media geochemical data into exposure or bioaccumulation information via multi-pathway bioaccumulation models is increasingly feasible. In their simplist applications such models might evaluate potential resource contamination under different conditions (for example methyl mercury or persistent chemical contamination of the flesh of edible species). Modeled bioaccumulation might replace the search for correlative agreement between water, sediment and tissue concentrations of contaminants and thus link hydrologic and geochemical monitoring to bioavailability and ecosystem effects .

Modeled or observed tissue concentrations of contaminants must ultimately be related to adverse effects on biota or communities to be maximally useful. A small body of work suggests that it will be possible to relate critical tissue residues of a contaminant (or some fraction of the tissue residue) to a decline in physiological

health within an organism itself (Luoma, 1996). It might also be possible to use residues in an indicator species to link observed pollutant concentrations with the expected disappearance of specific groups of species
The value of hydrologic and geochemical monitoring will expand as sophisticated biological interpretations are increasingly feasible.

SUMMARY

Although we are beginning to appreciate the complexities of contaminant effects in nature, important uncertainties remain. It is known that contaminant exposures in nature are complex but exposures are adequately studied only in rare circumstances. It is known that species differ widely in their vulnerability to contaminant effects but the role of basic biology in contaminant vulnerability is not adequately known. Generalizations about the groups of species most vulnerable to a specific type of contamination are rare. It is clear that contamination can simplify communities and affect population processes, but that knowledge is not yet sufficient to extrapolate over a variety of sites. As a result, consensus does not exist about the implications of changes in ecologically fundamental factors such as community composition. Long-term, interdisciplinary study of contaminated sites, a cornerstone of the Toxic Substances Hydrology Program of WRD/USGS, has been a key approach to providing some of the new advances in understanding contaminant implications. A great advantage of the long-term approach in field studies is that the hydrologic, geochemical and biological complexities that typify nature can be progressively resolved over critical temporal frequencies. Understanding the role of contaminants relative to other sources of stress requires such multi-disciplinary resolution. It will be critical in the future that laboratory studies that facilitate model development, and knowledge of sensitivities at different levels of biological organization be coordinated with persistent field studies characteristic of the Toxic Substances Hydrology Program.

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The San Francisco Bay-Estuary Toxics Study: Sustained Progress in a Unique Estuarine Laboratory

Like all major urbanized estuaries in the world, San Francisco Bay receives toxic substances from a variety of natural and anthropogenic sources. Once in the estuary, a network of interdependent physical, chemical and biological processes affect the transport and transformation of these substances over a wide range of spatial and temporal scales (Kuwabara and others, 1999). Inherent hydrodynamic complexities of estuaries coupled with chemical gradients and dynamics in benthic and planktonic communities generate a formidable interaction of processes relevant to the transport, transformation and potential biological effects of toxic chemical species. The San Francisco Bay-Estuary Toxic Substances Hydrology (Toxics) Study is therefore unique among estuarine studies in its long-term approach to quantitatively defining that process interdependence. In this study, the estuary is conceptualized as three interacting "sub-ecosystems" (the Delta, North Bay and South Bay; fig. 1), that operate differently, are controlled by different processes, and hence have environmental concerns that may be prioritized differently.

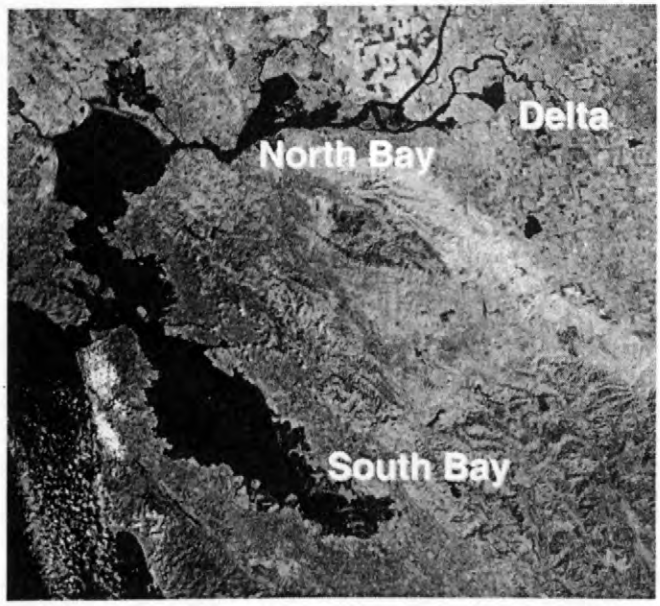

Figure 1. The San Francisco Bay-Estuary Toxics Study examines the inherent complexities of interacting estuarine subecosystems that affect the distribution, fate and toxicity of organic and inorganic contaminants (Landsat Thermatic Mapper image taken on September 16, 1993, and processed by Pat Chavez and his group, USGS, Flagstaff, AZ).

Subsequent reports are presented to give the reader a sense of the necessarily wide scope of research activities within this study. These collaborative studies continue to strengthen the scientific foundation that has kept the agency uniquely positioned for three decades to respond to specific problems related to imminent water-quality management decisions.

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Studies Relating Pesticide Concentrations to Potential Effects on Aquatic Organisms in the San Francisco Bay-Estuary, California

by Kathryn M. Kuivila

ABSTRACT

A variety of pesticides are applied in large quantities to agricultural and urban areas in the Central Valley of California and are transported into the San Francisco Bay-Estuary dissolved in water and associated with suspended sediments. These pesticides can have deleterious effects on aquatic organisms. Three studies that relate pesticide concentrations to potential effects on aquatic organisms are currently underway by the U.S. Geological Survey's San Francisco Bay-Estuary Toxic Substances Hydrology Project. These studies are (1) measuring the impacts of herbicides on phytoplankton primary production, (2) determining the exposure of Delta smelt to dissolved pesticides, and (3) assessing the effects of pesticides on the Asian clam, *Potamocorbula amurensis*.

INTRODUCTION

Large quantities of various pesticides are applied to agricultural and urban areas in the Central Valley of California that drain into the Sacramento-San Joaquin Delta (Delta) and then San Francisco Bay. Together, the Delta and San Francisco Bay are known as the San Francisco Bay-Estuary (Estuary). Several monitoring studies have measured elevated concentrations of dissolved pesticides in the Sacramento and San Joaquin Rivers, upstream of the Estuary (fig. 1) (MacCoy and others, 1995; Domagalski, 1996; Panshin and others, 1998). Fewer measurements of dissolved pesticides have been made in the Sacramento-San Joaquin Delta or farther downstream in the Estuary (fig. 1) (Kuivila and Foe, 1995; Kuivila and others, 1999). Pesticides also are transported into the rivers and Estuary associated with suspended sediments, but little is known about the concentrations and residence times of these pesticides (Domagalski and Kuivila, 1993; Bergamaschi and others, 1999; Bergamaschi and others, in press).

Once in the aquatic environment, pesticides can have deleterious effects on aquatic organisms; controlling factors include the concentration, exposure time, and bioavailability of the pesticide of concern. When assessing a biological effect, it is important to use the appropriate endpoint or indicator. Endpoints reflect responses at various levels—physiological, whole organism, population, and community. Field and laboratory studies can be used to assess effects of pesticides on aquatic organisms. Field studies take into account the complexity of the ecosystem, but that complexity makes it difficult to assign a single cause to an observed effect. In contrast, direct cause and effect are more easily shown in laboratory studies, but at the expense of oversimplifying the interrelations in the ecosystem. A combination of field and laboratory studies usually provides the most powerful approach.

The purpose of this report is to summarize continuing studies by the U.S. Geological Survey's (USGS) San Francisco Bay-Estuary Toxic Substances Hydrology Project (S.F. Bay Toxics Project) that relate measured pesticide concentrations to observed biological effects in the Estuary. These studies are (1) measuring the effect of herbicides on primary productivity and species composition of phytoplankton in the Sacramento-San Joaquin Delta; (2) determining the exposure of Delta smelt to dissolved pesticides during spawning and larval stages; and (3) assessing the effect of pesticides on the Asian

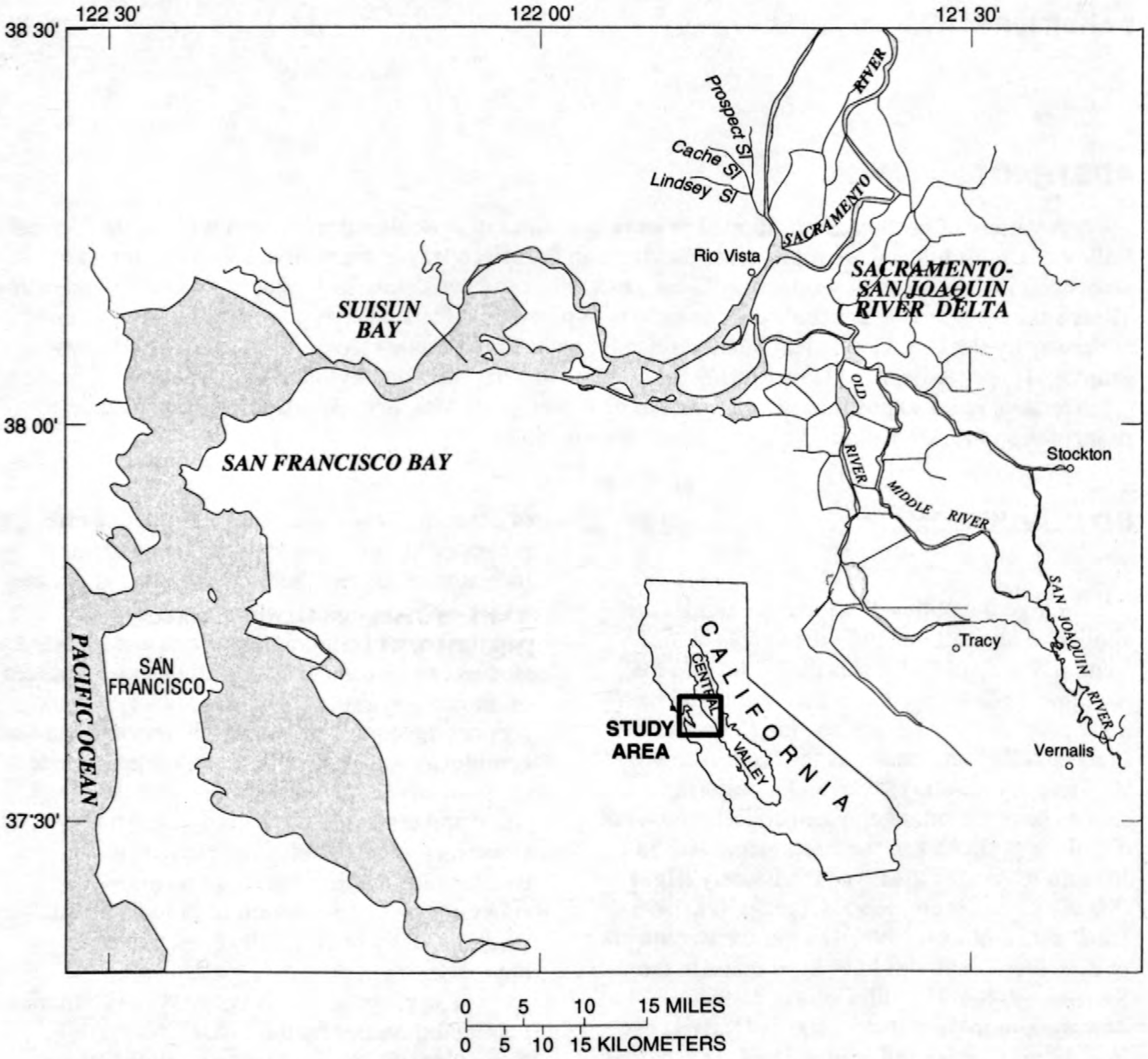

Figure 1. Location of the study area, Sacramento-San Joaquin Delta and San Francisco Bay, California

clam, *Potamocorbula amurensis* (*P. amurensis*), in field and laboratory studies.

HERBICIDES AND PHYTOPLANKTON

Half a million pounds of more than 30 different herbicides are applied on agricultural lands each year in the Delta and an additional 5 million pounds are applied upstream in other watersheds (California Department of Pesticide Regulation, 1996). Some of these herbicides are designed to kill plants by inhibiting photosynthesis. However, little is actually known about the effects of herbicides on primary productivity and species composition of phytoplankton in aquatic environments (Schneider and others, 1995).

A USGS study is evaluating the impairment of phytoplankton primary productivity in the Delta due to herbicides. The project is part of the S.F. Bay Toxics Project and is cooperatively funded by the Interagency Ecological Program for the Sacramento-San Joaquin Estuary (IEP). Herbicide concentrations and phytoplankton primary production rates (P_{max}) were measured concurrently in 53 Delta water samples from May through November 1997. The concentrations and possible sources of the herbicides are summarized in Kuivila and others (1999) and the results of the primary productivity measurements are discussed in Edmunds and others (1999). It is known that the concentration of an herbicide must exceed some minimum level before there is an "observable effect" on the phytoplankton's rate of photosynthesis. Although the herbicide concentrations in the Delta usually were less than a lowest observable effect concentration (LOEC), the lowest P_{max} measured during the study was observed in the one sample with an herbicide concentration that exceeded a LOEC (Edmunds and others, 1999). This sample from French Camp Slough on November 11 had a diuron concentration of 2,141 nanograms per liter. No correlation was found between measured values of P_{max} and herbicide concentrations in the other 52 samples. In these instances, measured herbicide concentrations were less than published LOECs.

Edmunds and others (1999) conclude that the study results do not indicate system-level impairment of primary production in the Delta, but caution that there are localized events when production is impaired. Of the herbicides that inhibit photosynthesis, diuron and hexazinone had the highest concentrations and most frequent detections; more sampling is needed to determine if these two herbicides frequently occur at concentrations high enough to impair primary production. The limited number of samples in this study made it difficult to determine the sources of diuron and hexazinone, and to predict herbicide concentrations.

Species composition also was measured in the study but the data analysis has not been completed. The sensitivity of different species of phytoplankton to herbicides can vary by as much as one or two orders of magnitude (Hollister and Walsh, 1973; Rand, 1995; Sabater and Carrasco, 1996, 1998; Peterson and others, 1997). This variability of response could influence species composition, allowing less sensitive species to dominate in locations and times of high herbicide concentrations. This shift in species composition could have an effect up the food chain because the phytoplankton species vary in their caloric value to higher trophic levels (Peterson and others, 1997).

EXPOSURE OF DELTA SMELT TO DISSOLVED PESTICIDES

Delta smelt are a threatened species in the Estuary. While the decline of some fish species have been attributed to factors such as low Delta outflow or number of spawners, the decline of Delta smelt cannot be explained by these factors (Bennett and Moyle, 1996; Kimmerer, 1998; Nobriga, 1998). Therefore, various other explanations for the decline of the Delta smelt are being explored, including chronic effects from exposure to dissolved pesticides.

A 2-year study (October 1998 – September 1999) in collaboration with California Department of Fish and Game (CDFG) is measuring the exposure of Delta smelt to dissolved pesticides during vulnerable egg and larval stages. The project was funded by the IEP and the USGS as part of the S.F. Bay Toxics Project. Sampling for dissolved pesticides was coordinated with CDFG's Delta-smelt surveys to concurrently determine pesticide concentrations and Delta smelt larval abundance.

Delta smelt spawn in early spring and one primary spawning ground is the northwestern part of the Delta (Cache, Lindsey, and Prospect Sloughs) (fig. 1). During some years, the Delta smelt also spawn closer to the central Delta. Very little data are available on concentrations of pesticides in the Delta during this time of year, but rice pesticides (molinate, thiobencarb, and carbofuran) have been detected in the Delta and at upstream sites (Domagalski and Kuivila, 1991; Crepeau and others, 1994; K.M. Kuivila, U.S. Geological Survey, unpublished data, 1997). During the spawning period (typically April-June), water samples for dissolved pesticide analyses are collected weekly at sites in the northwestern Delta and less frequently at central Delta sites concurrently sampled by CDFG.

About 1 month after spawning, Delta smelt larvae become motile and move into Suisun Bay. Larval fish sampling in Suisun Bay shows a distinct peak of larval Delta smelt at 2 parts per thousand (ppt) salinity, with considerably fewer larvae at higher and lower salinities. Water samples are being collected every 1-2 days at a site in Suisun Bay at approximately 2 ppt salinity during the ebb tide in the summer.

Related studies are being conducted by the University of California at Davis (U.C. Davis) to look at different indicators of contaminant effects on Delta smelt, including growth rates, histopathology, and DNA strand breaks (William Bennett, Swee Teh, and Susan Anderson, University of California at Davis, written commun., 1998). Recently, Delta smelt have been cultured in the laboratory which will allow for future laboratory studies of the direct effects of dissolved pesticides on Delta smelt.

BIOACCUMULATION AND STRESSES ON THE ASIAN CLAM

Contaminant effects on the Asian clam, *P. amurensis*, are being assessed in continuing studies by the USGS (Brown and others, 1999). The long-term field study measures bioaccumulation of trace elements, concurrent population biology, and physiological indicators of stress. Enzymatic, histopathologic, and biochemical biomarkers in these populations of *P. amurensis* are being studied by U.C. Davis to compare the biomarkers with the trends in contaminants (S.L. Clark, University of California at Davis, and C.L. Brown, U.S. Geological Survey, oral commun., 1998).

In contrast, very little data are available on the concentrations of organic compounds, especially pesticides, in *P. amurensis* in the Estuary. Recent studies in the Estuary (Domagalski and Kuivila, 1993; Pereira and others, 1996; Bergamaschi and others, 1999, in press) have shown elevated concentrations of some pesticides associated with suspended sediments. These results suggest that the potential exists for bioaccumulation in the filter-feeding *P. amurensis*. A 3-year study (October 1998 – September 2001), funded by the USGS Integrated Natural Resource Program, will measure bioaccumulation of a variety of organic compounds in *P. amurensis*. One site in Suisun Bay will be sampled monthly and several synoptic samplings will be done. The wide variety of pesticides used in the Central Valley will be measured in the clam tissues, in addition to typically-analyzed compounds such as polychlorinated biphenyls, polyaromatic hydrocarbons, and organochlorine pesticides.

Laboratory experiments exposing *P. amurensis* to a variety of contaminants under different conditions is another study by the S.F. Bay Toxics Project. For example, clams from populations with different toxicant-exposure histories will be exposed singly and then concurrently to low salinity and various dissolved pesticides to assess direct effects on respiration and glycogen concentration. Studies are continuing to determine the interactive effects between metals and dissolved pesticides on *P. amurensis* (Luoma, 1999). Finally, a related laboratory study is being proposed in collaboration with U.C. Davis to examine the effects of dissolved pesticides on early development of *P. amurensis*.

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Metal trends and effects in *Potamocorbula amurensis* in North San Francisco Bay

Cynthia L. Brown and Samuel N. Luoma

ABSTRACT

Long-term, multi-disciplined field sampling was used to assess both the fate and effects of trace metals in northern San Francisco Bay. Bioaccumulation in the bivalve *Potamocorbula amurensis* was measured at near-monthly intervals between 1990 and 1997. Three accumulation patterns were detected. One pattern indicates that biological regulation of Cu and Zn is an important control on tissue concentrations. This was evident by the absence of persistent spatial or temporal trends in the tissue concentrations of Cu or Zn, and the correlation of Cu and Zn tissue concentrations with the weight of the clam. The pattern in Cr, Ni, and V tissue concentrations was related to the combined influences of riverine inputs and local industrial inputs. Seasonally, the highest tissue concentrations of Cr, Ni, and V coincided with high delta inflows. Cadmium and Ag tissue concentrations were not clearly related to any obvious source. However, they were linked inversely to condition index, glycogen content, reproductive status, and histopathology. The histopathological changes are biomarkers indicative specifically of contaminant stress.

Trace metals can be an influential variable in ecosystem processes, affecting the well-being of organisms, populations and communities (Luoma, 1996). Metal bioaccumulation in macroinvertebrate tissues is an indicator of metal exposures that can either adversely affect the health of the organism, or be transferred up the food web to affect higher organisms. Bioaccumulation in the bivalve *Potamocorbula amurensis* has been used to assess both the fate and effects of trace metals in San Francisco Bay (Brown and Luoma, 1995). The present study began in 1990 and consists of monthly sampling at five sites in Northern San Francisco Bay (Figure 1). Four of these are deep water sites in the ship channel; near Chipps Island, near Roe Island, in Carquinez Strait near Martinez, and in San Pablo Bay northeast of Pinole Point. One site is in the shallow water of Honker Bay. Clams (60-100) are collected at each site and separated into replicate size composites (1mm shell length difference) of 12-15 clams each. Ag, Cd, Cr, Cu, Ni, V, and Zn are measured in the whole soft tissues of the clam. Condition index (mg dry weight for a standard shell length), glycogen content (% tissue dry weight), and reproductive status (Parchaso, USGS) are determined monthly to evaluate influences of metals and other environmental factors on the energetics and reproduction in *P. amurensis* as indicators of stress. Surface sediments are collected at the same time and analyzed for metals (Hornberger and Bouse, USGS). Collaborators from UC Davis (Drs. Hinton, Werner, Teh, Clark, Fan, Higashi, Kaufman) are simultaneously studying enzymatic, histopathologic, and biochemical biomarkers in these populations of *P. amurensis* to compare with the trends in contaminants.

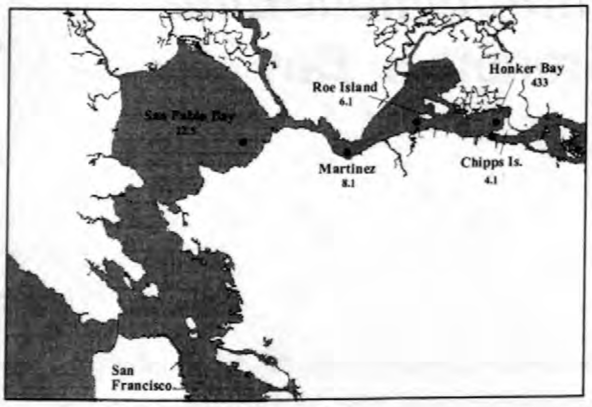

Figure 1. Map of sampling sites in Northern San Francisco Bay.

Since the beginning of this study, we have observed metal trends over a wide variety of hydrographic regimes. These include three very low flow years (1991, 1992, and 1994) where annual mean delta outflow was less than 220 m^3sec^{-1}, and years of moderate to high delta outflow (1993, 1995, 1996, and 1997) where annual mean delta outflow was between 760 - 1710 m^3sec^{-1} (Oltmann, USGS data). Each of the metals showed slightly different accumulation patterns in *P. amurensis* and thus indicated the variety of factors that control bioaccumulation. Three basic patterns were detected. One pattern indicates that biological regulation of Cu and Zn is an important control on tissue concentrations. The pattern in Cr, Ni, and V tissue concentrations is related to the combined influences of riverine inputs and local industrial inputs. The third pattern in Cd and Ag tissue concentrations is not clearly related to any obvious source, but is linked to patterns in biomarker indicators of metal stress.

Earlier studies (Luoma et al., 1990, Hornberger et al., 1999) showed that Cu contamination increases substantially in the industrialized regions of the Bay-Delta. However, there is no distinct spatial or temporal trend in the tissue concentrations of Cu (or Zn) in *P. amurensis* in the North Bay. Variability in Cu (and Zn) appears to be dominantly controlled by biological processes. Among all times at all sites, the amount of Cu in a 15mm shell length clam is strongly correlated ($r^2 = 0.53$) with the weight of tissue. The same relationship occurs within each site. As animals add (or lose) tissue mass, they add (or lose) Cu. Changes in weight, which occur seasonally and site-to-site, control 53% of the Cu variability in *P. amurensis.* It is known that the bioaccumulation response to environmental Cu and Zn contamination differs among species (Phillips and Rainbow, 1993). *P. amurensis* appears to be a species that biologically regulates its tissue burden of Cu and Zn, and thus does not appear to be a useful indicator of Cu and Zn contamination patterns (Brown and Luoma, 1995).

The second accumulation pattern shows that Cr, Ni, and V in the tissues of *P. amurensis* are related to both natural and anthropogenic inputs into the ecosystem. These metals are enriched in the ultramafic rocks that are common throughout the watershed and thus are naturally high in the sediments in the bay. Vertical cores of sediments indicate that the enrichment of Cr, Ni, and V extends back to before the Gold Rush and the acceleration of human activities in the area (Hornberger et al., 1999). These metals also have industrial sources in the North Bay. Chromium, Ni, and V concentrations in the tissues of the clams have a distinct temporal pattern that suggests the amount of freshwater flow into the bay from the delta affects their bioaccumulation. Vanadium has the strongest relationship with delta outflow. Vanadium concentrations increase during pulses of high inflows and are low in the tissues at all channel stations during low flow periods (Figure 2a). Tissue concentrations at the most landward site, near Chipps Island, are often as low as tissue concentrations at the most seaward site, San Pablo Bay, during low flows. However, when delta outflows increase, concentrations of V are higher in clams at Chipps Island than clams in San Pablo Bay. Chromium and Ni tissue concentrations behave similar to V during high flows (see also Abu-Saba and Flegal, 1997). But during low flow periods, tissue concentrations of Cr (and Ni) do not decrease to the lowest levels seen in San Pablo Bay (Figure 2b). Input of Cr and Ni from internal industrial or sedimentary sources appear to add to the source of Cr and Ni from the watershed; the influence of these internal sources is most evident when freshwater residence times increase (i.e. low flows). The trends of Cr, Ni, and V in the tissues of *P. amurensis* show how physical processes and natural sources within an ecosystem interact with anthropogenic inputs to affect bioavailable metal concentrations.

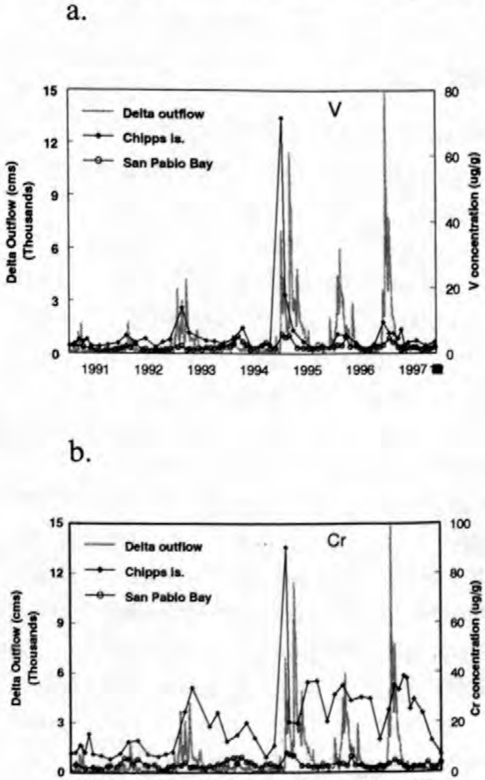

Figure 2. Vanadium concentrations (a) and Cr concentrations (b) in *P. amurensis* at Chipps Island and San Pablo Bay compared with delta outflow.

The third accumulation pattern is seen with Cd tissue concentrations which are linked to adverse effects in *P. amurensis*. Potential sources of cadmium include oceanic upwelling, urban/industrial effluents, and mining. There is not the distinct flow-related temporal trend in the Cd tissue concentrations that is seen in Cr, Ni, and V. However, there is a seasonally consistent and distinct spatial pattern (Figure 3): Cd concentrations are highest in clams near Chipps Island and in Honker Bay, and lowest in clams in San Pablo Bay. This pattern is consistent among all the years of the study. The source of the Cd contamination is not clear, but both geochemical processes and anthropogenic inputs could contribute.

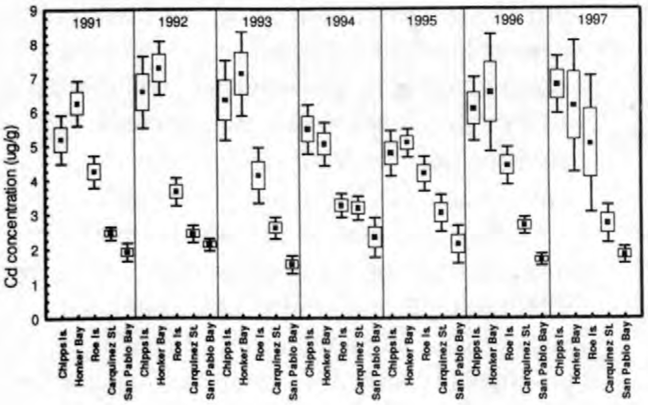

Figure 3. A seasonally consistent and distinct spatial pattern in Cd concentrations in *P. amurensis* occurs among years.

The biomarkers measured coincident with the metal data show significant correlations with the Cd tissue concentrations. Glycogen and condition index follow a pattern that is the inverse of the Cd tissue concentrations. The condition index is an indicator of overall fitness of the clam. Clams have their lowest condition index near Chipps Island and in Honker Bay. They have their highest condition index in San Pablo Bay with strong seasonal fluctuations that follow reproduction. Figure 4 shows that clams with the highest Cd in their tissues are unable to gain (and lose) weight, unlike the clams with lower tissue concentrations of Cd. Glycogen is used by an organism as a source of energy and is consumed during times of stress or reproduction when extra energy is needed. Initial time series show that glycogen levels in *P. amurensis* are lowest overall in the clams from the most contaminated site. Glycogen levels in the clams at both sites are depleted when the clams begin gametogenesis, but the depletion occurs earlier and lasts longer at the more contaminated site. The differences in reproductive status of *P. amurensis* among the stations also show a relationship with Cd tissue concentrations. Clams at the most contaminated site (lower condition, lower glycogen) have a lower proportion of reproductively mature individuals than clams at the least contaminated site. At least in the years of high Cd exposure (e.g. 1991-1993) reproduction is synchronous among animals in San Pablo Bay (least contaminated), but asynchronous at Chipps Island (most contaminated) (Thompson et al., 1996, Parchaso et al., 1997). Histopathologic biomarker alterations are also correlated with metal body burden. Preliminary analyses show stress occurring in the kidneys, gonad and digestive tract of *P. amurensis* at Chipps Island (Teh et al, 1999). Although other anthropogenic and natural stressors probably have some influence on the differences among the different populations in *P. amurensis* in North Bay, the correlations of general physiological indicators and the histochemical biomarkers with Cd body burdens indicate that metals must be considered a variable of potential importance when assessing the processes that influence this ecosystem.

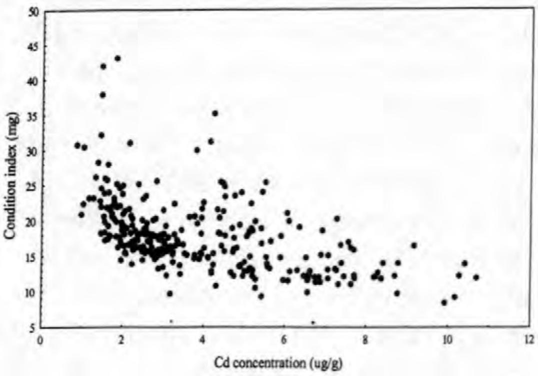

Figure 4. Cd concentration and condition data in P. amurensis for all months at all sites from 1991-1997. Clams with the highest Cd tissue concentrations are unable to gain (and lose) weight, unlike clams with lower Cd tissue concentrations.

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Pesticides Associated with Suspended Sediments in the San Francisco Bay During the First Flush, December 1995

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ABSTRACT

The majority of suspended sediments are transported into estuaries by the "first flush" – runoff from the first major storm of the water year. Pesticides associated with these sediments may represent a significant fraction of pesticides transported to estuaries, where they have different environmental effects than dissolved pesticides.

Water and suspended sediment samples were collected at the head of the San Francisco Bay during a peak in suspended sediment concentration, following the first major storm. These samples were analyzed for a variety of pesticides that span a wide range of hydrophobicity. For the 19 compounds analyzed, an average of 10 was found on the samples. Few pesticides were found dissolved in concurrent water samples and at concentrations much lower than would be expected from equilibrium partitioning between the aqueous and sedimentary phases.

DDT and its metabolites were observed in all suspended sediment samples in concentrations ranging from 5.1 to 11.1 nanograms per gram dry sediment weight. Other compounds were sporadic in occurrence, but did occur at higher concentrations. For example, oxyflurofen and endosulfan each exceeded 20 nanograms per gram in a single sample. However, there were no significant trends in the total or individual pesticide concentrations, or in the number of compounds observed as the peak in suspended sediment concentration passed the sampling site. Also, there were no significant relations between sediment-associated pesticide concentration and parameters such as K_{oc} of the pesticide, organic carbon content of the sediment, or amount of pesticide applied in the drainage basin, suggesting that observed sediment-associated pesticide concentrations may reflect disequibrium between sedimentary and aqueous phases resulting from long equilibration times at locations where pesticides were applied, and relatively short transit times for reequilibration to occur.

INTRODUCTION

The majority of suspended sediments are transported into estuaries usually after the first major storm of the year, during the ensuing high flow (Meade, 1972, Goodwin and Denton, 1991). Onset of the winter storms causes runoff that transports sediment from upstream areas downstream to the estuary. In the estuary, these sediments represent an important food source for filter-feeding organisms such as clams (Canuel and others, 1995).

Equilibrium partitioning models (DeToro and others, 1991; Liljestrand and Lee, 1992) and field studies (for example, Pereira and others, 1996; Domagalski and Kuivila, 1993) indicate that some pesticides may be concentrated on suspended sediments. Because suspended sediment may carry appreciable amounts of associated pesticides, sediment transport may be an important mechanism for introduction of pesticides into the San Francisco Bay (for example, Domagalski and Kuivila, 1993; Bergamaschi, and others, 1997).

Pesticides associated with suspended sediments may have different environmental effects in estuaries than dissolved pesticides because suspended sediments are a primary source of nutrition for filter-feeding organisms such as clams. Also, sediments have a longer residence time in estuaries than does water (Meade, 1972; Schubel and Carter, 1984), and inputs of sediments occur in a pulse, typically once or a few times a year in response to precipitation events (Meade, 1972; Goodwin and Denton, 1991). This introduction by sediments may increase exposure times to pesticides or provide a mechanism for exposure during biologically sensitive times.

Many factors may control the concentration of pesticides on sediments. For example, the short transit times between areas of pesticide application and the estuary during winter storm runoff may mean that pesticides associated with suspended sediments do not have sufficient time to reach equilibrium (Domagalski and Kuivila, 1993; Jepsen and others, 1995; Lick and Rapaka, 1996). If equilibrium is not achieved, higher concentrations of sediment-associated pesticides (SAPs) relative to aqueous concentrations may result. Thus, transport of suspended sediments from areas of pesticide use into estuaries may carry more pesticides than equilibrium models suggest.

Some of the possible factors that influence the distribution of pesticide concentrations, other than partitioning between the aqueous and sedimentary phases, are the timing and amount of pesticide applications, differences in the transit time between drainages and the Bay, variations in the time lag between pesticide application and onset of precipitation, the residence time of sediments within the drainage, and the effect of tidal cycles and river discharge on sediment transport and deposition (Schubel and Carter, 1984; Kratzer, in press).

We examine here the distribution of SAPs and dissolved pesticides following the first storm of the winter season at Mallard Island, a station near the head of the San Francisco Bay. The Sacramento and San Joaquin rivers drain California's Central Valley (fig. 1) through the Sacramento-San Joaquin Delta, and into San Francisco Bay. The Central Valley and Delta are areas of intense agricultural activity in which more than 500 different pesticides are applied (California Department of Pesticide Regulation, 1996).

The purpose of this study was to explore the variability in SAP concentrations in San Francisco Bay during the time suspended sediment concentrations are greatest, and the majority of sediment transport is expected to occur – during the “first flush,” the runoff of first major winter storm following the dry growing season (Goodwin and Denton, 1991, Schoellhamer, 1997). This report describes the pesticide content of suspended sediment samples collected at Mallard Island (fig. 1), near the head of the San Francisco Bay, following a storm on December 11-13, 1995 – the first major storm of the season, and during a period of the highest recorded suspended sediment concentrations of the water year (Schoellhamer, 1997).

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EXPERIMENTAL

Samples were collected from a gage house on Mallard Island, California, located just south the main shipping channel, 5 mi downstream of the confluence of the Sacramento and San Joaquin Rivers (fig. 1). A continuous record of optical backscatter data, stage height, and other ancillary parameters was maintained by the California Department of Water Resources and the U.S. Geological Survey during the study.

Suspended sediment samples were collected at times of predicted slack current – either slack after ebb tide or slack after flood tide. This sampling was developed to provide a twice-a-day strategy for suspended solids concentrations that best reproduces the residual near-surface suspended solids concentrations (Jennings, and others, 1997). Due to mechanical and operational difficulties, it was not possible to collect every sample indicated by the strategy.

Water for suspended sediment analysis (approximately 100-200 L, depending on

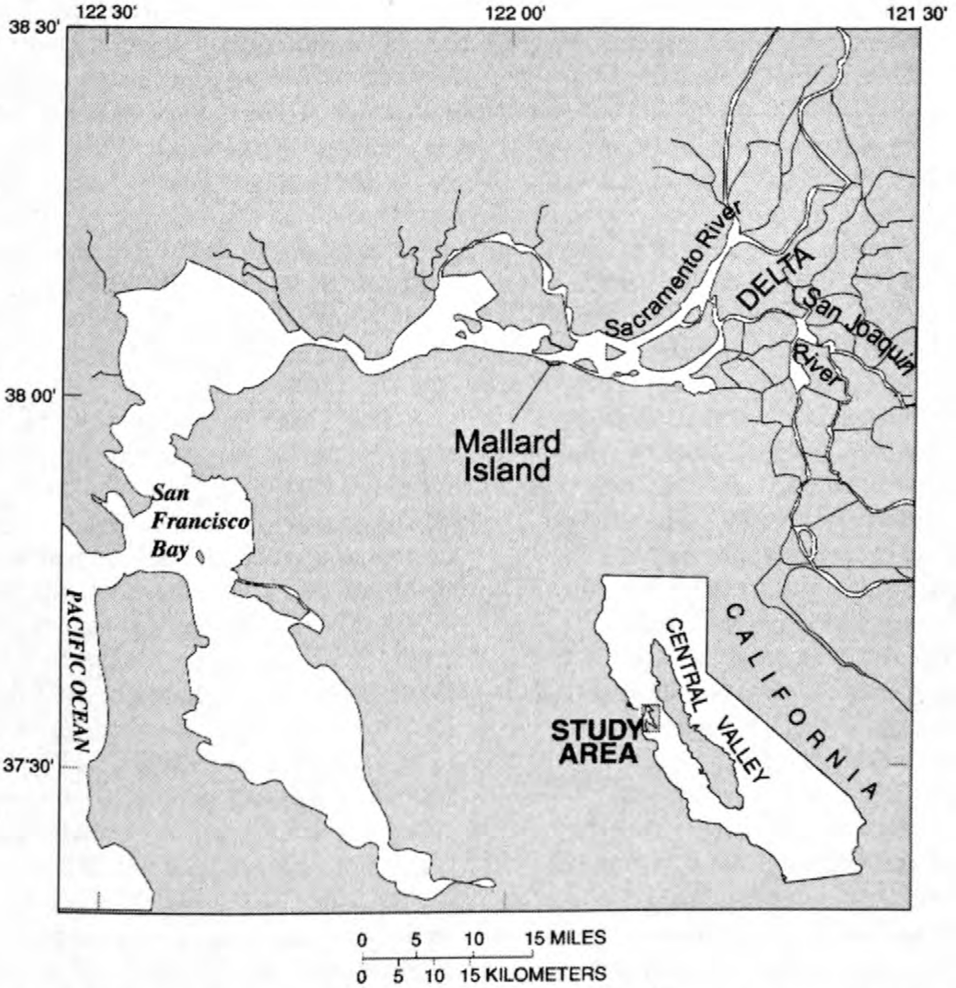

Figure 1. Location of sampling site in relation to San Francisco Bay, and the Sacramento and San Joaquin Rivers.

suspended sediment concentration) was collected by high volume peristaltic pump from 1 m below the surface into 37-L stainless steel containers, and transported to nearby facilities where sediments larger than 0.3 µm were isolated using a flow-through centrifuge into a 1 L slurry. Suspended-sediment slurries were dewatered on a high-speed centrifuge at 13,000 revolutions per minute in 50 mL Teflon centrifuge tubes, and frozen until analysis. (The use of brand names is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey.)

Suspended sediment samples were analyzed for pesticide content according to the method described by Bergamaschi and others (in press). Briefly, 1-10 grams (g) of wet sample were dried by grinding with Sodium Sulfate. Samples were extracted three times with fresh methlyene chloride in 200-mL Teflon bottles using an ultrasonic processor. The three extracts were composited and concentrated first by rotary evaporator, then under a stream of nitrogen to approximately 250 µL. Interfering coextracted compounds were removed by passing the sample through a 1 centimeter (cm) internal diameter (i.d.) 6 gram (g) column of 2 percent deactivated Florisil. The 250-µL sample extracts were loaded onto the cleanup column and eluted with 40 mL hexane:ethyl ether (4:1 by volume), then concentrated on a rotary evaporator and dried under nitrogen to a final volume of 1 mL. Finally, internal standards (deuterated acenapthene, pyrene and phenanthrene) were added and the sample reduced to a final volume of 100 µL for chromatographic analysis.

Water samples for dissolved pesticide analysis were collected using a peristaltic pump from 1 meter (m) below the surface, and analyzed according to the method described in Crepeau and others (1994). Briefly, 1 liter (L) of sample was filtered through a 142 millimeter (mm) diameter precombusted, 0.7 micrometer, glass fiber filter, and then extracted onto a 3 cubic centimeter C-8 solid phase extraction cartridge. Cartridges were dried under a stream of carbon dioxide and frozen until analyzed. On the day of analysis, the cartridges were thawed and sample extracts were eluted with 6 milliliters (mL) of a 1:1 (by volume) mixture of hexane and diethyl ether. Eluates were concentrated under a stream of nitrogen to about 200 microliters (µL), where upon internal standards (deuterated acenapthene, pyrene and phenanthrene) were added and the mixture concentrated to a final volume of 200 µL.

Chromatographic separations for dissolved pesticide samples and suspended sediment samples were accomplished using a gas chromatograph coupled to a ion-trap mass spectrometer, using a 30 m 5 percent-phenyl methylpolysiloxane bonded phase 0.25-mm i.d.-fused silica capillary column, and a oven temperature program providing full baseline separation of all analytes (Bergamaschi and others, in press). Samples were quantified by comparing the relative response of each analyte to the response of a known amount of the most closely eluting internal standard. The relative response of each analyte was calibrated daily by analyzing a suite of standards.

RESULTS

Hydrodynamics and suspended sediment concentrations

To evaluate the distribution of SAPs in the San Francisco Bay during the first flush, suspended sediment samples were collected at Mallard Island following the first major storm during the 1995-1996 water year. Measurements of stage height at the Mallard Island site indicate it is strongly tidally influenced. The stage height ranged nearly 2 m during the sampling period, with an average inter-tidal range of 0.4 m, and associated spring-neap variability (fig. 2c). The suspended-sediment concentration (SSC) ranged from a high of 126 milligrams per liter (mg/L) to a low of 23 mg/L, over the course of the sampling perios, and there was a large change in SSC between ebb and flood tides (fig. 2d). The inter-tidal change in suspended-sediment concentration ranged from a high of 70 mg/L to a low of 15 mg/L during the sampling period, with an average inter-tidal change of approximately 30 mg/L (fig. 2d).

Peaks in Delta outflow and SSC were observed (fig.2b, 2e) following heavy rainfall on December 11-13, 1995 (fig. 2a), the first storm of the winter season. Delta outflow is calculated on a daily basis, and represents the contribution of the tributaries of the Sacramento and San Joaquin Rivers as well as smaller and local inputs (California Department of Water Resources, 1986). Delta outflow began to rise from a level of 250 cubic meters per second (m^3/s) on December 11 and peaked at an outflow of 2,000 m^3/s. Following the increase in Delta outflow, the SSC increased (fig. 2d), and therefore the quantity of suspended sediment transported from California's Central Valley to the San Francisco Bay increased. Tidally averaged suspended sediment concentrations (Schoellhamer, 1996) in Delta outflow in the Sacramento River at Mallard Island increased to 97 mg/L following the storm (fig. 2e, 2f), from a base level of 30 mg/L.

Sediment Associated Pesticides

Total SAP concentrations ranged from 9.8 to 43.8 nanograms per gram (ng/g) dry weight sediment (table 1), which translates to 1-4 nanograms per liter (ng/L) SAP in whole water (unfiltered) samples. There was no apparent temporal variability in the total SAP concentrations associated with suspended sediments during the course of the peak in SSC following the December 11-13 storm. The observed lack of temporal variability stands in contrast to our previous study of sediments in the San Joaquin River (using the same methods) where total SAP concentrations were higher on the rising limb of the SSC peak than on the falling

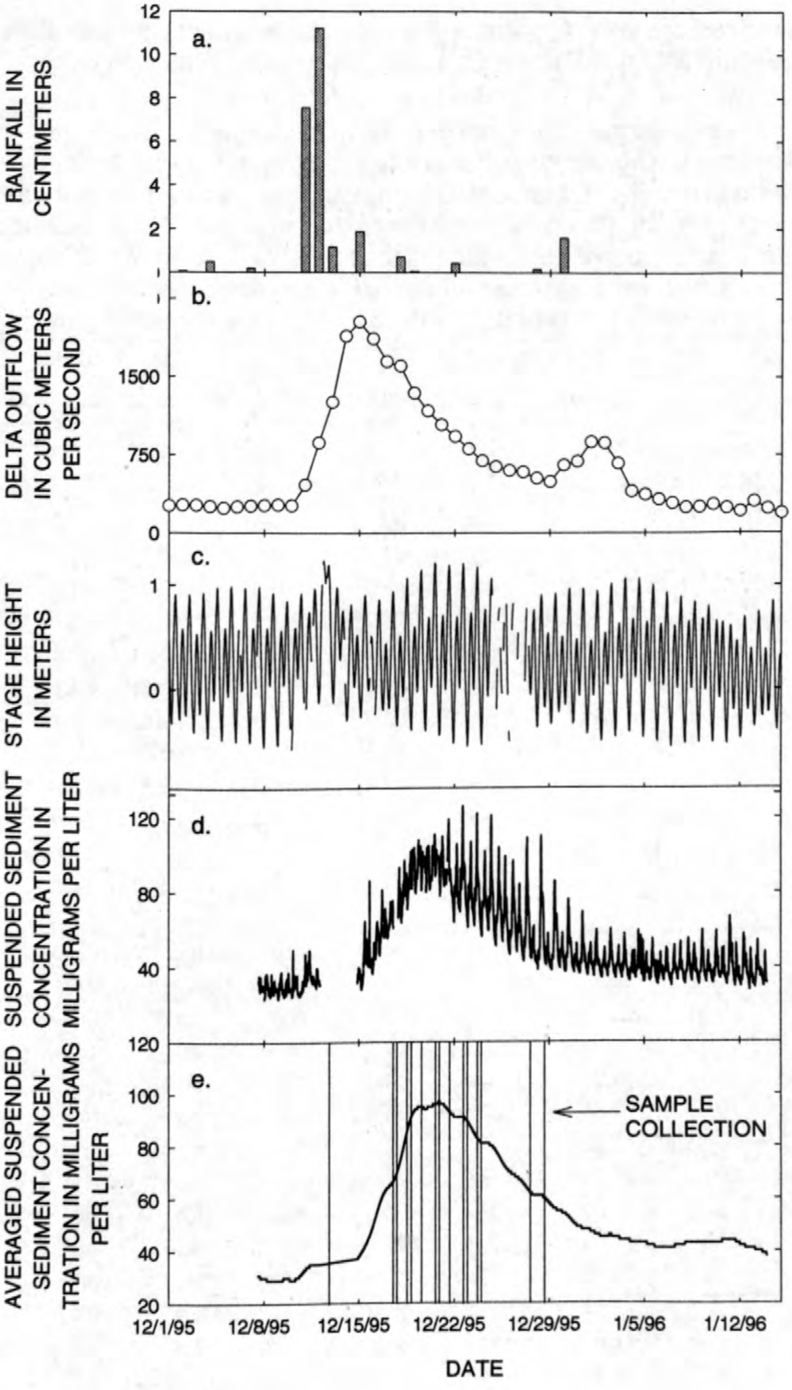

Figure 2. Graphs of Mallard Island local rainfall (a; Fairfield, California, National Oceanic and Atmospheric Administration, 1995), Daily calculated Delta outflow (b; California Department of Water Resources, 1986), stage height (c; California Data Exchange Center, 1998), suspended sediment concentration (d; Buchannan and Schoellhamer, 1998), and tidally-averaged suspended sediment concentrations calculated with a low-pass numerical filter(e; according to the method presented in Schoellhamer, 1996)

Table 1. Pesticide, organic carbon, total nitrogen, and suspended sediment concentrations in suspended sediment samples from Mallard Island study site, California, December, 1995

[Pesticide concentrations are in nanogram per gram dry weight of sediment. Endosulfan and chlordane values are the sum of all isomers. Organic carbon and total nitrogen concentrations are in weight percent. (C/N)a, atomic ratio of organic carbon to total nitrogen; SSC, sediment concentration in milligrams per liter at time of sampling (Buchanan and Schoellhamer, 1998); Total, sum of observed pesticide values; na, not available; no, not observed; ( ), concentration below method detection limit. Suspended sediment samples also were analyzed for the following pesticides, but none were observed (method detection limit in parentheses): dieldrin(2.1), ethalfluralin(0.1), fonofos(0.9), and malathion(1.2)]

Sample date and time	% C	% N	(C/N)a	SSC	Alachlor	Chlordane	Chlorpyrifos	Dacthal	DDD	DDE	DDT
12/12/95 19:00	2.59	0.19	15.9	na	no	(1.5)	1.7	(0.4)	2.1	2.6	(1.9)
12/17/95 12:10	2.63	0.17	18.0	61.2	no	(1.2)	no	(0.4)	3.3	6.9	(0.9)
12/17/95 19:00	2.17	0.16	15.8	na	(0.1)	(1.0)	0.6	(0.4)	1.5	3.5	(2.4)
12/18/95 12:50	2.33	0.16	17.0	75.5	(0.8)	(0.3)	no	no	1.5	3.7	(2.1)
12/18/95 20:20	2.09	0.15	16.2	89.2	no	(0.7)	(0.5)	(0.6)	1.6	3.9	(2.0)
12/19/95 14:05	2.42	0.17	16.6	87.2	no	(1.6)	(0.4)	(0.4)	1.1	2.6	(1.4)
12/20/95 14:30	2.03	0.14	16.9	88.7	no	(2.7)	1.0	(0.5)	2.0	4.2	(2.7)
12/20/95 21:40	2.37	0.17	16.3	89.3	no	no	1.7	(0.6)	2.1	5.1	(2.4)
12/21/95 15:20	2.30	0.16	16.8	93.3	no	(1.1)	0.7	(0.5)	1.6	3.8	(2.1)
12/22/95 16:00	2.23	0.17	15.3	101	no	(2.0)	1.3	(0.8)	1.9	5.2	(2.5)
12/22/95 23:10	2.20	0.16	16.0	64.5	no	(0.5)	1.4	(0.6)	2.1	6.3	(1.6)
12/23/95 16:50	2.04	0.14	17.0	107	no	(0.9)	(0.3)	(0.3)	1.0	3.0	(1.1)
12/23/95 23:50	2.23	0.15	17.3	58.9	no	no	2.1	(0.6)	2.4	4.5	4.4
12/27/95 14:50	2.25	0.17	15.4	46.9	no	(1.2)	1.0	(0.3)	1.0	3.3	(1.5)
12/28/95 15:30	2.59	0.20	15.1	48.0	no	(0.8)	0.8	(0.3)	1.3	3.9	(1.4)

Sample date and time	Endosulfan	Eptam	Molinate	Oxyfluorfen	Pebulate	Sulfotep	Thiobencarb	Trifluralin	TOTAL
12/12/95 19:00	no	(0.5)	(1.1)	1.2	3.3	no	no	no	16.3
12/17/95 12:10	17.7	(1.1)	(1.4)	6.2	no	no	no	1.3	40.6
12/17/95 19:00	no	no	(0.8)	(0.5)	3.4	no	no	no	14.2
12/18/95 12:50	no	(0.2)	(0.2)	no	(1.0)	no	no	no	9.8
12/18/95 20:20	no	no	(1.2)	21.3	3.2	no	no	no	35.0
12/19/95 14:05	no	(0.6)	(0.9)	(0.8)	(1.2)	no	no	no	10.9
12/20/95 14:30	no	no	(1.0)	no	3.3	no	1.6	no	18.9
12/20/95 21:40	no	no	(1.4)	2.2	3.7	no	no	no	19.4
12/21/95 15:20	no	no	(1.4)	1.2	2.3	(0.3)	(1.4)	(0.5)	17.0
12/22/95 16:00	no	no	(2.0)	3.8	no	no	2.4	(0.6)	22.6
12/22/95 23:10	24.6	(1.1)	(1.6)	no	no	no	3.9	no	43.8
12/23/95 16:50	no	no	(0.8)	1.7	no	no	(1.3)	(0.2)	10.7
12/23/95 23:50	no	no	(1.5)	no	4.7	no	2.4	no	22.6
12/27/95 14:50	no	no	(1.3)	1.4	(1.7)	no	1.6	no	14.2
12/28/95 15:30	no	(0.7)	(1.2)	2.1	2.6	no	1.8	no	16.9

limb (Bergamaschi and others, 1997, Bergamaschi and others, in press). Detailed studies of the San Joaquin River and its tributaries indicated that temporal variations in SAP concentrations in the main stem of the river were strongly influenced by the different travel times for water from various tributaries to the sampling site and differences in the SAP concentrations associated with sediment from each tributary (Kratzer and Biagtan, 1996; Kratzer, in press). The lack of temporal variability in SAP concentrations at the Mallard Island site may be the result of inter-annual variability in sediment transport or the result of a more complex mixing of suspended sediments at the Mallard Island study site. Samples form the Mallard Island site integrate inputs from the Sacramento and San Joaquin Rivers and their tributaries, as well as local inputs in the Delta, and bay sediments moved landward by tidal currents.

Of the 19 pesticides analyzed, an average of 10 were observed in each of the 15 samples. This frequency is higher than the number detected previously in San Joaquin River suspended sediments using the same method (Bergamaschi and others, 1997). There were no inter-tidal variations apparent in the frequency of occurrence. Most samples contained chlordane, chlorpyrifos, dacthal, DDD, DDE, DDT, molinate, oxyfluorfen, pebulate, and thiobencarb. Alachlor, endosulfan, eptam, sulfotep, and trifluralin were intermittant in their occurrence (table 1). DDT together with its metabolites were generally the most abundant of the commonly occurring compounds, ranging from 5.1 to 11.1 ng/g. Oxyfluorfen concentrations exceeded 20 ng/g in one sample (December 18 20:20; table 1), but was generally was much lower. Endosulfan occurred in concentrations greater than 17 ng/g in two samples (table 1), but was not observed in other samples. Of the compounds analyzed, only dieldrin, ethalfluralin, fonofos, and malathion were not found in any samples. Among the pesticides, only thiobencarb showed a temporal variation in occurrence; it was only found in samples collected after December 19 (table 1).

SAP concentrations of chlorpyrifos and DDE in the Mallard Island suspended sediment samples were similar to concentrations measured in other suspended sediments collected in San Francisco Bay after spring rains in 1991 (Domagalski and Kuivila, 1993), and similar to measurements of SAPs in Sacramento River (Bergamaschi, and others, 1997). Concentrations of organochlorine pesticides (DDD, DDE, DDT, chlordane) were much lower in the Mallard suspended sediments than in suspended sediments from the San Joaquin River and its tributaries in other years (Kratzer, in press; Bergamaschi, and others, 1997).

Dissolved pesticides

Water samples collected concurrently with the suspended sediment samples were analyzed for pesticide content. Although the analytical methods were considerably different, the 2 methods shared 11 analytes. In comparison to the suspended sediment samples, relatively few pesticides were found in the water samples; only three pesticides were observed. In part this lower frequency of pesticide occurrence may be the result of the higher concentrations required for observation in the much smaller water samples (1 L) in comparison to the suspended sediment samples (~200 L).

Table 2. Pesticide concentrations measured in water samples from the Mallard Island study site, California, December 1995

[Values are in nanograms per liter. no, not observed; ( ), concentration below method detection limit (Crepeau and others, 1994). Water samples also were analyzed for the following pesticides, but none were detected (method detection limit shown in parentheses): alachlor(22), atrazine(28), butylate (19), carbaryl(31), carbofuran(28), chlorpyrifos(35), cyanazine(50), dacthal(63), eptam(129), ethalfluralin (31), fonofos(25), malathion(44), methidathion(28), molinate(53), napropamide(28), pebulate(44), thiobencarb(60), trifluralin(60)]

Sample date and time	Diazinon	Metolachlor	Simazine
12/12/95 13:30	no	no	(23)
12/12/95 13:30	no	(1)	(33)
12/17/95 12:10	no	no	64
12/17/95 12:10	(2)	no	(55)
12/17/95 19:00	(6)	no	(38)
12/18/95 12:50	(9)	(4)	69
12/18/95 12:50	(5)	(2)	(57)
12/18/95 20:20	no	no	(25)
12/19/95 14:05	(2)	(5)	68
12/19/95 20:50	(2)	(3)	(32)
12/20/95 14:30	(7)	(4)	76
12/20/95 21:40	no	(4)	(53)
12/21/95 15:20	no	(6)	(59)
12/22/95 16:00	no	(4)	(47)
12/22/95 23:10	no	(3)	(15)
12/22/95 23:10	no	(4)	(29)
12/23/95 16:50	no	(6)	(48)
12/23/95 23:50	no	(5)	(34)
12/27/95 14:50	no	(4)	(28)
12/28/95 15:30	no	(5)	(24)

The only dissolved pesticides detected in the water sampled between December 12-28 were diazinon, metolachlor, and simazine (table 2). The following pesticides were not detected in the dissolved phase in any of the samples: alachlor, atrazine, carbaryl, carbofuran, chlorpyrifos, cyanazine, dacthal, diazinon-oxon, diethatylethyl, eptam, fonofos, malathion, methidation, molinate, napropamide, pebulate, sulfotep, thiobencarb, and trifluralin. Simazine concentrations ranged from 15 to 76 ng/L and were highest during the peak of suspended sediment concentrations (table 2).

DISCUSSION

The distribution of pesticides between water and suspended sediment at equilibrium commonly is described by the aqueous phase-sedimentary organic carbon partition coefficient, K_{oc}, which is defined as the concentration in a system at equilibrium of sorbed pesticide in ng/g of sedimentary organic carbon divided by the concentration of dissolved pesticide in nanograms per milliliter. Log K_{oc} values for pesticides in this study vary from 1.97 milliliters per gram (mL/g; molinate) to 5.38 mL/g (DDD and DDT) (table 3), indicating a wide range in expected equilibrium behavior for the suite of compounds analyzed.

K_{oc} values may be used to calculate equilibrium dissolved pesticide concentrations from SAP concentrations (for example, Domagalski and Kuivila, 1993; Kratzer, in press). Calculated dissolved pesticide concentrations for waters in equilibrium with the Mallard Island suspended sediment samples (table 4) were very different from measured dissolved pesticide concentrations (table 2). Calculated concentrations of alachlor, eptam, molinate, pebulate, and thiobencarb were well above the dissolved pesticide method detection limits, yet none of these compounds were observed in the Mallard Island water samples. Also, there is no significant positive correlation between individual SAP concentrations and K_{oc} in this study. Nor are there significant relations between SAP concentrations and sediment organic carbon content or the sediment carbon quality as indicated by C/N (table 1). Taken together, these observations indicate that pesticides are not in equilibrium between water and sediments, in agreement with what has been observed previously (Domagalski and Kuivila, 1993; Pereira and others, 1996; Brown, 1997; Kratzer, in press), and suggest that equilibration was not a determining factor for SAP concentrations.

Table 3. Pesticide log K_{oc} and usage data for the regions of the Delta and the Sacramento and San Joaquin drainages that drain into the Delta

[Log K_{oc} values are in milliliters per gram. Usage values are integrated amounts applied during registered activities for the portion of the Sacramento and San Joaquin River drainages, and the Delta that ultimately drain into the San Francisco Bay at Mallard Island for 1995. Usage values are in kilograms active ingredient applied]

Pesticide	Log K_{oc}	[7]Usage
Alachlor[1,2]	2.28	12,521
Chlordane[2]	5.15	53
Chlorpyrifos[1,3]	3.93	380,113
Dacthal[4]	3.70	8,968
DDD[5]	5.38	—
DDE[5]	5.29	—
DDT[1,2,6]	5.38	—
Dieldrin[3,5,6]	4.08	—
Endosulfan[5]	3.37	16,842
Eptam[1,5]	2.38	100,309
Ethafluralin[4]	3.60	15,069
Fonofos[4]	2.94	18,584
Malathion[3]	3.10	87,851
Molinate[5]	1.97	647,312
Oxyfluorfen[4]	5.00	70,632
Pebulate[1]	2.66	91,243
Sulfotep[5]	2.82	32
Thiobencarb[4]	2.95	260,624
Trifluralin[1]	3.96	230,712

[1] Kenega (1980)
[2] Environmental Protection Agency (1994)
[3] Howard (1991)
[4] Wauchope and others (1991)
[5] Montgomery (1993)
[6] Jury and others (1987)
[7] California Department of Pesticide Regulation (1996)

Another possible determining factor for SAP concentrations was mixing of sediments from varying inputs. The absence of temporal trends may be the result of tidal mixing of riverine suspended sediments before they reach the bay, or mixing with estuary sediments. Mixing would result from episodes of deposition and resuspension during transport, or shear effects in the flow. Mixing would obscure any temporal riverine signal. If tidal mixing obscured changing inputs, the expected result would be a gradual change in the amount and identity of SAPs. However, early SAP occurrences and concentrations (Dec. 12, table 1) were not different from later samples, suggesting that although mixing probably did occur, mixing of sediments from various inputs did not cause the observed distribution during the sampling period.

SAP concentrations also may have been influenced by the amount of pesticide applied in the drainage basin. However, there is no apparent relation between the amount applied in the Central Valley (fig. 1, table 3) and the SAP concentration (table 1).

What then controls the SAP concentration and distribution? The occurrence of molinate and thiobencarb may provide some insight. Molinate and thiobencarb are herbicides used in the cultivation of rice. They were applied in large amounts (table 3) to rice fields in April-June (California Department of Pesticide Regulation, 1996). In comparison with the other pesticide analytes, molinate has the lowest log K_{oc} (1.97), and thiobencarb has an intermediate value (2.95, table 3). Nevertheless, they are observed 6 months later on suspended sediments, suggesting that long equilibration times (in relation to transit times) may be an important factor.

One additional consideration is whether the observed SAP concentrations will have any effect on the health of the ecosystem. EPA chronic water quality criteria for protection of freshwater aquatic life contaminant chronic exposure levels have been established for four of the pesticides found in this study (chlordane, DDT, endosulfan, and chloropyrifos; Environmental Protection Agency, 1996). Of these four pesticides, only the SAP concentrations of ΣDDT approached the criteria of 1 ng/L. The observed ΣDDT ranged from 0.4 – 1.0 ng/L.

Despite the low concentrations of SAP, the SAP may still affect the estuary. Filter-feeding organisms have been shown to accumulate pesticides to levels far in excess of the associated sediment concentrations (for example, Pereira and others, 1996; Brown, 1997). Thus, annual flux of sediments with low concentrations of pesticides into the Bay may cause chronic exposure effects because of the long residence time of sediments.

Table 4. Calculated dissolved pesticide concentrations in water samples from the Mallard Island study site, California, December 1995

[Calculated from pesticide concentrations on suspended sediments using K_{oc} values in table 3. Values are given only for pesticides analyzed both in the dissolved and particulate phases. Values are in nanograms per liter. no, not observed associated with sediment; ( ), concentration below method detection limit (Crepeau and others, 1994)]

Sample date and time	Alachlor	Chlor-pyrifos	Dacthal	Eptam	Molinate	Pebulate	Thio-bencarb	Triflu-ralin
12/12/95 19:00	no	(9)	(4)	96	531	322	no	no
12/17/95 12:10	no	no	(3)	173	574	no	no	(6)
12/17/95 19:00	36	(3)	(4)	no	400	341	no	no
12/18/95 12:50	181	no	no	(37)	90	97	no	no
12/18/95 20:20	no	(3)	(6)	no	631	332	no	no
12/19/95 14:05	no	(2)	(3)	103	437	117	no	no
12/20/95 14:30	no	(6)	(4)	no	507	353	88	no
12/20/95 21:40	no	(8)	(5)	no	641	345	no	no
12/21/95 15:20	no	(4)	(5)	no	685	222	71	(3)
12/22/95 16:00	no	(7)	(8)	no	972	no	122	(3)
12/22/95 23:10	no	(7)	(6)	214	762	no	198	no
12/23/95 16:50	no	(2)	(3)	no	430	no	69	(1)
12/23/95 23:50	no	(11)	(5)	no	700	464	123	no
12/27/95 14:50	no	(5)	(3)	no	613	163	81	no
12/28/95 15:30	no	(4)	(3)	127	563	256	89	no

CONCLUSIONS

A large number of pesticides were found associated with suspended sediments entering the San Francisco Bay during the first storm of the 1995-1996 water year. The total amounts of pesticides associated with the sediments were similar to those measured previously in Sacramento River suspended sediment, but much lower than those previously measured in San Joaquin River suspended sediment. Few pesticides were found in concurrent water samples and their concentrations were much lower than may be expected from equilibrium partitioning between the aqueous and sedimentary phases.

There were no significant trends in the concentration or occurrence of pesticides found as the peak in suspended sediment concentration passed the sampling site. Nor were there significant relations between pesticide content and such parameters as K_{oc} of the pesticide, organic carbon content of the sediment, or amount of pesticide applied annually in the Central Valley. This finding suggests that observed sediment-associated pesticide concentrations may reflect disequibrium between sedimentary and aqueous phases resulting from long equilibration times at locations where pesticides were applied, and relatively short transit times for reequilibration to occur

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Evaluation of Polychlorinated Biphenyl Contamination in the Saginaw River Using Sediments, Caged Fish, and SPMDs

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ABSTRACT

The Saginaw River is contaminated with a number of industrial pollutants including polychlorinated biphenyls (PCBs). Understanding of the risk posed by PCBs in the aquatic system requires measurement of the dissolved, bioavailable levels of PCBs. In this study, three means of assessing bioavailability were compared: sediments, caged fish, and semipermeable membrane devices (SPMDs). Caged channel catfish and SPMDs were placed in the river for one month at five sites where sediments were also sampled. PCBs were analyzed by congener specific methods to determine PCB concentrations and patterns. Total PCB concentrations ranged 33 - 277 ng/g in sediments, 46 - 290 ng/g in caged fish, and 77 - 792 ng/g in SPMDs. SPMD and sediments provide complementary PCB information: sediments reflecting long term accumulation while SPMDs indicate what was present in the water at the site during the sampling period. Differences in PCB patterns in caged fish and SPMDs are due to lower chlorinated PCBs having reached steady state with the fish but not with the SPMD. Sediments were assumed to have reached equilibrium with PCBs in the water. Concentrations and patterns of dissolved PCB congeners were estimated from sediment data using an equilibrium model. The SPMD-based dissolved concentrations were estimated by using SPMD accumulation rates for 86 PCBs that were determined in an earlier SPMD calibration study. Steady-state bioconcentration factors for PCB congeners were used to estimate times required to reach steady state; congener concentrations in the fish were then normalized to steady state, and an equilibrium model was applied. The three methods indicated that similar patterns and concentrations of dissolved PCBs were present in the Saginaw River.

INTRODUCTION

Polychlorinated biphenyl (PCB) pollution in the Great Lakes region, in particular Saginaw Bay and Lake Huron has been documented for many years (Michigan DNR, 1988). Fish consumption advisories have been issued because of high concentrations of PCBs in sport fishes. Effects on wildlife have included increased reproductive failure in fish-eating birds and elevated mink kit mortality (Alan, 1991; Giesy and others, 1997). Comprehensive risk assessment of an aquatic system requires information about the bioavailable aqueous fraction of the contaminant burden– that concentration of a contaminant which is accessible to organisms at each trophic level. Currently there are no accurate procedures to assess the bioavailability of contaminants (Dickson and others, 1994). Exposure of organisms that have minimal capability for biotransformation approaches the criterea for defining bioavailability, but few organisms meet such criteria for a great number of contaminant classes. Therefore, regulatory agencies must rely on modeling efforts.

The objective of this study was to compare the semipermeable membrane device (SPMD) method of water sampling with sediment-based and fish-based biomonitoring methods. The SPMD method utilizes a passive sampler that combines membrane diffusion and liquid/liquid partitioning to concentrate low to moderate molecular mass (<600 Da) lipophilic compounds from water. (Huckins and others, 1996). Five sites along the Saginaw River were sampled using these methods and the resulting congener specific PCB concentrations in each of these sample types were interpreted so concentrations of bioavailable PCBs could be estimated. Concentrations of PCB congeners were estimated from previously determined SPMD uptake rates, bioconcentration factors (BCFs) and sediment partition coefficients.

METHODS

Caged fish, SPMDs, and sediments were used to sample PCBs at five sites along the Saginaw River as shown in Figure 1:

1) the terminus of the Shiawassee River,
2) terminus of the Tittabawassee River
3) Saginaw River- Zilwaukee Bridge,
4) near the wastewater treatment plant and industrial wastewater inlets at Middleground Island at Buoy 26 (site of dredge spoil disposal)
5) downstream of Bay City at Buoy 10

Figure 1. Map of Saginaw River Sampling Sites

SPMD Method

SPMDs (2.5-cm x 152-cm, 85 μm membrane thickness) with a total mass of 8.35 g each were made of a thin film of 95% pure triolein (1.64 g) sealed inside low-density polyethylene lay-flat tubing. Each analytical sample, consisting of four SPMDs, was transported to the field sites in new, airtight, hexane-rinsed, metal cans. At the site, SPMDs were placed inside stainless steel cages, and placed in the Saginaw River 2-10 meters above the sediment for 35 days. Field blank SPMDs accompanied the analytical samplers to the sampling sites, were replaced in their airtight containers, and returned to the laboratory.

Caged Fish Method

Juvenile hatchery-reared channel catfish (*Ictalurus punctatus,* 8-10 cm) were deployed in cages at each site for 30 days, at the same level as the SPMD cages. Negative control samples of the catfish were obtained at the beginning of the study as quality control matrix samples.

Sediment Sampling Method

Sediment samples were collected in triplicate from each site by stainless steel Ponar® dredge. Sediments were composited and homogenized from each site to insure a representative sample was analyzed.

Analytical Methods

Fish and sediment samples were extracted with methylene chloride, while SPMDs were dialyzed twice in hexane. Percent lipid determinations of fish samples were determined gravimetrically. Extracts and dialysates were treated by two-stage reactive cleanup, high performance gel-permeation chromatography (Feltz and others, 1995). Sediment extracts and SPMD dialysates were copper treated to remove sulfur. PCB congeners were analyzed by capillary GC/ECD to quantify 86 peaks. Total organic carbon in sediment was determined by coulometry.

PCB Water Concentration Estimations

Sediment-based water concentrations were calculated by the equation:

$$Cw_{sed} = C_{sed} f_{oc}^{-1} K_{oc}^{-1}, \tag{1}$$

where

Cw_{sed} is the sediment-based water concentration
C_{sed} is concentration in the sediment
f_{oc} is fraction of organic carbon in the sediment
K_{oc} is the sediment-water partition coefficient

The K_{oc} values for PCB congeners were estimated using K_{ow} values (Hawker and others, 1988) and the approximation that K_{oc} is $0.41K_{ow}$ (Karickhoff, 1981).

Caged fish PCB concentrations were used to estimate dissolved PCB concentrations by normalizing to steady state PCB concentrations:

$$Cw_{fish} = f_{ss}\ C_{fish}\ BCF^{-1}, \tag{2}$$

where

Cw_{fish} PCB water concentration is the caged fish-based
f_{ss} is the fraction of steady state reached in 28 days
C_{fish} is the calculated steady state PCB congener concentration in the fish
BCF is the steady-state bioconcentration factor for fish

Specific channel catfish steady-state *BCFs* for PCB congeners were not found in the literature, so selected *BCFs* from several studies with other species of fish were used (Mackay and others, 1992; Bruggeman and others, 1984; and Fox and others, 1994). The rates at which each PCB homolog group reached equilibrium was estimated from the *BCF* data.

SPMD-based water concentrations were calculated as follows:

$$Cw_{spmd} = C_{spmd}\, m_s R_s^{-1}\ t^{-1}, \tag{3}$$

where

Cw_{spmd} is SPMD-based PCB concentration in the water
C_{spmd} is the PCB concentration in the SPMD
m_s is mass of the SPMD
R_s is SPMD accumulation rate constant for the PCB
t is time

R_s values were obtained from a previous exposure study where SPMD uptake of 86 PCB congeners and dissolved PCB water concentrations were measured (Meadows and others, 1998).

PCB Pattern Analysis

Patterns of the PCB congener data were evaluated using SIMCA (Soft Independent Modeling by Class Analogy) principal components analysis (Schwartz and others, 1987; and Wold and others, 1984). PCB data was normalized to fractional composition and univariately transformed. A 95% confidence interval was used.

RESULTS AND DISCUSSION

Total-PCB concentrations in caged fish, sediments and SPMDs are presented in Table 1 as nanograms per gram of matrix. PCB congener data will be presented as SIMCA plots. All matrices suggest that Site 5 has the highest level of PCB contamination. Total PCB concentrations in the sediments at sites 1-5 were not directly related to variations in percent organic carbon values, which were 1.2, 1.0, 3.0, 2.8, 3.5, respectively.

Recoveries of PCBs ranged from 75% to 114% and variability was < 20%. Method detection limits were ~0.2 ng/g per congener or 25 ng/g in terms of total-PCBs.

Based on an SPMD uptake model, accumulation of PCBs remained in the linear phase during the one month period (Gale, 1998). The same situation existed for caged fish except for Cl_2 PCBs which reach steady in about 13 days. All other PCBs were well within the linear phase, with Cl_8 PCBs linear uptake into SPMDs being one and a half years.

Table 1. Total PCB concentrations in samples from Saginaw River (ng/g)

Sites	Sediment	Caged Fish	SPMD
1. Shiawasee	33	46	77
2. Tittabawasee	96	58	100
3. Zilwaukee	29	68	85
4. Middle Ground	98	71	156
5. Buoy 10A	277	290	792

PCB patterns were similar in each matrix but differed between matrices. SIMCA plots of the first two principle components are presented for all three matrices in Figure 2. The differences between SPMD and sediment PCB patterns reflect the long term accumulation of higher chlorinated PCBs and weathering of the lower chlorinated PCBs in the sediment. The differences between the SPMD and caged fish patterns can be the result of the lower chlorinated PCBs having reached steady state. Patterns of Cl_6 - Cl_{10} PCBs in the fish and SPMDs were similar. Feral fish from the Saginaw Bay, sampled near the mouth of the River, show slightly different patterns than caged fish, likely because PCBs homologs Cl_{3-5} have reached steady state.

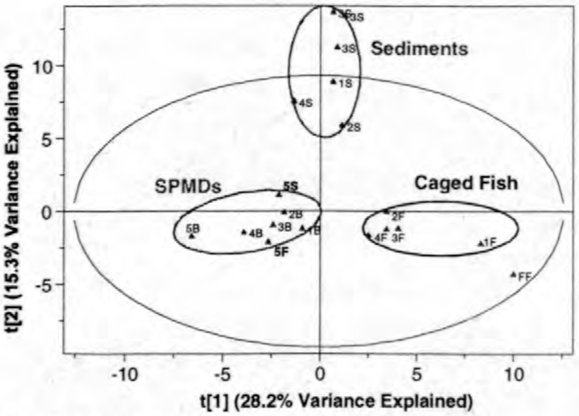

Figure 2. SIMCA plots of PCB congeners in sediments, caged fish and SPMDs. Score t[1]/t[2] using all sites. Large oval boundary line indicates 95% confidence interval.

Bioavailable aqueous concentrations of PCBs were estimated using the models described earlier in the text and are presented in Table 2 in terms of total PCBs. The PCB patterns are represented in the Figure 3 SIMCA plot. All estimated patterns of dissolved PCB are similar at all sites and fall within the 95% confidence interval. There is some separation between methods used to estimate the PCB patterns in the water, however the patterns are very similar.

Table 2. Bioavailable concentrations of total-PCBs in Saginaw River (ng/L) calculated from matrix*

Site	*Sediment	*Caged Fish	*SPMD
1. Shiawasee	8	4	1
2. Tittabawasee	8	5	1.4
3. Zilwaukee	3	6	1.2
4. Middle Ground	18	7	2.2
5. Buoy 10A	38	27	12

A filtered, large volume water sample that was taken two years earlier (Verbruegge and others, 1995) estimated similar dissolved PCBs concentrations (1.9 - 16 ng/L). This similarity may indicate that the PCB sources have not changed or dramatically increased at these sites over the two year period.

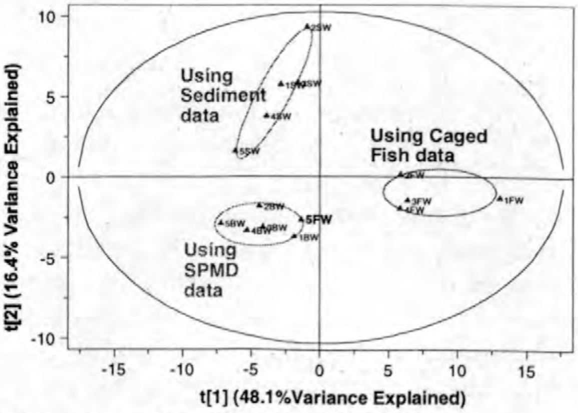

Figure 3. SIMCA plot of dissolved PCB congeners calculated from sediment, caged fish and SPMD PCB concentrations. Scores: t[1]/t[2]. Large oval boundary indicates 95% confidence interval.

CONCLUSIONS

The three models using SPMDs, caged fish and sediments predicted similar bioavailable concentrations and patterns of PCBs in the Saginaw River. PCB patterns in each matrix from sites 1-4 were distinctive. Patterns at at Buoy 10A near the mouth of the river (site 5) were slightly different, possibly indicating a different PCB source. All matrices at this site also indicated that PCB levels were higher than the other four sites. Concentrations and patterns of PCBs in sediments reflect longer term accumulation of higher chlorinated PCBs, whereas the fish and SPMDs reflect what is dissolved in the water column at a specific site over a period of time.

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Butyltin Contamination in Sediments and Lipid Tissues of the Asian Clam, *Potamocorbula amurensis*, near Mare Island Naval Shipyard, San Francisco Bay

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ABSTRACT

The former Mare Island Naval Shipyard, near Carquinez Straits in the northern reach of San Francisco Bay, California, has been a point source for introduction of butyltin compounds into the onshore and marine environment. Because tributyltin (TBT) is known to be a potent endocrine disrupting chemical, a study of butyltins in soil, benthic sediments, and lipid tussue of a common local bivalve, the Asian clam *Potamocorbula amurensis*, has been undertaken to evaluate the extent of the contamination. Soils from a sandblasting site at the shipyard contained low concentrations of mono-, di-, and tributyltin (0.3- 52 ng/g, total butyltin). Benthic sediments from nearby Mare Island and Carquinez Strait contained concentrations of total butyltin ranging from 1.3-8.1 ng/g. In contrast, clams accumulated much higher concentrations of di- and tributyltin (152-307 ng/g, total butyltin, with TBT and dibutyltin (DBT) making up from 54-85% and 15-46%, respectively, of the total butyl body burden of the clams). Biota Sediment Accumulation Factors (BSAFs) for butyltins in *Potamocorbula* were in reasonable agreement with literature values; they are greater than those of neutral hydrophobic compounds, suggesting that partitioning and binding processes may be involved in bioaccumulation. Therefore, there is potential for long-term chronic effects of TBT in San Francisco Bay.

INTRODUCTION

The Mare Island Naval Shipyard in San Francisco Bay, located at Mare Island, Vallejo, California (Figure 1), is a 6,000 acre U. S. Naval facility that repaired, overhauled, and maintained Navy vessels until April 1996, when the base was permanently closed. The Mare Island facility was the principal fleet supply base for the Pacific Coast. One important activity at the base was sandblasting and painting of submarine parts, ship hulls, vehicles, railroad cars, and small buildings. Over a period of years, a one-acre expanse of sandblast abrasives was generated from this activity at site IR04 on the northeastern shore of Mare Island (Figure 1). An estimated 400 cubic yards per year of spent sandblast abrasive material was stored and disposed of on the ground surface, in the tidal marsh areas along the shoreline of Mare Island Strait, and also in Mare Island Strait. The beach near site IR04 is referred to as "Green Beach" because of the intense green color of the sandblast abrasives. Site IR04 sluices directly into Mare Island Strait, which is contiguous with

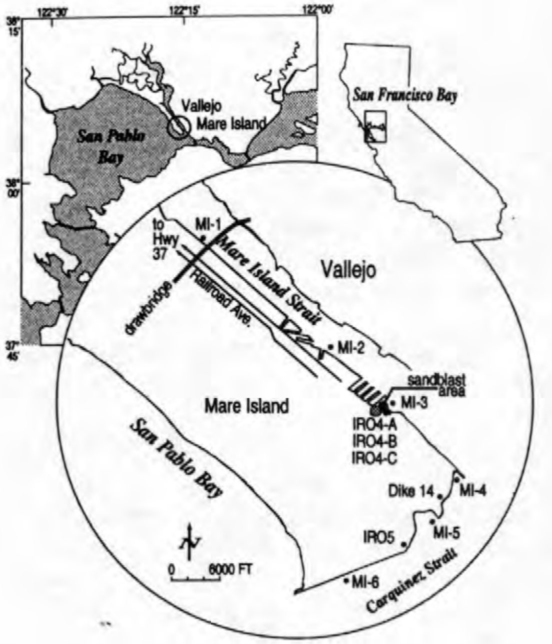

Figure 1. Map showing sampling locations near the former Mare Island Naval Shipyard, San Francisco Bay, California.

Carquinez Strait and San Pablo Bay. These sandblast abrasives and associated paint flakes contained the antifouling compound tributyltin (TBT).Tributyltin is the active ingredient of many bactericidal, fungicidal, insecticidal, acaricidal, and wood preservation products that have biocidal activity against a broad range of organisms. Tributyltin is used primarily as an antifoulant paint additive on ship and boat hulls, seawater piping systems, docks, fishnets, and buoys to prevent the growth of barnacles, algae, mussels, tube worms, and other marine organisms. Antifoulant agents discourage the growth of marine animals on ship-hulls by leaching out of the paint at a very slow rate, thus preventing attachment and growth of these organisms. About one million pounds of butyltin compounds are sold annually.

Prior to 1971, copper oxide was used as the primary antifoulant in marine paints. However, its use was discontinued because of its toxicity, and it was replaced by butyltin compounds. Since then, there have been numerous reports of contamination of aquatic and marine environments by butyltin compounds (Goldberg, 1986; Stang and Seligman, 1986; Clark and others, 1988; Gabrielides and others, 1990; Ritsema and others, 1991; Stewart and Thompson, 1994). Detrimental effects of TBT on biota include: high mortality on larvae and severe malformations of shells in oysters (Alzieu and others., 1981-1982), imposex (male genitalia imposed on females) in dogwhelk populations (Bryan and others, 1986), growth retardation in mussels (Salazar and Salazar, 1991) and micro-algae (Beaumont and Newman, 1986), and deformities in Fiddler crabs (Weis and others, 1987). Because of these environmental problems, several countries have adopted measures to control the usage and release of TBT into the environment. In the U.S. all of the organotins are regulated by the "Organotin Antifouling Paint Control Act of 1988".

It is estimated that in seawater 93% of the TBT exists mainly as the neutral hydroxide (TBTOH), with smaller amounts of the chloride (TBTCl; 2-3%) and cation (TBT^+; 4-5%) (Arnold and others, 1997). In addition, the TBT^+ cation may complex with other organic ligands such as naturally occurring carboxylic acids and amino acids in sediments, cellular components of microorganisms, and tissues. Because of its hydrophobic (Log K_{ow}=4.1; K_{ow} is the octanol/water partition coefficient) and ionic characteristics, TBT tends to accumulate in the lipid tissues of many aquatic species (Davies and McKie, 1987; Wade and others, 1988; Iwata and others, 1994; Kannan and others, 1995; Kannan and others, 1996). TBT also binds to macromolecules such as the protein glutathione in organs such as kidney and liver (Kannan and Falandysz, 1997). Because of the unique physicochemical properties of butyltin compounds, it has been postulated that significant amounts of TBT may be associated with particulate material and suspended sediments in coastal plain estuaries, and that TBT contaminated sediments may act as sources for dissolved TBT (Unger and others, 1987). Therefore, the potential exists for significant amounts of TBT to be leached out of contaminated sediments at the Mare Island site and to contaminate neighboring Mare Island Strait, Carquinez Strait, and San Pablo Bay. This area of the San Francisco Bay is the state's richest "nursery" for young Dungeness crabs, whose numbers have declined significantly in the last few decades. It is known that juvenile Dungeness crabs in San Francisco Bay feed on fish, crustaceans, bivalves, and other food sources, and also that they accumulate hydrophobic contaminants such as PAHs, DDE, and PCBs (California Department of Fish and Game, 1983). Because juvenile crabs may be vulnerable to toxic contaminants such as butyltins, it is important to assess the extent of contamination of sediments and benthic organisms in the vicinity of the sand-blasting site IR04.

The Asian clam *Potamocorbula amurensis* was introduced to San Francisco Bay in 1986, presumably in ballast water from cargo ships (Carlton and others, 1990). Since then the clam has spread throughout the Bay, displacing much of the former benthic community. It is now an important food source for San Francisco Bay biota such as diving ducks, sturgeon, and Dungeness crab. This suspension-feeder has the potential to alter completely the food-web dynamics in the Bay (Nichols and others, 1990). Because of this clam's grazing habits, and its ability to filter large volumes of water (1-5L per day), it also has the potential of being a biomonitor of pollutants in the Bay (Pereira and others, 1992). Bivalves, unlike fish and other species, are deficient in degradative enzymes such as microsomal oxidases and mixed-function oxidases which are responsible for the degradation of organic contaminants. Therefore, bivalves can serve as integrators and biomonitors of pollutants.

A study was initiated in 1995 to determine if TBT and its degradates have contaminated Mare Island Strait, Carquinez Strait, and possibly San Pablo Bay. The objectives of this investigation were to determine the extent of contamination of benthic sediments near Mare Island, and to investigate the bioavailability of TBT and its degradates to *Potamocorbula amurensis*.

FIELD AND LABORATORY METHODS

Sample collection

Soil samples (IR04-A, -B, -C, Dike 14, IR05) were collected at five locations on Mare Island (Fig. 1). Surficial bottom sediments (MI-1 to MI-6) were collected at six sites from Mare Island and Carquinez Straits (Fig. 1) using a Van Veen grab sampler. The upper 8 cm of sediment were sampled with a stainless steel pipe to include the depth to which the *Potamocorbula* clams burrow (~1-2 cm) and also any sediment immediately below this that may influence the clams, possibly from bioturbation and mixing of the underlying sediments. Sediments and soils were stored in a freezer at -15°C prior to analysis. Clams [MI-1(T) to MI-6 (T)] were collected in the same general vicinity as the sediment grab samples using the Van Veen grab sampler, followed by screening. Clams were allowed to depurate in ambient water for 48 hours to clear sediment from the digestive tract (Brown and others, 1995), rinsed with distilled water, and stored in a freezer at -15°C until analysis.

Sample preparation and analysis

Soils and sediments were air dried and ground using a clean porcelain mortar and pestle. The samples were passed through a 32 mesh stainless steel sieve. Clams were shucked, excess moisture removed with a clean filter paper, and the sample ground and homogenized. Sediment samples were extracted four times with 0.2% tropolone (2-hydroxy-2,4,6-cycloheptatrien-1-one) in methylene chloride by the method of Wade and others (1990) and McGee and others (1995). Tissue samples were extracted three times by maceration with 0.05% tropolone in methylene chloride in the presence of sodium sulfate by the method of Garcia-Romero and others (1993). A surrogate (tripropyltin chloride) was added to all samples prior to extraction and was used for the determination of analyte concentrations. The sample extracts were concentrated by Kuderna-Danish technique and the solvent exchanged to 10 ml of hexane. Organotin compounds were hexylated with Grignard Reagent (n-hexylmagnesium bromide) and the derivatized extract was purified using silica gel/alumina column chromatography. The hexylated organotin compounds were analyzed by high resolution capillary gas chromatography using flame photometric detection (GC/FPD) with a 610 nm filter for tin specific analyses. This method quantitatively determines tetrabutyltin (4BT), TBT, DBT, and monobutyltin (MBT). All concentrations are reported as ng Sn/g dry weight. Quality control samples (blanks, matrix spikes, and matrix spike duplicates) were processed with each batch of 20 or less samples in a manner identical to the actual samples. No butyltins were detected (peaks with area greater than three times the signal to noise ratio) in any of the blanks (tissues or sediment/soils). The recoveries of analytes in the matrix spike and matrix spike duplicates were acceptable (80-130 %), except MBT in sediments (22-46% recoveries). The detection limit, based on peaks with a signal/noise ratio of at least 3, is 0.2 ng Sn/g for sediments and 10 ng Sn/g for tissues.

TOC Analyses

TOC in soils and sediments was determined by MSI Analytical Laboratories, Santa Barbara, CA. The method involved acid treatment to remove inorganic carbon, followed by combustion to CO_2 with thermal conductivity detection on a Control Equipment Corporation instrument.

Lipid Analyses

Lipids in tissue samples were determined by methylene chloride extraction, followed by solvent evaporation, and determination of the weight of the extract.

RESULTS AND DISCUSSION

Total organic carbon and nitrogen in soils from Mare Island and sediments from Mare Island Strait and Carquinez Strait, along with the lipid content of *Potamocorbula amurensis* are shown in Table 1. In general, the organic carbon and nitrogen content of the benthic sediment were similar at the six sites, resulting in a relatively

Table 1. Total organic carbon and nitrogen in soils and sediment and lipid content of *Potamocorbula amurensis*, Mare Island, California.

Site	Weight %*		C/N ratio
	C	N	
Benthic Sediment			
MI-1	1.27	0.15	8.58
MI-2	1.34	0.15	9.21
MI-3	1.31	0.15	9.04
MI-4	1.23	0.13	9.64
MI-5	1.18	0.14	8.61
MI-6	1.18	0.14	8.49
Soils			
IR04A	0.29	0.03	11.30
IR04B	0.06	0.03	2.48
IR04C	0.04	0.03	1.30
IR05	0.30	0.02	13.2
Dike 14	1.36	0.10	13.3
Potamocorbula			% Lipid*
MI-1 (T)			19.1
MI-2 (T)			26.7
MI-3 (T)			6.7
MI-4 (T)			9.2
MI-5 (T)			8.7
MI-6 (T)			11.4

* On a dry weight basis

uniform C/N ratio that is characteristic of values in the range between terrigenous organic inputs [C/N 12~14, Prahl and others (1980)] and inputs from marine phytoplankton [C/N ~6, Muller (1977)]. The C/N ratios within the soils from sites IR04A, IR05, and Dike 14 gave values showing mainly terrigenous input. Soils from sites IR04B, and IR04C, which were very sandy and therefore had much lower organic content in general, gave much lower C/N values. Site IR04A had a much greater TOC value than sandy sites IR04B and IR04C, which, as will be seen later, resulted in sorption of greater amounts of butyltins. The lipid content of *Potamocorbula amurensis* varied between 6.7 and 26.7 % (dry weight basis); lipid content is dependent on the physiological condition of the clam communities at the different sites.

Soils: Concentrations of butyltin compounds in soils from Mare Island and in sediments and *Potamocorbula amurensis* from Mare Island Strait and Carquinez Straits are shown in Table 2. Soils from sites IR04A, IR04B, and IR04C within the sand-blasting zone all contain TBT and its degradates DBT and MBT. Of these three sites, site IR04A, which had the highest organic carbon content, also contained the highest levels of butyltins, suggesting that the sorption of these compounds, especially in the case of TBT, is controlled mainly by a partitioning process into the organic carbon of the soils (Chiou and others, 1983). At site IR04A, concentrations of the individual butyltins were in the order of decreasing lipophilicity, with TBT > DBT >MBT. The lipophilic t-butyl groups of TBT control sorption via a partitioning process into organic carbon. At sites IR04B and IR04C, the sandy soils, this order generally was reversed with concentrations of MBT > DBT > TBT at IRO4B, and MBT>TBT>DBT at IRO4C. This phenomenon occurs probably because the more ionic MBT and DBT are bound to the sand particles by ionic and ion-dipole bonds. Here, the ionic properties of the molecule may control the sorption process. It is reported that the dominant interaction of triorganotin (TOT) compounds with mineral surfaces is sorption of TOT^+ cations to negatively charged surfaces by cation exchange (Wiedenhaupt and others, 1997). These lipophilic and ionic properties of butyltins probably have important implications for the bioavailability and fate and transport of these compounds in estuarine systems. The presence of DBT and MBT also indicates that abiotic or microbiological degradation processes of TBT are occurring in soils at site IR04. Thus, TBT is progressively dealkylated and detoxified in soils, resulting in the formation of the less toxic DBT and MBT. Soil IR04-A from site IR04 also contained trace levels of 4BT, an impurity in commercial TBT. Site IR05, which is outside the sand-blasting zone, contained trace levels of TBT. Soil from the Dike 14 area, also outside the sand-blasting zone, contained no detectable amounts of butyltin.

Sediment: All the benthic sediments from Mare Island and Carquinez Straits (Table 2; MI-1 to MI-6) contained relatively low concentrations of TBT and its degradates, with concentrations of these compounds in the order TBT > DBT > MBT, except in the case of MI-3 where concentrations of butyltins were very low and in the order DBT > MBT > TBT. The reason

Table 2. Concentrations of butyltin compounds in soils, sediments, and the Asian clam *Potamocorbula amurensis,* from Mare Island (ng Sn/g dry weight).

Site	Monobutyltin	Dibutyltin	Tributyltin	Tetrabutyl tin	Total Butyltin
Soils					
IR04-A	13.6	15.5	22.0	0.5	51.6
IRO4-B	2.8	0.9	0.8	nd	4.5
IRO4-C	2.0	0.9	1.3	nd	4.2
Dike 14	nd	nd	nd	nd	-
IRO5	nd	nd	0.3	nd	0.3
Benthic sediment					
MI-1	1.6	2.2	2.8	nd	6.6
MI-2	2.0	2.5	3.6	nd	8.1
MI-3	0.4	0.6	0.3	nd	1.3*
MI-4	1.4	1.8	2.7	nd	5.9
MI-5	0.5	1.7	2.7	nd	4.9
MI-6	1.5	2.0	2.1	nd	5.6
Potamocorbula					
MI-1(T)	nd	29.4	123.0	nd	152.4
MI-2(T)	nd	46.0	142.5	nd	188.5
MI-3(T)	nd	26.9	158.5	nd	185.4
MI-4(T)	nd	141.0	166.0	nd	307.0
MI-5(T)	nd	39.3	151.7	nd	191.0
MI-6(T)	-	-	-	-	-

nd- not detected, * data point is suspect.

for this anomaly at site MI-3 is not understood at this time. In addition, as discussed later, an anomalously high Biota Sediment Accumulation Factor (BSAF) for TBT was obtained at site MI-3, in spite of the low concentration of butyltin in benthic sediment. In general, concentrations of butyltins in benthic sediment are controlled primarily by a partitioning process into the sediment organic carbon, with ionic interactions probably playing a minor role in the sorption process. Microbiological processes also may be responsible for transformation of TBT to DBT and MBT in marine benthic sediments. Ratios of TBT/DBT were relatively constant in all the benthic sediments, except for site MI-3, and ranged from 1.1-1.6 indicating relatively uniform and similar microbiological activity in benthic sediment at these sites. The data in Table 2 also indicate that TBT and its degradates, which are leached by precipitation and tidal action, are in the waters around Mare Island and into Carquinez Straits, and, by implication, possibly into San Pablo Bay. The benthic sediment data also suggests that a contamination gradient does not exist near Mare Island, and that the butyltin contamination problem is more diffuse in nature. The diffuse nature of this contamination probably is a result of the hydrodynamics of the Bay and remedial operations in progress at the Naval Base.

Biota: There is a paucity of information about butyltins in San Francisco Bay, and virtually no information available on the accumulation of butyltins in *Potamocorbula amurensis*. One study reported concentrations of TBT of 111-127 ng Sn/g dry weight in transplanted oysters (*Crassostrea gigas*) in the vicinity of our study area, whereas concentrations of TBT in these organisms and other bivalves ranged from 1-73 ng Sn/g in other locations of the Bay (San Francisco Estuary Institute, 1995). The National Oceanic and Atmospheric Administration (NOAA) National Status and Trends (NS&T) Mussel Watch Project reported in 1989-90 concentrations of total butyltins in bivalve mollusks in the range of 184-867 ng Sn/g and the concentrations of TBT in the range of 94-373 ng Sn/g at three locations in San Francisco Bay (Uhler and others, 1993). The concentrations of total butyltins for these three NS&T sites in 1995 ranged from 38-84 ng Sn/g (GERG, Texas A&M University, unpublished results), suggesting that the levels of organotins in biota in San Francisco Bay have decreased significantly since 1989 when limitations in the usage of TBT and (or) use of lower release rate paints were instituted. Concentrations of butyltins in lipid tissues of *Potamocorbula amurensis* from our study are shown in Table 2. Only TBT and lesser amounts of DBT were present in tissue samples. MBT was not detected in the tissues. The absence of MBT in the clam tissue probably indicates that this compound is bioaccumulated less efficiently or depurated more rapidly than TBT and DBT. Concentrations of TBT ranged from 123-166 ng Sn/g (ppb) and concentrations of total butyltins ranged from 152-307 ng Sn/g (ppb). TBT made up from 54-85% of the total butyltin burden of these bivalve mollusks, and DBT from 15-46% of the total butyltin burden. Ratios of TBT/DBT in *Potamocorbula amurensis* in the study area ranged from 1.2-5.9. The ratio of TBT/DBT in *Mytilus edulis* from the east and west coasts of the United States has been reported as 2.6 ± 1.0 (Uhler and others, 1993). The wider range in the ratios of TBT/DBT in our limited data set probably is due to various complex physiological processes that affect the uptake and depuration of butyltins in *Potamocorbula*. Although relatively small concentrations of butyltins were present in benthic sediment from Mare Island Strait at site MI-3 (which is in the vicinity of site IR04, the sand-blasting site near Green Beach), the largest concentrations of butyltins in *Potamocorbula* were found in sample MI-4(T). Concentrations in *Potamocorbula* at this site are on the order of about sixty times greater than those in sediment. This observation suggests that butyltins probably are gradually leached out from contaminated sediments into the water column. Because *Potamocorbula* filter very large volumes of water (about 5L/clam/day), the major routes of accumulation of TBT probably are by bioconcentration from the water column or uptake from suspended particulates including phytoplankton in the water column, with lesser amounts of accumulation from the benthic sediment.

Biota Sediment Accumulation Factor (BSAF): In order to describe the preference of hydrophobic organic contaminants for lipid tissues of the organism relative to sediment organic carbon, BASFs for butyltins were calculated using the method of Lee and others (1989) and McFarland and others (1994) (Table 3). BSAF is the ratio of the tissue contaminant concentration (normalized to the lipid content) of the organism to the sediment contaminant concentration (normalized to organic carbon) to which the organism is exposed, at equilibrium. Normalization of the sediment contaminant concentration to the organic carbon content reduces variability in sediment characteristics, and normalization of the tissue contaminant concentration to the lipid content reduces variability in tissue concentrations among individuals of the same species as well as between different species. The BSAF when multiplied by the organic carbon normalized sediment contaminant concentration

Table 3. Biota Sediment Accumulation Factors of butyltin compounds at Mare Island, San Francisco Bay, California.

Site	Dibutyltin	Tributyltin
Mare Island* (*Potamocorbula amurensis*)		
MI-1	0.96	2.32
MI-2	0.36	1.93
MI-3	8.7	96.3
MI-4	10.6	7.97
MI-5	3.19	7.78
**San Francisco Central Bay ** **		
(*Macoma nasuta*)	0.86	8.19
(*Mytilus edulis*)	3.48	5.85
**Oakland Inner Harbor Turning Basin ** **		
(*Macoma nasuta*)	0.09	0.11
(*Mytilus edulis*)	0.90	0.85

* Field study

** Data from Mc Farland and others, 1994 (28 day laboratory study) predicts the lipid normalized tissue residue concentration.

Data for Mare Island in Table 3 indicate that BSAFs of TBT ranged from 1.93-7.97 (except in the case of site MI-3), and those of DBT from 0.36-10.6. Because several grab samples of sediment had to be collected in order to obtain a sufficient number of clams, the anomalously high BSAF obtained at site MI-3 probably indicates that the clams from this site were not directly associated with this sediment sample or they were not in equilibrium with the sediment sample collected at this site. BSAFs determined in a 28-day laboratory bioaccumulation study in *Macoma nasuta* and *Mytilis edulis* (McFarland and others, 1994), are included in Table 3 for comparison. Differences in BSAFs, for example in the case of*Macoma nasuta* in San Francisco Central Bay and Oakland Inner Harbor Turning Basin, may be due to differences in sediment. The San Francisco Central Bay sediments were surficial sediments, whereas the Oakland Inner Harbor Turning Basin sediments were undisturbed virgin sediments that were collected from a depth of about 40 feet. However, several of the BSAFs reported in this field study for *Potamocorbula amurensis* are in closer agreement with the BSAF values reported in the laboratory study for San Francisco Central Bay than those reported for the Oakland Inner Harbor Turning Basin. These differences in BSAF values could be due to differences in the organisms under investigation (*Potamocorbula* vs. *Mytilus edulis* and *Macoma nasuta*), a field vs. laboratory study, or lack of equilibrium conditions in a dynamic estuary.

The BSAFs obtained in this field study also tend to be much larger than values reported for neutral hydrophobic compounds such as PAHs and PCBs (BSAF, ~ 0.9) in laboratory studies (McFarland and others, 1994). One possible explanation for the larger BSAFs of the organotin compounds over the neutral hydrophobic PAHs and PCBs may be due to processes other than simple partitioning of the neutral species into lipid, such as ionic binding to the -SH and =NH groups of proteins (Kannan and Falandysz, 1997) or ion pairing with other natural organic ligands which could enhance bioaccumulation.

In addition to the contamination problem from the Mare Island site, it is also possible that additional sources of TBT contamination are involved in the study area. These could include TBT from ships undergoing salvage operations at marinas in Mare Island Strait, as well as bay-wide transport of fine sediment containing TBT. Recent data from the San Francisco Estuary Institute (1995), and NS&T studies for mussels, clams and oysters also indicate that organotin contamination is widespread throughout San Franciso Bay; however, levels of organotin compounds in biota from San Francisco Bay have decreased significantly since 1989, when limitations on usage of butyltins and/or use of low release rate paints were instituted.

CONCLUSIONS

The results of this field study clearly indicate that soils from the sandblasting site at the former Mare Island Naval Shipyard, and benthic sediment and *Potamocorbula amurensis* from Mare Island and Carquinez Straits are contaminated with organotin compounds. This organotin contamination problem is now diffuse and has spread into Carquinez Strait and possibly into San Pablo Bay. Although benthic sediments contain low levels of these compounds, the clams bioaccumulate significant amounts of di- and tributyltin. Because *Potamocorbula amurensis* is an important food source for diving ducks, sturgeon, Dungeness crab, and other biotic species in San Francisco Bay, the organotin compounds have the potential for biomagnification in the food chain. Tributyltin is extremely toxic to marine organisms, and it is classified as a potent endocrine disrupting chemical. Further studies are necessary to determine the environmental effects of the organotin compounds on biota in San Francisco Bay.

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Forecasting Spring Discharge in the West: A Step Towards Forecasting Stream Chemistry

By David H. Peterson, Richard E. Smith, Michael Dettinger, Daniel R. Cayan, Stephen W. Hager, and Laurence E. Schemel

ABSTRACT

By linking climate to hydrology, correlations emerge that may be overlooked when studying hydrology alone. Forecasting the spring snowmelt discharge, of the Merced River, Happy Isles, Yosemite National Park is a first step towards that linkage. This approach looks promising for synthesis/interpretation of more complex chemical variations, perhaps including "pollutants", atmospheric or otherwise.

INTRODUCTION

A Conceptual Hydrological Cycle

One of the goals in hydrology is to be able to predict the behavior of dissolved substances (conservative, reactive, toxic, etc.) in the watershed/estuary/coastal ocean. This is often a source/transport/fate problem.

In the riverine transport part of the problem, to the extent that concentrations are related to river discharge, if we can predict river discharge, we can predict concentration. Similarly, if the major control on discharge is climate, which it is, if we can predict climate, we can predict discharge. Taking this a giant leap further, if the state of the ocean is a major control on climate, to the extent we can predict the state of the ocean, we can predict climate.

Our goals are deceptively simple, to link atmospheric circulation to discharge and discharge to chemistry. For reasons given below, we have started with the snowmelt discharge process. For reviews of this subject, see Morris (1985) and Gray and Prowse (1992).

YOSEMITE NATIONAL PARK

It may seem odd to pick as a starting point a relatively pristine region, Yosemite National Park (Fig. 1 photo of YNP), distant from obvious toxic problems. We have this luxury because, as stated earlier, we are taking a simplified view of the entire system and asking what are the linkages.

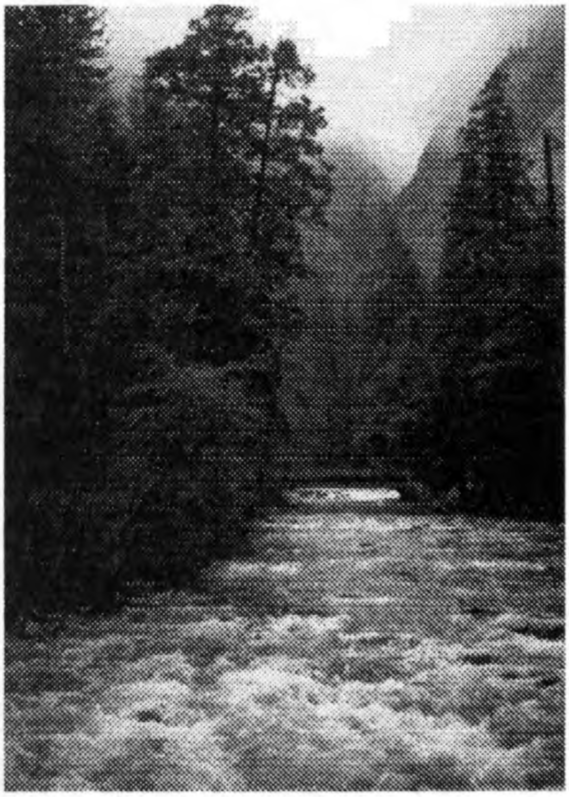


Figure 1. Merced River Yosemite National Park.

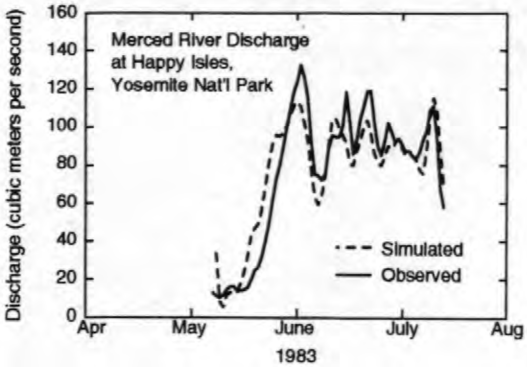


Figure 2. Merced River discharge, note abrupt rise in discharge (the spring pulse).

For a variety of reasons Happy Isles Bridge gage site, Merced River, Yosemite National Park is an excellent laboratory for studying high elevation hydrological processes. Attributes include that the discharge records are long and there is little human influence; discharge is largely snowmelt (simplifying the climate connection); Yosemite is an apparent bellwether site for the spring pulse (Fig. 2 Merced example) with large-scale atmospheric temperature correlations (Cayan and others, 1997) that have biological (S. Kammerdiener, University of California, San Diego, written communication, 1999), as well as hydrological implications (Dettinger and Cayan, 1995). The adjacent Tuolumne Basin (Fig. 3), a close twin of the Merced, is the major water resource for the city and county of San Francisco via the Tuolumne reservoir (Hetch Hetchy). As is typical of most reservoirs, there are water quality concerns associated with Hetch Hetchy. Because the pre-development major ion chemistry "finger print" of the Tuolumne and Merced are a close match (not shown), we can exploit their historical similarities. For example, the presumably changing chemistry of the Tuolumne can be measured against the more constant Merced chemistry. Further, the Merced River is upstream of one of the most important agricultural regions in the United States that is the source area for many contaminants.

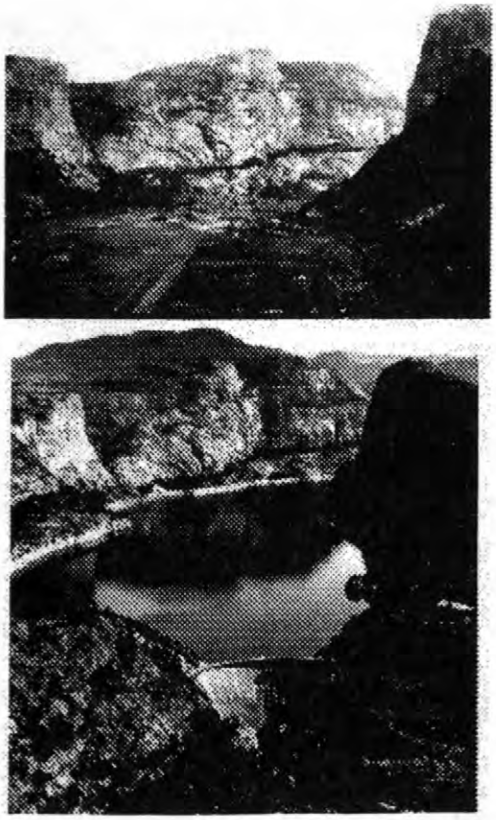


Figure 3. Tuolumne River before and after Hetch Hetchy reservoir, 1922.

WHY CLIMATE?

This may be best answered by example. Understandably, toxic research is focused on the source and disposition of toxic substances, natural or otherwise, and much less focused on the role of climate factors. One of the best documented examples of overlooking the role of climate was the use of a numerical groundwater salinization model to predict long-range salinization resulting from agriculture (Konikow and Patten, 1995). In revisiting the problem to see how successful their long-range forecasts were, the authors found they were off the mark. This led to the discovery that they had tuned their model with data collected in the middle of a three year period of declining discharge which contributed to the increased apparent rate of salinization (both human and climate effects were present). Perhaps this point illustrates why toxic researchers often remove or avoid climate effects in their results. The thesis here is that we may also learn by putting climate back into the equation (note, for purposes here weather and climate are used interchangeably).

With the above in mind, the Pacific Northwest and Southwest hydrology provides an introduction to our theme because this region is strongly influenced by the well-studied ENSO (El Niño Southern Oscillation) phenomena (see for instance Cayan, Redmond and Riddle, in press, and references cited). Not surprisingly, the strong ENSO-discharge correlation also includes a strong river chemistry correlation (simplified here as specific conductivity or total dissolved solids, Fig. 4).

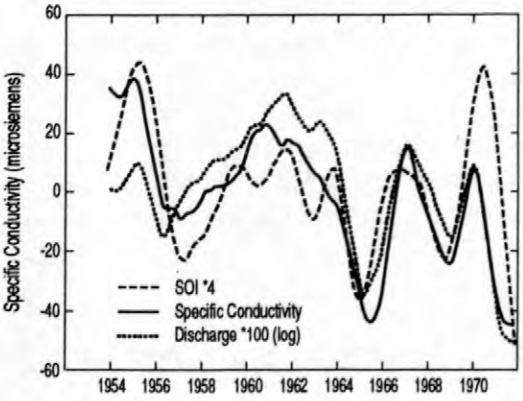


Figure 4. Low-pass filtered mean-monthly ENSO, Snake River discharge and specific conductivity, King Hill Idaho, deviations from long-term mean and discharge times minus one.

Our study area, Central and northern California, lies between these two centers of oscillating wet/dry ENSO patterns. In central and northern California, wetness or dryness in ENSO years varies, going with the northern regime in some years and the southern in others. This ambivalence tends to cancel out much of the atmospheric-hydrologic ENSO correlation for northern and central California over the period of instrumental record. Therefore the resulting atmospheric-discharge correlation is more regional in scope such as indicated by the atmospheric pressure anomaly index, the California Pressure Anomaly, or CPA, (Cayan and Peterson, 1989). CPA correlates with Merced River discharge, Happy Isles, Yosemite National Park (Fig. 5). On a regional scale, then, Merced River chemistry, although sparsely sampled, tends to follow CPA just as the Snake River discharge/chemistry follows ENSO to the North (Fig. 4).

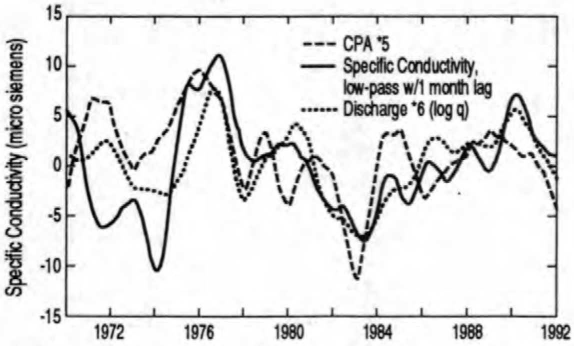


Figure 5. Low-pass filtered mean-monthly CPA and discharge with interpolated specific conductivity, Merced River, Happy Isles, Yosemite National Park, deviations from long-term mean and discharge times minus one.

FORECASTING SNOWMELT DISCHARGE

At the time of this writing we do not have time series of chemical variations to clearly link water chemistry to discharge for the Merced River (Fig. 6 shows a gappy pattern). We can however, provide initial results in the climate-discharge step, which of course proceeds the discharge-chemistry step.

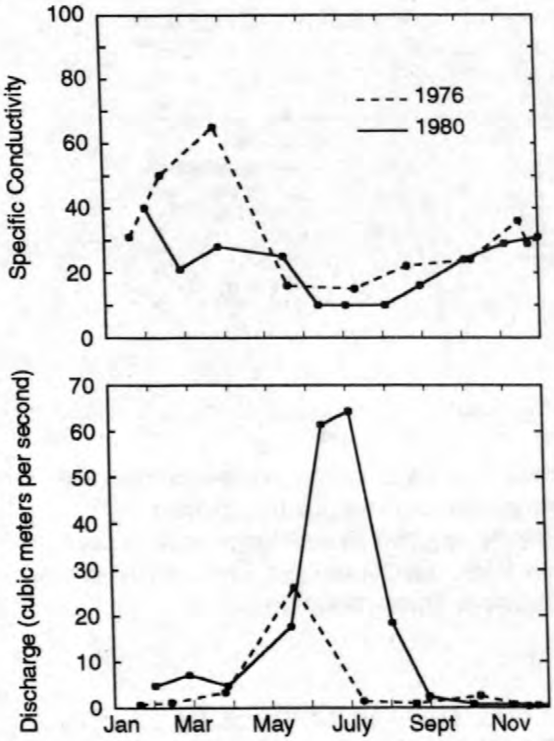


Figure 6. Specific conductivity and associated river discharge, Merced River Happy Isles, Yosemite National Park.

Our approach is to exploit the correlation between air temperature and snowmelt via statistical methods. Consider a time series of discharge observations and imagine discharge projected into the future (Fig. 7). To the extent that the observed and forecast wiggles in discharge correlate with the observed and forecast wiggles in air temperature (Fig. 8) we can predict discharge.

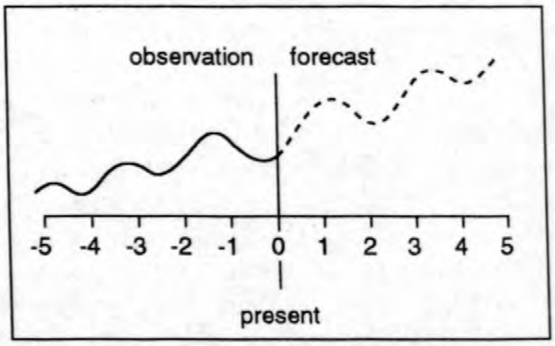


Figure 7. Cartoon of observation and prediction domains.

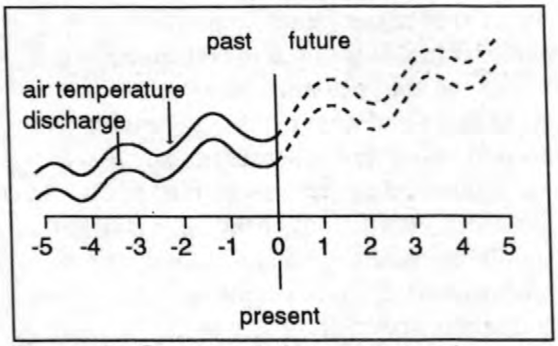


Figure 8. Cartoon of air temperature/ discharge correlation in the observational and prediction domains.

By predicting discharge in addition to measuring it, we may think about the system in ways we otherwise may not. Obviously, riverine toxic research will more fully benefit from a much more detailed knowledge of the riverine physics (and vice versa) than given here. Such research is proceeding by Dettinger, vanWagtendonk, Cayan and others.

Constant Parameter Snowmelt Model

A seasonal snowmelt cycle may be simplified in four phases (Fig. 9). In the first phase, discharge shows little response to changes in temperature (it's too cold). The second phase follows after the snowpack has stored sufficient heat. The snowpack is then ready to more strongly respond to an increase in temperature (that is, the spring pulse and rise in discharge). However, in phase three, the system is near temperature saturation, and the discharge response to temperature is nearly constant. Beyond this, phase four, discharge declines and air temperature is replaced by snowpack size as the major controlling factor. A major limitation of the simple temperature-driven model used here is that it does not describe phase four.

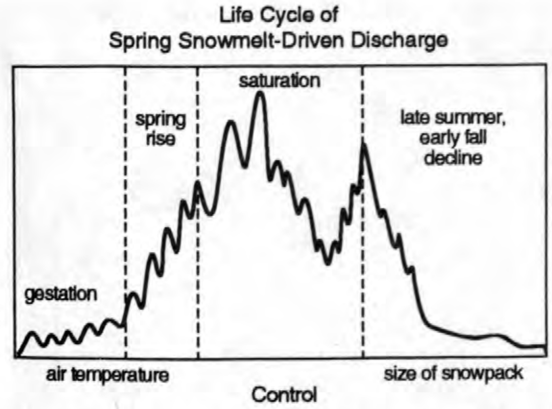


Figure 9. Cartoon of spring snowmelt discharge cycle.

Considering only the first three phases of the cycle, then, the discharge response to air temperature is first small, then increases, and last is nearly constant. To understand this more clearly, it is instructive to start with a constant parameter method. For response parameter estimation (air temperature as input, discharge as output) we used the instrumental variable method (Ljung, 1988, 1989). This method gives the average of response coefficients over the length of record (the response coefficients are constant). This method first overestimates and then underestimates the discharge when applied to the first three snowmelt phases (Fig. 10).

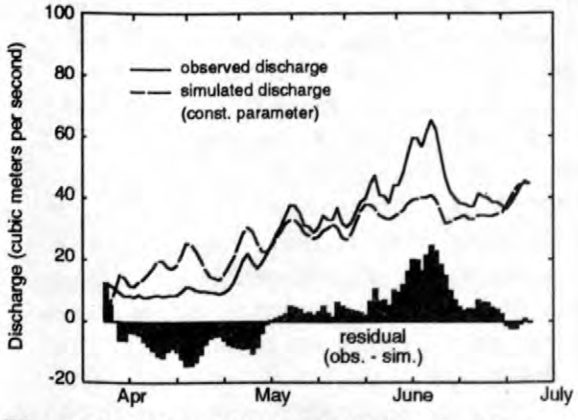


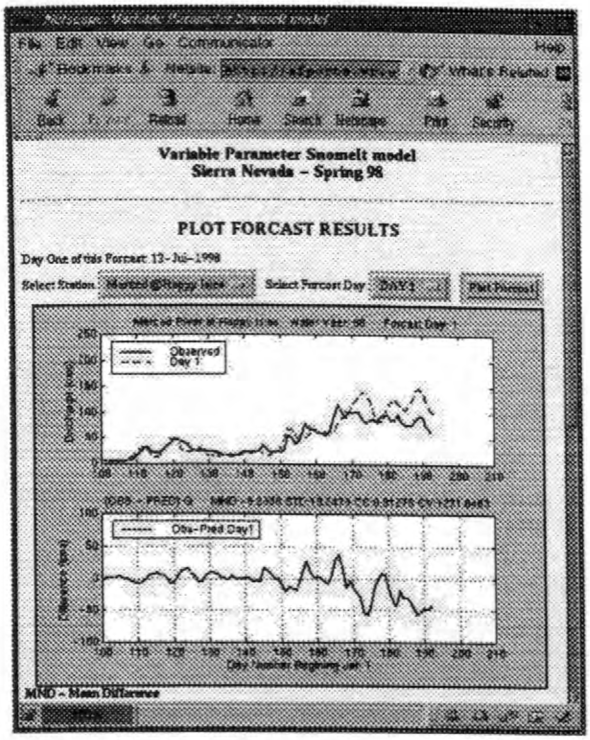
Figure 10. Observed and simulated discharge using a constant discharge response parameter to air temperature, Merced River, Happy Isles, Yosemite National Park.

Variable Parameter Model

The hypothetical cycle given above is better characterized using variable response coefficients. The details of the model are beyond the scope of this paper. Briefly the model uses air temperature as input (forecasts provided by the National Oceanic and Atmospheric agency) and discharge as output. The "system" model includes a Kalman filter to estimate the variable response coefficients (of discharge to temperature). In essence, the Kalman filter is a predictor/corrector scheme. The system model estimates the next discharge value and the time-equivalent discharge observation corrects the estimate. With various assumptions the optimal correction is estimated by minimizing the prediction error (the difference between observed and predicted value). An obvious difficulty, not discussed here, is the bootstrap nature of the method – we are feeding past forecasts into the loop under the guise that they are observations to extend the forecast horizon beyond one time step (one day). Examples of Kalman filtering in hydrology are given by Wood and O'Connel (1985) and Lettenmaier and Wood (1992). A step by step mathematical derivation of the Kalman filter is in Dutton and others (1997).

RESULTS/DISCUSSION

A variable parameter method experimental forecast website was maintained for the Merced (CA) and Gunnison (CO) Rivers in 1998 and will be continued in spring 1999 and beyond. For several reasons, still under study, in 1998 the Merced forecasts (Fig. 11) were not as good as for the Gunnison (Fig. 12). The most important reason was that the Gunnison air temperature forecasts were better. In other words, as air temperature forecast skill increases, which it will, discharge forecast skill will follow.



Variable Parameter Snomelt model
Sierra Nevada – Spring 98

PLOT FORCAST RESULTS

Day One of this Forcast: 13-Jul-1998

Select Station: Select Forcast Day: Plot Forcast

Figure 11. Observed and simulated discharge using variable discharge response parameters, Merced River, Happy Isles, Yosemite National Park.

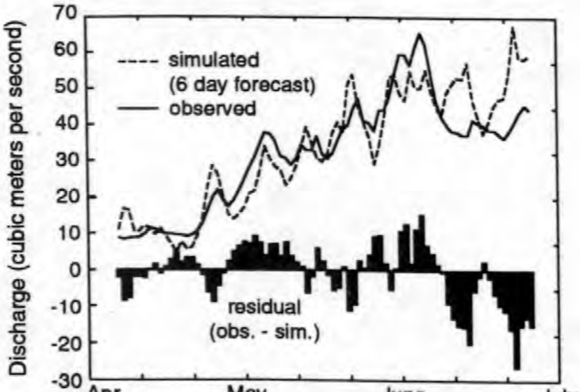


Figure 12. Observed and simulated discharge using variable parameters, Gunnison River, Colorado

Although we will be testing an in-situ continuous chemical analyzer this spring, 1999, we do not expect to get year-around records until the year 2000. This analyzer should provide the data needed for an experimental chemical forecast with the discharge model up front. Never-the-less, insight from our initial findings maybe relevant to toxic researchers even without the chemical observations.

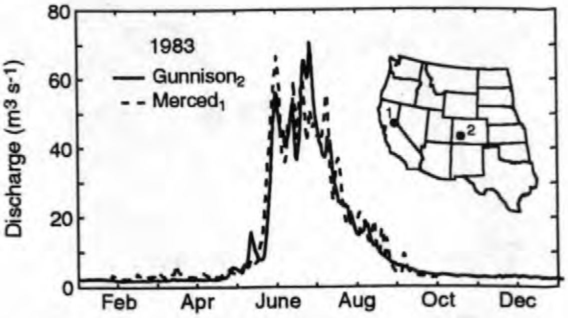


Figure 13. Merced River, Happpy Isles and Gunnison River, Colorado, 1983.

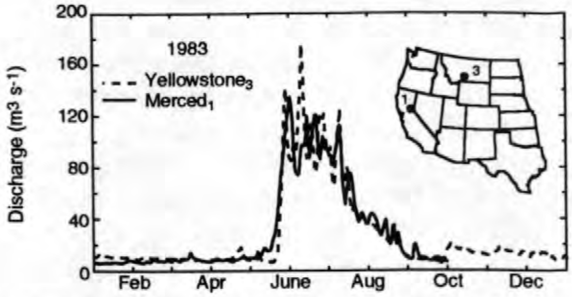


Figure 14. Merced River, Happy Isles and Yellowstone River, Wyoming, 1993.

Understandably, because each basin is unique, hydrologic researchers often are reluctant to transfer their findings from one watershed to another. However, individual basin characteristics/complexities may be less critical for snowmelt than rainfall discharge regimes, when interpreted in a broad climate context. For example, snowpack was unusually widespread in western United States in 1983. This, plus the fact that air temperature patterns usually are not only large scale but in 1983 air temperatures at distant locations also were very similar, and produced a remarkable synchronization of discharge history (time shifted and amplitude scaled) for three remote watersheds (Figs. 13 and 14).

Over a smaller scale, the matching detail in the average 1916-1922 daily discharge records Merced and Tuolumne River (Fig. 15) is also striking. Even the low-pass filtered residual, (the mean discharge differences between the two basins) appears to contain hydrologic information.

In fact, much of this residual may be explained by inter-basin differences in area vs. elevation (Fig. 16). Snowmelt starts at lower elevations, reflecting a space/time variability in both surface area and snowmelt as a function of elevation.

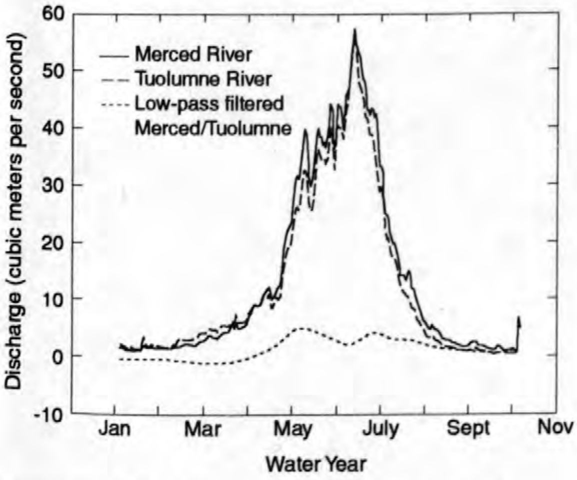


Figure 15. Daily average discharge over the annual cycle, 1916-1922 Merced River, Happy Isles and Tuolumne River, Hetch Hetchy, Yosemite National Park(Tuolumne discharge by 1/3.2) , and their low-pass filtered difference.

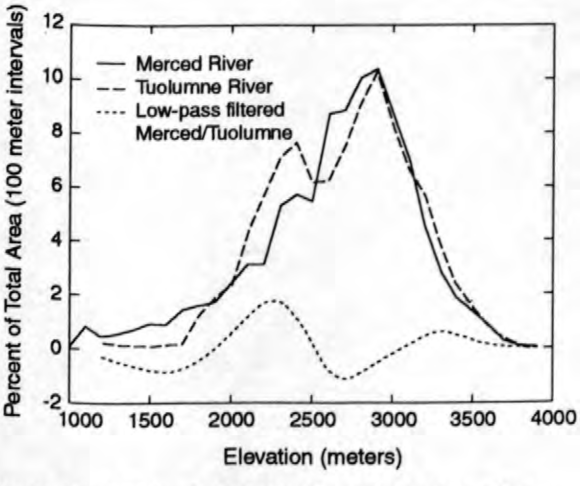


Figure 16. Areas of Merced and Tuolumne rivers as a function of elevation above the gage sites for discharge in Fig. 15. And their low-pass filtered difference.

In closing, it seems reasonable to assume that similar or new subtleties in chemical signatures also will be found when chemical hydrology is interpreted in the context of climate, including the behavior of toxic substances.

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Reduced Phosphate Loading to South San Francisco Bay, California: Detection of Effects in the Water Column

By Laurence E. Schemel, Stephen W. Hager, and David H. Peterson

ABSTRACT

Dissolved phosphate has been a useful tracer of municipal wastewater in many studies of South San Francisco Bay (South Bay), in part because water column concentrations are higher than background levels even after appreciable dilution. Over the last decade, however, wastewater loading of phosphate to South Bay has been reduced. Our objective was to identify changes in phosphate concentrations in the water column of South Bay that were caused by recent reductions in wastewater loading. The changes proved difficult to detect in this estuary, primarily because of strong effects of interannual and seasonal variability in climate- and weather-dependent processes. This case study for phosphate identified factors that should be considered when attempting to detect effects in the water column for substances that are not completely removed from wastewater discharges and have potentially complex interactions with biogeochemical cycles.

Water-column measurements of phosphate concentrations did not provide clear evidence of a response to the reduced loading. The apparent change in water-column concentrations in recent years could have been caused by dry hydrolgic conditions before 1993 and very wet conditions over 1995-1998. Although interannual and seasonal variations in climate and weather influence water-column properties in many ways, the most apparent was greater effects of dilution by freshwater inflow that persisted for many months during the wet years. During early fall of most years, however, wastewater was the dominant source of freshwater to South Bay, and longitudinal gradients in both salinity and phosphate were directly related to wastewater inflow. At those times, a simple mixing model was effective in estimating average concentrations of phosphate in the wastewater from the longitudinal gradients in the bay. This technique detected a reduction in the phosphate gradient in recent years that was consistent with the decrease in wastewater concentration and the reduced loading.

INTRODUCTION

South San Francisco Bay (South Bay), the largest tributary embayment of the San Francisco Bay estuarine system (fig. 1), is surrounded by densely populated urban areas (Davis and others, 1991). Consequently, South Bay has a long history of water-quality problems related to discharges of municipal wastewater (Nichols and others, 1986), particularly in the reach south of the San Mateo Bridge (defined here as the landward reach). Improvements in waste treatment and discharge strategies have reduced the impact of municipal wastewater on the landward reach over the last few decades, but wastewater remains the major source of many substances found in the water column and bottom sediments (Davis and others, 1991). One of these substances, dissolved reactive phosphate (phosphate), is not of particular concern in bay waters, but it has been a useful tracer of the dispersion of municipal wastewater in studies of South Bay because wastewater concentrations are high and receiving-water concentrations remain higher than natural background levels even after appreciable dilution (McCulloch and others, 1970; Conomos and others, 1979; Schemel and Hager, 1996). Over the last decade, decreasing use of phosphate in detergents and improvements in wastewater treatment have reduced the loading of phosphate to South Bay. Our objective was to detect effects of recent reductions in phosphate

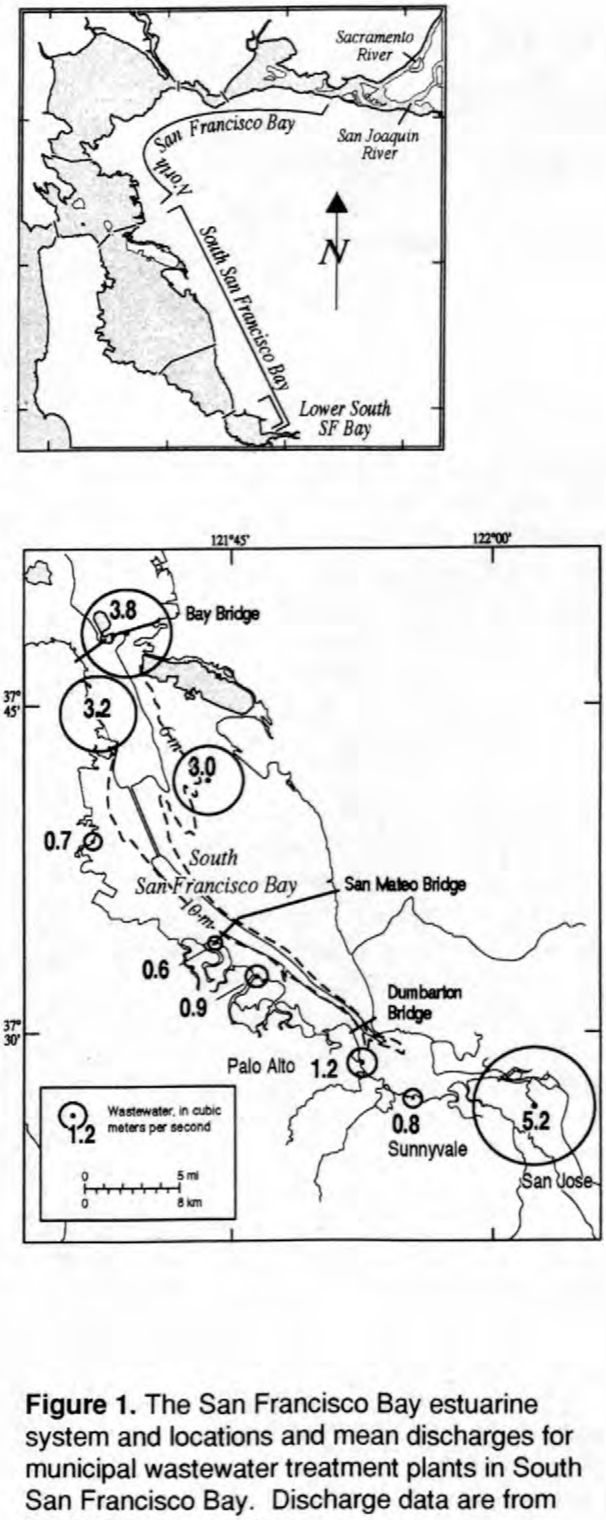

Figure 1. The San Francisco Bay estuarine system and locations and mean discharges for municipal wastewater treatment plants in South San Francisco Bay. Discharge data are from Davis and others, 1991.

loading to the landward reach by examining water-column concentrations and chemical gradients established by wastewater inflow. This case study for phosphate showed that effects in receiving waters can be difficult to identify in hydrologic systems that are influenced by large variations in climate- and weather-dependent processes. Lessons that were learned in the case of phosphate apply to other substances that might not be competely removed from wastewater discharge and have potentially complex interactions with biogeochemical cycles.

Since 1980, most of the major municipal wastewater treatment plants in South Bay have discharged into deep waters adjacent to North San Francisco Bay (North Bay), where the effluents are rapidly diluted and removed from South Bay by mixing processes (fig. 1). However, the San Jose-Santa Clara, Palo Alto, and Sunnyvale treatment plants discharge into the small, shallow basin below Dumbarton Bridge (lower South Bay), where dilution by bay waters is limited and tidally-driven circulation and mixing processes provide relatively slow seaward transport of waste-derived substances during most of the year (Walters and others, 1985). Discharges to lower South Bay from these three plants account for most of the total wastewater discharge to the landward reach (fig. 1). Because of the locations of these discharges and their magnitudes, longitudinal gradients of waste-derived substances are established in the landward reach during much of the year, with lowest concentrations near San Mateo Bridge and highest concentrations below Dumbarton Bridge.

The reduction in phosphate loading was most apparent in the final effluent data from the San Jose-Santa Clara treatment plant (fig. 2). Although mean annual loading varied over a wide range during the 1980's, decreases in the loading and the concentration of phosphate were substantial in 1992-1993. This coincided with improvements in the treatment process to control algal growth that also removed phosphate from the effluent. Phosphate loading for 1993-1997 was reduced to 49 percent of the mean value for 1990-1992 and 37 percent of the mean value for 1980-1989. Small reductions in phosphate loading by the Palo Alto and Sunnyvale treatment plants to 71 percent and 86 percent of their 1990-1992 values, respectively, also occurred over 1992-1993, but specific causes for these reductions were not identified. The combined reductions from these three plants decreased phosphate loading to lower South Bay to 58 percent of the mean value for 1990-1992.

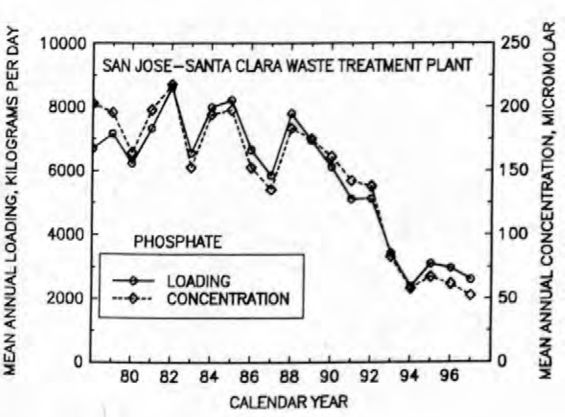

Figure 2. Mean annual loading and concentrations of phosphate in final effluent from the San Jose-Santa Clara waste treatment plant.

Although the reduction in phosphate loading was significant, the supply from wastewater remained greater than removal rates by phytoplankton and estimates of inputs from bottom sediments. Phosphate is not the limiting nutrient for plant growth in South Bay, and phytoplankton blooms typically remove only a small fraction of the water-column phosphate (Hager and Schemel, 1996). Estimated supply rates of phosphate from bottom sediments over the landward reach are equivalent to 8-34 percent of the wastewater supply to lower South Bay, with typical values most likely in the low range (Hammond and others, 1985).

Water-column concentrations of phosphate can be strongly influenced by seasonal inflows of fresh and brackish waters from local streams and North Bay, primarily because they dilute bay waters, thereby reducing concentrations of phosphate (Hager and Schemel, 1996). In addition, strong winds primarily associated with major storms during winter and spring move and mix waters in South Bay, which can rapidly change longitudinal gradients of waste-derived substances (Schemel and Hager, 1996). It is possible, however, to identify periods when freshwater inflows affect water-column concentrations of phosphate because salinity also is reduced in the bay. Dilution by local streamflow to the landward reach is usually limited to late fall through spring, but effects of inflows from North Bay can persist into summer (Schemel, 1998).

MEASUREMENTS IN SOUTH BAY

Interannual and seasonal variations in salinity and phosphate concentrations in South Bay waters between the San Mateo and Dumbarton bridges showed the influence of fresh and brackish water inflows (fig. 3) over the 1990's. Differences among the years primarily were related to the timing, duration, and magnitude of the inflows (Schemel, 1998).

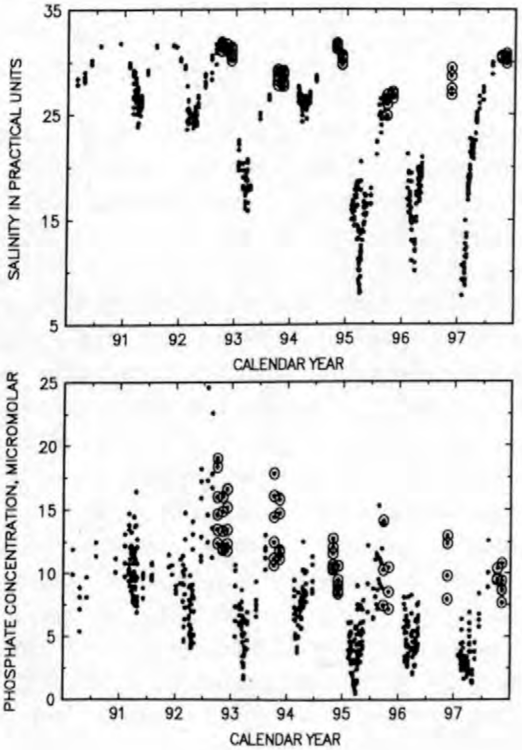

Figure 3. Salinity and phosphate concentrations in South Bay between the San Mateo Bridge and the Dumbarton Bridge. Circled points are measurements from early fall. These data are available from: URL http://sfbay.wr.usgs.gov/access/wqdata/index.htm

Salinity remained high and showed little seasonal variability during 1990-1992, which were drought years with few winter storms and low precipitation in northern California. In contrast, 1993 and 1995-1997 were years with greater-than-normal precipitation and substantial streamflow to the bay system, which caused large seasonal variations in salinity and phosphate concentrations in South Bay. Overall, the dilution of phosphate concentrations and salinity was much greater over the years since the reduction in phosphate loading. Consequently, effects of the reduction in phosphate loading could not be identified in South Bay simply by comparing water-column concentrations among the years.

During early fall of most years, effects of freshwater inflow and storm-related circulation and mixing during the previous winter and spring are at a minimum in South Bay. In general, highest annual values for salinity and concentrations of phosphate were observed during early fall (fig. 3). However, in some years since 1992 salinity during early fall showed residual effects of the previous storm season. Consequently, the apparent decrease in phosphate concentrations during early fall from 1992 to 1997 might have resulted from differences in hydrologic conditions among the years, reduced phosphate loading, or both.

Inflows from local streams are very low and wastewater is usually the major source of freshwater to the landward reach during early fall. Consequently, wastewater discharged into lower South Bay creates a longitudinal gradient in salinity when it mixes with the saline waters of the landward reach. This is analogous to the longitudinal gradient in phosphate caused by the wastewater discharge that was described earlier. Therefore, phosphate concentrations increase as salinity decreases in the direction of Dumbarton Bridge from the San Mateo Bridge (landward). Although mean salinity varied among the years, longitudinal gradients for measurements made during early falls of 1992-1996 showed consistent (inversely proportional) relations between salinity and phosphate concentrations, that in most cases were nearly linear (fig. 4).

The linearity of the relations indicated that the chemical gradients during early fall might be approximated by a simple, two-member mixing model (Liss, 1976). With this model, a linear function describing the relation between salinity and phosphate concentrations can be used to estimate the phosphate concentration of the wastewater mixing member. The zero-salinity intercepts for linear regressions of phosphate concentration as a function of salinity for 1991-1997 provided estimates which were compared to monthly average concentrations of phosphate in the combined wastewater discharge to lower South Bay (fig. 5). Although few field measurements were available the years before the reduction in phosphate loading, the decrease in the wastewater mixing members estimated from the longitudinal distributions was consistent with the decrease in phosphate concentration in the effluent from the three plants both in timing and magnitude.

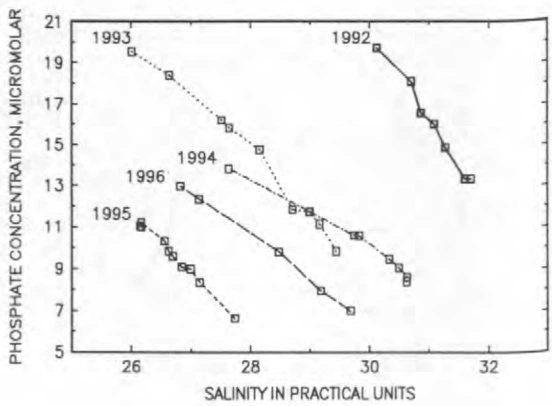

Figure 4. Phosphate-salinity gradients in the landward reach of South Bay from early fall 1992-1996.

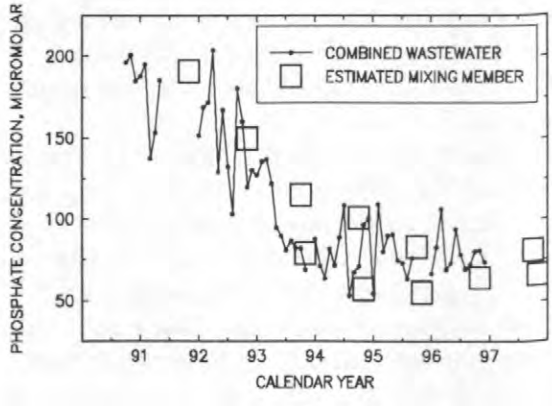

Figure 5. Estimated wastewater mixing-member concentrations during early fall and concentrations of phosphate in the combined wastewater discharge to lower South Bay.

As an additional comparison to recent values, wastewater mixing members were estimated from salinity and phosphate concentrations measured monthly in the landward reach during fall of 1980, a year with normal levels of rainfall and streamflow (Ota and others, 1989). The three estimates for the wastewater mixing member ranged from 161 to 200 micromolar, which is about twice the range measured during 1994-1998. This is consistent with the reduction in phosphate concentration shown by the San Jose-Santa Clara plant since 1980 (fig. 2).

DISCUSSION

Identification of effects of the reduction in phosphate loading in the water column was complicated primarily by large variations in interannual and seasonal climate- and weather-related factors. In addition to dilution by fresh and brackish water, storms and river inflows modulate density- and wind-driven processes that circulate and mix waters in South Bay (Walters and others, 1985; Schemel and Hager, 1996). It was apparent that salinity and phosphate concentrations in the landward reach often reflected current hydrologic conditions and showed residual effects from the previous storm season. Consequently, a response to the reduced loading could not be verified using water-column concentrations alone. Effects of seasonal processes that strongly influence salinity and phosphate concentrations in the landward reach were significantly reduced during early fall, which, in part, made possible the analysis of the longitudinal gradients with a simple mixing model.

An additional factor that made it difficult to detect changes in the water column was that loading and effluent concentrations were reduced to only about one-half of the previous value. Changes in the water column of South Bay were more easily recognized when a major improvement in treatment at the San Jose-Santa Clara plant in 1979 removed nearly all of the ammonium from its effluent (Hager and Schemel, 1996). Prior to this, ammonium had been the most concentrated nitrogen species in the effluent, and its concentration was reduced by more than an order of magnitude. After this large reduction, concentrations of ammonium were substantially lower in the water-column within a few months.

In some ways, the observed reduction in phosphate might be similar to what can be expected for many pollutants in municipal wastewaters that have been regulated in recent years. Concentrations and loadings of pollutants that are potentially harmful to San Francisco Bay, such as trace metals and toxic organic compounds, are typically much lower than for phosphate, but it is unlikely that they can be entirely removed from wastewater discharges (Davis and others, 1991). Consequently, it might be difficult to detect reductions of these more dilute pollutants in the water column, particularly if influences of bottom sediments and biogeochemical cycles are greater than for phosphate.

Although recent improvements in wastewater treatment have reduced the loading of many toxic pollutants to San Francisco Bay, there is concern that water-column concentrations might not be significantly reduced for many years because of sources within the bay that have become increasingly important. For example, pollutants have accumulated in the sediments of South Bay for many decades, and release to the water column through biogeochemical cycles appears to be an important influence on water-column concentrations of some trace metals and perhaps other substances (Flegal and others, 1996). Identification of the processes that control water-column concentrations for these substances is a great challenge. Results of our study indicate that evaluations of climate- and weather-related factors on interannual, seasonal, and perhaps shorter time scales are needed to identify appropriate hydrologic conditions and to select data sets that can be compared.

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A Marine Nowcast System for San Francisco Bay, California

By Chad A. English, Jeffery W. Gartner, Richard E. Smith, and Ralph T. Cheng

ABSTRACT

San Francisco PORTS (SFPORTS) uses an array of sensors at twelve locations in San Francisco Bay to provide real time observations of sea level (tides), tidal currents, water temperature and salinity, wind speed and direction, wind gust, air temperature and barometric pressure. A nowcast numerical model is used to fill spatial data gaps between observed data points and to provide tidal predictions and tidal current patterns. The SFPORTS system and the nowcast numerical model are combined as the San Francisco Bay Marine Nowcast System. The observed data and model results are presented to users on the Internet at http://sfports.wr.usgs.gov/sfports.html. The system was originally designed to aid in the prevention of and response to marine accidents and spills of toxic substances. Although the target users are navigators and spill-response authorities, scientists and educators have used the San Francisco Bay Marine Nowcast System as a resource to further develop our understanding of the bay ecosystem.

INTRODUCTION

Located near the middle of the California coast, the San Francisco Bay estuarine ecosystem is one of the most complex estuaries in the United States. The San Francisco Bay region is a center of commerce and recreation. Thus the bay waters are heavily traveled by tankers, cargo ships, and small craft serving the population around the bay and around the Pacific Rim. Despite preventive measures, vessel traffic accidents and spills of toxic substances will continue to be a threat to the bay and its users. To protect this fragile estuarine ecosystem, in 1995 National Oceanic and Atmospheric Administration (NOAA) initiated installation of Physical Oceanographic Real Time System (PORTS) in San Francisco Bay to aid safe navigation and hazard mitigation. Completed in 1998, the PORTS installation in San Francisco Bay (SFPORTS) provides real-time observations of tidal and meteorological conditions. The San Francisco Bay estuary is characterized by wide shoals and narrow channels, thus limited real-time observations are insufficient to adequately describe variations of the complex tides and tidal currents. In order to fill spatial gaps in the real-time observations the SFPORTS system is combined with a nowcast numerical model (Cheng and Smith, 1998) to compose the San Francisco Bay Marine Nowcast System (Cheng and others, 1998a). SFPORTS field observations and nowcast numerical model results are available to users through a user-friendly interface on the Internet at the World Wide Web address http://sfports.wr.usgs.gov/sfports.html (Cheng and others, 1998b). This paper gives a brief overview of the San Francisco Bay Marine Nowcast System.

SFPORTS SYSTEM

The data collection component of SFPORTS consists of five shore stations where water level (tides) and temperature, salinity, wind speed and direction, wind gust, air temperature, and barometric pressure are measured every six minutes. In addition, five Acoustic Doppler Current Profilers (ADCPs) have been installed to measure water velocity profiles every six minutes. The instrument sensor locations are shown in Figure 1. Characteristics of sensors, and precise sensor locations can be found on the SFPORTS Internet website.

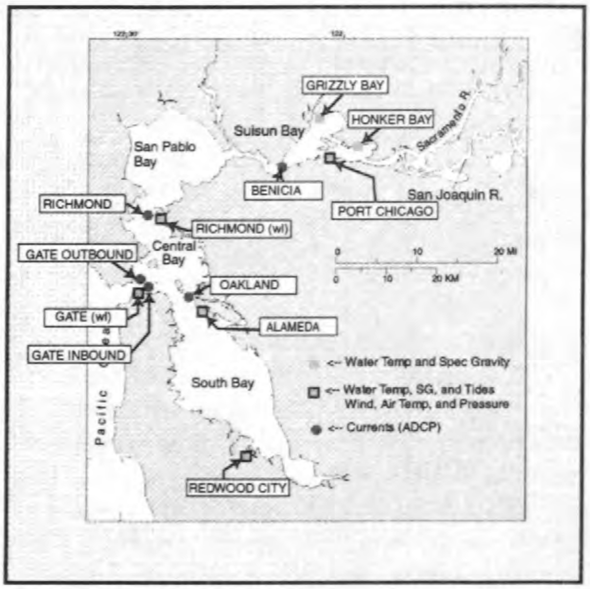

Figure 1. This map depicting SFPORTS data-collection stations is found on the SFPORTS Internet website.

The original sensor data are first sent to a "Data Acquisition System" (DAS) and subjected to NOAA's Continuous Operational Real-time Monitoring System (CORMS) to verify validity. These data are reformatted to a PORTS Uniform Flat File Format (PUFFF), and then sent to a server by e-mail whenever a new observation becomes available. E-mail was chosen as the messenger for transmitting field data (PUFFF) to the server because it utilizes a well-tested data buffering system to minimize data loss due to communication problems between computers. The observed field data received on the server are archived as time series. In addition to archived time-series data, a copy of "most-recent 24-hour time-series" data for each station and for each ADCP is maintained on the server. The "most-recent 24-hour time-series", which contains the observations for the immediate previous 24 hours, is updated every six-minutes on a sliding time scale. These data are used to generate display graphics, and to develop boundary conditions for the nowcast numerical model.

NOWCAST NUMERICAL MODEL

The nowcast hydrodynamic numerical model is designed to reproduce tides and tidal current distributions for the past 24 hours and to predict the tides and tidal currents for the next 24 hours. The field observations from the past 24 hours and the results of model simulations are examined by an assimilation algorithm to produce boundary conditions leading to an optimal numerical simulation of the tides and tidal currents for the next 24 hours. The hydrodynamic model, based on TRIM2D, solves the nonlinear, depth averaged, shallow water equations using a semi-implicit finite-difference scheme (Cheng and others, 1993). The assimilation algorithm and the over-all nowcast numerical modeling procedures are presented by Cheng and Smith (1998). Each hour the model outputs current-velocity patterns for the entire bay. The model also generates time series of salinity, water level and currents corresponding to each of the SFPORTS observation locations.

NOAA tide tables are calculations of astronomical tides and do not take into account effects of local weather variations. By using the actual water level measurements from the past 24 hours, more accurate short-term tidal variations are predicted for the future 24 hours. These "predicted tides", plotted along with NOAA tide table information and observed water levels, are very useful to those who work on or near the bay (Figure 2).

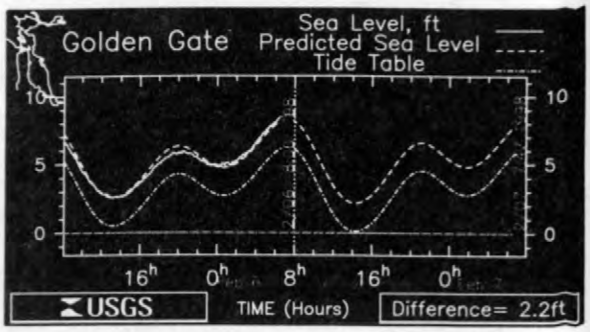

Figure 2. Time series graphic used on the SFPORTS Internet website to compare astronomical tides with measured tides. The difference of over 2 feet is significant to users of the bay.

PRESENTATION OF DATA

Recent advances in Internet technologies and the explosion in World Wide Web usage indicate that the Internet is the appropriate medium for data presentation in SFPORTS. This approach was successfully tested by making the real-time wind distribution over the San Francisco Bay region available on the Internet website http://sfbay7.wr.usgs.gov (Cheng and others, 1997). User feedback and usage greater than ten-thousand page-views a month provide further assurance that an Internet -based system is effective in delivering the information provided by the San Francisco Bay Marine Nowcast System.

Design of the SFPORTS user interface considers the balance of the computational efficiency, limitations in the rate of data transmission, and effective graphics presentation. Computational demands are kept to a minimum by judicious choice of the information to be displayed. Keeping individual page size less than 40 kilobytes and offering ASCII text alternatives to graphics accommodates those users limited by low data transmission rates. Finally, the graphics and page layouts deliver data in an easily understood, user-friendly manner.

The homepage of the SFPORTS website has direct links to all data display categories. In addition to a common header with links to both the homepage and a user feedback questionnaire, each page has links to other related data. For example, a link on the homepage connects to the "Winds and Tides" page where graphic displays of the wind and tide data from each station are superimposed on a map of the San Francisco Bay area (Figure 3).

Links connect to the "San Francisco Bay area Wind Patterns" and to the "Time-Series Plots". Clicking on the map will also link to the "Time-Series Plots" (Figure 2).

Figure 2 shows an example of a time series, taken directly from the SFPORTS website, of water levels during a storm in 1998, an extreme El Nino event. The solid line is a plot of observed data. The dot-dashed line shows NOAA tide table values, and the dashed line plots the predicted tide described earlier. The box in the lower right corner indicates that the difference between the NOAA tide table and the nowcast predicted tide exceeds two feet. A difference this large is unusual and important to navigators, disaster response teams, flood control officials, and those who work and live on or near the bay.

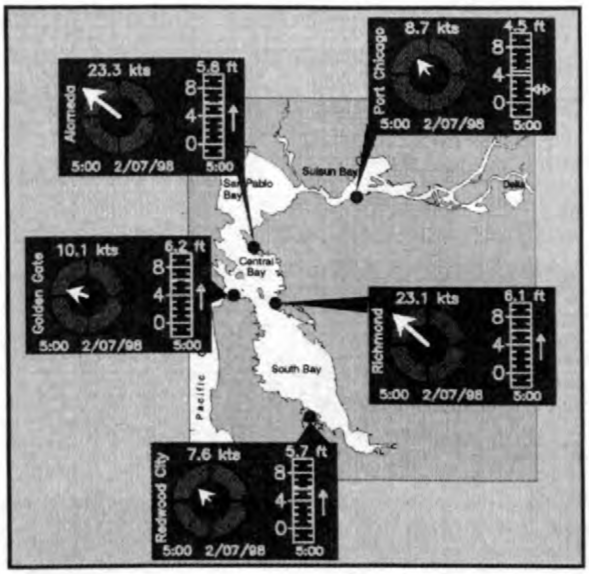

Figure 3. Wind roses and tide staffs for SFPORTS shore stations. The roses and staffs are linked to time-series plots like figure 2.

The model-generated current-velocity maps are another form of data display. The numerical model grid and water depths are defined using a coordinate system based on Mercator projection so the model outputs can be directly superimposed on NOAA navigation charts, a format that is familiar to the maritime community (Snyder, 1987). Figure 4 shows the model-generated current velocity vectors at the Golden Gate. Buttons around the image's edges link to adjacent images and a navigation bar allows links to time-series and current profiles at the nearest field observations.

SUMMARY

As the populations served by the San Francisco Bay estuary grow so do the demands upon the bay and the possibility of hazardous spills and toxic contamination. By combining the nowcast numerical model results, the real-time field observations, and Internet technologies, the San Francisco Bay Marine Nowcast System is a unique and potentially powerful resource to help prevent and aid response to such concerns. The combination of observed data and numerical model results gives a picture of the past, present and future state of the bay on an hourly time-scale. Beyond the immediate utility of these data for prevention of and response to contaminant spills, the availability of SFPORTS on the Internet invites constructive feedback from those who use and study the estuary.

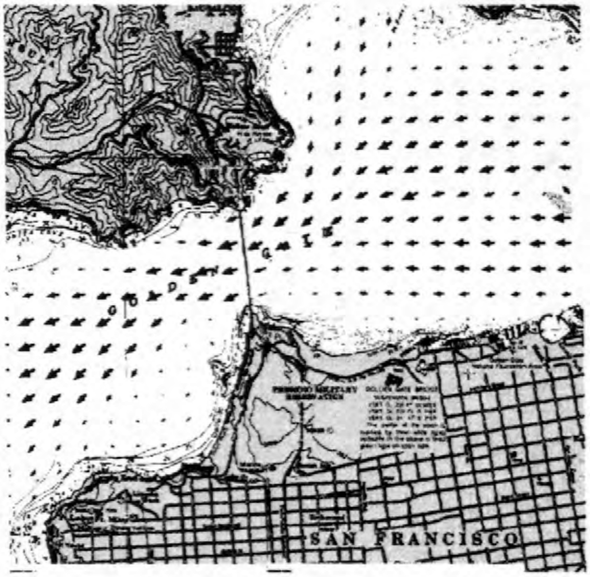

Figure 4. Graphic used on the SFPORTS website to display nowcast numerical model output of current patterns. Arrow size is proportional to velocity magnitude. The background is a NOAA nautical chart.

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Herbicide Concentrations in the Sacramento-San Joaquin Delta, California

By Kathryn M. Kuivila, Holly D. Barnett, and Jody L. Edmunds

ABSTRACT

The Sacramento-San Joaquin Delta watershed in California encompasses agricultural areas that receive intense applications of various herbicides, including some designed to inhibit photosynthesis. This study is to determine whether herbicides impair phytoplankton primary productivity in the Sacramento-San Joaquin Delta. The sampling strategy contrasted conditions in May-June, a time of expected high herbicide concentrations, with conditions in October-November, a time of expected low herbicide concentrations. Water samples from May through November 1997 were analyzed for herbicide concentrations and phytoplankton primary production rates. Thirteen herbicides were detected in one or more water samples. Herbicide concentrations varied considerably spatially and temporally. Diuron, metolachlor, and diethatyl-ethyl had the highest concentrations in the study. Two sites, Paradise Cut at Paradise Road and French Camp Slough at McKinley Road, had the most frequent detections and highest concentrations of herbicides.

The highest concentrations of molinate and thiobencarb were detected at the site receiving input from the Sacramento River watershed, following application of these herbicides on rice in May. The highest use of EPTC is in the San Joaquin River watershed and the highest concentrations were detected at the site representing this watershed. In contrast, the source of the other herbicides could not be attributed to a single watershed. Diuron and metolachlor had widespread detections that can be explained by their relatively high use in all the watersheds, whereas diethatyl-ethyl primarily was detected at the one site near the highest application in the Delta. The distributions of 2,4-D and hexazinone were more complex, and the amounts and timing of application do not readily explain the pattern of occurrence.

The results of this part of the study illustrate the complexity of herbicide concentrations in the Sacramento-San Joaquin Delta. In particular, the occurrence of diuron and hexazinone needs to be studied in more detail to determine their influence on primary production and phytoplankton species composition.

INTRODUCTION

The Sacramento-San Joaquin Delta (Delta) is a complex system of tidally-influenced, interconnected sloughs and channels (Oltmann, 1994). The hydrologic complexity is increased further by freshwater inputs to the Delta from several rivers and various sloughs. One-half million pounds of over 30 different herbicides are applied annually on agricultural lands in the Delta, and an additional 5 million pounds are applied upstream in three other watersheds: the Sacramento River, San Joaquin River, and French Camp Slough (fig. 1) (California Department of Pesticide Regulation, 1996). Herbicides enter the Delta waters from these external (upstream) and from local (Delta) inputs.

Herbicides have different modes of action (table 1). Some herbicides are designed to kill plants by inhibiting photosynthesis and can directly affect phytoplankton primary productivity; these are atrazine, cyanazine, diuron, hexazinone, simazine, and thiobencarb. Although little is known about the concentrations and residence times of these herbicides in the Delta, field studies have detected atrazine, simazine, and thiobencarb during the spring and summer (cyanazine, diuron, and hexazinone were not measured) (K.M. Kuivila, U.S. Geological Survey, unpublished data, 1996).

This study was designed specifically to determine whether herbicides impair phytoplankton primary productivity in the Delta. This report presents the herbicide concentrations measured in the Sacramento-San Joaquin Delta and their possible sources. Potential effects of herbicides on phytoplankton primary production

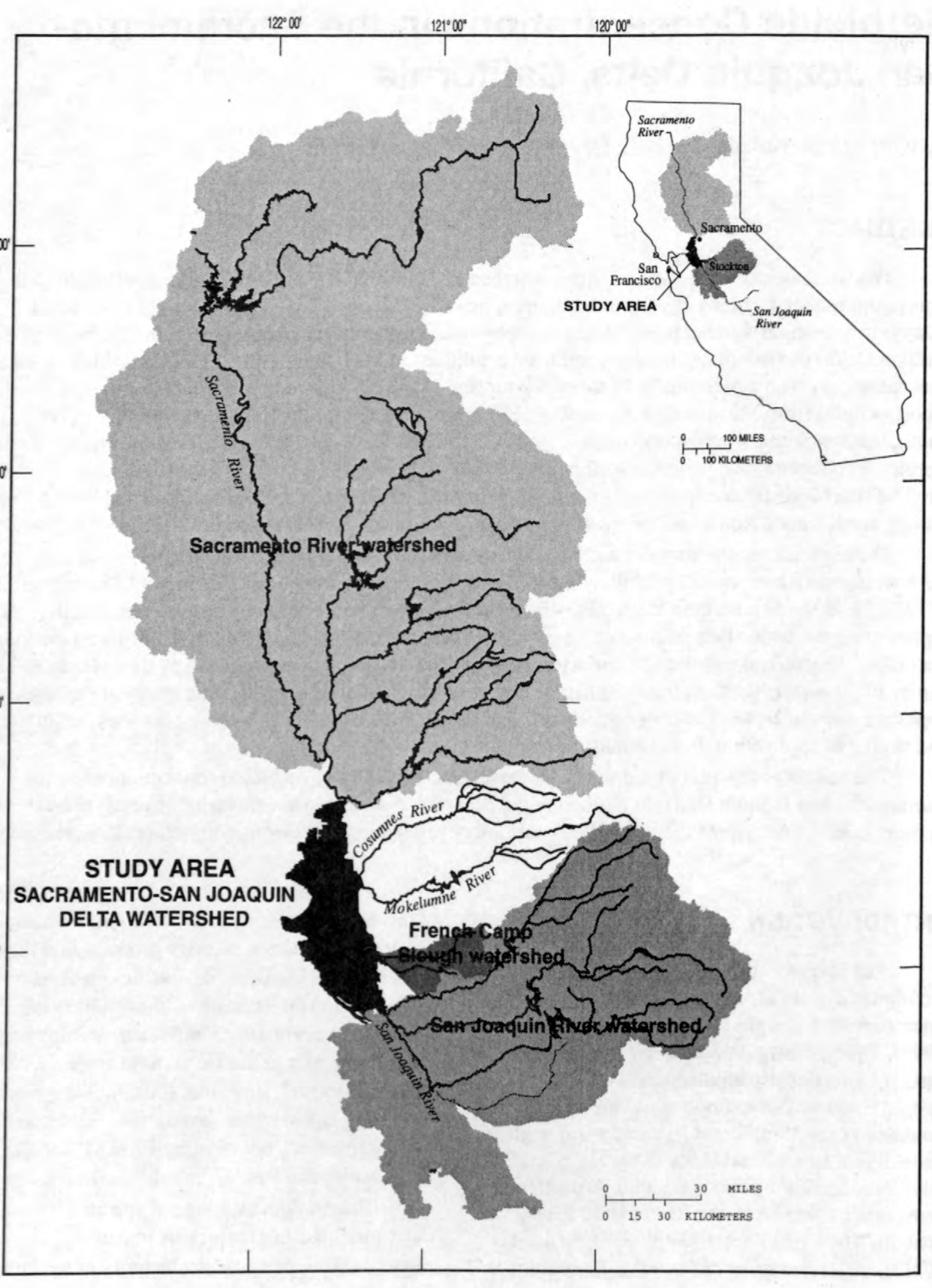

Figure 1. Location of the study area and the three adjoining watersheds, Sacramento-San Joaquin Delta, California.

Table 1. List of herbicides analyzed, mode of action, analytical method, method detection limit, and accuracy expressed as recovery from spiked water samples (in percent)

[Values in nanogram per liter; na, not available; HPLC, high-performance liquid-chromatography; GC/MS, gas-chromatography/mass spectrometry]

Herbicides	Mode of action	Method of analysis	Method detection limit	Recovery (percent)
2,4-D	Inhibits growth	HPLC	111	106
Atrazine	Inhibits photosynthesis	GC/MS	11	74
Cyanazine[1]	Inhibits photosynthesis	GC/MS	50	87
Dacthal	Inhibits germination	GC/MS	5	87
Diethatyl-ethyl	na	GC/MS	5	76
Diuron	Inhibits photosynthesis	HPLC	134	120
EPTC	Inhibits growth	GC/MS	25	72
Hexazinone	Inhibits photosynthesis	HPLC	45	71
MCPA	Inhibits growth	HPLC	28	103
Metolachlor	Inhibits germination	GC/MS	4	71
Molinate	Inhibits germination	GC/MS	24	113
Simazine	Inhibits photosynthesis	GC/MS	10	81
Thiobencarb	Inhibits photosynthesis	GC/MS	8	66

[1] Method detection limit and recovery data from Crepeau and others (1994).

are summarized by Edmunds and others (1999). This study is part of a larger project by the U.S. Geological Survey (USGS) Toxic Substances Hydrology Program that is studying the fate and transport of pesticides in San Francisco Bay. Additional funding was received from the Interagency Ecological Program for the Sacramento-San Joaquin Estuary. The authors wish to acknowledge the efforts of Brian Cole and Richard Millette for assistance in sampling, and Lucian Baker III, Kathryn Crepeau, and Keith Starner for the herbicide analyses.

SAMPLING DESIGN

The difficulties of tracking water flow, let alone contaminants, led to sampling at times and locations of expected high concentrations of herbicides and contrasting them with times and locations of expected low concentrations. There was no intent to calculate herbicide loads or track herbicides through the waterways of the Delta.

Nine sites (fig. 2) were sampled during five periods in 1997: May 27-29, June 10-12, June 24-26, October 14-16, and November 11-13. Results from past sampling suggested that herbicide concentrations would be the highest in late spring/early summer and the lowest in the fall. The nine sampling sites were chosen to represent a range of inputs and flow conditions. French Camp Slough at McKinley Road (French Camp Slough) and San Joaquin River at Vernalis (Vernalis) are sites that characterize external inputs from the French Camp Slough and San Joaquin River watersheds, respectively (figs. 1 and 2). Sutter Slough at Courtland (Sutter Slough) is a site that, although not on the mainstem of the Sacramento River, is primarily Sacramento River water (Rick Oltmann, U.S. Geological Survey, oral commun., 1996). The Sacramento River watershed (fig. 1) is the source of herbicides detected at the Sutter Slough site, but the integrated load from the Sacramento River watershed is divided among Sutter Slough, Steamboat Slough, and the mainstem Sacramento River. The site at Mokelumne River at New Hope Bridge (Mokelumne River) was chosen as a control site because previous samplings have detected few, if any, herbicides. The Old River at Bacon Island (Old River) and Middle River at Bacon Island (Middle River) sampling sites are on the two major flow paths of Sacramento River water through the Delta to the pumping plants (Oltmann, 1994) and represent a mixture of external and local Delta inputs. Beaver Slough at Blossom Road (Beaver Slough) primarily receives local input. Paradise Cut at Paradise Road (Paradise Cut) and Werner Slough at Orwood Road (Werner Slough) are dead-end sloughs that generally have less flushing and longer residence times than the other sites, and they represent local inputs.

In addition, the site at Middle River was sampled biweekly from May 28 through November 12. The purpose of this more frequent sampling was to measure the changes in herbicide concentrations

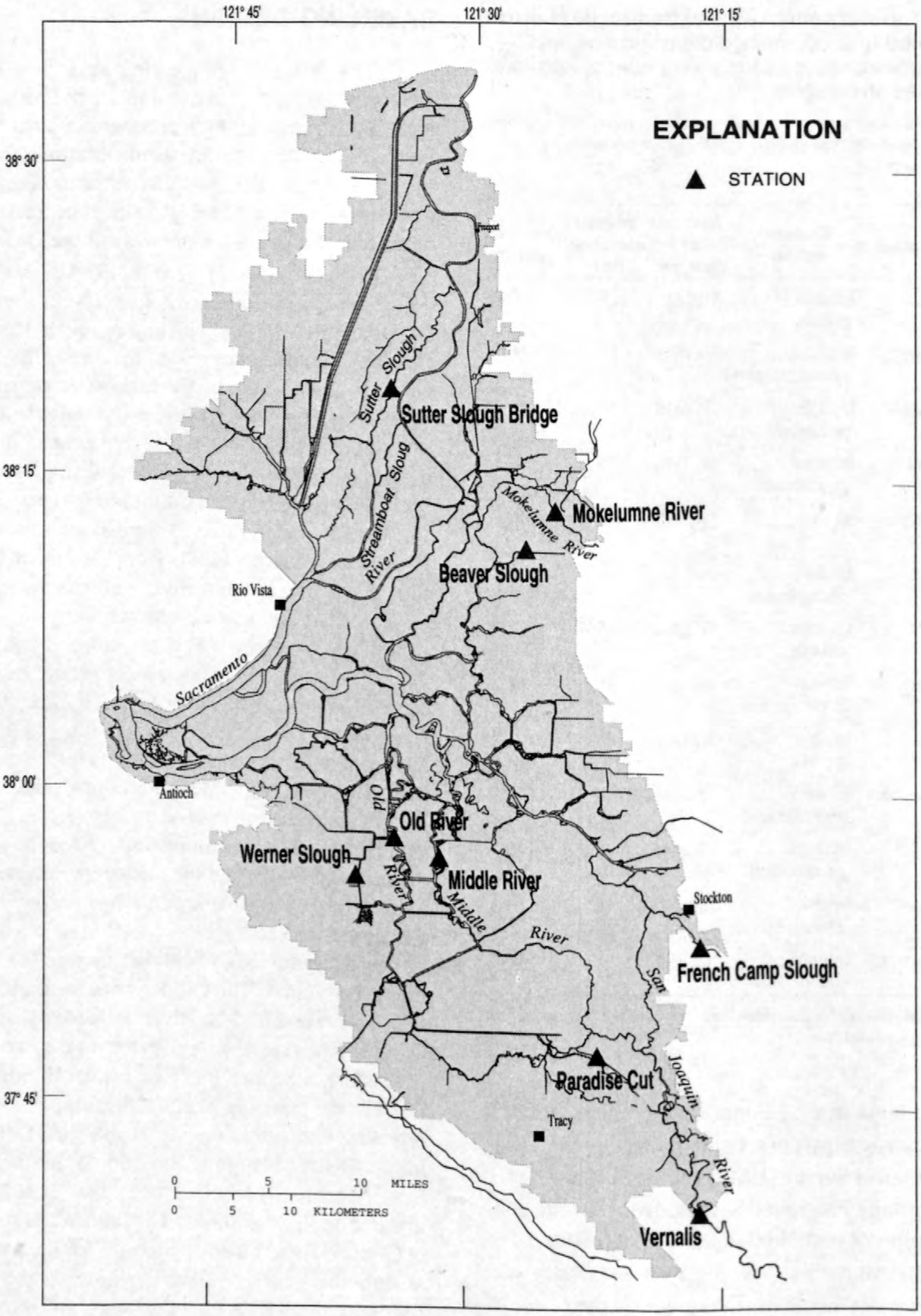

Figure 2. Locations of sampling sites in the study area, Sacramento-San Joaquin Delta, California. Table 2 gives full and abbreviated forms of site names.

throughout the entire sampling period and to determine the timing of the highest concentrations. Middle River was chosen as a site representative of the central Delta.

ANALYTICAL METHODS

Water samples were collected 1 meter (m) below the water surface using a 2.5-liter (L) Teflon-lined Niskin[1] bottle deployed horizontally. Water samples (8-10 L) were composited in a stainless-steel milk can and then split into aliquots using a Teflon cone-splitter (Shelton, 1994). Different aliquots were analyzed for herbicide concentrations, phytoplankton biomass, species composition, phytoplankton primary-production rates, and basic water-quality constituents. Two 1-L aliquots for herbicide analyses were filtered and analyzed by gas chromatography/mass spectrometry (GC/MS) with ion-trap detection and by high-performance liquid chromatography (HPLC) with photodiode-array detection at the organic-chemistry laboratory at the California District Office of the USGS.

For analysis by GC/MS, the filtered water sample was extracted onto a C-8 solid-phase extraction (SPE) cartridge. Complete details of the method, analysis, and quality assurance are given in Crepeau and others (1994). New method detection limits and recoveries from spiked water samples were determined in November 1995 using the same method as in Crepeau and others (1994) (table 1), except that eluates were concentrated to 100 microliters (µL), rather than to 200 µL. For analysis by HPLC, the filtered water sample was extracted onto a Carbopak-B SPE cartridge. More details of this method, analysis, and quality assurance are given in Werner and others (1996). One method change was the use of a longer HPLC gradient than listed in Werner and others (1996) to enable the separation of additional herbicides. Method detection limits and recoveries from spiked water samples are given in table 1.

RESULTS AND DISCUSSION

Thirteen herbicides were detected in one or more water samples. Concentrations varied considerably by site and with time (table 2). Three herbicides (diuron, hexazinone, and thiobencarb) that inhibit photosynthesis and five herbicides (2,4-D, diethatyl-ethyl, EPTC, metolachlor, and molinate) with different modes of action occurred frequently and at elevated concentrations. The other three herbicides (atrazine, cyanazine, and simazine) that inhibit photosynthesis and two other herbicides (dacthal and MCPA) occurred less frequently or at much lower concentrations and will not be discussed in this report.

Reported herbicide application in each watershed—Sacramento River, San Joaquin River, French Camp Slough, and the Delta—is listed in table 3. The application data is primarily agricultural use, most of the urban use is not included. Data from 1997 were not available at this time so data from 1995 were used as an approximation; application amounts may vary from year to year but are not expected to change radically. The areas of these four watersheds vary by two orders of magnitude so the applied amounts must be evaluated in relation to the scale of each watershed.

Molinate and Thiobencarb

Elevated concentrations of molinate and thiobencarb progressed over time from Sutter Slough through the Delta (fig. 3; table 2). The highest concentrations of molinate and thiobencarb were measured at Sutter Slough on May 29, 861 nanograms per liter (ng/L) and 313 ng/L, respectively. In subsequent samples, the concentrations of these herbicides were considerably lower. The molinate concentrations at Werner Slough, and Old and Middle Rivers peaked on June 25 at levels of 160, 267, and 290 ng/L, respectively. Concentrations of thiobencarb followed a similar pattern, but at much lower levels. The biweekly sampling at Middle River confirmed that the concentrations of molinate and thiobencarb decreased after June 25. The highest concentration of molinate (152 ng/L) at French Camp Slough was measured on May 27.

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[1] The use of trade names in this report is for identification purposes only and does not constitute endorsement by the USGS.

Table 2. Herbicide concentrations

[Concentrations are in nanograms per liter; values in parentheses are below the method detection limit; nd, not detected; —, not analyzed]

Site	Date, in 1997	2,4-D	Atrazine	Cyanazine	Dacthal	Diethatyl-ethyl	Diuron	EPTC	Hexazinone	MCPA	Metolachlor	Molinate	Simazine	Thiobencarb
Vernalis[1]	05/27	nd	(6)	nd	(1)	nd	182	(20)	nd	nd	92	nd	39	8
Vernalis	06/10	nd	(4)	(7)	(1)	nd	217	(13)	53	nd	38	nd	13	(4)
Vernalis	06/24	nd	(4)	(17)	(1)	nd	160	222	nd	nd	89	(5)	21	nd
Vernalis	10/14	nd	nd	nd	(1)	nd	nd	387	nd	nd	8	nd	(9)	nd
Vernalis	11/11	nd	nd	nd	nd	nd	nd	nd	nd	nd	9	nd	nd	nd
French Camp Slough[2]	05/27	nd	(3)	nd	(2)	nd	387	38	275	nd	502	152	39	9
French Camp Slough	06/10	nd	(3)	(27)	1	nd	241	(6)	nd	nd	160	(13)	19	(6)
French Camp Slough	06/24	nd	nd	128	(1)	nd	176	101	nd	nd	106	(7)	27	nd
French Camp Slough	10/14	nd	nd	nd	nd	nd	434	nd	nd	nd	nd	nd	71	nd
French Camp Slough	11/11	495	nd	nd	73	nd	2141	nd	—	—	251	nd	nd	nd
Paradise Cut[3]	05/27	nd	(8)	nd	(2)	(2)	1012	40	669	nd	916	nd	32	8
Paradise Cut	06/10	nd	(5)	(30)	(1)	57	487	41	nd	nd	1,019	nd	17	8
Paradise Cut	06/24	110	(8)	127	nd	56	589	110	77	nd	1,107	(1)	31	nd
Paradise Cut	10/14	nd	(3)	nd	nd	1041	139	85	nd	nd	393	nd	25	nd
Paradise Cut	11/11	356	nd	nd	nd	150	309	46	nd	nd	146	nd	nd	nd
Old River[4]	05/28	nd	25	nd	(2)	(1)	(112)	(9)	190	nd	18	(13)	36	8
Old River	06/11	nd	17	(1)	nd	nd	nd	(6)	179	nd	13	84	12	17
Old River	06/25	132	14	(15)	nd	nd	nd	(2)	82	135	23	267	18	26
Old River	10/15	nd	nd	nd	nd	65	nd	nd	61	nd	nd	nd	nd	nd
Old River	11/12	190	nd	(5)	nd	(1)	378	(3)	nd	nd	12	(4)	(6)	nd
Middle River[5]	05/28	nd	29	nd	nd	(1)	497	(14)	nd	nd	21	nd	33	nd
Middle River	06/11	nd	18	(5)	nd	(1)	(120)	(8)	(43)	nd	23	95	17	19
Middle River	06/25	nd	16	(29)	nd	nd	(72)	(12)	67	nd	38	290	20	35
Middle River	07/08	(74)	(9)	nd	nd	nd	nd	nd	nd	nd	nd	108	nd	nd
Middle River	07/22	nd	nd	(9)	nd	nd	nd	(6)	65	nd	27	(20)	(7)	10
Middle River	08/05	(44)	nd	(12)	(1)	(4)	nd	(14)	342	nd	25	(15)	(3)	(8)
Middle River	08/20	(67)	(3)	(10)	nd	nd	nd	nd	120	nd	58	nd	nd	nd
Middle River	09/03	nd	nd	nd	nd	nd	nd	(8)	291	nd	19	(19)	nd	13

Table 2. Herbicide concentrations—Continued

Site	Date, in 1997	2,4-D	Atrazine	Cyanazine	Dacthal	Diethatyl-ethyl	Diuron	EPTC	Hexazi-none	MCPA	Meto-lachlor	Molinate	Simazine	Thioben-carb
Middle River[5]	09/17	nd	nd	nd	(1)	(1)	nd	(6)	301	nd	12	(19)	nd	nd
Middle River	10/01	nd	nd	nd	nd	nd	nd	nd	288	nd	nd	nd	nd	(4)
Middle River	10/15	nd	nd	nd	nd	nd	nd	(5)	nd	nd	11	(3)	(2)	nd
Middle River	10/29	nd	nd	nd	nd	nd	nd	(10)	nd	nd	11	nd	(3)	nd
Middle River	11/12	nd	nd	nd	(1)	(1)	nd	(8)	nd	nd	7	(4)	(3)	nd
Werner Slough[6]	05/28	(77)	24	nd	nd	nd	153	(9)	nd	nd	51	nd	34	(3)
Werner Slough	06/11	nd	17	(5)	nd	(2)	145	(7)	(31)	nd	29	57	15	9
Werner Slough	06/25	116	22	(19)	(1)	nd	nd	(3)	nd	nd	21	160	19	30
Werner Slough	10/15	nd	nd	(12)	nd	nd	nd	nd	111	nd	nd	nd	nd	nd
Werner Slough	11/12	840	nd	(4)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Beaver Slough[7]	05/29	(91)	(3)	nd	nd	nd	nd	(7)	193	nd	17	nd	37	(6)
Beaver Slough	06/12	nd	(2)	nd	nd	nd	nd	(2)	nd	nd	20	52	13	15
Beaver Slough	06/26	nd	(6)	(21)	nd	(1)	(38)	(2)	nd	nd	78	42	51	(7)
Beaver Slough	10/16	nd	nd	nd	nd	nd	nd	nd	305	nd	nd	nd	nd	nd
Beaver Slough	11/13	nd	nd	nd	nd	(1)	nd	(3)	nd	nd	nd	nd	nd	nd
Mokelumne River[8]	05/29	nd	(2)	nd	(2)	nd	nd	nd	nd	nd	(1)	nd	10	(3)
Mokelumne River	06/12	nd	nd	nd	nd	nd	nd	nd	nd	nd	(1)	nd	(5)	nd
Mokelumne River	06/26	nd	nd	nd	nd	nd	nd	nd	nd	nd	(1)	nd	(8)	nd
Mokelumne River	10/16	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Mokelumne River	11/13	nd	nd	nd	(1)	nd	nd	(2)	nd	nd	nd	nd	(2)	nd
Sutter Slough[9]	05/29	nd	15	nd	nd	nd	(36)	(2)	217	83	9	861	11	313
Sutter Slough	06/12	nd	(4)	nd	nd	nd	nd	nd	78	nd	(3)	117	(8)	24
Sutter Slough	06/26	nd	nd	nd	(1)	nd	nd	nd	122	nd	(3)	47	(5)	(7)
Sutter Slough	10/16	nd	nd	nd	(3)	nd	nd	nd	nd	86	nd	(7)	nd	nd
Sutter Slough	11/13	nd	nd	nd	(1)	(2)	nd	(1)	nd	53	4	(14)	nd	nd

[1] San Joaquin River at Vernalis.
[2] French Camp Slough at McKinley Road.
[3] Paradise Cut at Paradise Road.
[4] Old River at Bacon Island.
[5] Middle River at Bacon Island.
[6] Werner Slough at Orwood Road.
[7] Beaver Slough at Blossom Road.
[8] Mokelumne River at New Hope Bridge.
[9] Sutter Slough at Courtland.

Table 3. Total agricultural herbicide application in 1995 in each watershed: Sacramento River, San Joaquin River, French Camp Slough, and Sacramento-San Joaquin Delta, California. (California Department of Pesticide Regulation, 1996)

[lbs. a.i., pounds per active ingredient. Shaded values indicate highest application of the 13 herbicides in watershed shown. Figure 1 shows watershed locations]

Herbicides	Sacramento River (1,000 lbs. a.i.)	San Joaquin River (1,000 lbs. a.i.)	French Camp Slough (1,000 lbs. a.i.)	Sacramento-San Joaquin Delta (1,000 lbs. a.i.)
2,4-D	200	60	21	61
Atrazine	6	0	<1	7
Cyanazine	13	56	1.7	6
Dacthal	<1	<1	1.5	<1
Diethatyl-ethyl	1	<1	<1	2
Diuron	35	33	11	35
EPTC	68	78	17	55
Hexazinone	16	20	3	6
MCPA	172	19	7	11
Metolachlor	58	25	11	29
Molinate	1,400	20	9	1
Simazine	36	48	15	13
Thiobencarb	570	5	4	<1
Total watershed acres, in thousands	17,161	4,683	258	679

The concentrations of molinate and thiobencarb were much lower at all the other sites. These herbicides are applied only to rice in the Sacramento River watershed in April through June (table 3). A much smaller quantity (two orders of magnitude) of the two herbicides are applied to rice in the French Camp Slough watershed (table 3). Sutter Slough receives drainage from rice fields in the Sacramento River watershed via the Sacramento River; rice-field water typically is released at the end of May.

EPTC

The highest concentrations of EPTC were detected at Vernalis on October 14 (387 ng/L) and June 24 (222 ng/L). Elevated concentrations also were measured on June 24 at French Camp Slough (101 ng/L) and Paradise Cut (110 ng/L). EPTC was not detected above the method detection limit at any of the other sites. This is in agreement with previous studies where elevated concentrations of EPTC were detected at Vernalis in the summer but were not detected at the Sacramento River at Sacramento (MacCoy and others, 1995; Panshin and others, 1998). In the San Joaquin River watershed, EPTC had the highest use of the 13 herbicides detected [78 thousand pounds (table 3)]. In the French Camp Slough and Sacramento-San Joaquin Delta watersheds, EPTC is the second most commonly used herbicide and is used near Paradise Cut.

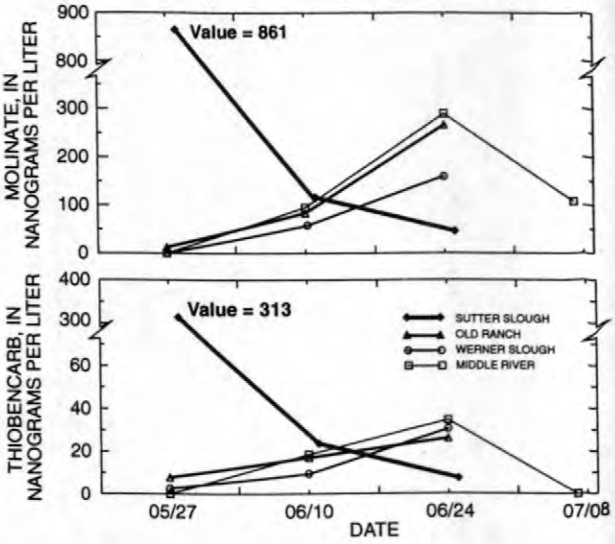

Figure 3. Concentrations of molinate and thiobencarb at the sampling sites Sutter Slough, Old River, Werner Slough, and Middle River detected biweekly between May 27-July 8, 1997, Sacramento-San Joaquin Delta, California. Table 2 gives full and abbreviated forms of site names.

Diuron, Metolachlor, and Diethatyl-ethyl

The highest concentrations of diuron and metolachlor were detected at Paradise Cut and French Camp Slough. In comparison, diethatyl-ethyl also had the highest concentrations at Paradise Cut, but was not detected at French Camp Slough in all the samples.

The highest concentration of diuron (2,141 ng/L) was measured at French Camp Slough on November 11, the day following rainfall. The next highest concentration (1,012 ng/L) of diuron was measured at Paradise Cut on May 27. At both French Camp Slough and Paradise Cut, concentrations of diuron were above the detection limit in all the samples (table 2). Concentrations of diuron above the method detection limit also were measured at Vernalis, Old River, Middle River, and Werner Slough. Other studies (California Regional Water Quality Control Board, 1995; Panshin and others, 1998) have detected high concentrations of diuron in the Sacramento and San Joaquin River watersheds in the winter following application and rainfall. Diuron is applied primarily on rights-of-way in the winter. In the Delta, diuron also is applied on alfalfa and asparagus in December-March near Paradise Cut.

The highest concentrations of metolachlor were measured at Paradise Cut; 916 ng/L on May 27, 1,019 ng/L on June 10, and 1,107 ng/L on June 24 (table 2). The site with the next highest concentrations was French Camp Slough (106-502 ng/L). Metolachlor concentrations at Vernalis were less than 100 ng/L and are in agreement with previous studies (MacCoy and others, 1995; Panshin and others, 1998). Detectable concentrations were measured at all other sites except Mokelumne River. Use of metolachlor primarily is on corn and beans during April-June. Within the Delta, metolachlor is applied in the vicinity of Paradise Cut.

Four of the five samples at Paradise Cut contained detectable concentrations of diethatyl-ethyl; the highest concentration was on October 14 (1,041 ng/L). The only other site with concentrations above the method detection limit was Old River on October 15 (65 ng/L). The use of diethatyl-ethyl is low in all the watersheds and within the Delta (table 3), but diethatyl-ethyl is applied directly to sugar beets adjacent to Paradise Cut. The application is in May but the highest concentration was detected in October.

The variable spatial distribution of diuron and metolachlor could be the result of the relatively high use in all the watersheds. But, for diuron, the timing of detection is not easily explained from the timing of application. In contrast, metolachlor was detected shortly after application. Diethatyl-ethyl was detected almost entirely at the site closest to application in the Delta, Paradise Cut; however, the timing of detection (October) was much different than application (May).

2,4-D and Hexazinone

The distribution of both 2,4-D and hexazinone varied considerably temporally and spatially. The highest concentrations of 2,4-D were measured during November 11-12 at French Camp Slough (495 ng/L) and at three sites receiving local inputs: Werner Slough (840 ng/L), Paradise Cut (356 ng/L), and Old River (190 ng/L). The next highest concentrations were measured on June 24-25 at the same three local-input sites but not at French Camp Slough. Only limited measurements of 2,4-D have been reported in previous studies; concentrations at Vernalis were not detected in samples from March to December 1993 (Panshin and others, 1998). The herbicide 2,4-D is commonly used in all the watersheds and is applied to a variety of crops several different times of the year. Applications are in February on wheat, almonds, and barley, and in May-July on corn and asparagus. In the Sacramento Valley, the predominant use of 2,4-D is on rice, but the application is in June and July, after the release of the rice field water.

The pattern of hexazinone concentrations is even more complex. High concentrations were measured during three different times in different areas of the Delta. During May 27-29, the highest concentrations were detected at Paradise Cut (669 ng/L), French Camp Slough (275 ng/L), and Sutter Slough (217 ng/L). At Middle River, the site sampled biweekly, hexazinone concentrations were elevated from June-September (fig. 4) with the maximum concentration on August 5 (342 ng/L). Finally, on October 16, the highest concentration was measured at Beaver Slough (305 ng/L). The use

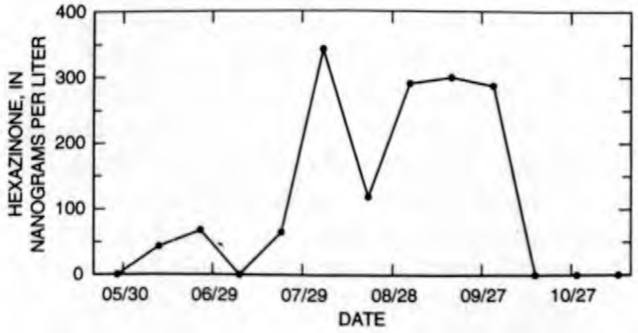

Figure 4. Concentrations of hexazinone at Middle River at Bacon Island, May 27-November 11, 1997, Sacramento-San Joaquin Delta, California. Table 2 gives full and abbreviated forms of site name.

of hexazinone is relatively low in all four watersheds, with applications in October-February on alfalfa and forests. The pulse of hexazinone observed at Middle River cannot be explained with this data set or application patterns.

Both 2,4-D and hexazinone had complex patterns of concentration. There are few previous measurements of 2,4-D in the Delta and adjoining watersheds, and no other reported measurements of hexazinone. The limited data in this study and other studies make it difficult to determine the sources and transport of these herbicides.

CONCLUSIONS AND POSSIBLE EFFECTS ON PHYTOPLANKTON

The measured concentrations of 13 herbicides in the Sacramento-San Joaquin Delta varied considerably temporally and spatially. The most frequent detections and some of the highest concentrations were measured in May and June. Some samples in November also had unexpectedly high concentrations; this could be due to rainfall near the time of our sampling. Paradise Cut and French Camp Slough were the sites with the most frequent detections and highest concentrations. For some herbicides, elevated concentrations could be attributed to one of the watersheds as a source. Molinate and thiobencarb could be attributed to use on rice in the Sacramento River watershed; elevated concentrations were detected at Sutter Slough in late May, and then at central Delta sites in late June. The primary source of EPTC was the San Joaquin River watershed in the summer, with the highest concentrations detected at Vernalis. In contrast, explaining the distribution of other herbicides was not so simple. Diuron concentrations were highest at Paradise Cut and French Camp Slough, but diuron was detected at a variety of sites at different times. The ubiquitous nature of diuron applications on rights-of-way could explain the widespread detection. Metolachlor was measured at concentrations above the method detection limit at all sites except Mokelumne River, and the highest concentrations occurred shortly after application. Diethatyl-ethyl was detected at the site closest to its Delta application (Paradise Cut), but the timing of detection cannot be explained by the timing of application. The pattern of 2,4-D and hexazinone concentrations was complex, and cannot readily be explained by times or locations of application.

In the 53 samples collected for this study, Edmunds and others (1999) found little correlation between the concentrations of the six herbicides that inhibit photosynthesis and the maximum rate of phytoplankton primary production (Pmax). But the one sample with the highest diuron concentration (2,141 ng/L at French Camp Slough on November 11) had the lowest Pmax (0.9 milligrams carbon per milligram chlorophyll per hour). Edmunds and others (1999) concluded that there is no indication of system level impairment of primary production in the Delta, but caution that there are localized events when production is impaired.

Of the six herbicides that inhibit photosynthesis, diuron and hexazinone had the highest concentrations and most frequent detections and therefore, are the herbicides most likely to impair primary production in localized events. More sampling is needed to understand the source(s) of these two herbicides and to predict the location and timing of the highest concentrations in the Delta. Species composition also was measured in this study; this data will be analyzed to assess any effect of herbicide concentration on the phytoplankton community structure.

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Do Herbicides Impair Phytoplankton Primary Production in the Sacramento-San Joaquin River Delta?

By Jody L. Edmunds, Kathy M. Kuivila, Brian E. Cole, and James E. Cloern

ABSTRACT

The effect of herbicide concentration on the maximum rate of phytoplankton primary production (P_{max}) was examined for 53 water samples collected at 9 sites in the Sacramento-San Joaquin River Delta between May and November, 1997. Samples were analyzed for P_{max} and the concentrations of diuron, atrazine, cyanazine, simazine, thiobencarb, and hexazinone. The herbicide concentrations ranged between 0 µg/L and 2.1 µg/L, with 50% of the values (n=318) between 0 and 0.018 µg/L. Herbicide concentrations in 52 of the water samples were well below the lowest observable effect concentrations (LOECs) that have been reported in laboratory experiments to inhibit primary production. P_{max} ranged between 2 and 11 milligrams of carbon per milligrams of chlorophyll a per hour (mg C (mg chla-h))$^{-1}$ for the 52 samples where the herbicide concentrations were less than any reported LOEC. However, for the one sample where the diuron concentration (2.1 µg/L) exceeded the reported LOEC of 2.0 µg/L, P_{max} was the lowest observed during the study, 0.9 mg C (mg chla-h)$^{-1}$. Herbicide concentrations we observed throughout the system do not appear to limit production; however, localized occurrences of elevated herbicide concentrations exist and may affect primary production.

INTRODUCTION

The Sacramento-San Joaquin Delta is at the confluence of the Sacramento and San Joaquin rivers. In the early 1800's, this conjunction comprised approximately 700,000 acres of Delta freshwater marshes (Atwater and others, 1979). However by 1920, 95% of the marshes were leveed for farming. Today, the Delta is a maze of levees that create small sloughs, vast lakes, and meandering rivers surrounding over twenty islands dominated by agricultural use. Over 500,000 acres of Delta farms grow alfalfa, corn, grapes, safflower, sorghum, sugarbeets, tomatoes, winter wheat, and orchards.

Each year, 500,000 pounds of over 30 different herbicides are applied on the agricultural lands within the Delta (California Department of Pesticide Regulation, 1996). Unwanted plant species that decrease crop production and crowd rights-of-way are treated with herbicides that have specific mechanisms designed to inhibit plant growth and primary production. These compounds have been detected within nontarget areas such as Delta waterways, yet the effect of these compounds on natural aquatic plant and phytoplankton communities in unknown.

Phytoplankton are the base of the food web thus providing much of the food resource to grazers in lower trophic levels. If primary production is significantly depressed by herbicide exposure, the food resource to higher consumers such as zooplankton and fish may be reduced.

In this study, we focused on a particular class of herbicides that inhibit photosynthesis. This class includes the triazines (atrazine, simazine, cyanazine, and hexazinone), the substituted urea-based compound diuron, and the thiocarbamate, thiobencarb. Laboratory experiments with algal cultures have demonstrated that these compounds can be potent inhibitors of phytoplankton photosynthesis (Day, 1993; Tubbing and others, 1993; Brown and Lean, 1995; Schneider and others, 1995; Peterson and others, 1997).

We designed this study to explore the hypothesis that phytoplankton primary production in an agriculturally impacted freshwater ecosystem is inhibited by herbicides. Our objective was to answer three specific questions relevant to the Sacramento-San Joaquin River Delta: (1) What is the range of concentrations of the herbicides which inhibit photosynthesis? (2) Are there times/locations when these compounds are present at concentrations known to inhibit algal photosynthesis in the laboratory? (3) Are there negative correlations between measured rates of phytoplankton photosynthesis and measured concentrations of these herbicides in the Delta ecosystem? To answer these questions we used two different approaches. First, we measured herbicide concentrations from a field study and compared them to the lowest observable effect concentrations (LOEC) for primary production found in the literature. Second, we performed a bioassay of photosynthetic performance on natural phytoplankton communities while simultaneously measuring herbicide concentrations at the same location. Data were then analyzed to determine if there were significant (negative) correlations between herbicide concentrations and photosynthetic performance of the natural phytoplankton populations.

We used a modified primary productivity method (Lewis and Smith, 1983) to measure phytoplankton photosynthetic performance. We used this method as a bioassay to directly determine effects of herbicides on photosynthesis. The method uses radiocarbon ($NaH^{14}CO_3$) as a tracer for measuring the incorporation rate of CO_2 into algal cellular carbon. The rate of photosynthesis varies with light intensity, so the procedure includes measurement of $H^{14}CO_3$ assimilation rate across a range of light intensity. Photosynthesis-irradiance (P/I) functions are hyperbolic, and are described with empirical functions such as the one described by Platt and others (1980):

$$P^B = P_{max} \left[1 - \exp\left(-\alpha I / P_{max}\right)\right] \qquad (1)$$

Here, P^B is the rate of carbon fixation normalized to chlorophyll a concentration; P_{max} is the maximum rate of carbon fixation at saturating irradiance levels; α is the slope of the P/I function at low irradiance, and I is the instantaneous irradiance (photon flux density, PFD, of photosynthetically active light (PAR)). Herbicides that inhibit photosynthesis directly affect the light reaction of photosynthesis by blocking the electron transport chain in photosystem II (Arsalane and others, 1993). The energy generated by the light reaction is lost as fluorescence and therefore not transferred to the dark reaction (Calvin cycle) for carbon uptake. We focused on the parameter P_{max}, which measures the efficiency and performance of both the light and dark reactions, including the photosystems and Calvin cycle.

Results of this study were not consistent with the hypothesis that system wide phytoplankton primary production is impaired by herbicides within the Delta. However, our results do indicate that there may be localized events when production is reduced.

METHODS

Experimental design

Sampling was designed to maximize the information content from each sample. Sampling occurred at nine sites to determine spatial variability and one site to determine temporal variability across the network of sloughs and primary channels throughout the Delta. Spatial sampling was designed to characterize the potential effects of herbicide compounds and concentrations in areas of varying water retention times and phytoplankton production. Stations D1 (Vernalis), and D2 (French Camp Slough) and D9 (Sutter Slough) represent inputs from the San Joaquin and French Camp slough, and Sacramento watersheds, respectively (for a map of the stations, please see Kuivila and others, 1999). Water flow through the central Delta was sampled at Stations D4 (Old River) and D5 (Middle River), representing a mixture of both external and internal inputs. Stagnant flow with a variety of high concentrations of pesticides was sampled at D3 (Paradise Cut), D6 (Werner), and D7 (Beaver Slough). Station D8 (Mokelumne River) was chosen as a control site because few herbicides in low concentrations have been detected previously at this site. The nine sites were sampled at five times through the sampling period. High herbicide concentrations were expected during the first three sampling dates May 27-29, June10-12, and June 24-26. In contrast, low herbicide concentrations were expected during the later sampling dates, October 14-16 and November 11-13. In addition, Middle River (D5) in the central Delta was sampled bi-monthly to better characterize temporal variability of water quality, herbicide concentrations, and phytoplankton primary production in May through November.

Analytical methods

A 10 L water sample was collected from 1 meter depth at each site using a 2.5-L Teflon-lined Niskin bottle. The sample was collected in a stainless steel milk can and then split equally into eight 1-L bottles. Three kinds of measurements were made; chemical indicators of water quality and origin, herbicide concentrations, and the bioassay of phytoplankton photosynthetic performance.

Water quality

The water quality parameters included specific conductivity, temperature, suspended particulate matter (SPM), chlorophyll a, nutrients, dissolved inorganic carbon, and phytoplankton community composition. Ambient water temperature (°C) was measured using a glass mercury thermometer during the water collection at the sampling site. Suspended particulate matter was determined gravimetrically, as described by Hager (1993). Using 47-mm polycarbonate membrane filters 100-500 mL of sample was filtered onto preweighed 0.4-µm, and allowed to air dry for 48 to 72 hours before being re-weighed. Triplicate chlorophyll a samples of 500 mL were filtered through a Gelman A/E glass fiber filter and immediately frozen on dry ice. Filters were transferred to a freezer at the lab and allowed to dry for 72 hours. The dried filters were ground in 90% acetone and replaced in the freezer for 24 hours for chlorophyll extraction. Centrifuged samples were then analyzed on a Hewlett Packard 8452A diode array spectrophotometer. Chlorophyll a concentrations were calculated using Lorenzen's equations (1967). Nutrient samples were collected in an acetone-rinsed high-density polyethylene bottle, filtered through a 0.4-µm nuclepore filter, with the filtrate collected into a 30-mL high-density polyethylene bottle, and frozen until analyzed. Concentrations of ammonium (NH_4), nitrate plus nitrite (N+N), nitrite (NO_2), dissolved reactive phosphate (DRP), and dissolved silica were measured simultaneously on a Technicon AutoAnalyzer II system. Total dissolved inorganic carbon was measured by injecting 4 mL of sample into a sealed 20-mL serum bottle, acidified with 0.1-mL 6N HCl, and analyzed on a Perkin Elmer Sigma 2000 gas chromatograph. Samples of 100 mL were preserved with Lugol's solution and analyzed for phytoplankton species composition, density and cell volume.

Herbicide concentrations

Herbicide concentrations were determined by gas chromatography/mass spectrometry (GC/MS) and high-performance liquid chromatography (HPLC) at the organic chemistry laboratory at the U.S. Geological Survey California District Office (see Kuivila and others,1999).

Bioassay

The water sample was split within 30 minutes after collection and phytoplankton production was measured immediately. Fifty milliliters of sample were spiked with ~5.0 µCi/mL $NaH^{14}CO_3$ and thoroughly stirred. Seventeen scintillation vials each were filled with 2-mL aliquots and incubated in the photosynthetron at ambient water temperature for 30 minutes over a light range of 0-1600 µEinst/m^2/s (Lewis and Smith, 1983). Three 2-mL aliquots were immediately acidified with 0.3 ml 0.2N HCl to account for any initial carbon uptake before incubation (time zero - T_0). Three 2-mL aliquots were fixed with 6N NaOH to determine total ^{14}C initially available. At the end of the incubation, the 17 samples were immediately acidified with 0.2N HCl under a ventilation hood and shaken for 1 hour to drive off the ^{14}C still in solution. Ten milliliters of OptiPhase 'HiSafe' scintillation cocktail were then added to each sample and analyzed in a 1209 Rackbeta scintillation counter. Irradiance measurements were made using a Biospherical QSL-100 4π light probe placed in a modified scintillation vial inside the aluminum light tubes (Lewis and Smith, 1983).

Production and irradiance data were fit to the hyperbolic function (equation 1) to determine estimates of P_{max}. Negative correlation between P_{max} and herbicide concentration was determined using the software package Systat ($p = 0.05$)

RESULTS

Comparisons to published LOEC's

In 317 out of 318 samples, herbicide concentrations fell below reported LOECs. Atrazine, simazine, and cyanazine concentrations ranged from undetectable to very low throughout the sampling period (table 1). Atrazine concentrations ranged between 0 and 0.03 µg/L, well below the lowest observable effect concentration (LOEC), 26 µg/L (Caux and others, 1996).

Table 1. Maximum herbicide concentrations measured in the Sacramento-San Joaquin Delta during May-November 1997, compared to lowest observable effect concentrations (LOEC) reported in the literature.

Herbicide	Maximum concentration measured in the Delta (µg/L)	LOEC (µg/L)	Reference
atrazine	0.03	26	Caux and others, 1996
cyanazine	0.13	145	Caux and others, 1996
diuron	2.14	2	Edmunds, U.S. Geological Survey, unpublished data, 1998
hexazinone	0.67	22.5	Schneider and others, 1995
simazine	0.07	50	Bryfogle and McDiffett, 1979
thiobencarb	0.31	17	Sabater and Carrasco, 1996

Simazine and cyanazine concentrations ranged between 0 – 0.07 µg/L and 0 – 0.13 µg/L, respectively, and also were well below the reported LOECs, 50 µg/L and 145 µg/L, respectively. All three herbicides were used in low quantities and were generally applied on rights-of-way throughout the Delta (Kuivila and others, 1999.

The highest thiobencarb concentration was detected (0.31 µg/L) on May 29 following the application to rice fields in the Sacramento River watershed. This concentration also was well below the LOEC for thiobencarb, 17 µg/L (Sabater and Carrasco, 1996).

The highest concentrations of herbicides detected were hexazinone (0.67 µg/L) and diuron (2.14 µg/L). Hexazinone and diuron are both applied to alfalfa, while diuron is also applied to rights-of-way and asparagus. The maximum hexazinone concentration was lower than the LOEC of 22.5 µg/L, however, the maximum diuron concentration slightly exceeded the diuron LOEC of 2.0 µg/L.

Bioassay

P_{max} estimates for the 53 water samples ranged between 0.9 and 11 mg C (mg chl a-h)$^{-1}$ (Figure 1).

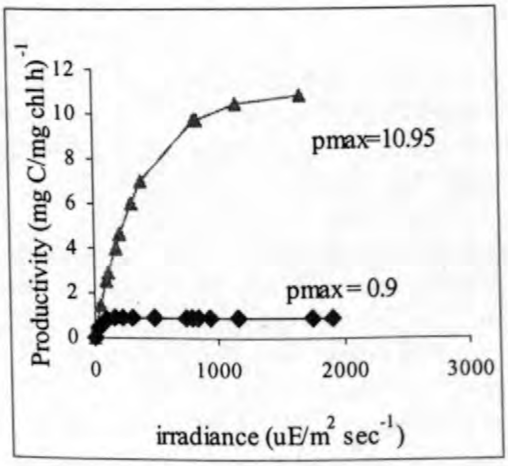

Figure 1. Highest and lowest P_{max} estimates depicted by productivity versus irradiance curves.

There was no negative correlation between herbicide concentrations and P_{max} for any of the six herbicides (Table 2). However, the lowest P_{max} was measured when the diuron concentration exceeded the reported LOEC, 2.0 µg/L (Edmunds, U.S. Geological Survey, unpublished data, 1998).

Table 2. Correlation coefficients between measured P_{max} (bioassay of photosynthetic performance of natural phytoplankton communities) and concentrations of six herbicides that inhibit photosynthesis. In all cases, sample size n = 53

Herbicide	r
atrazine	0.19
cyanazine	0.25
diuron	0.32
hexazinone	0.31
simazine	0.08
thiobencarb	0.06

DISCUSSION

In our first approach, we measured concentrations, of atrazine, diuron, cyanazine, simazine, thiobencarb, and hexazinone, that typically were orders of magnitude lower what has been determined in laboratory experiments to inhibit algal photosynthesis. Only once was the herbicide concentration above the reported LOEC, indicating possible inhibition during localized or transient events.

High herbicide concentrations were expected during the spring application period, while low concentrations were expected during late fall. However, the highest herbicide concentration was measured after a small rain event in mid-November. Based on these initial results, we concluded that herbicide concentrations found within the Delta should not impair total ecosystem primary production, but it may be impaired on a localized episodic basis.

Our second approach consisted of a bioassay to determine P_{max} rates with simultaneous measurements of herbicide concentrations at the nine sites. This approach reaffirmed the initial conclusion that production was not significantly affected by herbicide concentrations in the Delta. A negative correlation between P_{max} and herbicide concentrations was not detected. The range of P_{max} measured varied by a factor of ten, but this variability is apparently due to factors other than herbicide concentration, such as available inorganic carbon. However, the one sample with a measured herbicide concentration that exceeded the LOEC, also had the lowest P_{max}. This illustrates that the bioassay technique we used was effective at demonstrating the inhibitory effects of herbicides when a LOEC was exceeded.

The temporal and spatial variability of herbicides and the generally low concentrations of herbicides seen in the Delta waterways may be due to a number of factors. Although more than 500,000 pounds of herbicides are applied annually to crops grown in the Delta, there are more than 30 different herbicides applied to over a dozen crops. Consequently, the concentration of individual herbicides is generally lower in the Delta even though the total use of herbicides is high. This situation differs from the dynamics of other agricultural regions found within the United States. For example, in the Midwest, one or two crops (usually corn and sorghum) dominate large agricultural regions. In the Delaware River Basin, located in southeastern Nebraska and northeastern Kansas, it is estimated that 240,000 pounds of atrazine alone are applied to corn and sorghum yearly (Stamer and others, 1994). Atrazine concentrations are found regularly within the Delaware River at levels exceeding the LOEC indicating that system-wide production in the Delaware River may be impaired.

The scarcity of observed effects also may be due to the peat rich soil common in the Delta. The Delta farmlands are valued in part because of their high organic carbon content. However, herbicide compounds sorb more readily to soil composed of high organic matter (Celis and others, 1998; Nkedi-Kizza and Brown, 1998). Herbicides also degrade due to light and bacterial metabolism (Schneider and others, 1995). The occurrence of a sample with a high concentration of diuron in the fall indicates that herbicides may be applied throughout the year and flushed from the fields during rain events. Our study design may have missed potentially high herbicide concentrations found during the rainy season.

Episodic high concentrations and persistent low-level concentrations of herbicides found in the Delta do not appear to impair the photosynthetic rate of the resident phytoplankton, but may alter the species composition. Other studies have shown large differences among phytoplankton species in their sensitivity to herbicide toxicity (for example, Day and Hodge, 1996). If persistent low levels of a wide variety of herbicides in the Delta alter the phytoplankton community composition, then the quantity and quality of food available to higher trophic levels may be impaired even though ambient herbicide concentrations do not exceed LOECs.

CONCLUSIONS

We did not observe system wide reduction in phytoplankton primary production due to herbicide concentrations measured throughout the Sacramento-San Joaquin River Delta. However, this data is unique to this system. Herbicides have been detected in other systems at concentrations that exceed reported LOECs, and may impair production at the system level. Our results have introduced a useful assay to detect herbicide effects on natural phytoplankton populations and may be applied to these other systems where system-wide production may be reduced.

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Degradation Rates of Six Pesticides in Water from the Sacramento River, California

By Keith Starner, Kathryn M. Kuivila, Bryan Jennings, *and* G. Edward Moon

ABSTRACT

The degradation rates of six pesticides were measured in water samples collected from the Sacramento River, California, in April and August 1996. Samples were spiked with the pesticides carbaryl, malathion, atrazine, simazine, methidathion, and diazinon, and the concentrations were measured at various intervals. The laboratory experiments were done in the dark in incubators at 10°C or 25°C to represent a range of conditions. Carbaryl and malathion degraded rapidly, with half lives of 2-3 weeks at 10°C and of 1-5 days at 25°C. In contrast, atrazine and simazine were stable under all conditions. For methidathion and diazinon, the degradation rates were quite variable, with half lives ranging from 9 days to no observed degradation during the 34–37 days of the experiment. Differences between the water collected in April and August were more important than temperature in controlling the degradation rates of methidathion and diazinon. A more complete understanding of the factors that control this variability is necessary for accurate modeling of environmental fate.

INTRODUCTION

A variety of pesticides is applied in large quantities to the Central Valley of California. These pesticides are transported as runoff from agricultural fields into the Sacramento and San Joaquin Rivers, which flows into the San Francisco Bay Estuary. One part of the assessment of the fate and effects of pesticides is the study of their degradation rates in water from the Sacramento River under ambient conditions. The biological effects of a pesticide in an aquatic environment depend on several factors, including the concentration of the pesticide in water and the length of time that the organism of interest is exposed. Information on the degradation rates of pesticides under ambient conditions is integral to estimating their aquatic environmental significance.

This study, which is part of the U.S. Geological Survey (USGS) San Francisco Bay-Estuary Toxic Substances Hydrology Program, is designed to determine the degradation rates of six pesticides in the Sacramento River. Water samples collected in April and August 1996 were spiked with the six pesticides; carbaryl, malathion, atrazine, simazine, methidathion, and diazinon. Concentrations of the pesticides were measured throughout the 34-37-day experiment, and the degradation rates were calculated. This report describes the results of this study.

METHODS

Experimental Design

Water samples were collected from the Sacramento River during April and August 1996. Each sample was passed through a Westphalia continuous-flow centrifuge (centrifuged) to remove particulate matter and was stored in two 33-liter (L) stainless steel milk cans. Each sample was spiked with a mixture of pesticides (table 1). The pesticides were combined in ethyl acetate in a glass bottle and the mixture was evaporated to dryness with nitrogen to minimize the addition of solvent to the water sample. The bottle was rinsed several times with a centrifuged water sample, the rinsates were added to the milk can, and the entire sample was thoroughly mixed. Finally, approximately 20 L of the spiked sample was put into a clean Teflon bag that was collapsed to eliminate any headspace, sealed, and stored in the dark in an incubator. With this experimental design, the potential mechanisms

Table 1. Pesticides studied, class, and amount applied, Central Valley, California, 1995

Pesticide	Class	1995 Central Valley use[1], in pounds of active ingredient
Carbaryl	Carbamate insecticide	1,307,078
Malathion	Organophosphate insecticide	308,534
Atrazine	Triazine herbicide	22,085
Simazine	Triazine herbicide	673,439
Methidathion	Organophosphate insecticide	300,932
Diazinon	Organophosphate insecticide	1,988,987

[1] California Department of Pesticide Regulation (1996).

of degradation are abiotic hydrolysis and possibly aerobic microbial degradation. Two samples were incubated at a time, with one incubator maintained at 10.0°C±0.5°C and the other at 25.0°C±0.5°C. The water sample was mixed continuously with a motorized paddle stirrer throughout the experiment. Temperature, pH, and dissolved oxygen also were monitored with Campbell electrodes and a CR10 data logger. (The use of trade names in this report is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey.) Several days after spiking, and once the temperature had equilibrated, two or four 500-milliliter (mL) aliquots were pumped from each sample and analyzed for the starting concentration C_0 of each pesticide. Subsequently, duplicate aliquots of 500 mL of water were collected for analysis at varying sampling intervals. Aliquots were collected more frequently at the beginning of each experiment. As each sample was removed, the Teflon bag was further collapsed to eliminate headspace. Experiments were terminated after 34-37 days.

Analytical Method

The analytical method consisted of solid-phase extraction (SPE) and analysis by gas chromatography/ion trap mass spectrometry (GC/MS). At each sampling interval, duplicate 500-mL water samples were spiked with a surrogate compound (terbuthylazine) and extracted into C-8 SPE cartridges at a flow rate of 20 mL per minute (mL/min). Each cartridge was dried with carbon dioxide and stored in a freezer until analysis began. The SPE cartridges were eluted with three 2-mL aliquots of hexane:diethyl ether (1:1), internal standards were added, and the extracts were concentrated under nitrogen to approximately 100 microliters (µL). The concentrated samples were analyzed by GC/MS with ion-trap detection. A more detailed description of the analytical method is given in Crepeau and others (1994).

Each sample was injected twice on the gas chromatograph. When the calculated concentration for duplicate injections differed by more than 20 percent, the injections were not used in the data analysis. In addition, samples with surrogate recovery that was outside the control limit of 1.5 standard deviations from the mean were not included in the data analysis. Finally, when the calculated concentrations for replicate samples differed by more than 25 percent, those replicates were not included in the data analysis.

HALF-LIFE CALCULATIONS

Generally, the rate of abiotic hydrolysis for organic compounds in water is directly proportional to the concentration of the organic compound (Lyman and others, 1990). Assuming this relation, abiotic hydrolysis can be described using a first-order degradation curve:

$$C_t = C_0 e^{-kt} \qquad (1)$$

where C_t is the concentration of the organic species at time t,
C_0 is the initial concentration of the organic species, and
k is the rate constant (Wang and Hoffman, 1991).

A plot of ln (C_t/C_0) versus time yields a straight line with slope equal to k. The rate constant can then be used to derive the half life $t_{1/2}$:

$$t_{1/2} = (\ln 2)/k \qquad (2)$$

The rate of hydrolysis of organic compounds increases with temperature as described by the Arrhenius equation:

$$k = Ae^{-E_a/RT} \qquad (3)$$

where k is the reaction rate,
E_a is the activation energy,
R is the gas constant,
T is the temperature, and
A is a constant characteristic of the reaction (Vollhardt, 1987).

Reformulating the equation by taking the natural logarithm of both sides gives:

$$\ln k = \ln A - E_a/RT \qquad (4)$$

Thus, a plot of ln k against 1/T results in a straight line of slope $-E_a/R$ and y-intercept equal to ln A. Therefore, when reaction rate information is available at two or more temperatures, the activation energy E_a can be determined. The activation energy indicates the sensitivity of the reaction to temperature changes; the larger the activation energy value, the greater the increase in the reaction rate with a given increase in temperature.

The rate of hydrolysis of organic compounds in water also can exhibit a dependence on pH (Lyman and others, 1990). The total reaction rate k_T is the sum of acid- and base-catalyzed and neutral hydrolysis reactions:

$$k_T = k_H [H^+] + k_{H_2O} [H_2O] + k_{OH} [OH^-] \qquad (5)$$

In aqueous systems, the concentration of water essentially is constant and so, for acid-catalyzed hydrolysis below pH 7, the hydrolysis rate will decrease as the pH increases. Conversely, for base-catalyzed hydrolysis above pH 7, the hydrolysis rate will increase proportionately to the pH.

RESULTS AND DISCUSSION

Time-zero concentrations (C_0) for the different pesticides in the April and August samples varied from about 300-1,500 nanograms per liter (ng/L). Temperature and pH remained relatively constant and the samples remained aerobic throughout the experiments. Under these conditions, the potential mechanisms of degradation include abiotic hydrolysis and possibly aerobic microbial degradation (if not affected by removal of particles during centrifugation). No attempt was made to estimate the contribution of microbial degradation to the overall degradation rate. Photolysis and loss caused by volatilization were not measured because of the lack of sunlight and headspace.

For this study, first-order kinetics was assumed. The slope of the line was calculated using linear regression analysis, and the half life of each pesticide was calculated using eq. 2. The calculated half lives for each pesticide at 10°C and 25°C in the samples collected in April and August 1996 are given in table 2. When the calculated half life was shorter than the experiment (34-37 days), that value was reported. If the concentration of a pesticide decreased during the experiment, but the calculated half life was longer than the experiment, the half life was extrapolated and reported as an estimate (table 2). When no measurable decrease in concentration of a pesticide occurred during the experiment, the designation "no degradation" was used.

The half lives of pesticides in this experiment varied greatly (table 2), ranging from 1 day to no degradation. The degradation rate of some pesticides varied considerably with temperature and between the samples from April and August. The pH values for the sample collected in April ranged from 7.9 to 8.1 and those for the sample collected in August ranged from 7.3 to 7.7. As reported in the literature, pH affects the rate of hydrolysis for some of the pesticides studied here. Carbaryl is readily hydrolyzed under alkaline conditions and is substantially more stable under acidic conditions (Aly and El-Dib, 1971; Sharom and others, 1980; Chapman and Cole, 1982; and Fisher and Lohner, 1986). For malathion in water with a pH value of 4 or greater, the hydrolysis rate increases proportionately with the pH (Wolfe and others, 1977; Freed and others, 1979). For atrazine and simazine, hydrolysis is not significant at pH 4–10 (Erickson and Lee, 1989). For diazinon, hydrolysis is slow at pH 7 and increases rapidly with decreasing pH, and increases somewhat slower with increasing pH (Gomaa and others, 1969).

Table 2. Calculated half lives of pesticides, Sacramento River, California, 1996

[Half lives are reported in number of days. R^2 value is from linear regression analysis of plot of $\ln(C_t/C_0)$ as a function of time and is shown in parentheses. PH for samples collected in April ranged from 7.9 to 8.1, and pH for samples collected in August ranged from 7.3 to 7.7. Est., estimated degradation where half life exceeded length of experiment; No, no degradation where there was no measurable decrease in concentration. .

	April		**August**	
Pesticide	**10**°C	**25**°C	**10**°C	**25**°C
Carbaryl	22 (0.85)	2 (0.89)	16 (0.97)	1 (0.99)
Malathion	16 (0.95)	5 (0.99)	16 (0.95)	2 (0.96)
Atrazine	No	No	No	No
Simazine	No	No	No	No
Methidathion	33 (0.94)	21 (0.94)	No	18 (0.99)
Diazinon	9 (0.95)	16 (0.92)	Est. 134 (0.40)	Est. 103 (0.82)

Carbaryl and Malathion

At a given temperature, the degradation rates for carbaryl and malathion were similar in the April and August water samples (table 2; figs. 1, 2). Additionally, both pesticides degraded more rapidly at the higher temperature. The half life of carbaryl at 10°C was 16-22 days, and at 25°C decreased to 1-2 days. These values generally are in agreement with past studies (table 3). Szeto and others (1979) reported a half life of 20 days for carbaryl in pond water at 9°C. Lartiges and Garrigues (1995), reported a half life of 45 days for carbaryl at 6°C in filtered river water, and a half life of <2 days in the same water at 22°C. Armbrust and Crosby (1991) reported a half life of 1 day for carbaryl in filtered seawater at 24°C. The calculated activation energy E_a for carbaryl was 29 kilocalories per mole (kcal/mol) in the April and August samples. This is about twice the reported value of 15 kcal/mol for filtered river water (Lartiges and Garrigues, 1995).

The half life for malathion was 16 days in the April and August samples at 10°C and 2 and 5 days, respectively, at 25°C (fig. 2). These half-life values are in agreement with past studies, especially at the higher temperature (table 3). Lartiges and Garrigues (1995), reported half lives of 53 and 7 days for malathion in filtered river water at 6°C and 22°C, respectively. Wang and Hoffman (1991) reported a half life of 2 days at 28°C. The calculated activation energy for malathion was 16 kcal/mol for the April sample and 25 kcal/mol for the August sample. Freed and others (1979) reported a similar activation energy for malathion of 22 kcal/mol.

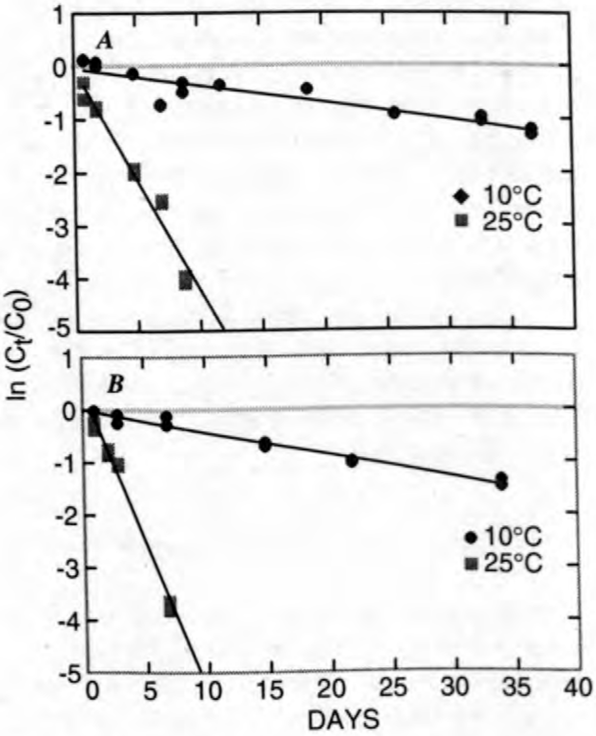

Figure 1. Degradation of carbaryl in water samples from the Sacramento River, California, ***A,*** April and ***B,*** August 1996.

Atrazine and Simazine

In contrast to carbaryl and malathion, atrazine and simazine did not degrade during the experiment in the April or August sample or at 10°C or 25°C. Similar stability has been reported previously at temperatures ranging from 4°C to 40°C (table 3). Lartiges and Garrigues (1995) reported that atrazine showed no sign of degradation after 180 days in

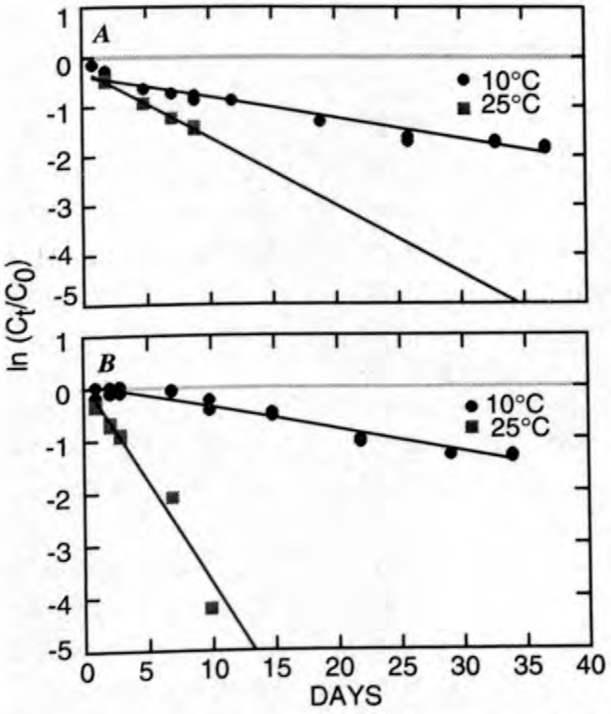

Figure 2. Degradation of malathion in water samples from the Sacramento River, California, ***A,*** April and ***B,*** August 1996.

filtered river water at 6°C and had an estimated half life of 130 days in the same water at 22°C. Widmer and others (1993) reported that atrazine showed no signs of degradation after 133 days in well water at 4°C and 30°C. Noblet and others (1996) reported that simazine and atrazine were stable after 43 days in sterilized creek water at 40°C.

Methidathion and Diazinon

The degradation rates of methidathion and diazinon were different in the April and August samples (table 2; figs. 3, 4). The half life of methidathion was 33 days at 10°C in the April sample but no degradation was observed in the August sample at 10°C. In contrast, the degradation rates in the April and August samples were similar at 25°C, with half lives of 21 and 18 days, respectively. These results compare well with those reported in the literature (table 3), with the exception of the rapid degradation observed in the April sample at 10°C. Frank and others (1991) reported a half life for methidathion of 139 days in river water at 4°C and 20 days at 21°C at pH 8.0-8.2. This pH range is similar to that of our April sample (7.9-8.1, table 2), therefore, the observed difference in degradation rate is not likely because of pH. Our experiment, at 10°C, had a higher temperature, whereas that by Frank and others (1991) had a temperature of 4°C. Using the calculated E_a value for methidathion in the April experiment, (5 kcal/mol), the theoretical half life of methidathion in our April sample at 4°C would be approximately 42 days, which is significantly shorter than the reported half life of 139 days. This difference suggests that some factor other than temperature or pH may have increased the rate of methidathion degradation in our study.

For diazinon, the differences in the April and August samples had an even greater influence on the degradation rate than for methidathion. Degradation was much more rapid in the April sample than the August sample, with the half lives differing by one order of magnitude. In contrast, temperature didn't have a large influence on degradation. The April sample had a half life of 9 days at 10°C and 16 days at 25°C. This difference of 7 days is statistically significant (at $\alpha = 0.05$, the calculated half lives ± standard deviation do not overlap). However, the decrease in degradation with an increase in temperature cannot be explained by abiotic hydrolysis. In contrast, diazinon degraded very slowly in the August sample with estimated half lives at 134 days at 10°C and 103 days at 25°C (table 2).

Half life values for diazinon reported in the literature also vary considerably, depending on the pH and type of water (table 3). At lower temperatures, literature values for the half life of diazinon in river water range from 45 days at 4°C and pH 8.0-8.2 (Frank and others, 1991) to 132 and 181 days in filtered and unfiltered river water, respectively, at 6°C and pH 7.3 (Lartiges and Garrigues, 1995). The half-life values at 10°C in the August sample agree with these values, but the 9-day half life in the April samples at 10°C is considerably shorter (table 2). This could be due to a difference in the water chemistry or some other unmeasured parameter of the April sample.

Similar variability in half lives for diazinon has been reported at temperatures around 20°C. The half life of diazinon in river water was reported as 14 days at 21°C and pH 8.1 (Frank and others, 1991). At pH 7.3, the half life of diazinon in filtered and unfiltered river water was much longer—52 days and 80 days, respectively, at 22°C (Lartiges

Table 3. Comparative values of half lives and related experimental conditions, Sacramento River, California

[Half lives are reported in number of days. <, actual value is less than value shown; na, not available]

Compound	Temperature (°C)	Half life	pH	Water Type	Light conditions	Reference
Carbaryl	6	45	7.3	Filtered river water	Dark	Lartiges and Garrigues (1995)
	9	20	7.5-7.8	Pond water	na	Szeto and others (1979)
	22	<2	7.3	Filtered river water	Dark	Lartiges and Garrigues (1995)
	24	1	7.9	Filtered seawater	Dark	Armbrust and Crosby (1991)
	24	1	8.2	Filtered seawater	Dark	Armbrust and Crosby (1991)
Malathion	6	53	7.3	Filtered river water	Dark	Lartiges and Garrigues (1995)
	22	7	7.3	Filtered river water	Dark	Lartiges and Garrigues (1995)
	28	2	8.2	River water	Dark	Wang and Hoffman (1991)
Atrazine	6	[1]180	7.3	Filtered river water	Dark	Lartiges and Garrigues (1995)
	4	[1]133	7.8	Well water	Dark	Widmer and others (1993)
	22	130	7.3	Filtered river water	Dark	Lartiges and Garrigues (1995)
	30	[1]133	7.8	Well water	Dark	Widmer and others (1993)
	40	[1]43	8.0	Sterilized creek water	na	Noblet and others (1996)
Simazine	40	[1]43	8.0	Sterilized creek water	na	Noblet and others (1996)
Methidathion	4	139	8.0-8.2	River and well water	Fluorescent light	Frank and others (1991)
	21	20	8.0-8.2	River and well water	Fluorescent light	Frank and others (1991)
Diazinon	4	45	8.0-8.2	River and well watrer	Fluorescent light	Frank and others (1991)
	6	132	7.3	Filtered river water	Dark	Lartiges and Garrigues (1995)
	6	181	7.3	Unfiltered river water	Dark	Lartiges and Garrigues (1995)
	20	185	7.4	Distilled water	Diffused light	Gomaa and others (1969)
	21	14	8.1	River water	Fluorescent light	Frank and others (1991)
	22	52	7.3	Filtered river water	Dark	Lartiges and Garrigues (1995)
	22	80	7.3	Unfiltered river water	Dark	Lartiges and Garrigues (1995)

[1] No degradation after number of days shown.

and Garrigues, 1995). Temperature and pH are not the only factors effecting degradation; for example, at a similar temperature (20°C) and pH (7.4), the half life in distilled water was much longer—185 days (Gomaa and others, 1969). This range of reported half life values is similar to the values determined in our study.

For the August samples, the calculated E_a for diazinon was 3.2 kcal/mol, which is similar to the value of 3.5 kcal/mol reported by Lartiges and Garrigues (1995) for unfiltered river water. Lartiges and Garrigues (1995) reported a higher E_a of 7.6 for the same water when filtered, suggesting that some component of natural water that is lost during filtering can affect the temperature dependence of degradation.

The variability in degradation rates for methidathion and diazinon in the April and August samples suggests that differences in other parameters, such as microbial populations or water chemistry, could be responsible for the variance. Sethunathan (1972) reported that certain

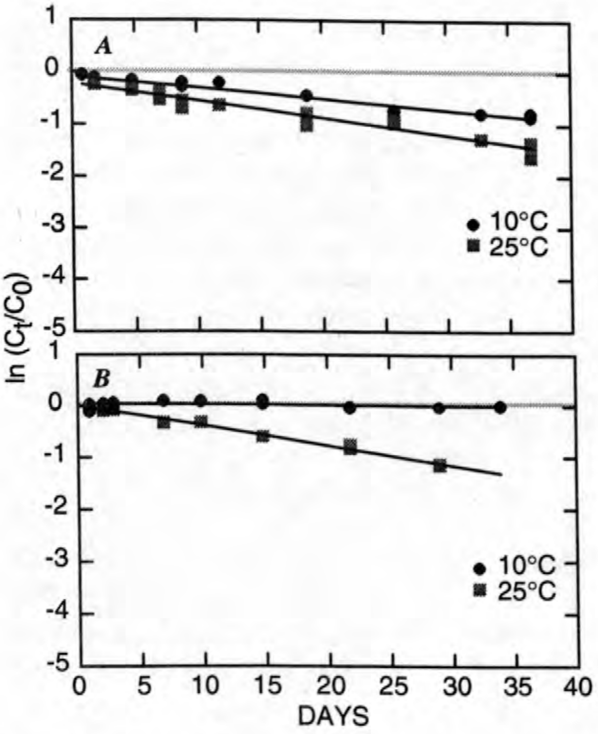

Figure 3. Degradation of methidathion in water samples from the Sacramento River, California, ***A,*** April and ***B,*** August 1996.

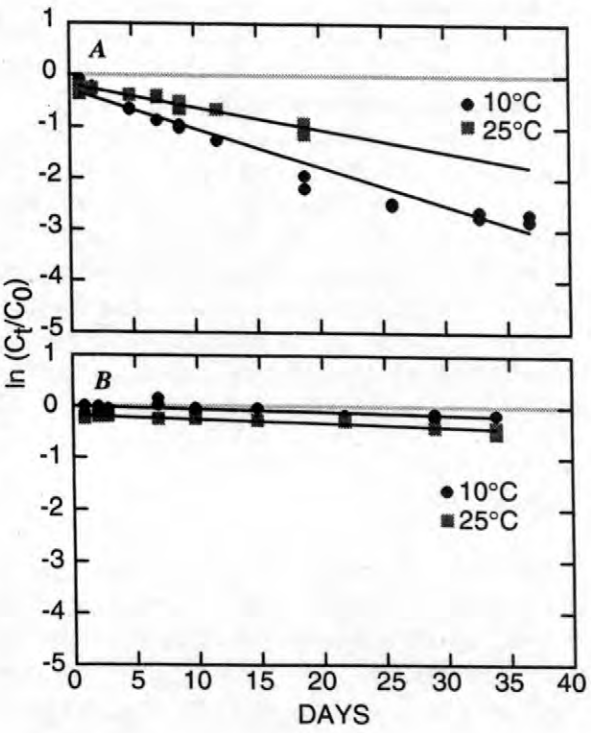

Figure 4. Degradation of diazinon in water samples from the Sacramento River, California, ***A,*** April and ***B,*** August 1996.

microorganisms are highly efficient at metabolizing diazinon in water, and the degradation rate is highly dependant upon the presence or absence of such microorganisms. In addition, Lartiges and Garrigues (1995) reported faster degradation of diazinon in filtered versus nonfiltered subsamples of the same water, suggesting that some component that is lost during filtration can effect degradation rates. Other studies indicate that hydrolysis of diazinon in aqueous solution can be catalyzed by divalent metal ions such as copper (II) (Smolen and Stone, 1997) or influenced by dissolved organic carbon (Macalady and others, 1989).

IMPLICATIONS AND FUTURE WORK

The findings from this study generally compare well with the available environmental data for pesticide levels in the Sacramento and San Joaquin Rivers. Carbaryl and malathion degraded relatively rapidly in Sacramento River water. These compounds have been detected in the Sacramento River only infrequently (MacCoy and others, 1995). Measurement of the degradation products of these compounds may, therefore, be important in assessing biological impacts. In contrast, simazine has been detected in a large number of samples in both the Sacramento River (MacCoy and others, 1995) and the San Joaquin River (Panshin and others, 1998). Given its resistance to degradation and high use in the Central Valley (table 1), this frequency of detections is not surprising. Atrazine, which also was shown to be resistant to degradation, is found less frequently than simazine (MacCoy and others, 1995; Panshin and others, 1998). This most likely is due to the much lower usage of this compound (table 1). Methidathion and diazinon are detected on a seasonal basis in the Sacramento River (MacCoy and others, 1995) and the San Joaquin River (Panshin and others, 1998). For these two compounds, degradation rates can vary significantly. An understanding of the factors that control this variability is critical for the development of accurate environmental fate models.

Future work will involve similar degradation studies with measurement of additional water-chemistry parameters, such as dissolved organic carbon and select trace metals such as copper (II).

Measurement of expected degradation products will be added to the experiments. Currently, little is known about the occurrence, behavior, and fate of pesticide degradation products in the aquatic environment. It has been noted that studies of surface waters in the United States done during the past 35 years have targeted degradation products representing only 15 of the more than 600 pesticides used in agriculture (Larson and others, 1997). While degradation products typically are less toxic than the parent pesticide, some may remain biologically active and potentially could have significant effects on water quality.

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The Carbon Isotopic Composition of Trihalomethanes Formed from Chemically Distinct Dissolved Organic Carbon Isolates from the Sacramento-San Joaquin River Delta, California, USA

By Brian A. Bergamaschi, Miranda S. Fram, Roger Fujii, George R. Aiken, Carol Kendall, and Steven R. Silva

ABSTRACT

Dissolved hydrophobic and hydrophilic acids were isolated from samples collected at five channel sites within the Sacramento-San Joaquin Rivers and Delta, California, USA, and from a peat island agricultural drain to examine the relationship between the chemical composition of dissolved organic carbon and the formation of trihalomethane, and to test whether peat island-derived dissolved organic carbon contributed substantially to reactivity. The chemical composition of the isolates was quite variable, as indicated by significant differences in carbon-13 nuclear magnetic resonance spectra and carbon to nitrogen concentrations ratios, though the variability was not consistent with simple mixing of river- and peat-derived dissolved organic carbon. The lowest propensity to form trihalomethane observed was in the peat island agricultural drain sample, suggesting the addition of peat island waters into Delta channels did not increase the amount of trihalomethane formed from channel water dissolved organic carbon. Changes in the chemical and isotopic composition of the isolates suggest the source of the trihalomethane precursors was different among samples and between isolates. The carbon isotopic composition of the trihalomethane formed from the isolates generally tracked the carbon isotopic composition of the isolates themselves, but variability in the carbon isotopic composition of the trihalomethane was higher.

INTRODUCTION

Dissolved organic carbon (DOC) present in source water for drinking water forms a variety of disinfection byproducts when chlorinated during treatment (Rook, 1974). Some of these byproducts are known to be carcinogenic, and the final concentrations are regulated in finished drinking water (U.S. Environmental Protection Agency, 1994). The largest class of these byproducts is trihalomethane (THM), which is comprised of the sum of chloroform, and bromodichloromethane, dibromochloromethane, and bromoform. The environmental factors determining the extent to which a particular source water forms THM upon chlorination are the concentration of bromide, the amount of DOC present in the source water, and the molar reactivity of the DOC with respect to THM formation (Reckhow and others, 1990). It is commonly held that the predominant pathway for THM formation is by reaction of chlorine with aromatic structures present in dissolved humic material (Reckhow and others, 1990). However, available data show no simple molar relation between ultraviolet (UV) absorbance at 254 nanometers, the common surrogate for DOC aromaticity, and the amount of THM formed upon chlorination of waters from the Sacramento-San Joaquin Delta (Delta) (Fujii and others, 1998). Thus, the amount of THM formed upon chlorination of any given water must depend on the composition as well as the concentration of the DOC.

THM formation is of particular concern for managers of the water-treatment facilities that supply drinking water, originating in the tidal reaches of the Delta, to 22 million people. The Delta is a region largely composed of below-sea-level, peat-rich islands maintained in agricultural production by a network of levees and pumps (California Department of Water Resources 1993). It is commonly believed that the DOC content and composition of river water changes as it flows through the Delta before being diverted into the California Aqueduct system for use as drinking water in southern California (California Department of Water Resources 1990, 1994a; Fujii and others 1998). The changes are thought to result from the addition of water that is high in DOC because this water drains peat-rich Delta islands. High DOC concentrations in Delta water combined with high bromide levels from tidal mixing of brackish water frequently results in source water that potentially forms THM in excess of regulated levels upon chlorination (California Department of Water Resources 1990, 1994a; Fujii and others, 1998).

The purpose of this study is to investigate the relationship between biogeochemical processes in the Delta, composition of the DOC, and the molar capacity of DOC to form THM by examining the chemical and isotopic variability in DOC isolates from water samples collected in the rivers and Delta. This report presents the data obtained in this study and a discussion of the results. Based on the chemical and isotopic variability of the DOC isolates, we conclude that the DOC in Delta channel water does not represent a simple mixture of river- and peat-derived DOC, and that the THM formation potential of Delta water is not increased by additon of peat-island drainage waters. Based on the variability in the carbon isotopic composition of THM formed upon chlorination of the DOC isolates, we conclude that THM precursors in various fractions of the DOC are different, and that in-channel processes may contribute to the observed variability in the composition of the DOC. This study was done as part of the USGS Toxic Substances Hydrology Program and the USGS National Drinking Water Initiative.

EXPERIMENTAL METHODS

Samples of about 140 liters (L) were collected by pump from below the surface, near mid-channel at five sites in the Sacramento and San Joaquin Rivers and main Delta flow channels. Additionally, an agricultural drainage water sample was collected from a drainage ditch on Twitchell Island. The experimental design used the Sacramento and San Joaquin Rivers and the Twitchell Island Drainage samples, respectively, as representatives of upland river and Delta island sources of DOC transported to Delta channel water. The Old River and Middle River sites are located within the Delta and represent a mixture of riverine DOC and DOC added or modified within the Delta. The Export site is located just upstream of the facility that pumps water from the Delta into the California Aqueduct, and other drinking-water supply aqueducts.

Analytical methods are described in detail elsewhere (Bergamaschi and others, in press). Briefly, DOC was isolated and concentrated from the water samples by sequential extraction on nonionic macroporous resins (XAD-8 and XAD-4; Aiken and others, 1992). Materials eluted with base from the XAD-8 and XAD-4 resins are termed hydrophobic acids (HPoA) and hydrophilic acids (HPiA), respectively. DOC measurements were made with a Shimadzu TOC-5000A total organic carbon analyzer. (The use of trade names in this report is for identification purposes only and does not constitute endorsement by the U. S. Geological Survey.) Elemental composition was determined using a Perkin-Elmer 2400 Series II CHNS/O analyzer. UV measurements were made on filtered samples in a PE Lambda 3B UV/VIS spectrophotometer using a 1 centimeter (cm) cell.

Nuclear magnetic resonance (NMR) spectra for the DOC isolates were obtained by carbon-13 solid-state, cross polarization, magic angle spinning spectroscopy (^{13}C-CPMAS) using a 200 megahertz Chemagnetics CMX spectrometer with a 7.5 millimeter diameter probe. The spinning rate was 5,000 hertz, the pulse delay was 1 second, the pulse width was 4.5 microseconds for the 90º pulse, and contact times were 1 milliseconds in duration. Data were collected over approximately 2000 transients, and a line broadening of 100 hertz was applied in Fourier transformation of the free induction decay data. We assign resonance in the regions 0-60 parts per million (ppm) as aliphatic, 60-90 ppm as heteroaliphatic, 90-110 ppm as anomeric, 110-160 ppm as aromatic, and 160-190 as carboxylic. Integrated areas in these regions were normalized to the aggregate area and the proportion of each functional assignment expressed as a percentage of the whole.

Aliquots of the reconstituted XAD isolates were reacted with chlorine to form THM using a modified version of U.S. Environmental Protection Agency (EPA) Method 510.1, following the reactivity-based dosing method (Krasner and Sclimenti, 1993; California Department of Water Resources, 1994b). THM concentrations were measured using a Tekmar ALS2016 and LSC200 purge and trap, and a Hewlett-Packard 5890 II gas chromatograph with an electron capture detector, following a modified version of U.S. EPA Method 502.2. Results are reported as millimoles of THM formed per mole carbon (mmol/mol). Carbon isotopic ratios of THM were measured using purge and trap introduction into a gas chromatograph coupled with a combustion furnace and a Micromass Optima continuous flow stable-isotope mass spectrometer. Results are reported in standard δ notation, where $\delta^{13}C = 1000*[(^{13}C/^{12}C)_{samp} - (^{13}C/^{12}C)_{std}]/(^{13}C/^{12}C)_{std}$ and V-PDB is the C standard, and atmospheric nitrogen is the N standard.

Mean deviation between replicate THM isotopic measurements was 0.30 per mil (‰) (standard deviation=0.2 ‰). Replicate analyses for isolate samples were within 0.05‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$. All replicate sample analyses were within 1% for concentrations of C and N.

RESULTS

Whole Water

The DOC content of Delta water is a concern for municipalities using the Delta as a source for drinking water. In this study, DOC concentrations in Delta water varied over a relatively narrow range, from 4.4 milligrams per liter of carbon (mg/L C) for the Old River sample, to 6.1 mg/L C for the Middle River sample (table 1). The DOC concentration of the Export sample was 4.6 mg/L C, and the San Joaquin River sample had a DOC content of 4.2 mg/L C. For these samples, therefore, the water did not become appreciably elevated in DOC content by passage through the Delta, even though the Twitchell Island Drainage sample possessed a high DOC concentration of 17.1 mg/L C.

Table 1. Dissolved organic carbon content and specific ultraviolet absorbance at 254 nanometers of whole water samples from locations in the Sacramento-San Joaquin Delta, California, USA

[Dissolved organic carbon (DOC) concentrations reported in milligrams of carbon per liter of water (mg/L C); specific ultraviolet absorbance at 254 nanometers (SUVA) reported in absorbance units per milligram of carbon per liter of water (abs/mg/L). UV absorbance measurements made in a 1 centimeter cell]

Sample	DOC (mg/L)	SUVA (abs/mg/L)
Sacramento River	3.7	0.015
San Joaquin River	4.2	.032
Twitchell Is. Drainage	17.1	.049
Middle River	6.1	.028
Old River	4.4	.042
Export	4.6	.038

UV absorbance at 254 nm is a common parameter used to infer the amount of humic material in river waters, and predict the amount of THM likely formed during water-treatment. The carbon-normalized UV absorbance (SUVA) is used to compare water with different DOC contents to infer the proportion of aromatic humic material within the DOC pool. SUVA varied by less than a factor of two within the study (table 1), with the Delta sample yielding 0.028 and 0.042 absorbance units per centimeter per milligram per liter of carbon (abs/mg/L) at Middle River and Old River respectively. The Drainage water sample, as expected, had the highest SUVA (0.049 abs/mg/L), indicating a higher aromatic content. The San Joaquin River sample SUVA value, 0.032 abs/mg/L, was between those of the two Delta channel samples, and lower than the

Table 2. Carbon and nitrogen isotopic compositions, carbon-nitrogen ratios, and specific trihalomethane formation potentials of the hydrophobic and hydrophilic acid fractions of isolated dissolved organic carbon, and carbon isotopic compositions of trihalomethane formed upon chlorination of these dissolved organic carbon isolates from water samples from locations in the Sacramento-San Joaquin Delta

[HPoA, hydrophobic acid fraction; HPiA, hydrophilic acid fraction; carbon (C) and nitrogen (N) isotopic ratios reported as permil relative to standards (‰); subscript BULK refers to the solid isolate material; subscript THM refers to the trihalomethane; carbon to nitrogen ratio (C:N) reported in molar units; STHMFP, specific trihalomethane formation potential reported in millimole trihalomethane per mole dissolved organic carbon (mmol THM/mol DOC); na, not analyzed]

Sample	$\delta^{13}C_{BULK}$ (‰)	$\delta^{13}C_{THM}$ (‰)	$\Delta\delta^{13}C_{BULK-THM}$ (‰)	$\delta^{15}N_{BULK}$ (‰)	C:N (molar)	STHMFP (mmol THM/ mol DOC)
			HPoA Isolate			
Sacramento River	-27.1	-31.1	4.0	1.7	31	9.7
San Joaquin River	-26.9	-31.3	4.4	0.4	52	9.0
Twitchell Is. Drainage	-27.1	-31.5	4.5	-1.7	31	8.5
Middle River	-26.7	-31.3	4.6	.9	33	9.2
Old River	-26.9	-32.0	5.1	2.7	30	9.2
Export	-26.5	-29.6	3.1	.8	62	8.6
			HPiA Isolate			
Sacramento River	na	na	na	na	na	na
San Joaquin River	-25.8	-30.3	4.5	3.0	33	8.0
Twitchell Is. Drainage	-26.5	-31.0	4.5	.2	25	6.9
Middle River	-25.8	-30.4	4.6	.8	23	6.9
Old River	-25.7	-30.5	4.8	1.3	24	8.2
Export	-25.9	-30.3	4.4	9.7	35	7.4

Export sample (0.038 abs/mg/L), suggesting that the DOC may have increased in UV absorbance and aromatic humic content during passage through the Delta.

Dissolved Organic Carbon Isolates

Carbon to nitrogen ratio measurements (C:N) of HPoA and HPiA isolates in this study revealed that the DOC isolates were universally low in N. HPiA C/N values ranged from 23 in the Middle River sample, to 35 in the Export sample (table 2). HPoA C/N values were more depleted in N than their HPiA counterparts, ranging from 30 in the Old River sample to 62 in the Export sample. The highest values were observed in samples from San Joaquin River and the Export site. C:N can be indicative of the diagenetic state and source of DOC because terrestrial plants generally have C:N greater than 15 while planktonic algae have C:N about 6.5 (Hedges and others, 1988a), and diagenesis of organic matter generally results in increasing C:N (Hedges and others, 1988b).

The $\delta^{13}C_{BULK}$ values of each type of isolate varied only slightly among the six samples, but the HPoA and HPiA isolates were distinctly different from each other (t-test of difference between two means, $\alpha = 0.05$, $P < 0.001$; Zar, 1984) (fig. 1; table 2). The $\delta^{13}C_{BULK}$ of the HPiA isolates averaged -25.9 ‰, while HPoA isolates, averaging -26.9 ‰, were 1‰ lighter. The lighter $\delta^{13}C_{BULK}$ value for HPoA isolates may be a result of the higher aromatic carbon content: lignin, the

Table 3. Percentages of carboxylic, aromatic, anomeric, heteroaliphatic, and aliphatic carbon in the hydrophobic and hydrophilic acid fractions of isolated dissolved organic carbon from water samples from locations in the Sacramento-San Joaquin Delta as determined by carbon-13 nuclear magnetic resonance spectroscopy

[Values reported as percentages. HPoA, hydrophobic acid fraction; HPiA, hydrophilic acid fraction. Sacramento River isolates were not analyzed]

Sample	Carboxylic	Aromatic	Anomeric	Heteroaliphatic	Aliphatic
			HPoA Isolate		
San Joaquin River	13	21	9	21	36
Twitchell Is. Drainage	15	25	7	18	35
Middle River	14	21	9	20	37
Old River	13	21	10	21	36
Export	12	16	8	19	44
			HPiA Isolate		
San Joaquin River	13	14	8	24	40
Twitchell Is. Drainage	18	18	8	22	34
Middle River	17	13	9	26	35
Old River	16	12	9	25	38
Export	17	13	10	23	38

putative primary precursor for aromatic moieties in humic materials, is isotopically lighter than bulk humic material by about 2 ‰ (Benner and others, 1987). The variability in $\delta^{13}C_{BULK}$ values of the isolates among sites may reflect small variations in the sources of the DOC. The lightest isotopic ratios for both the HPoA and HPiA isolates were observed in the Twitchell Island Drainage sample. The peat soils were formed largely from the decayed remains of *Scirpus,* a C-3 marsh plant endemic to the Delta prior to cultivation of this area by man in the mid 19th century (Mason, 1957; Atwater, 1980; Fujii and others, 1998). The Export sample HPoA isolate $\delta^{13}C_{BULK}$ value was heavier than average, perhaps indicative of the contribution of small amounts of heavier, algal- or C-4 plant-derived carbon.

The $\delta^{15}N_{BULK}$ values of HPoA and HPiA isolates varied considerably, ranging over 10 ‰ between samples, in contrast to the $\delta^{13}C_{BULK}$ results (table 2). The Export sample HPiA isolate exhibited the highest $\delta^{15}N$ value (9.7 ‰), while the HPoA isolate from the Twitchell Island Drainage sample exhibited the lowest value (-1.7 ‰). $\delta^{15}N_{BULK}$ values did not vary systematically among the samples from the six sites nor between the HPiA and HPoA isolates. The Export sample, however, exhibited a nearly 9 ‰ difference between HPoA and HPiA isolates, indicating distinct sources for the organic nitrogen found in each isolate.

^{13}C-CPMAS NMR analysis of the isolate samples yielded information about the distribution of functional groups within the bulk material, and provided a basis for comparison of the compositional differences between isolates and among samples. As expected, the aromatic carbon content of the HPoA isolates, which averaged 21%, was distinctly higher than the higher aromatic carbon content of the HPiA isolates, which averaged only 14% (t-test of difference between two means, $\alpha = 0.05$, $P < 0.001$; Zar, 1984) (fig. 1; table 3). The HPiA isolates were relatively enriched in heteroaliphatic and carboxylic carbon compared to the HPoA isolates (table 3). The enrichment in heteroaliphatic and carboxylic carbon, and depletion in aromatic carbon in the HPiA, compared with the HPoA isolate, may indicate that the HPiA material is more degraded.

The highest proportions of aromatic and carboxylic carbon for both the HPoA and HPiA isolates were found in isolates from the Twitchell Island Drainage sample (table 3). This result is consistent with a peat origin for the material, as peats in other environments have been shown to be enriched in aromatic and carboxylic carbon (Spiker and Hatcher, 1988). Despite the high aromatic carbon content of DOC derived from the Delta islands, the Delta channel samples from Old River and Middle River did not contain higher proportions of aromatic carbon than the San Joaquin River sample. This result indicates that either DOC derived from the peat islands may not be a substantial contributor to the bulk DOC in the channel waters, or that the aromatic carbon may be degraded by the time it reaches the channels.

The Export sample HPoA isolate was distinctive; it contained more aliphatic and less aromatic carbon than all of the other isolates. This chemical difference may result from the addition of algal-, agricultural-, or sewage-derived organic material to the water, or from in-channel degradation of organic material.

Trihalomethanes

The specific trihalomethane formation potential (STHMFP) is a measure of the potential of the organic material in the DOC isolates to form THM when treated with chlorine. The reactivity of the HPoA isolates averaged 1.5 millimoles THM per mole DOC (mmol/mol) higher than that of the HPiA isolates (t-test of difference between two means, $\alpha = 0.05$, $P = 0.003$; Zar, 1984) (table 2; fig. 1). STHMFP values of the HPoA isolates ranged from 8.5 to 9.7 mmol/mol (average = 9.0 mmol/mol) while the STHMFP values of the HPiA isolates were more variable, ranging from 6.9 to 8.2 mmol/mol (average = 7.5 mmol/mol). Surprisingly, the HPoA and HPiA isolates from the Twitchell Island Drainage sample exhibited the lowest reactivity, forming 8.5 and 6.9 mmol/mol, respectively. The fact that the Twitchell Island Drainage isolates were the least reactive is curious in view of the fact that they were the most aromatic of the isolates (table 3), and aromatic structures are the putative precursors for THM compounds.

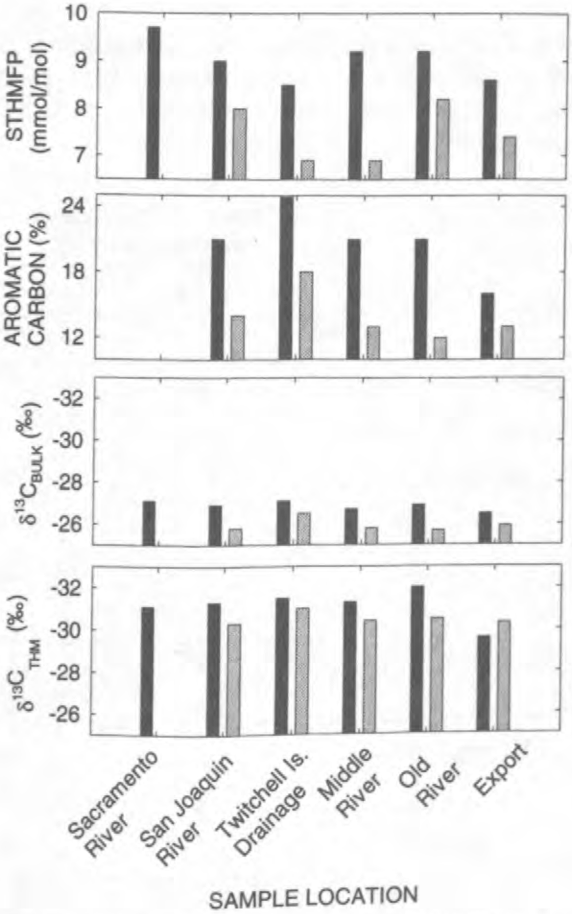

Figure 1. Specific trihalomethane formation potential, percentage aromatic carbon, and carbon isotopic compositions of solid material and trihalomethanes formed upon chlorination for the hydrophobic (dark bars) and hydrophilic (light bars) acid fractions of isolated dissolved organic carbon from water samples from locations in the Sacramento-San Joaquin Delta (data from tables 2 and 3).

$\delta^{13}C_{THM}$ values of the THM formed from the isolates averaged 4.4 ‰ lighter than that of the bulk material (table 2; fig. 1), which is similar to the average difference of 3.5 ‰ found for 60 other samples of DOC isolates (Bergamaschi and others, in press). The average $\delta^{13}C_{THM}$ formed from the HPoA isolates was 0.6 ‰ lighter than that from the HPiA isolates, but this difference was not statistically significant (t-test of difference between two means, $\alpha = 0.05$; Zar, 1984). The carbon isotopic fractionations observed between HPoA and HPiA isolates, between THM derived from HPoA and HPiA isolates, and between THM and bulk materials suggest strong intermolecular differences within the DOC. Different compounds within the DOC carry distinct $\delta^{13}C$ values, and the chlorination reactions are highly selective in which compounds are attacked and broken down to form THM (Bergamaschi and others, in press; Fram and others, 1998). The THM formation reactions occur preferentially on isotopically lighter compounds within the DOC.

The difference between the $\delta^{13}C_{THM}$ and $\delta^{13}C_{BULK}$(defined as $\Delta\delta^{13}C_{BULK-THM}$) does vary among the six samples, which may reflect distinct sources or diagenetic histories of the THM precursors in them. HPiA isolate $\Delta\delta^{13}C_{BULK-THM}$ values ranged from 4.4 to 4.8 ‰, and HPoA $\Delta\delta^{13}C_{BULK-THM}$ values ranged from 3.1 to 5.1 ‰ (table 2). The highest values for both isolates were in the Old River sample, while the lowest values for both isolates were in the Export sample. The $\Delta\delta^{13}C_{BULK-THM}$ values for Twitchell Island Drainage and San Joaquin River samples were similar, and averaged 4.5‰ for both the HPoA and HPiA isolates. The Export sample had the greatest difference between values (1.3 ‰) for the HPoA and HPiA isolates.

DISCUSSION

Mixing and In-Channel Processes

The results presented here are in disagreement with the general view that the DOC in Delta channel waters represents conservative mixing between riverine and peat island-derived DOC. This simple scenario does not fully account for the chemical variability among samples in this study. No systematic differences were observed between the Sacramento and San Joaquin River samples and the Old River, Middle River, and Export site Delta channel samples in DOC content, proportion of aromatic carbon, C:N, $\delta^{15}N_{BULK}$, STHMFP, $\delta^{13}C_{BULK}$, or $\delta^{13}C_{THM}$. The compositions of the Old River, Middle River, and Export site Delta channel samples cannot be accounted for by combinations of the Sacramento River, San Joaquin River, and Twitchell Island Drainage samples (tables 1-3; fig. 1). C:N, $\delta^{13}C_{BULK}$, $\delta^{15}N_{BULK}$, and ^{13}C-NMR measurements of the isolates indicate substantial differences in DOC source and composition, and differences among HPoA and HPiA fractions of the DOC isolates.

The Export sample was chemically quite distinct from other samples in the study, suggesting that in-channel processes may be more influential than previously thought in determining the DOC content, composition, and THMFP of Delta channel waters. Although the Export sample was relatively high in UV absorbance (table 1), the isolates had relatively low proportions of aromatic and high proportions of aliphatic carbon (table 3). The $\delta^{13}C_{BULK}$ of the Export sample HPoA isolate, and the $\delta^{15}N_{BULK}$ of the Export HPiA isolate, were the heaviest C and N isotopic values measured, respectively, and the $\delta^{13}C_{THM}$ of the THM formed from Export sample HPoA isolate was 1.5 ‰ heavier than the average (table 2). These results suggest that material with a heavier isotopic composition had influenced the Export. Possible sources for this material include: algal, sewage, or agricultural source. The high C:N values for the isolates (table 2), however, suggest fresh algal or sewage material was not the source of the heavier carbon.

Specific Trihalomethane Formation Potential and Carbon Aromaticity

The results presented here are in disagreement with the generally held view that aromatic carbon content of Delta water determines its STHMFP (California Department of Water Resources, 1994a). The Twitchell Island Drainage sample, with the highest aromatic carbon content had the lowest STHMFP, while the HPoA isolate of the Export sample had the lowest aromatic content, but higher STHMFP (table 2; fig. 1). This decoupling between aromatic carbon content and THMFP was the probable cause for the general failure of UV absorbance 254 nm to predict STHMFP in Delta water (Fujii and others, 1998). Another indication that aromatic carbon content does not determine STHMFP in these samples is the difference between HPoA and HPiA isolates. HPiA isolates contained 33 percent less aromatic carbon than the HPoA isolates, and HPiA isolates also were less likely to form THM on chlorination. However, STHMFP of HPiA was only 16 percent less than STHMFP of HPoA isolates, or only about half the decrease expected based on the difference in aromatic carbon content.

One possible explanation for why these samples do not react to form THM in expected molar ratios may be related to the differences in the $\Delta\delta^{13}C_{BULK-THM}$ values between the samples. We have previously found isotopically distinct pools of organic material within isolates that react to form THM at different rates, the combination of which determines the final $\delta^{13}C_{THM}$ and the $\Delta\delta^{13}C_{BULK-THM}$ values (Fram and others, 1998). $\Delta\delta^{13}C_{BULK-THM}$ results from fresh plant decoctions suggest that the THM precursers removed during diagensis are isotopically the lightest precursers, so that diagenesis yields residual samples with lower $\Delta\delta^{13}C_{BULK-THM}$ values (Bergamaschi and others, in press). The reactivity of organic compounds to diagenesis and chlorination are not likely related to the aromaticity of the carbon in the same way. Thus, in samples of various diagenetic maturities, as indicated by the $\Delta\delta^{13}C_{BULK-THM}$, a correlation between aromatic carbon content and STHMFP would not be expected.

CONCLUSIONS

1. The variability in chemical composition of the Delta channel water isolates, as indicated by significant differences in ^{13}C CPMAS NMR spectra and C:N measurements, was not consistent with simple mixing of river- and peat-derived DOC.

2. For these samples, there was no significant increase in DOC concentration, or STHMFP and aromatic carbon content of DOC isolates between the Sacramento and San Joaquin River samples and the Delta channel samples (Old River, Middle River, and Export), even though SUVA did increase. These results indicate that changes in UV absorbance may be decoupled from THMFP and aromatic carbon contents in this environment.

3. The lowest STHMFP observed was in the peat island agricultural drainage sample, even though this sample had the highest SUVA and aromatic carbon content, suggesting that the addition of peat island waters into Delta channels will not increase the channel water STHMFP.

4. The higher aromatic carbon content and STHMFP of the HPoA isolates, and lower $\delta^{13}C$ value of THM formed from the HPoA isolates suggests that THM have different chemical sources in HPoA and HPiA isolates.

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Understanding the Human Influence on the San Francisco Bay-Delta Estuary Ecosystem - The Toxic Substances Hydrology Program and USGS Place-based Studies Program Provide Complementary Approaches and Results

By James S. Kuwabara, Frederic H. Nichols, Kathryn M. Kuivila, and Jeanne S. Dileo

ABSTRACT

The San Francisco Bay-estuary Toxic Substances Hydrology (Toxics) Study is unique among estuarine studies in its long-term approach to quantitatively defining the processes that affect contaminant transport and distribution in major urbanized estuaries. These studies create the scientific foundation from which specific, management oriented problems may be addressed. The necessary balance between maintaining this scientific foundation and responding effectively to critical management issues is demonstrated by the complementary nature of Toxics and USGS Place-based studies in San Francisco Bay. Examples of this programmatic linkage are provided that span wide spatial and temporal scales.

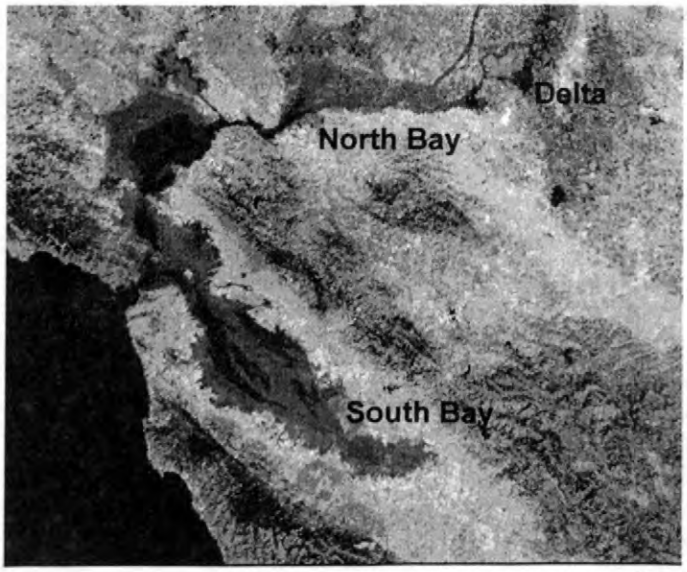

Figure 1. Landsat photograph (processed by Pat Chavez and his group, USGS, Flagstaff, AZ) depicting how, like most major estuaries, San Francisco Bay represents a complex integration of circulation patterns that generate a wide range of ecosystem types.

INTRODUCTION

For nearly three decades, the U.S. Geological Survey (USGS) has methodically developed a unique network of interdisciplinary and interdependent studies in the San Francisco Bay-Estuary, one of the most economically and ecologically important estuaries in the United States (Conomos, 1979; Hollibaugh, 1996). Sustained progress through these long-term studies has maintained the USGS in a leadership role in responding to shorter-term (that is, less than 5 years), regional management issues, as well as in transferring information about fundamental processes that affect the transport of toxic substances in all major estuaries. Since its inception in 1991, the San Francisco Bay-estuary Toxic Substances Hydrology Study has provided critical support to this role. A clear example of the application of long-term studies supported by the San Francisco Bay Toxics Study to detailed issues regarding resource management is the complementary nature of the Toxics Study to the USGS Place-based Studies Program in San Francisco Bay. Since 1995, the USGS Place-based Studies Program [formerly the "Integrated Natural Resource Science (INATURES) Program"] funding has been used to augment Toxics studies for additional research, monitoring, and assessment that focus on critical management issues, and improve the accessibility of scientific information produced by USGS about the estuary.

TEMPORAL-SCALE DISTINCTIONS

Processes controlling the transport of inorganic and organic toxic substances and associated biological response are particularly complex in an estuarine setting (fig. 1). They operate from sub-second (for example, certain complexation and redox reactions) to decadal (for example, sediment quality changes and basin-scale meteorological effects) time scales. The ability to examine the interdependence of these fundamental processes within an estuarine laboratory is unique to the Toxics Program. Given the short-term management-oriented goals of the USGS Place-based Studies Program, trends over seasonal and shorter time scales can be intensively observed and then placed within the context of longer term trends established by sustained Toxics studies.

Both the Toxics and USGS Place-based Studies Programs currently support the development of sediment-transport, and two- and three-dimensional numerical models that build on our present knowledge. Existing models have been applied to provide easily-accessible " real-time" descriptions of current and wind patterns as tools for spill response, navigation, and recreation (fig. 2). In the San Francisco Bay/Delta system, water-resource demands generate continual interest and need on the part of regional managers and the general public for unbiased scientific information of the highest quality that describe relationships among flow, circulation, contaminant distribution and effects, and habitat alteration.

Figure 2. Population growth surrounding major estuaries generates new challenges for management of environmental quality. Hydrodynamic studies over more than two decades in San Francisco Bay have developed sophisticated yet practical models that provide "now-casting" (real-time) information about wind and circulation patterns (Cheng and others,; http://sfports.wr.usgs.gov/sfports.html).

SPATIAL-SCALE DISTINCTIONS

Contaminants enter the estuary in municipal and industrial sewage, urban and agricultural runoff, and weathering processes. There is currently a strong interest in quantifying the linkage between fluxes of these contaminants and the health and abundance of biological resources in the estuary. Within the San Francisco Bay Toxics Study, the estuary is viewed as an interaction between several " subecosystems"

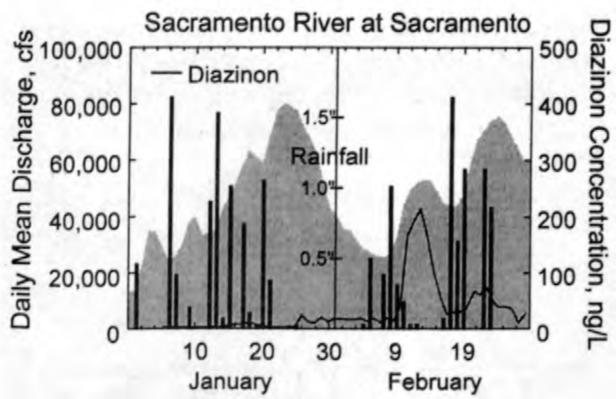

Figure 3. Intense application of pesticides and herbicides in the Central Valley of California obviously has important positive implications on the agricultural productivity of our country. Conversely, inputs of these chemicals into the Delta and North Bay during winter flushing events also can generate a toxicological response. Current collaborative work examines the effects of multiple stressors when exposure to elevated pesticide/herbicide concentrations can be coincident with elevated heavy-metal and suspended sediment concentrations, in addition to changes in salinity (National Research Program and California District, 1998).

which operate distinctly, are controlled by different processes, and are faced with different problems. Those distinctions between interacting subecosystems, the Delta, the northern component of San Francisco Bay (North Bay; see fig. 1) and southern component (South Bay) are the basis for major study themes in our Toxics study workplan. For example, research elements associated with the Delta examine the " Influences of Riverine Inputs, Local Land Use and Managed Flows" (fig. 3). Work in the North Bay (including San Pablo and Suisun Bays) focuses on long-term transport of sediment and toxic substances, and associated biological response by benthic and planktonic organisms (fig. 4). In the South Bay, where municipal point dischargers dominate freshwater inflow during the summer and fall dry season, " Interactions between Toxic Substances, Phytoplankton and Nutrients" is the theme for biological response, modeling, and benthic flux studies (fig. 5).

As a result of anthropogenic modifications to the San Francisco Bay region since the California Gold Rush 150 years ago, historic tidal marshes have been levied and freshwater flow into the estuary has been diverted to support agriculture and population growth in California. Dredging practices have generated primary effects on dredged and disposal sites as well as secondary effects on flow patterns and water-quality gradients. The programmatic effort of the USGS Place-based Studies Program in the San Francisco Bay-estuary has addressed questions in three areas: (1) the relations among freshwater flows, suspended sediments, and contaminants (including biological effects); (2) sedimentologic and contaminant factors related to wetland restoration (fig. 6); and (3) the development of new tools and procedures to make existing and new information more widely

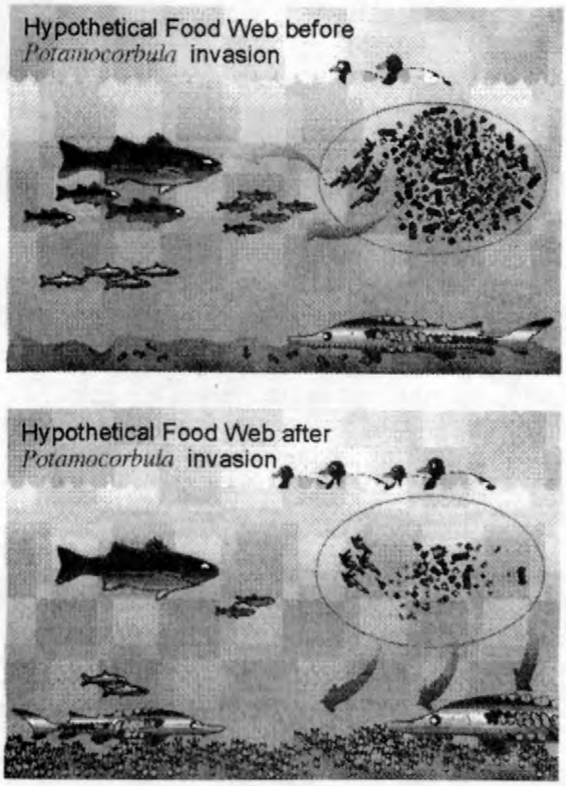

Figure 4. Cartoons depicting how aquatic systems such as San Francisco Bay can be dramatically affected by the proliferation of invader species. Our sustained studies of the estuary's water column and benthos quantitatively describe how the transfer of toxic substances and energy between major biological groups can signifcantly change in response to the rapid domination of an invader species like the Asiatic clam, *Potamocorbula amurensis* (Thompson and others, 1998).

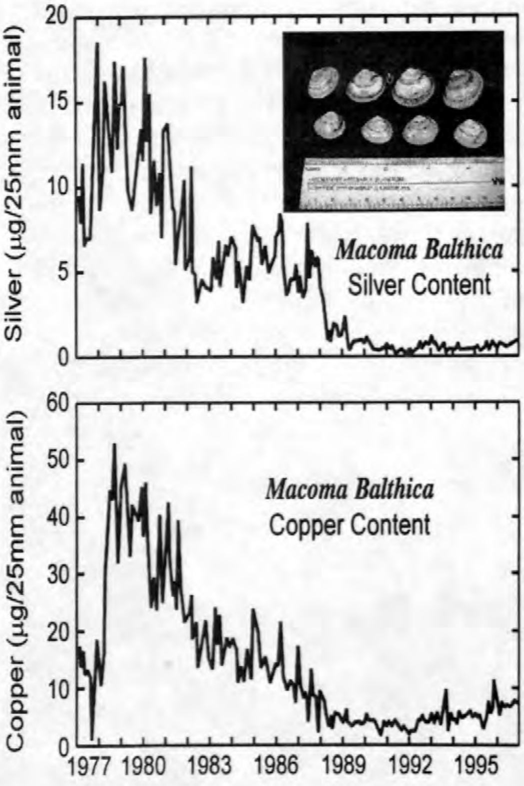

Figure 5. A significant fraction of the freshwater input to the southern component of the bay constitutes receiving waters from municipal water-treatment facilities. A unique long-term study of the condition of benthic organisms in the South Bay has motivated and monitored the positive effects of a series of improvements in sewage treatment practices over past decades. These two slides depict decadal-scale improvement in silver and copper bioaccumulation (Hornberger and others, 1999b).

available via the Internet (Nichols, 1998). Beginning in FY1999, the USGS Place-based Studies Program activities in San Pablo and Suisun Bays will be focused on two areas relevant to restoring ecosystem health and improving water management: (1) the relations between freshwater inflows, sediment movement, and contaminant effects; and (2) the hydrology and ecology of abandoned salt-evaporating ponds.

Work supported by the Toxic Substances Hydrology Program and the USGS Place-based Studies Program also is complemented by ongoing and upcoming studies within other collaborative programs administered by the Geologic Division such as the Global Change Program and the Marine and Coastal Geologic Surveys Program.

For more detailed information on the Toxic Substances Hydrology Study and the USGS Place-based Studies Program in San Francisco Bay, please visit the following Internet sites:

http://wwwrvares.er.usgs.gov/toxics/

http://sfbay.usgs.gov.

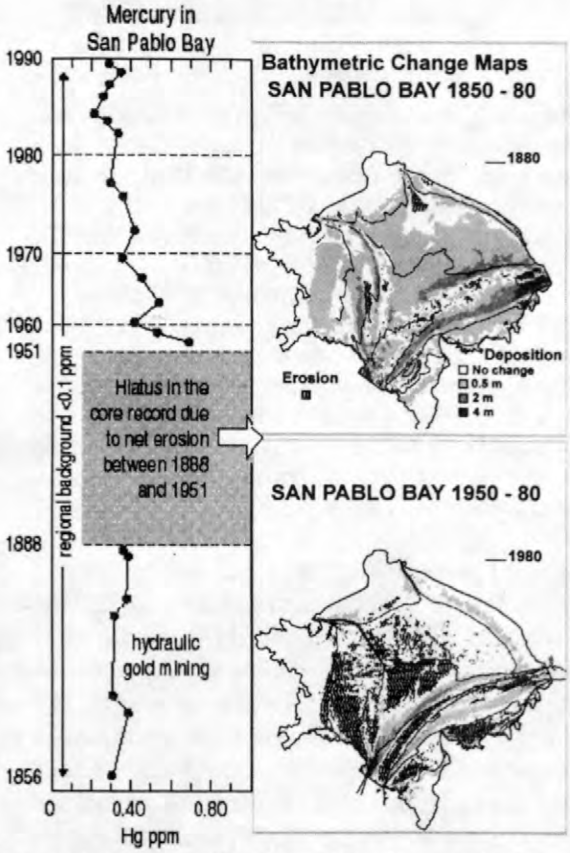

Figure 6. Studies of bathymetric changes in the estuary, resulting from anthropogenic practices (for example, levying and dredging), modify the relative areal coverage of different ecosystem types. The USGS Place-based Studies Program in San Francisco Bay examines how historical trends in contaminant deposition (shown here for sediment-associated mercury) have been affected by changes in wetland morphology and sediment distribution patterns (Nichols, 1998; Hornberger and others, 1999a; Fuller and others, 1999; Smith, R.E. and Jaffe, B.E., written communication, 1999).

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Processes Affecting the Benthic Flux of Trace Metals into the Water Column of San Francisco Bay

By James S. Kuwabara, Brent R. Topping, Kenneth H. Coale, and William M. Berelson

ABSTRACT

Locally regulated point sources represent a significant input of trace metals to San Francisco Bay, especially in the southern component (South Bay) during the dry seasons and periods of drought. However, with recent recognition of the importance of non-point sources, a variety of approaches are being used to evaluate sediment remobilization and subsequent benthic flux of trace contaminants. The extent to which chemical processes couple with diffusive and advective physical processes in regulating benthic flux is also being examined. In terms of metal-speciation controls on solute remobilization and benthic flux, we are investigating the importance of metal-sulfide complexation relative to complexation with dissolved organic substances by determining both metal and ligand fluxes simultaneously. Consistent with previously reported water-column measurements, dissolved pore-water sulfide concentrations, measured at two South Bay sites in July, 1997 and September, 1998, increased with sediment depth from around 10 nM near the sediment-water interface to as high as 980 nM within the top 10 cm, suggesting a source to the water column. Diffusive flux for sulfides estimated from pore-water profiles ranged from 11 to 45 nmoles-m^{-2}-h^{-1}. As a complement to the pore-water approach, core-incubation experiments were used to directly measure sulfide benthic flux which ranged from 92 to 480 nmoles-m^{-2}-h^{-1} over the same period. The significant difference between these two approaches was attributable to biologically enhanced advection (bioturbation/irrigation). Although sulfide benthic flux has been found to be consistently positive (that is, out of the sediment as much as 948 nmoles-m^{-2}-h^{-1}), benthic fluxes for dissolved organic carbon (0.2 µm filtered, DOC), copper and cadmium are temporally variable in direction across the sediment-water interface, suggesting the importance of DOC complexation in regulating metal benthic flux. Quantifying and understanding processes that affect the variability of these fluxes would enhance water-quality modeling for this estuary.

INTRODUCTION

Although locally regulated point discharges of metals represent a significant source to San Francisco Bay, recent studies have consistently indicated that non-point sources of trace contaminants are also significant (Flegal and others, 1991, Flegal and Sanudo-Wilhelmy, 1993, Wood and others, 1995; Caffrey and others, 1996, Kuwabara and others, 1996). Water-quality managers have, therefore, been motivated to develop regional monitoring studies to quantitatively describe the fate of these sources in " sources and loadings" models (that is, solute input-output box models) for the estuary. Three approaches have been used in complementary studies, within and outside the USGS, to determine benthic fluxes: diffusion gradient calculations based on pore-water profiles, direct flux measurements by core-incubations, and *in-situ* flux chamber experiments. A comparison of values measured by these different approaches can help identify processes that regulate benthic fluxes of toxic substances as well as substances that affect their chemical speciation, partitioning, and toxicity. This paper provides descriptions of the spatial and temporal variations in benthic flux for trace metals and reactive ligands. Those ligands are represented by dissolved organic carbon, as a model for complexation with natural organics, and dissolved sulfides.

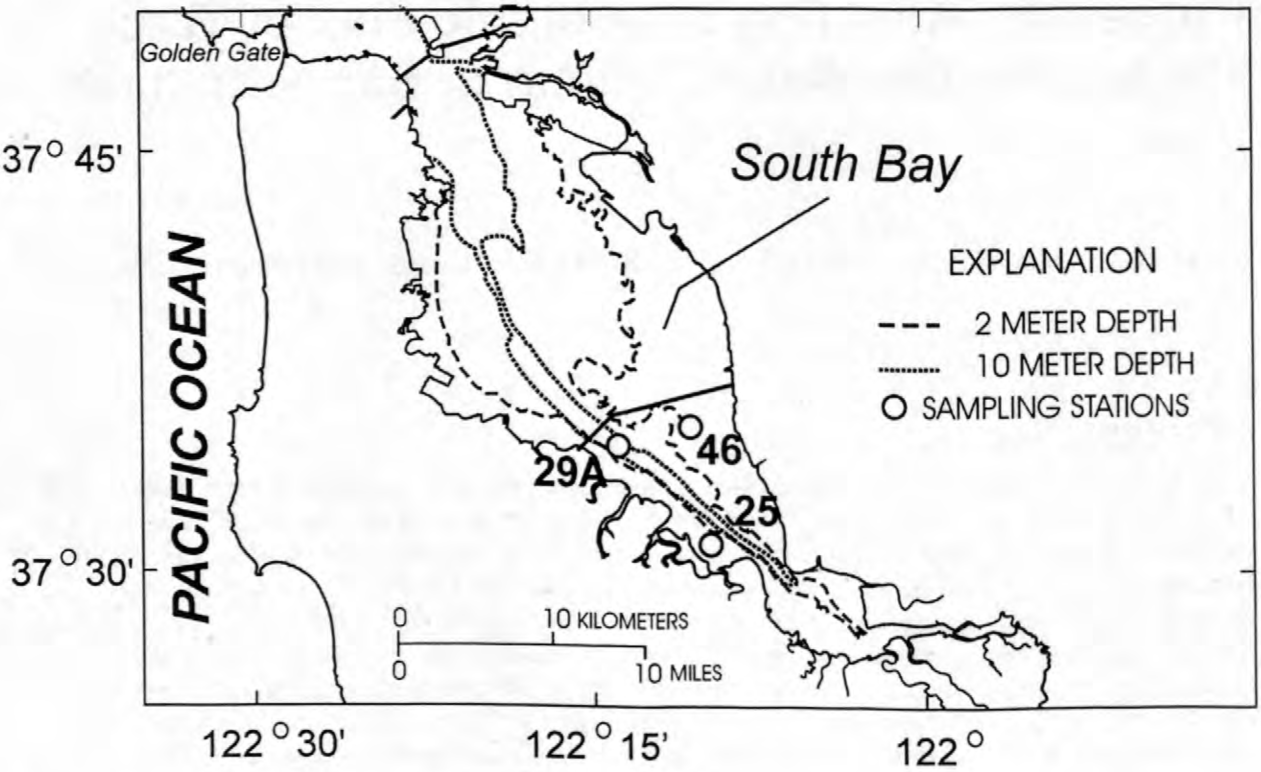

Figure 1. Site map showing three sampling stations in the southern component of San Francisco Bay (South Bay) where benthic flux measurements of trace solutes and nutrients have been made.

METHODS

Three sampling stations in the southern component of San Francisco Bay (South Bay; fig. 1) were used to determine benthic flux for dissolved (0.2 micron filtered) constituents by core-incubation experiments (Caffrey and others, 1996) and by diffusive flux calculations (Kuwabara and others, 1999). One site was located in the main channel (station 29A; approximately 14 m depth), while the other two represented shallow stations (< 5 m depth), one on the eastern side of the main channel (station 46), and the other on the western side (station 25).

Dissolved organic carbon (DOC) was measured by low-temperature, persulfate/oxygen/ultraviolet oxidation (Hunter and Kuwabara, 1994) and by high-temperature non-catalytic oxidation (Qian and Mopper, 1996) within one day of sampling. Dissolved sulfide concentrations were determinated by square-wave voltammetry (Kuwabara and Luther, 1993) typically within two hours of sampling. Samples for trace metal analysis were filtered, acidified (pH 2) and stored in darkness at 3°C. Trace-element concentrations were determined by atomic absorption spectroscopy (Kuwabara and others, 1996) and by flow-injection inductively-coupled plasma mass spectrometry (Topping and Kuwabara, 1999).

RESULTS AND DISCUSSION

Processes that control the biological availability of trace inorganic solutes in the oxic water column of South San Francisco Bay have been investigated (Kuwabara and others, 1989; Donat and others 1994; Kuwabara and others, 1996). Previous water-column studies have suggested the importance of competitive complexation reactions involving dissolved sulfides and dissolved organic substances (Kuwabara and Luther, 1993). Concentrations of certain dissolved metals (e.g., copper and zinc) correlate with concentrations of dissolved organic substances in San Francisco Bay (Kuwabara and others, 1989). Dissolved sulfide typically is more concentrated at depth, suggesting a primary benthic source, and no longitudinal sulfide gradients or water column sources have been observed. In contrast,

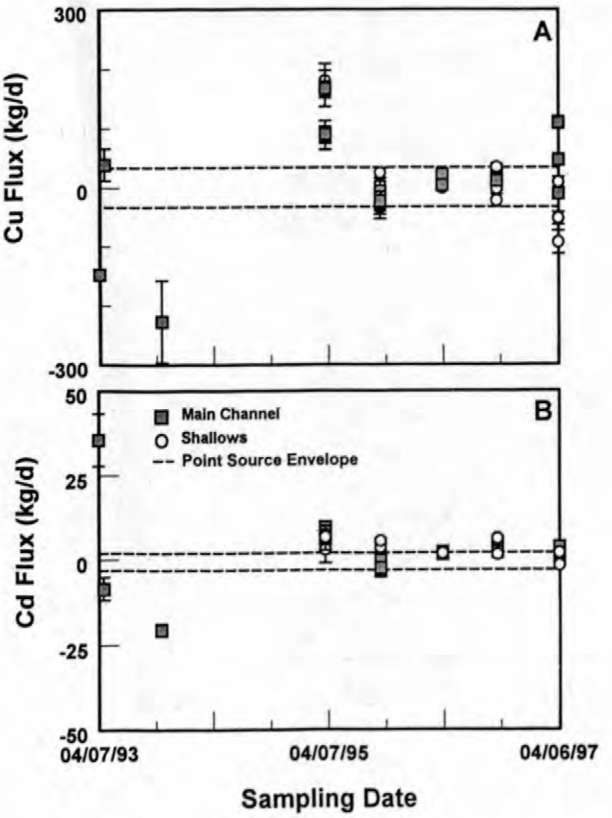

Figure 2. Benthic flux of dissolved copper (Cu; plate A) and cadmium (Cd; plate B) at main channel station 29A and eastern shallow station 46. Dashed lines represent the magnitudes of regulated point sources into the South Bay.

longitudinal gradients for dissolved organic carbon (DOC) have been routinely observed in the estuary due to freshwater sources of natural organic material (Kuwabara and Luther, 1993). Furthermore, vertical gradients for DOC were observed only during periods of salinity stratification. Because the presence of metastable dissolved-sulfide species in the Bay could affect trace metal speciation over ecologically significant concentration ranges (Kuwabara and Luther, 1993), one might therefore expect that the benthic flux of metals, like the flux for dissolved sulfides, would be consistently positive (that is, out of the sediment). However, unlike benthic flux for dissolved sulfide, benthic fluxes for DOC, dissolved copper, and cadmium are temporally variable in direction across the sediment-water interface, suggesting the importance of DOC complexation in regulating metal benthic flux. Note in Figure 2, that the direction of the trace metal fluxes is not always positive as some previous transport models would suggest (Wood and others, 1995). It should also be noted that for both copper and cadmium, the magnitudes of these fluxes are significant relative to regulated point sources. However, because of intra-annual direction shifts, it is not clear how the net flux over annual time scales or longer would compare to point-source inputs to the South Bay.

Concordant with previous water-column studies, experiments using incubation cores and *in-situ* benthic flux chambers indicate consistent sulfide flux out of the sediment. This flux is generated by redox gradients depicted in pore-water profiles for dissolved sulfide (fig. 3). Diffusive flux calculations, based on these pore-water profiles, suggest a benthic sulfide flux of 11 to 45 nmoles-m^{-2}-h^{-1}, with consistently steeper concentration gradients at western shallow station 25 than at eastern shallow station 46 (fig. 3). Station 25 is characterized by clay and silt bottom sediment, in contrast to unconsolidated sandy sediment at station 46. A comparison of sulfide flux determined directly in core-incubation studies and those calculated from pore-water gradients indicate orders-of-magnitude underestimation by diffusive flux calculations (fig. 4A). This suggests the importance of biologically enhanced advection (bioturbation/irrigation). We are also compelled to examine microbial and macroinvertebrate benthic processes, because simple geochemical relationships raise some clear inconsistencies. For example, the timing of shifts in benthic flux direction for trace metals is not always consistent with shifts in DOC flux direction (fig. 4B), particularly for cadmium which does not form strong complexes with dissolved organic ligands compared to other trace metals like copper (Perrin, 1983). Our goal is to quantify and understand processes that affect the variability of trace-contaminant benthic fluxes, and thereby improve water-quality modeling for this estuary. Even with recent additional interest in benthic flux studies, this remains a formidable challenge.

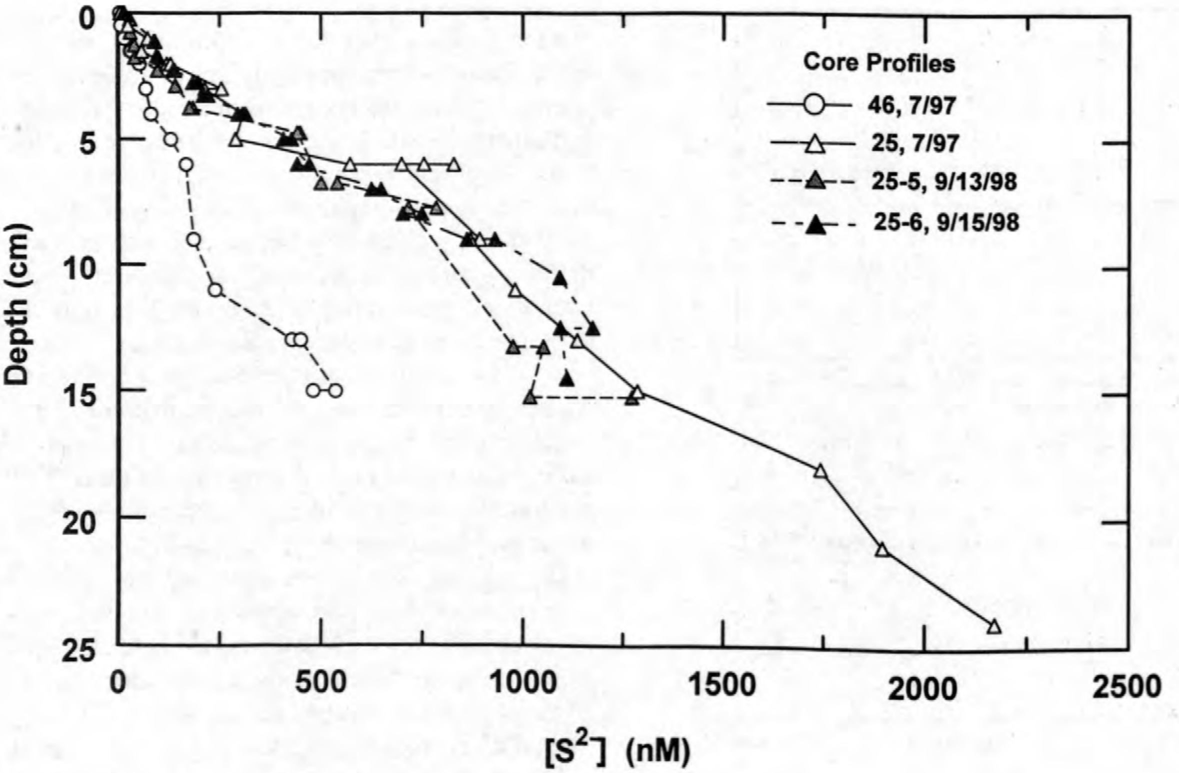

Figure 3. Initial pore-water profiles for dissolved sulfides from cores taken at eastern shallow station 46, and western shallow station 25. Cores 25-5 and 25-6 were replicate cores taken two days apart in September, 1998.

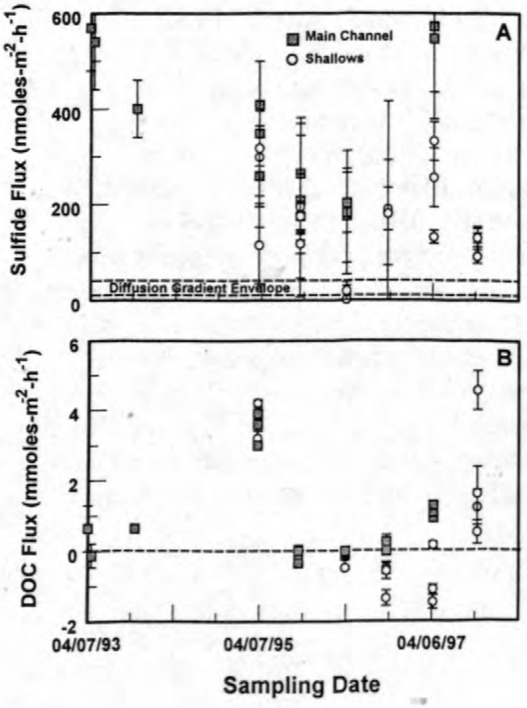

Figure 4. Benthic flux of dissolved sulfides (plate A) and dissolved organic carbon (DOC; plate B) directly measured from core-incubation studies. The diffusion gradient envelope depicted in plate A results from pore-water gradients shown in Figure 3.

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The critical support provided by Francis Parchaso and Byron Richards in coring activities is much appreciated. Fred Murphy's consultation, advice and fabrication of field equipment for trace contaminant studies are gratefully acknowledged. The authors also wish to acknowledge the dedicated work of John Haskins, Jocelyn Douglas, Brynie Kaplan, Encarni Trueba, and John Douglas in depolying in situ flux chambers and coring devices, as well as processing the pore-water samples that were analyzed for this study.

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Redox Gradients in the Vicinity of the Santa Barbara Basin: Application of Techniques Developed within the San Francisco Bay Toxics Study

By James S. Kuwabara, Alexander van Geen, Daniel C. McCorkle,
Joan M. Bernhard, Yan Zheng, and Brent R. Topping

ABSTRACT

Methodology for trace sulfide determinations, developed within the San Francisco Bay-Estuary Toxics Study, was applied in an investigation of redox gradients in the suboxic Santa Barbara Basin. Water-column distributions of dissolved oxygen, sulfide and nitrate, and pore-water distributions for dissolved sulfide and iron indicate very different redox conditions between the coring sites within the Santa Barbara Basin and its vicinity, and provide a contrast to the more-oxidizing conditions just north of the basin's sill. For example, pore-water sulfide concentrations exhibited a range of 5 orders of magnitude, indicating a primary sulfide source from the deep basin (that is from depths greater than 500 m). An estimate for sulfide flux out of the basin using water-column data (9 to 138 nmoles-m^{-2}-h^{-1}) is consistent with spatial and temporal variability of pore-water gradients near the sediment-water interface (-4 to 13,000 nmoles-m^{-2}-h^{-1}). This variability in pore-water gradients affects the authigenesis and mobility of trace elements in the Santa Barbara Basin.

INTRODUCTION

A number of reasons motivate interest in coastal-zone redox gradients. First, there is paleoceanographic interest in how these redox gradients are established as a result of changes in water column productivity and subsequent deposition, and also as a result of ocean ventilation due to natural hydrodynamic events (Thunell and others, 1995; Zheng, unpublished doctoral dissertation). Of related interest are the effects of redox gradients on nutrient cycling (Murray and others, 1978; Klump and Martens, 1981; Thamdrup and others, 1994a). Yet another reason is to understand how the authigenic formation of metals in the ocean sediments proceeds (Calvert and Pederson, 1993; van Geen and others, 1995; Piper and Isaacs, 1996; Zheng, unpublished doctoral dissertation). Finally, there has been some consideration of waste disposal strategies (some involving radionuclides) for deposition on the sea floor. One obvious management question would be, "What processes are going to control the mobility of any toxic or hazardous substance?" One might expect that the partitioning and hence mobility of many inorganic and organic solutes would be affected by the range of redox conditions in these environments.

Santa Barbara Basin is considered "suboxic," that is, intermediate between conditions in permanently anoxic systems such as the Black Sea and oxygenated open-ocean water. Water-column hydrogen sulfide (H_2S) concentrations range from <1 to ~20 µM in seasonally anoxic marine systems such as Saanich Inlet (Richards, 1965; Emerson and Huested, 1991). In previous studies in the Santa Barbara Basin using standard colorimetric procedures, micromolar sulfide concentrations were measured in pore waters in the basin sediments at a distance of <10 cm below the sediment-water interface (Reimers et al, 1990). Such observed gradients suggest a sulfide flux into the water column.

In this study, a voltammetric technique developed for use in the oxic water column of San Francisco Bay (Kuwabara and Luther, 1993) was used to measure total dissolved (0.2

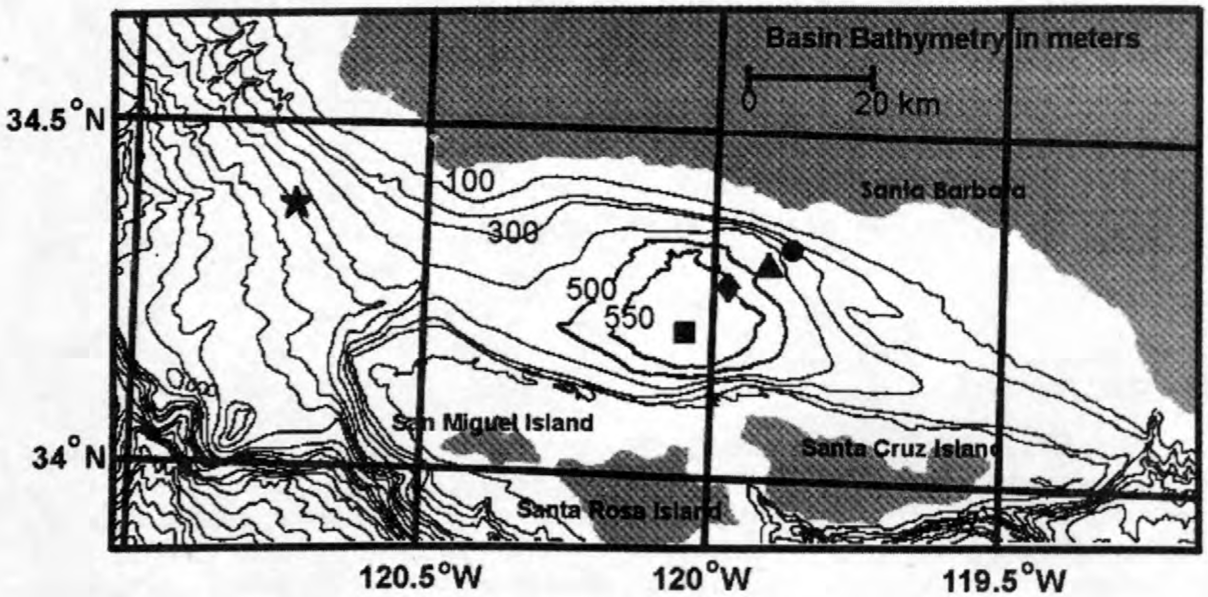

Figure 1. Bathymetric map of the coring and hydrocast stations within the basin at 590 m (square), 550 m (diamond), 430 m (triangle), and 340 m (circle), and outside the basin (590 m, star). Symbols on this map are used in later figures to indicate data from corresponding sampling locations.

µm-filtered) sulfides in pore waters and in the water column aboard ship during three separate cruises conducted between February 1995, and April 1997. Dissolved-oxygen and nitrate profiles in the water column inside and outside (northwest) of the basin, as well as iron pore-water profiles also are presented to facilitate the intepretation of the sulfide data. Association of sulfide gradients with authigenic metal production is discussed. It is, to our knowledge, the first instance where both water-column and pore-water sulfide gradients are presented together, thus allowing closer examination of interfacial processes.

METHODS

In February 1995, the Research Vessel (R.V.) Robert Gordon Sproul was used to obtain Soutar box cores from sites within the Santa Barbara Basin at 590 and 550 m (fig. 1). In November and December 1995, and later in April 1997, the R.V. Pt. Sur was used to sample 4 sites (590, 550, 430 and 340 m) within the basin and an exposed site to the northwest of the basin's sill (fig. 1). From the Pt. Sur, a multicorer was used to collect 8 simultaneous cores at a site with each deployment. The cores were processed under nitrogen in a refrigerated container at 6 ± 2 °C. Hydrocasts were also taken at the 590 m deep-basin site and northwest of the basin to provide comparative water column information.

Dissolved oxygen (DO) was determined using a dissolved oxygen probe mounted on the sampling rosette on R.V. Point Sur cruises only. To complement oxygen-probe determinations, discrete micro-Winkler DO analyses were also performed on bottom waters (Broenkow and Cline, 1969). Seawater density was calculated from pressure, temperature, and salinity measurements (i.e., data from the probe assemblage or CTD) taken from additional probes on the rosette (Fofonoff and Millard, 1983). Dissolved sulfide concentrations were determined immediately after pore-water samples were processed by square-wave voltammetry (Kuwabara and Luther, 1993) and by mixed diamine spectrophotometry (Cline, 1969). The voltammetric technique provided nanomolar detection limits in contrast to micromolar detection limits of more conventional spectrophotometric methods. Due to the concentration range of interest in this suboxic environment (nanomolar to millimolar), and the decision not to store samples, the method that was used here did not descriminate between dissolved sulfide species (sample manipulation would otherwise be required).

Flow injection analysis (Johnson and Petty, 1983) was used to measure dissolved nitrate+nitrite concentrations (hereafter referred to as nitrate) for all water-column samples. Pore-water iron concentrations were measured by graphite-furnace atomic absorption spectroscopy using standard addition methods. Particulate molybdenum concentrations were determined by isotope-dilution inductively coupled plasma-mass spectrometry after sediment digestion in concentrated $HClO_4$ (Zheng, unpublished doctoral dissertation). Sediment trap analysis for molybdenum was performed on subsamples collected by Thunell and others (1995). Precision of the dissolved iron, nitrate and particulate molybdenum measurements was ± 5, 5, and 4%, respectively.

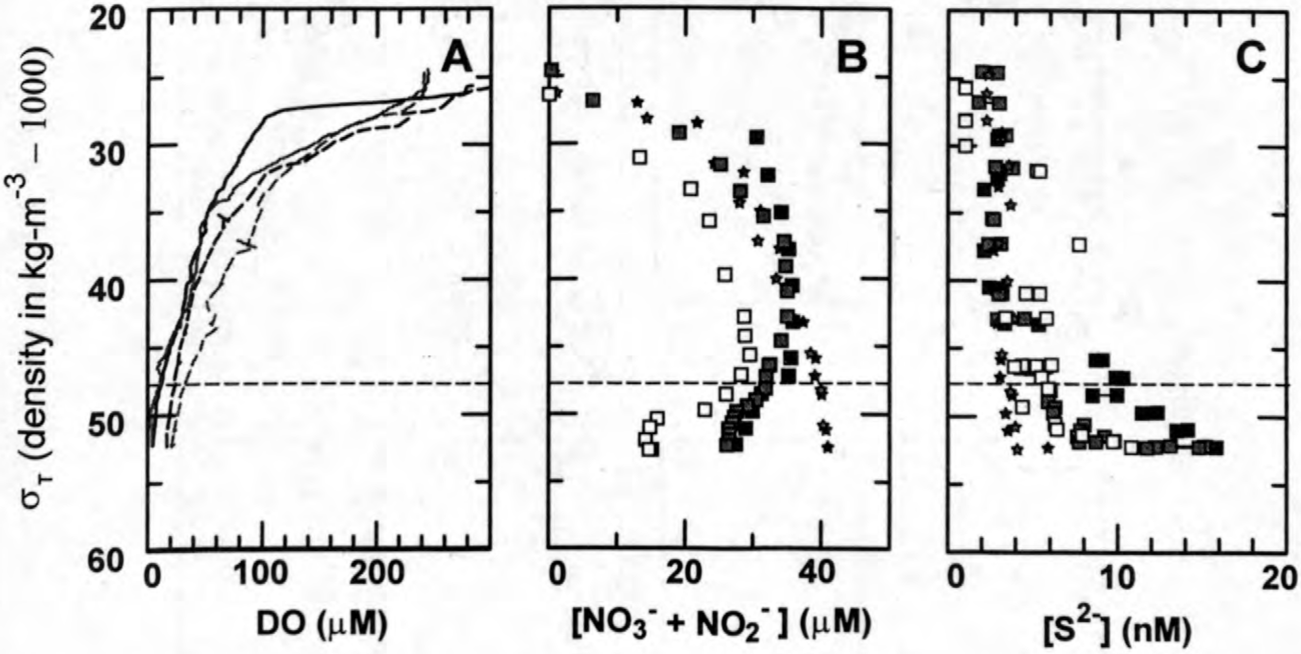

Figure 2. A comparison of water-column redox conditions at hydrocast stations within and outside the basin relative to seawater density for: 1) dissolved oxygen (DO), 2) nitrate and nitrite, and 3) sulfides (note nanomolar units). Open, shaded, and dark symbols or lines denote data from February 1995, December 1995 and April 1997 cruises, respectively. Squares and stars denote concentrations at the deep basin site and outside the basin, respectively. For DO measurements by oxygen probe, solid and hatched lines represent data from sites within and outside the basin, respectively. The horizontal hatched lines at a σ_T (density in kg-m^{-3} - 1000) of 48 indicate the density at the 480 m sill depth. In the absence of CTD (probe assemblage) data for February 1995, nitrate and sulfide profiles for February 1995 are plotted against estimated density from April 1997 CTD profiles.

RESULTS

Sedimentary Attributes: At the 590 m site, the cores in February, 1995 had a brown surface layer of ~1cm thickness. In December, a thin orange veneer overlaid that brown surface layer. In contrast, the cores in April, 1997 exhibited tufts of the sulfide-oxidizing bacterium *Beggiatoa,* which had not been seen during the February and December 1995 cruises. These structures have been hypothesized to reflect an attempt by the colony to move into the more oxidizing environment just above the sediment-water interface (Møller and others, 1985). Surface material overlaid anoxic varved sediments. Formation of these varves from seasonal changes in biogenic and lithogenic sources has been previously described (Sholkovitz and Gieskes, 1971; Reimers and others, 1990; Thunell and others, 1995). By contrast at shallower sites, cores exhibited thick (>2 cm), brown, oxidized layers without evidence of varving. The sedimentation rate in the basin is approximately 0.4 to 0.7 cm-yr^{-1} (Reimers and others, 1990).

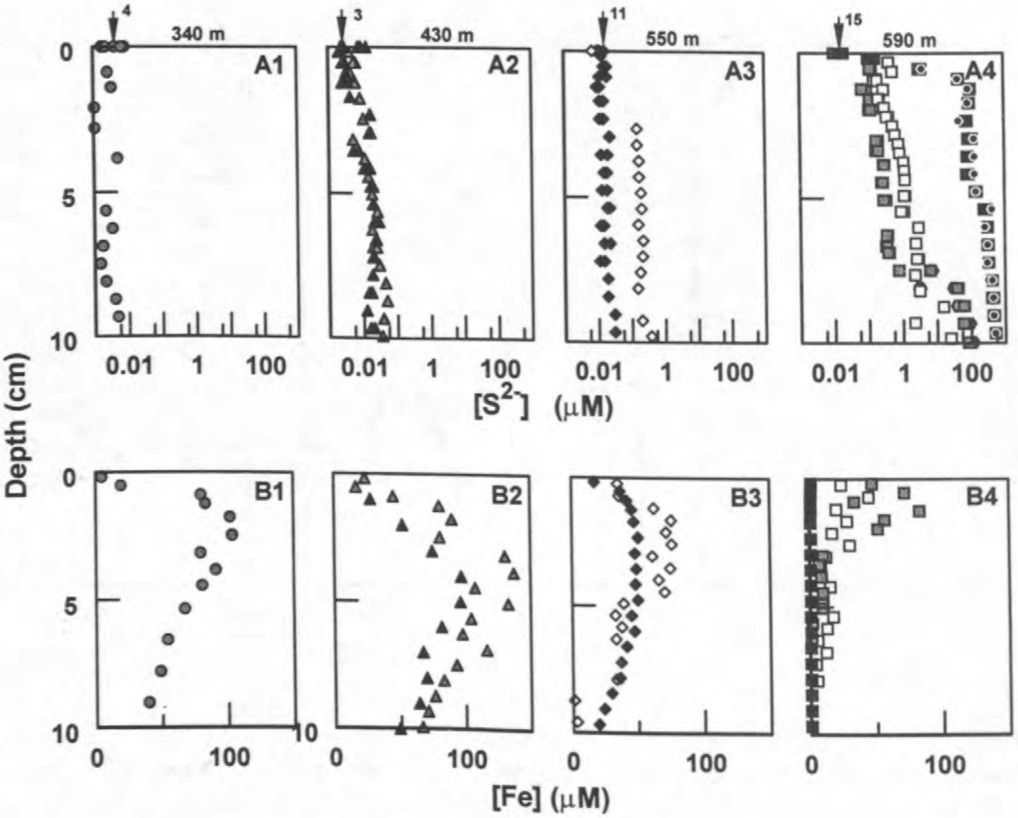

Figure 3. Pore-water profiles in the Santa Barbara Basin for dissolved sulfides (note log scale, panel A), and iron (panel B) from cores taken at various basin depths. Using symbols consistent with Figure 1, the panel columns 1, 2, 3, and 4 denote coring site depths 340 m (circle), 430 m (triangle), 550 m (diamond), and 590 m (square), respectively. Consistent with previous figures, open, shaded, and dark symbols denote data from February 1995, December 1995 and April 1997 sampling cruises, respectively. In plate A4, comparative colorimetric sulfide data at elevated concentrations (>1 μM) are shown as hexagons. Arrows and adjacent numbers on the top border of each plot indicate mean bottom-water sulfide concentrations (nM). Bottom-water iron concentrations are not available.

Water-column: Contrasting redox conditions were evident in water-column profiles observed within and outside the Basin on the same density plane (fig. 2). The density of deep water within the basin (σ_T of 52.2 at 580 m) closely corresponds to a depth of approximately 575 m outside the basin. Dissolved oxygen (DO) concentrations within the basin decreased from >240 µM at the surface to approximately 5 µM near the sediment-water interface. Although water-column DO concentrations outside the basin also decreased with depth, bottom-water (580 m) DO concentrations were 21 and 16 µM in December 1995 and April 1997, respectively. In December 1995, Microwinkler DO analysis for the bottom water at 580 m, 430 m, and 340 m going upslope were 5, 15, and 23 µM, respectively. That is, the bottom water DO concentration at the shallowest sampling site along the basin slope (340 m) was similar to that outside the basin at 580 m. Similarly in April 1997, bottom water DO concentrations at 590 m, 550m, and 430 m were 3, 5, and 11 µM, while the bottom-water concentration just to the northwest of the basin was 16 µM. The decrease in water-column nitrate concentration to <15 µM in February 1995 (fig. 2B), and to 26 µM in November 1995 inside the basin, relative to 40 µM on the same density surface outside the basin, is another indication of additional oxidant consumption within the basin due to organic matter decomposition (i.e., denitrification by facultative anaerobes or by anaerobes in water-column microenvironments (Robertson and Kuenen, 1984; Lloyd and others, 1987; Robertson and others, 1989).

During all three sampling periods, dissolved sulfide concentrations within the basin (fig. 2C) consistently showed a gradual increase below the sill depth (>480m). Elevated dissolved sulfide concentrations observed in April 1997 below the sill depth relative to the other sampling dates is consistent with elevated pore-water profiles for sulfides observed in April 1997 (fig. 3A). By contrast, the water-column profiles outside the basin showed no evident increase in dissolved sulfides below sill depth (fig. 2C).

Core profiles: At the 590 m site, within the top 10 cm of sediment depth, dissolved sulfide concentrations increased by 3 orders of magnitude from tenths to >100 µM (fig. 3A). Intercalibration between voltammetric and colorimetric methods for sulfide analyses during all three sampling trips showed good agreement ($r^2 = 0.93$) over the effective concentration range for the colorimetric technique (>1 µM). Sampling at shallower stations within the basin generated profiles that were typically orders of magnitude lower in sulfide concentrations for comparable core depths. Pore-water iron concentrations provide supporting evidence for this spatial variability within the basin (fig. 3B), with negligible concentrations at 590 m relative to shallower, more bioturbated sites (Kuwabara and others, 1999). The pore-water profiles consistently show the dynamic nature of the basin geochemistry even in the deepest areas (fig. 3).

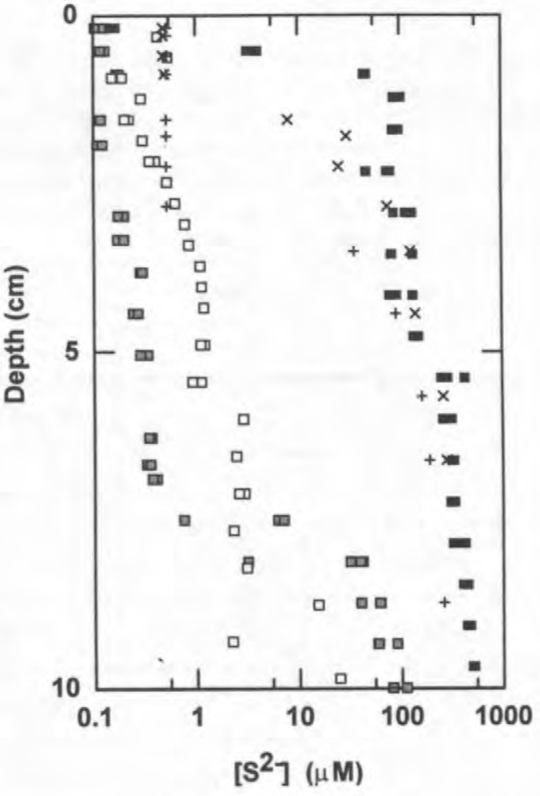

Figure 4. Temporal variability in pore-water sulfide gradients near the sediment-water interface at 590 m are shown with profiles from 2 replicate cores taken in October, 1988 by Reimers and others (1990) shown with cross and "x" markers. Open, shaded and dark squares represent data from February 1995, December 1995, and April 1997 sampling cruises, respectively. The mean bottom-water

concentration from three sampling cruises at this site was approximately 15 nM (fig. 3A4).

DISCUSSION

Water Column: Integrated studies of water-column and pore-water gradients for redox-sensitive solutes provides boundary conditions from which interfacial processes can be identified that regulate solute transport and distribution. An estimate of the dissolved sulfide flux from the basin can be made using water column data (fig. 2C). Dissolved sulfide concentrations outside the basin were consistently $\leq$ 5nM, while at the 590 m deep basin site, concentrations increased to between 10 and 15 nM below 500 m with highest water-column concentrations observed in April 1997. Using bathymetric contours, the basin volume below 500 m is approximately 29 km^3. Mean water residence times between 0.8 to 6 years have been determined for the basin (Berelson and others, 1987; Hammond and others, 1990; van Geen and others, 1995). A flux from the basin can therefore be crudely estimated as:

$$\text{Flux} = (\Delta C/\Delta t)(V/A),$$

where $\Delta C/\Delta t$ is equivalent to the solute concentration change over the period of residence, V is the basin water volume where the solute is elevated, and A is area of exchange, assumed here to be 300 km^2, the projected area at 500 m. The calculation yields a sulfide flux range of 9 to 138 nmoles-m^{-2}-h^{-1}.

Table 1. Sulfide flux for various locations within the Santa Barbara Basin based on pore-water gradients. A diffusion coefficient of 1 x 10^{-5} cm^2-sec^{-1} (Li and Gregory, 1974) was used with the pore-water gradient determined from an exponential fit of data near the sediment-water interface (<1 cm depth). Estimates for October 1988 are from representative pore-water profiles (upper 5 cm due to micromolar colorimetric detection limits for sulfides) from replicate cores by Reimers and others (1990) taken near our 590 m site. The number of data points (n) and coefficient of determination (r^2) for each non-linear gradient determination are also tabulated.

Date	Core[1]	n; r^2	Gradient (uM-cm^{-1})	Flux (nmoles-m^{-2}-h^{-1})
10/88A	-	10; 0.89	36	13000
10/88B	-	10; 0.59	16	6000
2/16/95	B590-1	3; 1.00	2.36	900
2/17/95	B550-1	3; 1.00	0.49	200
12/1/95	M580-2	9; 0.93	1.54	600
11/30/95	M430-2	8; 0.83	6 x 10^{-3}	2
12/2/95	M340-2	9; 0.10	-0.01	-4
4/27/97	M550-3	8; 0.01	0.01	4
4/26/97	M430-3	9; 0.08	9 x 10^{-3}	3

[1] Cores are indexed with the first letter designating the core type (B for Soutar box core, and M for multicore). The next three digits correspond to the site depth in meters. Finally the number after the hyphen indicates the cruise number (1 for February 1995, 2 for December 1995, and 3 for April 1997).

Pore-water: Sulfide flux from the sediments can alternatively be determined from pore-water gradients near the sediment-water interface using an exponential model (Klump and Martens, 1981; Table 1). Clearly, benthic flux determinations for sulfides based on diffusion gradient methods yield widely disparate estimates (-4 to 13,000 nmoles-m^{-2}-h^{-1}) based on easily observable differences in the pore-water gradients between 1988, 1995, and 1997 profiles (Table 1). This variability, consistent with profiles for other redox-sensitive solutes (Kuwabara et al, 1999), suggests that the deep basin was characterized by more reducing conditions in 1988 and 1997 relative to 1995. It should be noted that ^{210}Pb and ^{234}Th profiles suggest a removal of about 12 cm of sediment from the 590 m core a few months to weeks before the April 1997 cruise at the 590 m site (Zheng, unpublished doctoral dissertation). However, the surficial microbial population was already reestablished by April 1997.

Temporal and spatial differences in sulfide flux within the basin (fig. 4) have been linked to an iron-sulfide trapping mechanism (Reimers and others, 1996; Kuwabara and others 1999). Further retardation of sulfide flux near the sediment-water interface is indicated by lower water-column based flux estimates (9 to 138 nmoles-m^{-2}-h^{-1}) relative to pore-water gradient estimates at the 590 m site (600 to 13,000 nmoles-m^{-2}-h^{-1}). This interfacial retardation has been attributed to sulfide oxidation by the benthic microbial layer (Kuwabara and others 1999). Sulfate reduction rates are consistently >2mM -y^{-1} at sediment depths <10 cm near our deep-basin site (Reimers and others, 1996). If this rate even approximated the net microbial sulfide production rate, then it would take only a few weeks to shift the shallow gradient observed in February and December 1995 to that observed in October 1988 and April 1997. The composition of benthic biota within the basin is regulated by bottom-water redox conditions (Bernhard and Reimers, 1991). It is therefore reasonable to assume that physical and biological events that alter redox gradients at the sediment-water interface could result in periods of transition for the benthic community. Using colorimetric techniques, Reimers and others (1996) could not detect pore-water sulfides (<0.5 µM) near the sediment-water interface in spite of high rates of sulfate reduction. Results presented herein provide resolution of the redox gradient in this ecologically and geochemically significant zone.

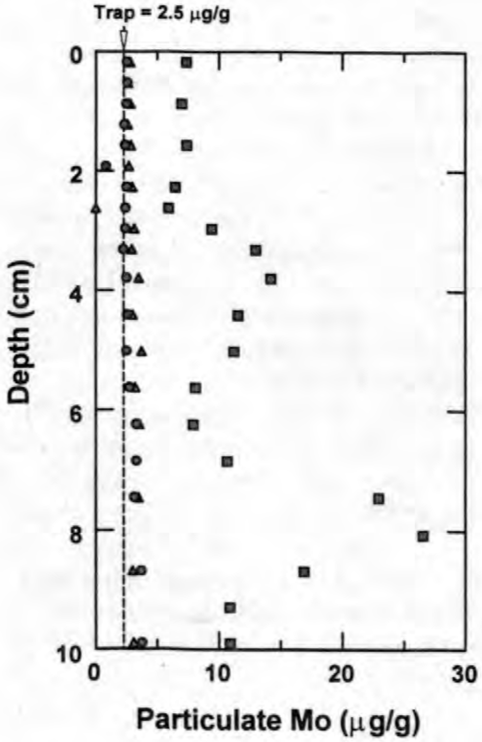

Figure 5. Authigenesis of molydenum in Santa Barbara Basin sediments. Site markers are consistent with previous figures: 590 m (square), 430 m (triangle), and 340 m (circle). The vertical hatched line at 2.5 µg-g^{-1} indicate the particulate molydenum concentration in the sediment trap.

Implications on Metal Authigenesis: Redox gradients can have pronounced effects on metal authigenesis. For example, the authigenic formation of molybdenum minerals has been thought to be controlled by the reduction of molybdate (that is, Mo(VI)) and the formation of thermodynamically stable disulfide precipitates, Molybdenite and Jordisite (Emerson and Huested, 1991; Calvert and Pederson, 1993). Recent sediment analyses by Extended X-ray Absorption Fine Structure, EXAFS, have indicated a Mo - Fe interaction that results in the formation of Mo-Fe sulfides (Helz and others, 1996). Only at the deep basin site is molybdenum authigenesis clearly indicated by the elevated particulate molybdenum concentrations in the sediment profile relative to sediment trap concentrations (i.e., particulates advected from the water column to the bottom-sediment surface;

fig. 5). Authigenic formation is therefore favored under the more reducing conditions of the deep basin relative to the shallower basin-slope sites (fig. 3A and 5; Zheng, unpublished doctoral dissertation). Helz and others (1996) have suggested that the sulfidation of molybdate would place bisulfide in the role of a geochemical switch, shifting molybdenum between conservative and particle-reactive species. Reimers and others (1996) observed a pH shift from 7.5 to 8.0 in the top 2 cm near our 590 m deep-basin site. Over this pH range, Helz and others (1996) indicated a bisulfide switch point of the order of 10^{-4}M, where the activities of MoO_4^{2-} and the particle-reactive MoS_4^{2-} would be equivalent. It is therefore noteworthy that the observed porewater pH transition was coincident with an increase in dissolved sulfides to $>10^{-4}$M in 1988 and 1997, and also in 1995 at a lower depth (fig. 4). The enrichment of other metals (e.g., Cd) in suboxic sediments also has been linked to the formation of insoluable metal sulfides, but without a valence change (Calvert and Pederson, 1993).

Summary Points:

(1) Distributions of dissolved oxygen, sulfide and nitrate in the water-column, and dissolved pore-water sulfide and iron indicate very different redox conditions between the coring sites within the Santa Barbara Basin and its vicinity, and certainly provide a contrast to the more-oxidizing conditions just north of the basin. A concentration range of 5 orders of magnitude for pore-water sulfide was observed.

(2) Our results indicate more oxidizing conditions in the basin in 1995 than in 1988 when previous pore-water studies were conducted. Temporal variability between studies can be explained by net sulfide-reduction rates.

(3) In contrast to the deep basin, micromolar pore-water sulfide peaks are absent at the shallower sites below observed peaks for dissolved Fe and appear to reflect changes in bottom-water chemistry at these more-oxidized sites as well as the magnitude of bioturbation.

(4) Observed spatial and temporal variability in pore-water gradients affect the authigenesis and mobility of trace elements in the Santa Barbara Basin.

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Flow-Injection-ICP-MS Method Applied to Benthic Flux Studies of San Francisco Bay

By Brent R. Topping and James S. Kuwabara

ABSTRACT

An automated low-pressure flow-injection (FI) sample introduction method for use with an inductively-coupled plasma mass spectrometer (ICP-MS) has been applied to determine trace-element benthic fluxes in the San Francisco Bay. Due to metal concentrations of the order of 10 nM and lower, dilution is not an applicable method for circumventing the high ionic-strength (up to 0.7M) of the samples, which inhibits accurate analysis. Due to low volume constraints (as low as 60 ml per sample) presented by the design of the benthic flux experiment, high-throughput concentration methods are not viable. Using the FI method, small (~4 ml) aliquots are buffered on-line to pH 5.2 and are passed through a chelating resin. Following a deionized water flush, the sorbed metals are eluted with 1M quartz-distilled HNO_3, and carried into the plasma. This process represents advancement over the previously employed method involving off-line preconcentration followed by graphite furnace atomic absorption spectroscopy (GFAAS) analysis. Improvements are observed in detection limits, confidence intervals, viable analytes, reduction of contamination sources, and analysis time. For copper and nickel, metals whose estuarine transport mechanisms in the Bay are presently of much interest, detection limits are <0.2 nM, which are less than those for similar samples by GFAAS (<2 nM). Whereas GFAAS allows for effective analysis of only copper and cadmium under our experimental constraints, the FI method has thus far been found appropriate for analysis of Cu, Cd, Ni, Zn, Pb, Mn, Co and V. The FI-ICP-MS method, while still in its relative infancy, represents an invaluable addition to our analytical capabilities, and could benefit any study requiring multi-element analysis in low-volume samples of high ionic strength.

INTRODUCTION

To more effectively model the benthic fluxes of the San Francisco Bay (Wood and others 1995, Kuwabara and others 1996), more efficient methods for trace metal determinations in estuarine waters are required. Analysis of dissolved organic carbon and dissolved sulfide can be performed within a week of the benthic flux experiment. However, GFAAS trace metal analysis, with appropriate replication and quality control, requires 3 months to effectively complete. This process involves excessive handling, which can contribute to inconsistent results due to contamination. Only copper (Cu) and cadmium (Cd) data have been consistent and reliable using this method, inhibiting a more comprehensive study of solute benthic fluxes.

New methods involving chelating resins for trace metal analysis have recently been developed (Sturgeon and others1981, Akatsuka and others1992). Unfortunately, since these were designed for seawater studies, they typically require large sample volumes (>1L). McLaren and others, 1993 provided the outline for an inductively coupled plasma mass spectrometry (ICP-MS) based method using on-line preconcentration of small-volume, high-ionic strength on chelating resin. In later work (Willie and others, 1998), the method was applied to a commercially available flow-injection autosampler (FIAS), with the sample volume required for each replicate less than 5ml. In addition to the low-volume, high-salinity conditions under which it could operate, this method allowed for simultaneous multi-element analysis. The goal of this study is to validate this method so that it can be applied to the benthic flux studies, thus improving both the scope and efficiency of the project as a whole.

MATERIALS AND METHODS

Analysis is performed on a Perkin-Elmer Sciex Elan 6000 ICP-MS. The instrument is optimized daily and operated as recommended by the manufacturer. The on-line preconcentration is performed with a Perkin-Elmer FIAS-400MS. Both the ICP-MS and the FIAS are controlled by Elan software, version 2.0.

All fittings, non-metallic and acid-washable, were selected to minimize metal contamination, mostly ETFE (Tefzel), with some polyetheretherketone (PEEK). Acid-washed PharMed tubing is used on the peristaltic pumps of the FIAS. All other tubing applications are performed with acid-washed flouroethylene polymer (FEP) tubing. The chelating resin, Toyopearl AF-Chelate-650M (Tosohaas, Montgomeryville, PA), is packed into an FEP Perkin-Elmer column. The column has frits on each end and is push-fit into a threaded sleeve.

The buffer, ammonium acetate, is made with quartz-distilled acetic acid (Fisher Optima) and double-distilled ammonium hydroxide (Aldrich). The pH is adjusted to 5.6 ± 0.2. The elutant, 1M HNO_3 is made with quartz-distilled nitric acid (Fisher Optima). MQ water is used for all dilutions and reagents, as well as for a rinse step. High-purity 10ppm standards are diluted in 0.1% nitric acid to create the calibration curve. The National Research Council of Canada certified estuarine reference material SLEW-2 (St. Lawrence Estuary) is used for quality control and to assess the accuracy of the method.

All sample types (samples, standards, reference materials, and blanks) are passed throught the FIAS. Due to the pH sensitivity of the resin, the pH for all must be equal. Software-calculated linear regressions of the calibration curves are used to derive all final concentrations.

A four-step sequence program, designed to optimize the system, controls two pumps and two different valve settings on the FIAS. First, the sample is mixed on-line with the buffer and pumped over the resin column. This is followed by a DI water rinse to remove salts. The elutant passes over the resin, removing the analytes and carrying them to the instrument for analysis. A fourth step rinses the resin again with DI water, removing any acid and neutralizing the pH.

The use of trade names within the text is for identification purposes only and does not constitute and endorsement by the United States Geological Survey.

DISCUSSION

Eight different elements (Cu, Cd, Ni, Zn, Pb, Mn, Co, and V) are linear throughout the desired range of concentrations (Figure 1). The calibration curves are set to encompass the values of the certified reference material SLEW-2 and the expected values of our samples. Each range covers nearly three orders of magnitude, allowing for a wide range of unknown concentration levels. This also creates data for six additional elements besides Cu and Cd, which were performed using the GFAAS method. Moreover, the simultaneous analysis of all of these, combined with efficient sample throughput, allows for the trace metal determinations required for each experiment to be completed within a month.

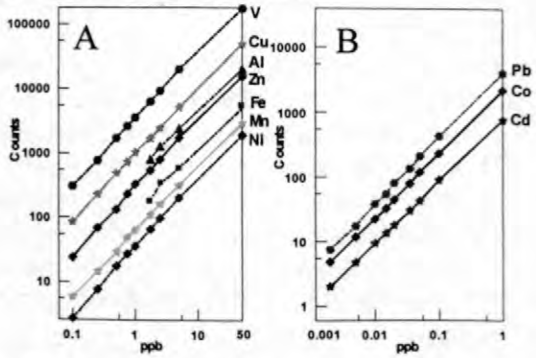

Figure 1. Standard curves for 10 elements using the FIAS-ICP-MS method. Note concentrations are ppb, not nM.

Unfortunately, iron (Fe) and aluminum (Al) (Figure 1A) are inaccurate at concentrations <10 ppb. While Al is ineffective due to inconsistent values, the interferences for Fe are mostly created by argon (Ar). Ar is the carrier gas for the ICP-MS. It mixes with the sample in the nebulizer and forms polyatomic species with oxygen, nitrogen and hydrogen in the plasma. The mass values of the interferences overlap those of Fe. This creates a massive background signal through which it is difficult to isolate the Fe signal. A recently acquired, but as yet untested, ultrasonic nebulizer promises to significantly reduce this background signal.

Cadmium, cobalt (Co), and lead (Pb) (Figure 1B) show method detection limits on the order of 1 ppt (parts per trillion). Although not necessary for our needs, other elements show better method detection limits than those shown, including Cu and vanadium (V) (~1ppt), and Ni and zinc (Zn) (~10ppt).

To confirm the method's effectiveness in analyzing salt waters, SLEW-2 is used as a measure of recovery for each element. (Table 1) The recoveries are derived from a ratio of the determined value and the certified value. Al and V are not certified, and Fe is certified at a concentration that is too low to be measured effectively due to interferences explained above. Also, manganese (Mn) must be diluted at least three-fold to be accurately measured. Mn seems to be suppressed above 10 ppb (SLEW-2 Mn is 17ppb) in high-ionic strength solutions. The overall results are very good, allowing for reliable analysis of our own estuarine samples.

Table 1. Percentage recoveries with error (average of six analysis dates) for seven elements certified in SLEW-2.

	% Recovery		
Cu	96.5	+/-	1.5
Ni	102.6	+/-	4.0
Zn	106.8	+/-	4.1
Mn	100.5	+/-	6.4
Co	110.4	+/-	3.2
Pb	82.3	+/-	9.4
Cd	92.4	+/-	7.0

A comparison of benthic flux curves derived from the GFAAS method and the FIAS method is shown to indicate that it is prudent to continue with the development and implementation of this method. (Figure 2). The general trends for the benthic fluxes are similar. The FIAS method shows significant improvement in confidence intervals, especially for Cd.

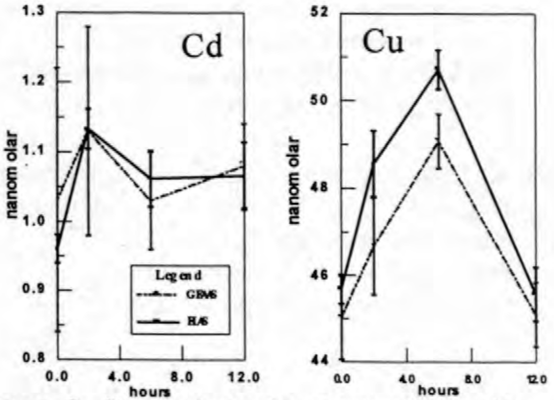

Figure 2. Comparison of concentrations using GFAAS method and FIAS-ICP-MS method for analysis of benthic fluxes. Error bars are 95% confidence intervals.

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Aspects of the Exxon Valdez Oil Spill--A Forensic Study and a Toxics Controversy

By Frances D. Hostettler, Keith A. Kvenvolden, Robert J. Rosenbauer, and Jeffrey W. Short

ABSTRACT

After the *Exxon Valdez* oil spill in Prince William Sound in March, 1989, much work has been done to track the spilled oil and study its fate and its affect on the environment. Our studies involved developing and applying methods to identify and track the spilled *Exxon Valdez* oil (EVO) as it weathered, as well as to differentiate it from other petrogenic hydrocarbon input sources in PWS. Application of these methods to the study area has yielded two important findings. First, it was discovered that not all the oil or tar on the beaches was EVO. Instead, tarry residues of oil from the Monterey Formation, Southern California, also were prominent on some of the Prince William Sound beaches along with EVO. Monterey Formation oil was used in the early development of Alaska, before the discovery of North Slope Crude, and most likely was disbursed throughout the Sound from the port of Old Valdez in the Great Alaskan Earthquake of 1964. The second study also involves differentiating petrogenic sources. A third petroleum input source to PWS, namely natural oil seeps in the Gulf of Alaska, has been claimed by others to contribute a substantial, and potentially toxic, polycyclic aromatic hydrocarbon (PAH) background to the Prince William Sound benthic sediments. If this claim is true, it would have the effect of mitigating the long-term effects of the oil spill. Our studies and cooperative work with NOAA provide evidence that it is coal and not oil that contributes this PAH background. Unlike the case with oil, PAHs in coal would not be bioavailable and thus would be considered contaminants but not pollutants capable of causing adverse effects on exposed biota. Resolution of the source of the PAHs is, therefore, an important environmental issue.

This work in identifying petroleum sources, both natural and anthropogenic, has a great deal of transfer value to other estuarine systems. The geochemical information obtained in Prince William Sound can be extrapolated and applied to the study of oil residues on the California coastline and in San Francisco Bay. Current studies are attempting to correlate or differentiate spills in these two areas and sort out the origin of the petroleum input sources.

INTRODUCTION

On 24 March 1989, the T/V *Exxon Valdez* grounded on Bligh Reef in Prince William Sound (PWS), Alaska (Fig. 1), causing the largest oil spill in U.S. history. About 41 million liters (11 million gals = 258,000 bbls) of North Slope crude oil spilled into the water and eventually reached about 16% (500 km) of the shorelines of the sound. The oil spill was an environmental and visual disaster as crude oil spread throughout the sound and washed up on the pristine beaches. Massive efforts were undertaken to clean up the oil-contaminated beaches and remove oil from the water. Over the years many studies have been undertaken to document the effects of the spill. Some studies analyzed the biological effects (for example, Maki, 1991, and Rice and others, 1996); others focused on tracking the spilled oil and studying its distribution and fate in the environment (for example, Page and others, 1995; Bence and others, 1996; O'Clair and others, 1996; Short and others, 1996; Short and Heintz, 1997). Starting two months after the spill and continuing through 1997, the U.S. Geological Survey conducted a series of investigations to look at the geological fate of the spilled oil (Kvenvolden and others, 1993; Hostettler and Kvenvolden, 1994; Carlson and others, 1997). Various aspects of the oil spill aftermath have been pursued, with the focus particularly on two areas: a forensic study to determine the source of oil(s) present on the PWS beaches, and a study to help determine the source of background hydrocarbons, particularly polycyclic aromatic hydrocarbons (PAHs), many

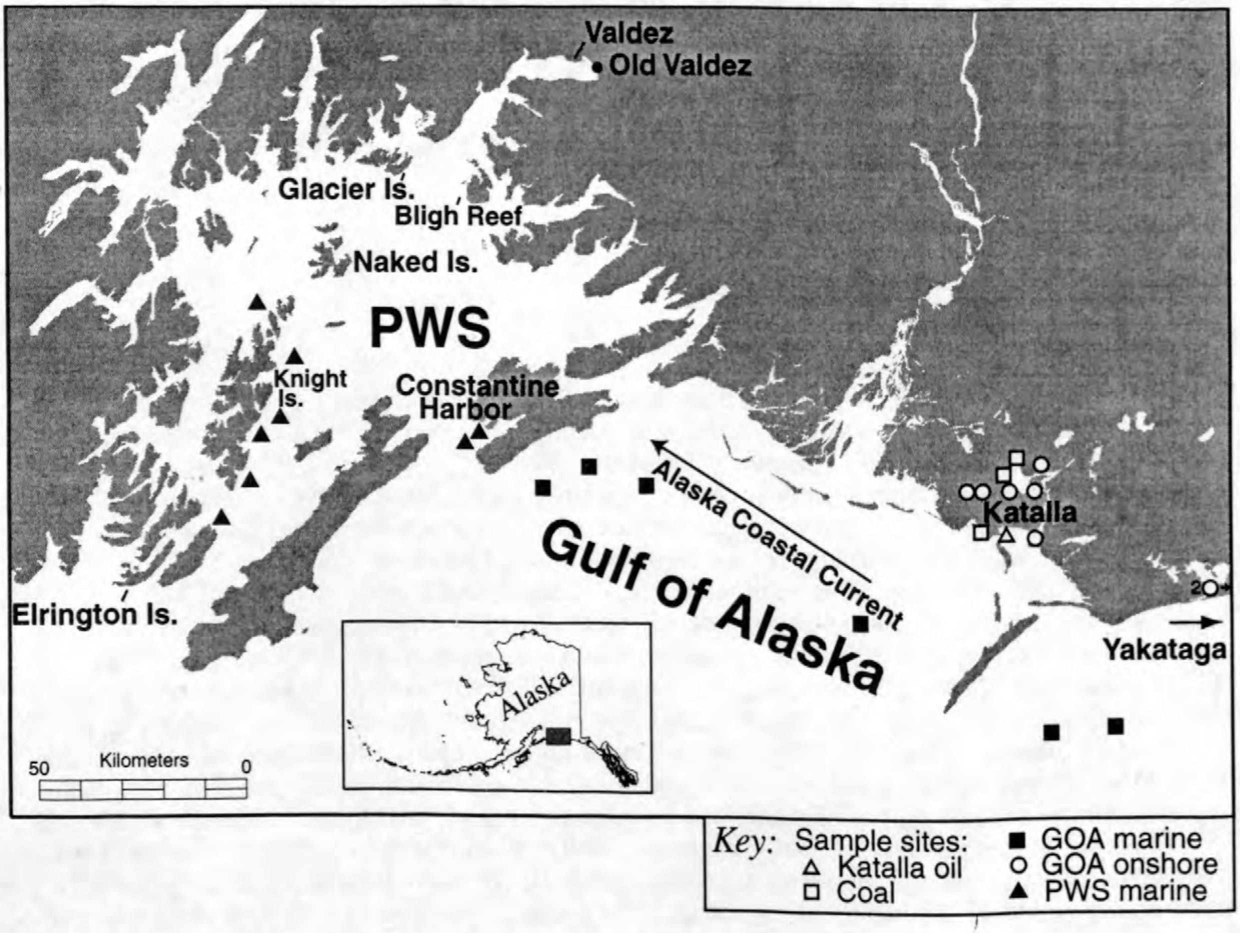


Figure 1. Map of Prince William Sound and Gulf of Alaska (GOA) study area.

of which are toxic, in the benthic sediments of PWS. This paper summarizes these two efforts.

The forensic study was undertaken after it was discovered that not all of the oil and tar residues on the beaches of PWS were attributable to *Exxon Valdez* oil (EVO) (Kvenvolden and others, 1993). Any tar or oil prominent in the recent geologic record of PWS would be relevant as the responsibilities and repercussions of the oil spill were sorted out.

The second focus of our work has been to investigate the source(s) of the background hydrocarbons in the benthic sediments of PWS. In general, these sediments, especially deeper water sediments, have been shown not to contain any residual EVO (Bence and others, 1996). However, the sediments have been shown to contain somewhat elevated levels of PAHs relative to the eastern reach of the Gulf of Alaska (Short and others, 1999). Many of these PAH are potentially toxic to native biota. These PAHs have been attributed to petrogenic sources (the term "petrogenic" is used here in its generic sense, meaning organic matter originally sourced from rocks; as such, then, it can refer to oil, coal, and sediments containing oil and/or coal constituents). The PAHs are assumed to have originated in the Gulf of Alaska and to have been transported into PWS by the Alaska Coastal Current. There is a significant dispute as to which petrogenic source is responsible for the PAHs. A large body of work by Exxon (for example, Page and others, 1997, Bence and others, 1996) has claimed that the PAHs are from natural oil seeps in the Gulf of Alaska, particularly at Katalla and Yakataga (see Fig. 1). In contrast, this study and a previous study by Short and others (1999) presents evidence that the source of the PAHs is coal from the same region. Whether the PAHs are from oil or coal is an extremely relevant environmental issue in PWS. Even though oil and coal contain many of the same PAH constituents, PAHs from oil are bioavailable and capable of causing adverse effects to exposed biota, whereas PAHs from coal are very tightly bound within the coal matrix and generally not bioavailable (Chapman and others, 1996). If oil from natural seeps were a chronic PAH source to PWS, then biota in the marine ecosystem would have already adapted to these hydrocarbons. Long-term effects of an oil spill would, therefore, be mitigated, and once the catastrophic impacts immediately after the spill had passed, there would be no further impact attributable to the spill oil. On the other hand, if the PAH are derived from coal, there is no adaptive benefit to biota with respect to PAH input from anthropogenic sources, and long-term environmental effects of the oil spill can be more readily evaluated.

In both of these studies, the identification and differentiation of sources of hydrocarbons in a sedimentary environment is critical. A variety of molecular and isotopic techniques can be applied. For individual oils from a single source, carbon isotopic analysis of the whole oil can be a defining characteristic, even when the oil is in a weathered state. Another analytical approach is individual component analysis. Oils, tars, and sediments are very complex mixtures that may contain a wide variety of organic constituents. This paper will focus on petrogenic hydrocarbons. In sediments these organic compounds can derive from very diverse sources, both natural and anthropogenic. In addition, there is broad overlap among specific compounds that might originate from any of these sources. Finally, the sedimentary system is generally dynamic, in that its components are subject to change through the processes of diagenesis and weathering.

Weathering adds a further dimension to the problem of identifying hydrocarbon input in the environment. Weathering can involve water-washing, chemical degradation (e.g., photo-oxidation), microbial degradation, solubilization, evaporation, mechanical redistribution, and other such processes (Hunt, 1996). Thus, in addition to the high degree of complexity of sedimentary constituents, the system is in a constant state of flux because these components are continually exposed to weathering. Furthermore, weathering effects can vary. Petroleum PAHs, for example, have been noted to be more subject to weathering than those in coal (Short and others, 1999) and those from pyrogenic sources (Bouloubassi and Saliot, 1993) due to differences in matrix effects and particle associations. Weathering of PAH in petroleum has been shown to follow a progression in the number of rings, in which compounds like naphthalene with two fused aromatic rings are degraded faster than three-ring compounds such as phenanthrene, with the rate of degradation being 2>3>4>5 aromatic rings. In addition, parent PAHs degrade faster than their alkylated homologs, and C1>C2>C3>C4 (Volkman and others, 1984).

There are several approaches to identifying sources of hydrocarbons that are subject to weathering. The first approach, referred to here as *weathering invariant*, involves identifying a specific analyte or group of analytes, either not subject to weathering or not yet weathered, which is known to come from a given source. The use of ratios of aliphatic hopane and sterane biomarkers to differentiate specific crude oils in PWS, Alaska, is one example of this approach (Kvenvolden and others, 1995). Biomarkers, which are molecular fossils, are highly refractory and have been shown to resist postdepositional alteration in the environment until extreme weathering has occurred (Volkman and others, 1984). A second approach, *weathering variant*, considers the weathered system and mathematically extrapolates backward from the current levels of constituents to the original concentrations of the pure source material at the time of deposition. This approach was used by Short and Heintz (1997) in their work in following EVO as it weathered in the environment. A third approach, *weathering covariant*, utilizes ratios of analytes assumed to weather at the same rate. This approach has been used by Page and others (1995) and Douglas and others (1996) who utilized ratios of alkyl dibenzothiophenes to alkyl phenanthrenes in tracking EVO in PWS and differentiating it from background aromatic hydrocarbons. All three approaches provide powerful tools for establishing hydrocarbon sources. However, of the three approaches, *weathering invariant* may be the most robust, because it utilizes constituents that have not yet been altered in the environment and hence carry the chemical signature of the original source most reliably.

Our study also introduced a new parameter, the PAH refractory index (RI), to apply to correlations of petrogenic systems. The RI is a ratio of specific members of two classes of the most refractory PAH compounds present in crude oils, namely triaromatic steranes (T) and monomethyl chrysenes (C) (T/C, see Fig. 2 and Table 1). Triaromatic steranes are perhaps the most refractory components of oil; they are present even when almost no traces of other PAHs are resolvable through analysis by gas chromatography-mass spectrometry (GC/MS) (for example, Hostettler and Kvenvolden, 1994). They also persist in sediments as some of the last of the resolvable PAH components of weathered petroleum contamination (Killops and Howell, 1988; Hostettler and others, 1992). In general, however, triaromatic steranes are either absent or less common in coal than in oil, (Lu and Kaplan, 1992) due to differences in formation pathways between crude oil and coal. Chrysene and its alkylated homologs are also highly refractory. As the highest molecular weight PAH family typically present in an analytical profile of petroleum (for example, Page and others, 1995), the chrysenes are, therefore, among the most refractory of the regular PAHs. Alkylated chrysenes are common in both crude oils and coals (Barrick and others, 1984). Monomethyl chrysene, rather than chrysene, was chosen for the refractory index because chrysene can have a combustion as well as a petroleum source. The refractory index, then, falls into the *weathering invariant* category, like hopane and sterane biomarkers, and, as such, should be a useful parameter for source correlation. It has been shown to work well in differentiating specific oil families in PWS, specifically EVO, California (Monterey) oil, and Katalla seep oil, but to give values close to 0 for several Alaska coals (Hostettler and others, 1999).

METHODS

Sample collection. Samples and sampling procedures are described in Carlson and others (1997) and Short and others (1999). Samples for this study include EVO and other oils and tars from PWS beaches, a composite sample of unweathered Katalla oil, a surface seep sample of exposed Katalla oil, offshore sediments from the northern Gulf of Alaska and PWS, and terrestrial riparian sediment samples from the Duktoth, Copper, and Bering Rivers. Coal samples were collected from outcrops in the Bering River coal field and from the beach at Katalla.

Sample analysis. Bulk carbon isotopic analysis was done on the tars and oils from the beaches as described in Carlson and others (1997). Individual component analysis on all samples also is described in the same reference. Briefly, this analysis consisted of air drying the sediment samples and grinding to pass a 32 mesh screen, or, in the case of the tars, weighing out an aliquot, extracting with or dissolving in dichloromethane, treating with HCl-activated copper to remove sulfur, and fractionating by alumina/silica gel column chromatography into a hexane fraction, containing aliphatic hydrocarbons, and a 30% benzene- or dichloromethane-in-hexane fraction containing PAHs and other aromatic compounds. These fractions were then analyzed by GC/MS in the full-scan and selected-ion-monitoring (SIM) modes. Biomarker parameters used in the identification of the various petrogenic sources were calculated and are discussed elsewhere (Kvenvolden and others, 1993, 1995; Carlson and others, 1997). Full-scan GC/MS analysis of the aromatic fraction was used for calculation of the refractory index and a semi-quantitative comparison of naphthalenes, phenanthrenes, dibenzothiophenes, chrysenes, and triaromatic steranes. The refractory index was calculated

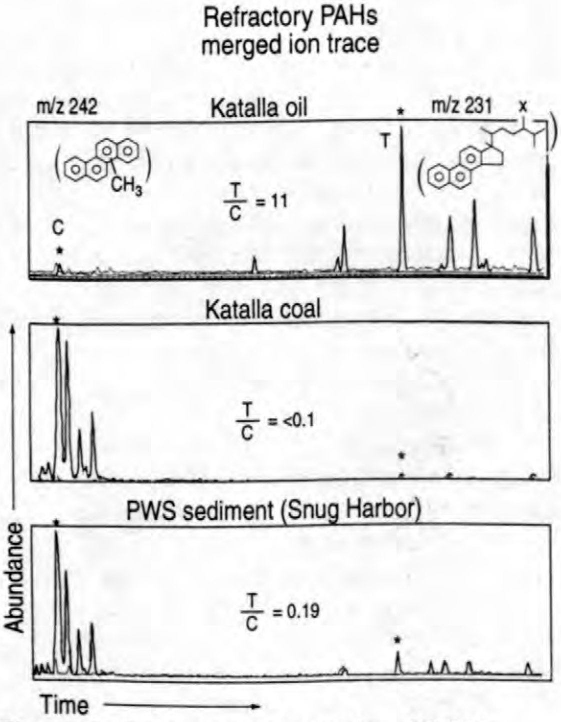
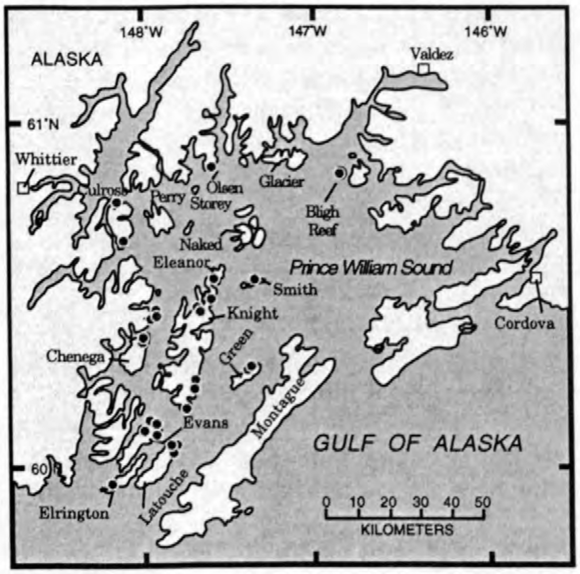


Figure 2. Refractory index ratio: GC/MS extracted ion profiles from the aromatic fraction of Katalla seep oil, Katalla coal, and a typical PWS benthic sediment. The merged trace utilizes m/z 231 for the suite of C_{26}-C_{28} triaromatic steranes (X = H, CH_3, C_2H_5) and m/z 242 for methyl chrysenes. The major peak (*) for each suite was used to calculate the refractory index.

from the ratio of the peak heights of the major peak of the C_{26} to C_{28} triaromatic sterane suite (m/z 231 extracted ion chromatogram, peak identified as consisting of the $C_{26}R$ and $C_{27}S$ epimers) to the major peak of the monomethyl chrysene suite (m/z 242 extracted ion chromatogram, first and dominant peak), as shown in Figure 2. Values for calculated parameters are given in Table 1.



A. Prince William Sound showing locations where residues of *Exxon Valdez* oil have been found.

B. Prince William Sound showing locations of tar balls related to Monterey Formation (Miocene) source rocks of California.

Figure 3. Locations of oil and tar balls.

RESULTS AND DISCUSSION

Forensic study: In the analysis of tar ball and oil samples found on the beaches of PWS it was found that the beached oils and tarballs had two distinctly different chemical signatures. Figure 3 shows the distribution of the two different families of tarry residues of these oils in our sample sets. The parameters that best identified and correlated these tars and oil residues were carbon isotope compositions ($\delta^{13}C_{PDB}$) of the whole-oil samples and selected terpene biomarker ratios. Of particular value is the biomarker ratio [C_{26}-tricyclic terpane (S?+R?)]/C_{24}-tetracyclic terpane, called the Triplet (T) because of how it appears on m/z 191 mass chromatograms (Fig. 4). This parameter was first noticed in an earlier study of North Slope

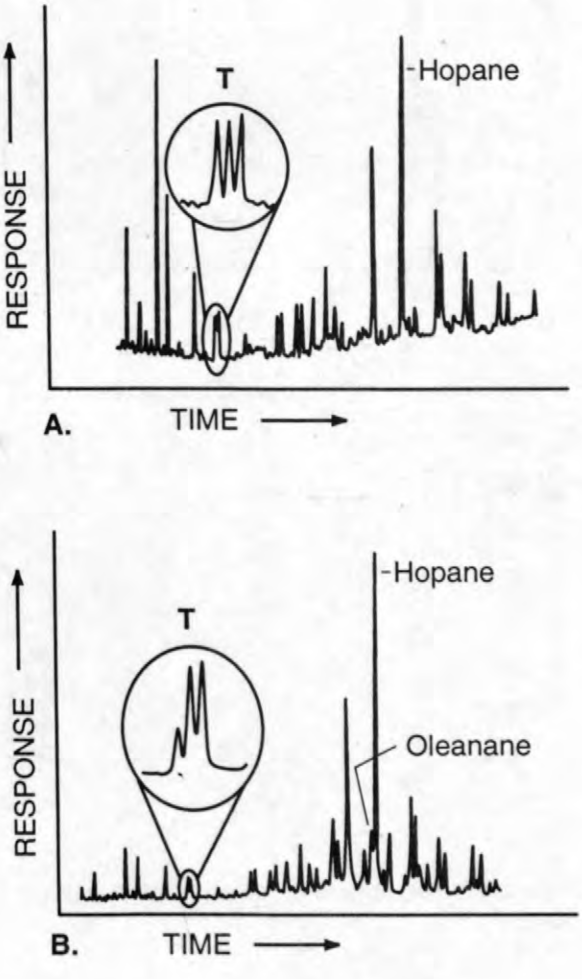


Figure 4. m/z 191 mass chromatograms showing the peaks used for the Triplet ratio and those used for the Oleanane index.

crude oils (Kvenvolden and others, 1985). Studies on PWS show that T = 2.0 ±0.1 in 25 samples of EVO (n=1) and residues (n=24) (Fig. 5A); the $\delta^{13}C$ values for 28 samples of EVO (n=1) and residues (n=27) are –29.4 ±0.1 ‰ (Fig. 5B). These parameters clearly correlate the EVO and its residues. In contrast, T = 5.1 ±0.5 in 39 samples of tar balls (Fig. 5A), and the $\delta^{13}C$ values for 61 samples of tar balls are –23.7 ±-0.2 ‰ (Fig. 5B). These values, particularly the distinctively heavy $\delta^{13}C$, are within the range of oil products sourced from the Monterey Formation of southern California (Curiale and others, 1985).

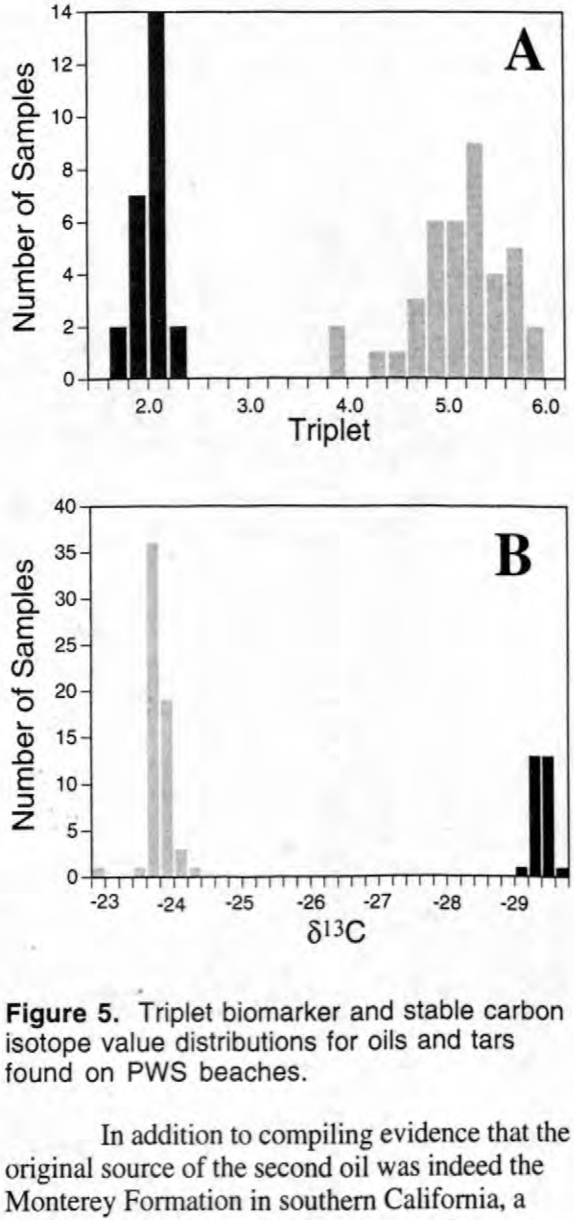


Figure 5. Triplet biomarker and stable carbon isotope value distributions for oils and tars found on PWS beaches.

In addition to compiling evidence that the original source of the second oil was indeed the Monterey Formation in southern California, a plausible explanation for its widespread distribution in PWS was necessary. The waters of PWS flow counterclockwise through the sound and out to the southwest, driven by the Alaska Coastal Current. Therefore, Valdez, the northernmost point in PWS, is an obvious possible origination point for these oils (Fig 3B). In 1964, 25 years before the EVO spill and also on Good Friday, the Great Alaskan Earthquake occurred. Huge storage tanks of Monterey Formation California oil, which was used in the development of Alaska before North Slope Crude or Cook Inlet oil was discovered, located in the port of Old Valdez, were ruptured and destroyed in this earthquake. However, in the aftermath of the chaos caused by the 9.1 magnitude earthquake, the fate of the California oil was not documented. Kvenvolden and others (1995) and Carlson and others (1997) have suggested that this oil was disbursed throughout PWS after the earthquake and that it is the tarry residues of this oil that is retained in the recent geological record, often in the same locations as the EVO.

Background hydrocarbons in benthic sediments. Several approaches have been used to address the question of the source of the PAH background to the PWS sediments. Some of the geochemical parameters are listed in Table 1 and discussed below. Exxon's long-held claim that natural oil seeps from the Katalla/Yakataga region of the Gulf of Alaska contribute this background is based on three major geochemical correlations: the correspondance of the ratios of C2D/C2P (D = dibenzothiophene and P = phenanthrene), the presence of oleanane, a C_{30} biomarker commonly found in oils of the late Cretaceous or Tertiary and not present in EVO, and the similarity of PAH fingerprints given by the background PAH in benthic sediments and the water/suspended sediment PAH from seep streams (Bence and others, 1996). Coal is discounted as a potential source primarily due to a reported lack of coal deposits in the drainage areas of streams east of the Bering River.

An alternate source suggested by Short and others (1999) acknowledges the above-mentioned similarities, but shows that the alkylated Db/Ph ratios as well as the presence of oleanane (shown in Table 1 as an oleane/hopane index) fall within the same range for coal as well as seep oil and benthic sediment samples. These ratios are therefore inadequate discriminants. As for the purported dearth of coal sources in the area, references in Short and others (1999) show that the literature details extensive coal deposits along this reach of the Gulf of Alaska coast.

Table 1. Ranges of parameter values. See text for parameter definitions.

Parameters	PWS Background	Gulf of Alaska			
	Marine sediment (n = 6 - 10)	Marine sediment (n = 6)	Coal (n = 3)	Onshore (n = 8)	Seep Oil f = fresh; w = weathered
C2D/C2P(a)	0.11 - 0.21	0.22 – 0.25	0.17 – 0.59	0.07 – 0.31	0.18(f,w)
Oleanane Index(a)	0.19 – 0.37	0.13 – 0.14	0.08 – 0.12	0.01 – 0.22	0.12(f,w)
MSE (Kat)(b)	1.68 – 4.41	1.55 – 1.82	3.25 – 7.20	2.07 – 8.46	0.05(w)
MSE (CH)(b)	0.19 – 0.54	0.20 – 0.30	0.60 – 1.14	0.86 – 1.77	1.21(w) 2.68 (f)
RI (T/C)(b)	0 – 0.2	≤ 0.1	0 - 0.02	0(most)–0.3	11(w), 13(f)

(a) Parameter used by Bence and others (1996) as evidence of naturally occurring oil seep contribution to benthic sediments

(b) Parameter used by Short and others (1999) as evidence of coal contribution to benthic sediments

Whereas Bence and others (1996) claim that there are numerous natural oil seeps in the same region and assume these seeps have sufficient outflow to deliver at least 360 to 1200 tons of petroleum per year in order to account for the loadings of PAH in PWS, Short and others (1999) claim that the seeps are small, with low productivity, and limited accessibility to Gulf of Alaska waters (for example, no oil slicks documented from these seeps into the Gulf waters, and no published reports of offshore seeps). However, coal particles eroded by natural processes would be widespread and accessible to transport to PWS. Indeed, finely divided coal particles can be seen in the intertidal area and all along the beach at Katalla. Short and others (1999) then examined other geochemical parameters. They also considered the PAH fingerprints of the oils, coals, and sediments and looked more closely at the weathering characteristics of the PAH in the seep oil and in benthic sediments. Using pattern similarity analysis, they calculated two parameters of the mean square error (MSE) of logarithmically transformed proportions of the 14 most persistent PAHs in the respective samples (Table 1). The first parameter, MSE(CH), compared patterns in samples with patterns characteristic of intertidal sediments at Constantine Harbor (CH), PWS. This site was chosen as a reference because PAH concentrations in CH sediments have remained unchanged since 1977 and are representative of deeper subtidal sediments elsewhere in PWS (Short and Heintz, 1997). The second parameter, MSE(Kat), compared the patterns of relative PAH concentrations with those in fresh Katalla seep oil, but in this case, corrected for weathering losses in the samples. Both parameters are natural log functions, with smaller numbers indicating greater similarity and larger numbers indicating less similarity. The value 0.05 of MSE(Kat) for the weathered Katalla seep oil indicates how well the weathering model accounts for weathering losses. Table 1 shows that the PAH fingerprints in PWS and Gulf of Alaska benthic sediments are very similar (MSE $\leq$ 0.54) to that at CH; coal is also close (e.g., 0.60 for coal at Katalla Beach), whereas Katalla seep oil, both fresh and weathered, are very dissimilar (MSE(CH) = 2.68 and 1.21, respectively). The PAH fingerprints of the PWS benthic sediments are quite dissimilar to those of Katalla seep oil (MSE(Kat) = 1.68-4.41), even accounting for weathering.

The last parameter utilized is the refractory index. Katalla seep oil has a RI of 11 and 13.

However, the benthic sediments and coal all give very low values and differ from the RI of Katalla oil by one to two orders of magnitude. The lack of a substantial presence of triaromatic steranes in the GOA and PWS benthic sediments, as reflected in the RI, is another contraindication for substantial oil input in PWS, particularly oil related to seep oil from Katalla.

One last factor considered by Short and others (1999) was the bioavailability of the PAHs in the respective potential sources. Juvenile coho salmon, collected 100 m downstream from an oil seep, were found to contain significant levels of petrogenic PAHs. However, in a comparable study, mussels further down stream and very near particulate coal outcrops had none of the petrogenic PAHs, indicating that the coal PAHs were not bioavailable and also that the oil productivity upstream was so small, or the weathering so complete, that the PAHs did not reach biota this distance away.

CONCLUSIONS

Geochemical parameters described in this study have enabled us to differentiate petroleum input sources to the recent geologic record in PWS, Alaska. First, it was found that oil washed up on the beaches in the Sound comes from two different sources—EVO from the oil spill of March 1989, and Monterey Formation, California, oil from the aftermath of the Great Earthquake of 1964. Second, evidence is contributed to the ongoing controversy as to which of two petrogenic sources of PAH is responsible for the PAH background in the benthic sediments of PWS, natural oil seeps from the coastline of the Gulf of Alaska or coal from the same area. Our studies, and cooperative work with NOAA, provide evidence that coal is the more likely source. This evidence includes a geographic and geologic framework for the coal origin and transport, geochemical parameters including weathering extrapolations and ratios of refractory constituents of the petrogenic PAH, and bioavailability experiments, all of which correlate better with a coal-sourced PAH background in the benthic sediments of PWS.

In addition to contributing to the body of information after the *Exxon Valdez* oil spill, the geochemical information and procedures obtained in these studies have implications and applications that can be carried over to other estuarine systems. For example, EVO is representative of a mixture of North Slope crude oils, and the Monterey tar balls are representative of crude oils produced from the Monterey Formation source rocks of California. Both of these types of oils are commonly transported along coastal California and into San Francisco Bay. Application of the work in PWS can help differentiate or correlate these oils and tars from spills and natural outcrops, as well as answer questions as to whether they have natural or anthropogenic origins.

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SECTION B

Mercury Contamination of Aquatic Ecosystems

The potential consequences of mercury contamination of aquatic food webs were first recognized in the 1950s and 1960s in Minamata and Niigata, Japan, where human consumers of contaminated fish were severely poisoned. These and other tragic incidents prompted widespread reductions in direct releases of mercury into surface waters in many countries. Mercury levels in fish in affected waters typically declined during the years after point-source reductions, leading to a widespread perception that the "mercury problem" had been solved. Since about 1985, however, widespread mercury contamination of aquatic biota has become evident in systems remote from obvious anthropogenic mercury sources. Investigations at these sites have shown that in most cases atmospheric transport and low rates of mercury deposition are responsible for the observed mercury contamination levels, and virtually any aquatic ecosystem is potentially affected. In some cases, concentrations in fishes from these remote sites have equaled or exceeded those in fishes from waters heavily contaminated by direct industrial discharges. Mercury concentrations in aquatic biota are often elevated, for example, in fish from low-alkalinity or humic freshwaters, newly flooded reservoirs, and surface waters that adjoin wetlands. However, we lack sufficient information to predict reliably which aquatic ecosystems will contain mercury-contaminated biota.

Unlike most contaminants, some natural processes in the environment actually increase mercury toxicity by the conversion to methylmercury. Methylmercury comprises nearly all the mercury found in the top levels of aquatic food webs, yet it rarely exceeds 10 percent of the total mass of mercury in sediment or water. Gaps in our understanding of the processes and factors controlling exposure to methylmercury (methylation, demethylation and biotic uptake) are a key challenges facing scientists investigating the mercury problem. Several information gaps currently exist in the scientific basis for addressing the environmental mercury problem. These include: (1) availability of reliable, multi-media (biota, sediment and water) mercury data from diverse ecosystem settings, including methylmercury determinations; (2) knowledge of the relative importance of factors controlling mercury methylation and bioaccumulation (mercury loading rates, mercury source type, ecosystem setting, and water and sediment chemistry); and (3) a better understanding of the toxicological significance of methylmercury exposure on wildlife.

The U.S. Geological Survey (USGS) has the multidisciplinary expertise, technical capabilities, and nationally distributed workforce to address many critical information gaps concerning the mercury problem. The Toxic Substances Hydrology Program recently initiated the National Mercury Project to help catalyze mercury research efforts. Many of these efforts are the result of scientific interactions of several USGS Divisions, or other USGS programs. Papers presented in this session will identify current UGSG activities that are helping to fill some of these information gaps, including areas where the USGS is uniquely qualified to carry out the needed work.

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A National Pilot Study of Mercury Contamination of Aquatic Ecosystems along Multiple Gradients

By David P. Krabbenhoft, James G. Wiener, William G. Brumbaugh, Mark L. Olson, John F. DeWild, and Ty J. Sabin

ABSTRACT

Mercury (Hg) contamination of aquatic ecosystems is a global problem. However, databases for Hg in environmental samples at regional-to-national scales are few, especially for multi-media sampling that include determination of methylmercury (MeHg). A national scale pilot study to examine relations of total Hg (Hg_T) and MeHg in water, sediment and fish was conducted in the summer and fall of 1998. Samples were collected at 106 sites from 21 basins across the United States, including Alaska and Hawaii. The data showed wide ranges in concentrations, which were expected given the diverse array of environmental settings, water chemistry, and Hg loading represented by these sites. Wetland density was the single most important basin-scale factor controlling MeHg production. At low concentrations, total Hg in sediment may also influence MeHg production, but at high total Hg concentrations (>1,000 nanograms per gram; ng/g) in sediment there was little evidence of increasing MeHg production with increasing total Hg. An atmospheric Hg accumulation index was developed for differentiating areas where atmospheric Hg deposition was the dominant Hg source from areas with significant on-site sources. Four study basins along the east coast of the United States had the greatest methylation efficiency, as reflected by the MeHg/HgT ratio in sediments. Nationwide, sub-basins characterized as mixed agriculture and forest cover types had the highest methylation efficiency, whereas areas affected by mining had the lowest efficiency. This study represents a first step toward a national assessment of Hg contamination of aquatic ecosystems in the United States, however, additional data are needed to improve our resolution of the factors controlling MeHg production and bioaccumulation.

INTRODUCTION

Methylmercury is the most toxic and widespread contaminant affecting our Nation's aquatic ecosystems. Methylmercury contamination has prompted steadily increasing numbers of fish-consumption advisories in 40 states, now accounting for more than eighty percent of all such advisories in the Nation (1,782 advisories for mercury of 2,196 total advisories nationwide; U.S. Environmental Protection Agency, 1998). Eleven states have statewide advisories for Hg in fish from lakes and/or rivers, and five have statewide advisories for coastal waters. In addition, some tribal representatives report that adherence to fish-consumption advisories has adversely affected the social, economic, and cultural well being of certain Native American tribes (for example, Wheatley and others, 1997; Wheatley, 1997). Methylmercury readily crosses biological membranes, can accumulate to harmful concentrations in exposed organisms, and biomagnifies to concentrations of toxicological concern in aquatic food webs, posing a threat to humans (Grandjean and others, 1997) and an increasing, potentially severe threat to fish-eating wildlife (Heinz and Hoffman, 1998).

For most aquatic ecosystems, atmospheric deposition of inorganic Hg (about 0.3 to 30 micrograms per square meter per year; U.S. Environmental Protection Agency, 1997) is the primary source of contamination. Generally only a small fraction of this atmospheric Hg load to aquatic ecosystems exists as MeHg (Rudd, 1995). In addition, the masses of MeHg found in sedimentary and biological compartments of most aquatic ecosystems cannot be accounted for by direct inputs of MeHg (Branfireun and others, 1998), and mass effluxes of MeHg from watersheds generally far exceed total inputs (Hurley and others, 1995). The bridge between the seemingly incongruent observations that inorganic Hg is the dominant form released to the environment, and MeHg is the dominant form of Hg found in edible fish (Bloom, 1992; Wiener and Spry, 1996) is the process of methylation. It is generally accepted that in natural settings mercury methylation is mediated through microbial sulfate reduction (Gilmour, 1991). Over the past 10 years, a great deal of scientific attention has been placed on trying to better understand mercury methylation. Yet, a complete understanding of the factors controlling methylation has remained elusive.

Management and regulatory responses to the growing mercury problem have been greatly impeded by a lack of information from a wide range of locations and environmental settings on the sources, transport, biogeochemical transformations, biological exposure, and toxicological consequences of mercury in the environment. Although there is a growing body of literature on mercury in the environment, to date there has not been a coordinated effort using consistent sampling and analytical methods conducted at the national scale. This paper presents the initial results of a pilot study to evaluate whether trends in Hg accumulation and MeHg production can be identified at national or regional scales across the United States. The study was designed so that a range of factors known to affect contamination levels, such as Hg loading rates, Hg source types, water chemistry, and land use and cover, could be evaluated.

THE USGS NATIONAL MERCURY PILOT STUDY

The National Mercury Pilot Study was conducted though the collaborative efforts of the Toxic Substances Hydrology program and the National Water Quality Assessment (NAWQA) program of the U.S. Geological Survey (USGS). The overall objective of this study is to identify ecosystem characteristics that favor the production and bioaccumulation of MeHg. Ultimately, we would like to be able to construct predictive models indicating environments of most concern for MeHg contamination, such as that depicted in figure 1. Ecosystems that have low methylation efficiency may exhibit low or moderate bioaccumulation even under high Hg loads, such as the Poplar Creek watershed, near Oak Ridge, Tennessee (Turner and others, 1993). On the other hand, significant bioaccumulation of methylmercury can result even when very low Hg loading rates exist if methylation efficiency is high, such as low-pH lakes in northern Wisconsin (Wiener and others, 1990; Lamborg, and others, 1995). Other ecosystems, such as newly constructed reservoirs, may experience a perturbation that increases methylation efficiency without increasing the mercury load (Bodaly and others, 1997). In this case, after flooding, the methylation efficiency of the reservoir would be expected to show a vertically upward trend across this conceptual model.

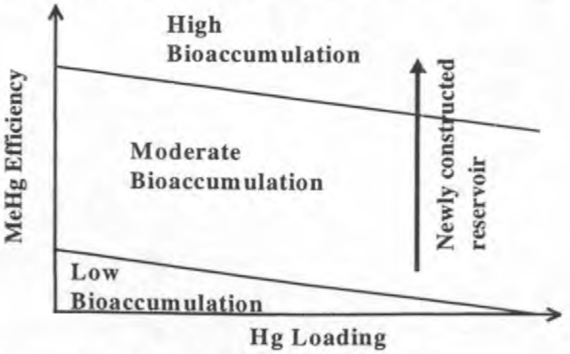

Figure 1. Hypothetical model for the relations between Hg loading, methylation efficiency and boaccumulation.

Regional and national scale fish surveys have been done in the past for mercury and other bioaccumulative contaminants. This is the only known study, however, in which national-scale, multi-media sampling (water, sediment, and fish) was conducted in concert with low-level Hg speciation analysis. Recent studies in Wisconsin have shown that several basin-scale factors influence the relative sensitivity of ecosystems to MeHg production (Hurley and others, 1995). These basin-scale factors include the relative abundance of wetlands (a site known to favor Hg methylation), soil types, and land use and cover. In Wisconsin, these factors serve as good predictors of mercury methylation efficiency, Hg and MeHg yields, and levels of MeHg in water. We caution that when viewed from a national perspective, however, the variation in atmospheric Hg-deposition rates across Wisconsin are small (U.S. Environmental Protection Agency, 1997), point-source contributions were presumably few and moderate in strength, geologic sources were negligible, and the range of ecosystem types were limited. Thus, we can not reliably transfer these results to a national scale.

Study Design

The major characteristics of this study were its (1) national scope, (2) emphasis on multi-media sample collection (water, sediment, and predator fish), (3) consistent use of trace metal clean sampling methods, and (4) low-level Hg and MeHg analytical procedures. By collecting and analyzing all three media, much more can be ascertained about controls of Hg and MeHg partitioning, accumulation, bioconcentration, and methylation efficiency across the vast diversity of sites that comprise our Nation's freshwater ecosystems.

The NAWQA program has scientists located throughout the United States who are trained in the procedures for the collection of water, sediment and biological samples; this greatly facilitated field efforts for this study. An additional benefit of conducting the pilot study at NAWQA study basins is the substantial amount of ancillary information available to strengthen the interpretations of the data generated via this study.

Sampling was conducted from June to October, 1998 at multiple locations (3 to 8) in each of 21 NAWQA study basins (figure 2, table 1) for a total of 106 sampling sites. Nationally, these basins spanned the dominant east-to-west mercury deposition gradient (figure 3) and represented a wide range of environmental settings. Individual study basin teams were asked to choose sites spanning gradients of wetland density, surface water pH, sulfate (SO_4), total organic carbon (TOC), and suspected or known Hg loading. In most cases, the sites sampled were streams, and every attempt was made to sample during baseflow conditions. Several of the chosen basins had known point sources of mercury from mining activity, and were representative of very high mercury loading conditions.

Table 1. NAWQA study basins from which samples were collected for this study.

Abbrev.	Study Basin Name
ACAD	Acadian-Pontchartrain
ALMN	Allegheny and Monongahela Basins
CKIB	Cook Inlet Basin
DELR	Delaware River Basin
GRSL	Great Salt Lake Basins
LINJ	Long Island and N. J. Coastal Drainages
LTEN	Lower Tennessee River Basin
MIAM	Great and Little Miami River Basins
MOBL	Mobile River and Tributaries
NECB	New England Coastal Basins
NROK	Northern Rockies Intermontane Basins
NVBR	Nevada Basin and Range
OAHU	Oahu Island
SACR	Sacramento Basin
SANA	Santa Ana Basin
SANT	Santee Basin and Coastal Drainages
SOFL	Southern Florida
TRIN	Trinity River Basin
UCOL	Upper Colorado River Basin
UIRB	Upper Illinois River Basin
YELL	Yellowstone Basin

Sampling Methods for Water, Sediment, and Fish

Aqueous Hg and MeHg samples were collected with trace-metal clean methods (Fitzgerald and Watras, 1989). All sample containers were Teflon (any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government), which had been rigorously cleaned in hot acid, partially filled with one percent HCl for storage, and then double bagged in Ziploc bags. Sampling crews wore plastic gloves, and executed clean-hands, dirty-hands techniques (Olson and DeWild, 1999) to remove the sample bottle from the two bags. Almost all of the sampling locations were streams, whereby grab samples were taken from the centroid of flow by submersing the bottle while wearing arm-length

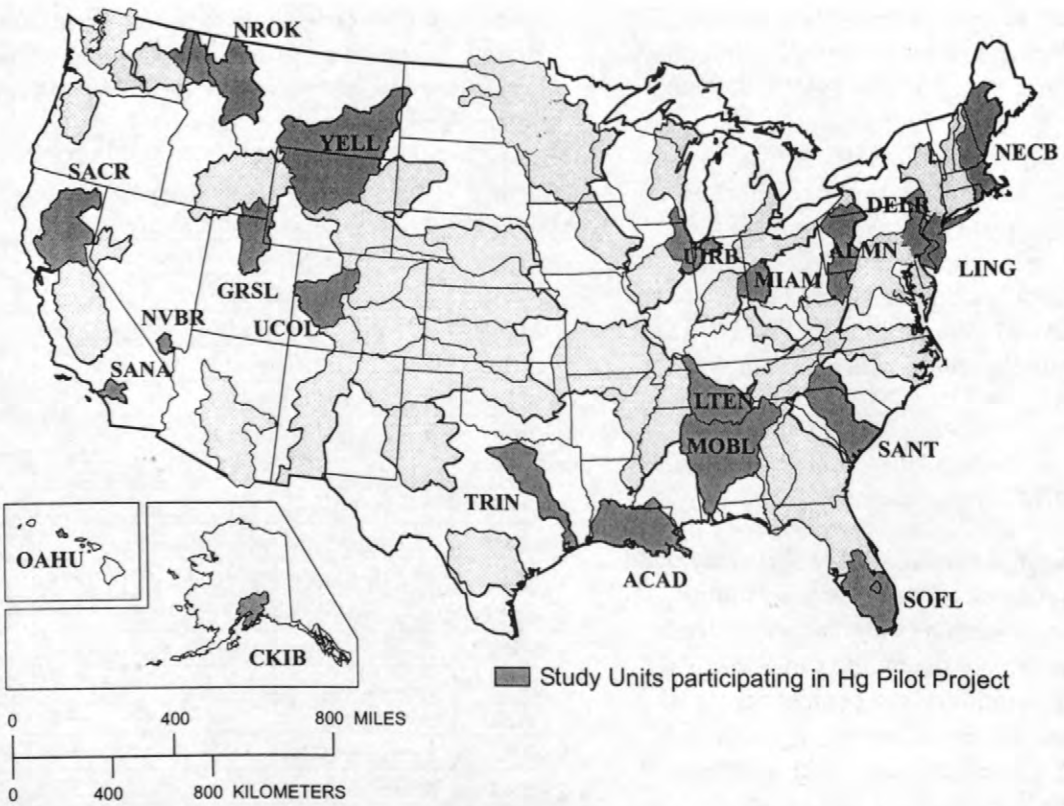

Figure 2. Locations of the twenty-one NAWQA study basins from which samples were collected. See Table 1 for the definitions of the basin names.

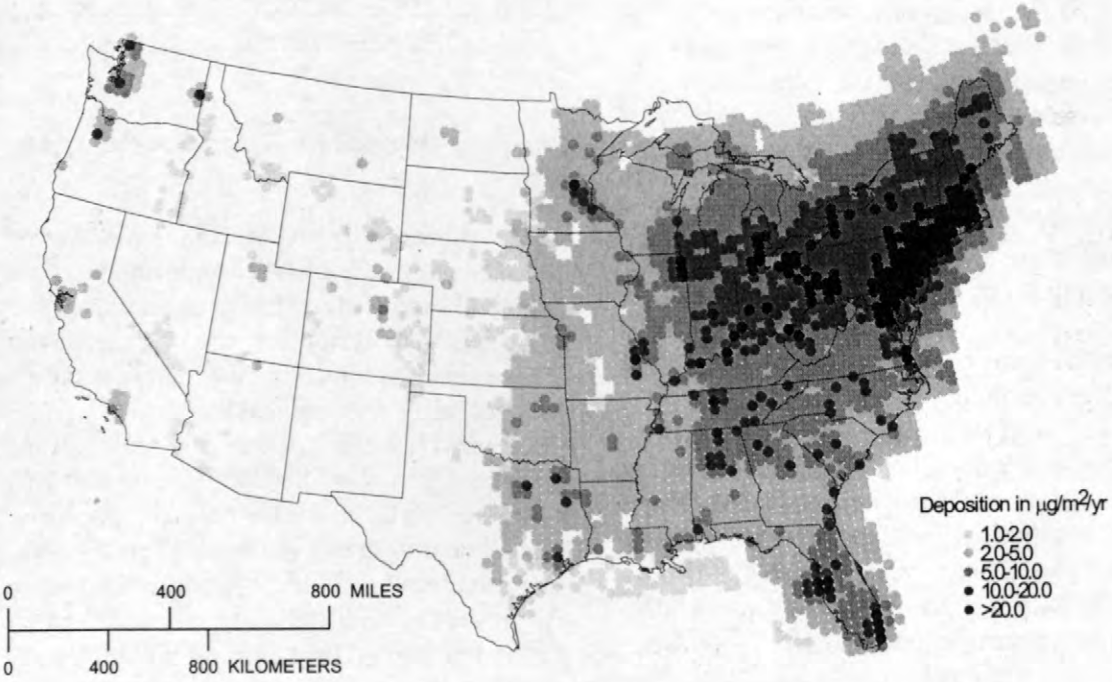

Figure 3. Estimated total mercury deposition(wet plus dry) for the contiguous United States. Source: USEPA, 1997.

gloves. Total-Hg (Hg_T) samples were acidified to one percent HCl by volume, while MeHg samples were immediately placed in a darkened cooler until they could be frozen.

Bed sediment samples were collected using the NAWQA program's trace-element sampling protocols (Shelton and Capel, 1994). Briefly, field personnel wore plastic gloves and used a clean Teflon or plastic scoop to collect the top few centimeters of sediment. Generally, samples were taken from multiple points (about 5 to 10) at each site, which were then pooled, homogenized, and then subsampled. A Teflon vial was used for the total Hg and MeHg subsample, while a second subsample was taken in a polypropylene vial and used for other analyses. Sediment samples were frozen as soon as possible.

Fish were collected by the most efficient means available, provided it did not jeopardize the specimen with regard to mercury contamination. Up to five individuals of a single, top-predator species were collected from each site. Field crews were asked to focus sampling efforts on largemouth bass of age 2-3 years, but in the absence of these fish to collect the most common predator species inhabiting the basin. If at all possible, the same species was collected from each sampling site within a study basin. Each fish was rinsed in stream water, measured for length and weight, placed in Ziploc bags, and frozen as soon as possible.

Anaytical Methods for Sediment and Water and Wetland Delineation

Details of the analytical procedures for Hg_T and MeHg in sediment and water are given in Olson and DeWild (1999) and Olson and others (1997). Samples collected for this study were shipped to the USGS Mercury Research Laboratory in Middleton, Wisconsin. Total mercury in aqueous samples was determined by cold vapor atomic fluorescence spectroscopy (CVAFS) following oxidation with BrCl at 50°C, reduction by $SnCl_2$, and purge and trap of the evolved Hg^0 onto gold-coated glass bead columns. Total mercury in sediments was determined with the same procedure, but samples were pre-digested with nitric and sulfuric acids in sealed Teflon bombs at 125°C. Methylmercury in sediment and water was analyzed with the distillation and aqueous phase ethylation method of Horvat et al. (1993), and detection by CVAFS. Analytical results for fish tissue samples were not available at the time of this paper, so the procedures are not given. Ancillary chemical parameters were determined by the following methods: pH was measured in the field with a calibrated probe; SO_4 by ion chromatography; DOC by a carbon analyzer that employs acidification and persulfate/UV oxidation; dry weight percent by drying wet sediment at 105°C; and loss on ignition (LOI) by heating dried sediment samples to 550°C.

A Geographic Information System (GIS) was used to quantify wetland areas within the study basins. Sampling locations for each study basin were plotted, and the sub-basin areas upstream of each point were delineated. Percent wetland area for each of the sub-basins was determined by overlaying a GIS coverage of the National Wetlands Inventory (Greg Allord, USGS, unpub. data, 1999).

RESULTS AND DISCUSSION

The study results are presented in three ways: across individual sites, among basins, and among site types.

All analytically determined parameters varied considerably within and among sites (table 2). This was expected given the extreme ranges in environmental settings (climate, geology, land use, and land cover), water chemistry, and Hg loading represented by this array of sampling sites. Generally, the Hg_T and MeHg concentrations in water and sediment were similar to the range of values from other recent freshwater studies that employed clean sampling and low-level Hg analysis. Very high concentrations of Hg_T and MeHg in water and sediment observed at the mining affected sites created a highly skewed distribution of concentration data. As a result, the mean values were much larger than their respective median values (table 2).

Overall, stronger correlations were observed between sediment MeHg and the

Table 2. Study basin names, type, percent wetland area of sub-basin, and analytical results from the sediment (dry weight) and water samples.[-- indicate no data, ng/L (nanograms per liter); ng/g (nanograms per gram); mg/L (milligrams per liter)]

				Water					Sediment		
Study Basin	Site Name	Site[1] type	% Wet-Land[2]	MeHg (ng/L)	Hg_T (ng/L)	TOC (mg/L)	pH	SO_4 (mg/L)	MeHg (ng/g)	Hg_T (ng/g)	LOI[3] (%)
ACAD	Mermentau R. @ Mermentau	Urb.	--	0.09	1.11	8.9	7.5	3.6	0.06	157.0	7.87
ACAD	Calcasieu River @ Kinder	A/F	--	0.15	3.68	5.2	6.8	3.7	0.05	1.9	0.26
ACAD	Bayou Boeuf	Urb.	--	0.05	1.66	8.6	8.9	21.06	0.10	53.5	5.93
ACAD	Turtle Bayou nr B. Penchant	Bkg.	--	0.03	1.03	8.6	7.4	29.07	0.41	83.6	32.46
ACAD	Bayou Lacassine	Ag.	--	0.46	1.61	11.1	6.7	3.03	0.26	88.1	10.06
ALMN	Youghiogheny R.	Mine	2	0.02	1.16	2.1	7.6	94	0.52	96.78	4.01
ALMN	Allegheny R. @ New Kensing.	Urb.	1	0.01	0.85	1.8	7.8	86	0.27	56.51	4.10
ALMN	Dunkard Crk. @ Shann.	Mine	0	0.04	0.64	2.6	7.5	195	0.15	12.8	4.26
ALMN	Tenmile Crk @ Amity	Bkg.	0	0.10	2.48	3.9	7.7	31	0.38	20.7	3.04
ALMN	Clarion River	Mine	1	0.09	8.09	4.2	7.4	39	1.08	76.5	2.81
CKIB	Chester Creek	Urb.	0	0.02	2.96	3.8	8	28	0.38	109.9	3.24
CKIB	SF Campbell Creek	Bkg.	0	0.02	2.50	1.6	7.8	9.3	0.67	200.0	2.83
CKIB	Deshka River	Bkg.	39	--	--	8.4	6.8	0.2	5.10	21.0	4.07
CKIB	Johnson R. abv Lateral Glacier	Bkg.	0	0.02	9.78	0.7	7.7	--	0.01	50.4	0.79
CKIB	Costello Creek	Bkg.	0	0.02	4.97	0.7	8.1	--	0.04	169.1	3.45
DELR	Little Neshaminy Ck.	Ag.	0	0.10	4.08	4.9	7.8	39	0.38	40.1	3.04
DELR	Tulpehocken Creek	Ag.	1	0.09	2.14	2.7	7.9	28	0.57	45.8	6.46
DELR	Hay Creek nr Birdsboro, PA	Ag.	1	0.04	0.77	1.4	7.8	24	1.64	36.4	5.45
DELR	Manataway Creek	Urb.	0	0.06	1.37	3.3	8.7	17	1.08	62.8	8.85
DELR	Raccoon Ck. @ Swedesboro	Ag	5	0.05	1.11	2.9	7.3	21	0.20	33.9	4.49
GRSL	Cub River nr. Richmond, UT	Ag.	0	0.03	2.60	2.1	8.5	9.9	0.14	11.3	1.80
GRSL	Weber River nr. Coalville, UT	Mine	13	0.10	21.76	3.1	8.5	14.0	4.02	1041	7.13
GRSL	Jordan River @ Salt L. City, UT	Urb.	2	0.03	4.80	3.3	7.5	217.6	3.20	116.3	3.97
LINJ	Passaic River, Millington, NJ	Urb..	0	0.24	2.72	5.5	6.9	15	2.67	89.8	15.20
LINJ	Swan River	Urb..	37	0.06	2.13	2.4	6	11	10.85	161.4	34.69
LINJ	Muddy Run	Ag.	30	0.06	2.25	3.9	7.3	10	6.03	252.3	43.48
LINJ	Great Egg @ Sicklerville	A/F	19	0.35	12.26	3.4	5.8	8.4	5.36	31.8	8.96
LTEN	Seqwatchie R. @ Whitnell, TN	A/F	--	0.01	1.38	1.4	7.6	6.7	0.18	10.4	2.32
LTEN	Buffalo River, Flatwoods, TN	Bkg.	--	0.01	1.45	0.9	7.2	6.1	0.64	47.7	2.88
LTEN	Indian Creek nr Madison, AL	Urb..	--	0.03	3.40	1.6	7.2	7.2	1.14	66.4	6.18

Table 2. Site names, type and analytical results from the sediment and water samples—Continued
[-- indicate no data]

Study Basin	Site Name	Site[1] type	% Wet-Land[2]	Water					Sediment		
				MeHg (ng/L)	Hg_T (ng/L)	TOC (mg/L)	pH	SO_4 (mg/L)	MeHg (ng/g)	Hg_T (ng/g)	LOI[2] (%)
MIAM	Stillwater R. on Springfield Rd	Ag.	1	0.05	2.10	3.3	7.6	56.87	0.17	34.0	1.04
MIAM	Great Miami R.	Ag.	1	0.05	2.52	3.4	7.9	64.2	0.34	31.4	1.41
MIAM	Mad R., Hwy 41, Springfield	Ag.	0	0.08	0.79	2.1	8.0	61.5	1.54	30.8	2.27
MIAM	Holes Creek	Urb..	0	0.05	1.10	2.5	7.8	37.23	0.26	10.5	1.02
MIAM	Great Miami R., Hamilton, OH	Urb..	1	0.19	3.00	5.0	8.4	73.5	0.67	87.2	2.36
MIAM	Whitewater R. @ Nulltown, IN	Ag.	0	0.03	0.81	1.9	7.7	33.67	0.43	11.7	1.51
MIAM	Little Miami R. @ Milford, OH	Urb.	0	0.05	1.57	3.5	8.3	49.3	0.45	9.2	1.12
MIAM	EF L. Miami R., @ Williamsburg	Ag.	0	0.07	2.09	4.4	8.3	44.9	0.24	13.3	1.03
MOBL	Shades Ck. @ Homewood, AL	Urb..	--	0.04	1.31	2.6	8.2	--	0.17	15.2	1.42
MOBL	Cahaba Valley Creek	Urb..	--	0.02	0.97	1.7	6.7	--	0.31	34.8	3.54
MOBL	Satilpa Ck.nr Coffeeville, AL	A/F	--	0.07	2.09	3.8	7.5	--	0.18	11.8	1.06
MOBL	Chickasaw Creek	A/F	--	0.21	2.27	5.5	6.3	--	0.65	11.1	2.11
MOBL	Alabama River @ Clairborne	Ag.	--	0.04	1.78	4.4	7.8	--	0.15	19.5	2.38
MOBL	Coosa River @ Rome	Ag.	--	0.04	4.68	2.4	7.5	--	0.15	33.2	1.74
MOBL	Tombigbee R. @ Coffeeville	Urb..	--	0.04	2.74	4.3	7.9	--	0.06	26.3	2.81
NECB	Stillwater River, Sterling, MA	Bkg.	2	0.25	0.53	2.1	6.6	10.9	7.02	72.9	17.45
NECB	Neponset R. @ Norwood, MA	Urb..	16	0.28	4.40	6.6	6.6	7.9	7.93	2477	20.23
NECB	Ipswich R. nr S. Middleton, MA	Urb..	27	0.44	2.72	7.0	6.6	15.6	9.91	380.0	22.54
NECB	Saugus R. @ Saugus Iron.	Urb..	34	0.11	2.79	4.4	7	19.5	4.41	309.2	16.70
NECB	Aberjona River	Urb..	2	0.08	9.11	4.0	6.5	24.4	7.14	1488	17.36
UCOL	Red Mountain Creek	Mine	0	0.02	1.68	0.4	3.3	484	0.13	107.2	6.40
UCOL	Dry Creek @ Begonia Road	Ag.	1	0.15	6.05	3.9	8.2	467	1.68	37.4	3.82
UCOL	Snake River @ Peru Creek	Mine	0	0.02	0.48	1.3	6.7	46	0.28	56.2	7.45
UCOL	French Gulch nr Breckenridge	Mine	0	0.02	0.64	1.4	7.8	63	0.21	113.2	5.76
UCOL	Colorado River @ Baker Gulch	Bkg.	13	0.05	0.57	1.3	7.8	6.2	2.84	27.4	6.18
NROK	Flathead River @ Perma, MT	Ag.	1	0.01	1.14	1.7	8.1	2.7	0.18	19.2	1.62
NROK	Clark Fork @ Turah Bridge	Mine	0	0.09	5.57	2.0	8.6	39.6	3.75	337.7	3.27
NROK	Clark Fork@ St. Regis, MT	Ag.	1	0.02	1.53	1.5	8.1	12.8	3.39	41.4	1.85
NROK	MF Flathead R. nr W. Glacier.	Bkg.	0	0.01	1.63	0.7	8.5	4.5	2.10	24.0	3.53
NROK	S. Fork Coeur d'Alene	Mine	0	0.01	8.91	0.6	7.0	61	8.21	4517	5.73
NVBR	Carson @ Dresslerville	Mine	0	0.16	3.42	1.5	8.3	24	2.73	66.2	5.53

Table 2. Site names, type and analytical results from the sediment and water samples—Continued
[-- indicate no data]

Study Basin	Site Name	Site[2] type	% Wet-Land[2]	Water					Sediment		
				MeHg (ng/L)	Hg_T (ng/L)	TOC (mg/L)	pH	SO_4 (mg/L)	MeHg (ng/g)	Hg_T (ng/g)	LOI[2] (%)
NVBR	Carson @ Markleeville	Bkg.	0	0.08	4.74	1.3	8.31	--	0.55	45.3	5.69
NVBR	Carson @ Deer Run Rd.	Ag.	3	0.68	31.08	6.9	8.1	52	1.21	78.4	1.73
NVBR	Carson @ Fort Churchill	Mine	2	0.51	1106	4.7	8.2	77	4.20	4130	3.33
NVBR	Carson @ Tarzan Rd.	Ag.	4	1.34	204.57	5.5	8.4	145	1.34	778.3	0.82
OAHU	S. Fork Lake Wilson	Urb..	--	0.12	1.93	3.3	6.8	--	1.18	300.2	17.02
OAHU	Kawainui Canal	Bkg.	--	0.02	1.00	3.1	7.0	--	0.64	106.2	41.79
OAHU	Ali Wai Canal	Urb..	--	0.01	1.17	0.6	8	--	0.34	255.7	21.62
OAHU	Nuuanu Reservoir	Ref	--	0.10	24.27	2.5	7.8	--	0.46	291.2	23.97
OAHU	Waikele Stream	Ag.	--	0.01	1.27	0.8	7.2	--	3.55	186.0	15.95
SACR	Bear River @ Hwy 70	Mine	0	0.24	17.82	3.5	7.4	5.6	0.55	176.8	2.89
SACR	Putah Creek @ Davis	Mine	0	0.05	4.10	2.3	8	28	0.27	275.6	3.86
SACR	Cottonwood Creek	Mine	0	0.03	1.02	1.5	8.5	10.8	0.36	26.3	2.61
SACR	Sacramento Slough	Ag.	2	0.15	10.19	3.2	7.9	7.9	2.84	128.5	7.07
SACR	Colusa Basin Drain	Ag.	2	0.08	6.90	5.9	7.9	55.3	0.52	53.3	6.85
SANA	Santa Ana R. blw Prado Dam	Urb..	0	0.14	14.67	4.8	7.8	105	1.47	45.6	5.77
SANA	Mill Ck. @ Chino-Cor. Rd.	Urb..	--	0.08	2.99	6.9	10.0	53.8	1.29	30.3	7.75
SANA	Santa Ana R. @ Hamner Rd.	Urb..	--	0.03	3.71	3.0	8.2	94.4	1.70	24.3	5.02
SANA	Santa Ana R. @ MWD Cross.	Urb..	--	0.05	2.28	2.3	8.1	87.6	3.42	27.3	5.81
SANA	S. Fork, Santa Ana River	Bkg.	0	0.02	0.61	0.6	7.7	1.4	3.65	28.8	17.22
SANT	NF Edisto R. nr Fairview Cross	A/F	16	0.32	3.77	4.8	6.1	1.1	6.80	86.6	12.04
SANT	NF Edisto River nr Branchville	A/F	16	1.48	9.41	3.8	6.4	2.9	2.50	6.1	15.46
SANT	SF Edisto River @ Springfield	A/F	16	0.41		3.0	6.3	--	2.70	41.5	28.09
SANT	SF Edisto River, Canaan	A/F	20	0.40	7.27	5.9	6.4	2.1	3.10	69.4	31.03
SANT	Edisto River, Givhans	A/F	36	1.36	8.18	4.6	6.6	4	5.80	70.1	21.31
SANT	Saluda River, Silverstreet, SC	Bkg.	0	0.09	1.05	2.9	7.1	5.5	0.70	23.9	6.67
SOFL	WCA 2, site U3	Bkg.	50	0.61	3.50	24.0	7.5	25.6	2.70	194.0	91.00
SOFL	WCA 2, site 2BS	Bkg.	50	0.45	2.10	18.0	6.9	10.2	3.90	234.0	90.00
SOFL	WCA 3, site 3A15	Bkg.	50	0.50	1.90	16.0	7.1	0.5	7.80	288.0	92.00
SOFL	Evergld s. Natl. Pk., site TS7	Bkg.	50	0.20	2.38	12.0	6.8	0.5	5.80	145.0	90.00
TRIN	Trinity River nr Crockett, TX	Urb.	0	0.02	6.50	5.8	7.5	--	0.28	30.6	4.42
TRIN	Lake Livingston	Urb.	1	0.02	1.34	6.0	--		0.20	45.2	7.59
TRIN	White Rock Ck. Dallas, TX	Urb.	0	0.04	1.65	4.9	--	--	0.14	8.6	
TRIN	White Rock Lake, Dallas	Urb.	0	0.02	1.24	5.6	7.9	--	0.52	55.4	6.24

Table 2. Site names, type and analytical results from the sediment and water samples—Continued
[-- indicate no data]

Study Basin	Site Name	Site[1] type	% Wet-Land[2]	Water					Sediment		
				MeHg (ng/L)	Hg_T (ng/L)	TOC (mg/L)	pH	SO_4 (mg/L)	MeHg (ng/g)	Hg_T (ng/g)	LOI[2] (%)
TRIN	Clear Crk. @ Sanger	Bkg.	0	--	--		8.0	55	0.23	5.9	2.18
UIRB	Des Plaines River	Ag.	15	0.10	4.18	11.4	7.5	94	3.58	11.6	1.40
UIRB	Nippersink Ck. bv Wonder L.	Ag.	0	0.04	1.42	3.5	7.8	64	0.08	8.7	2.30
UIRB	Salt Creek @ W. Springs, IL	Urb..	0	0.13	9.26	6.3	7.3	89	1.10	46.8	3.29
UIRB	Pitner Ditch nr LaCrosse, IN	Ag.	0	0.03	0.27	3.1	7.8	89	0.09	9.1	1.66
UIRB	Mukwanago R. @ Mukwanago	Ref	3	0.06	1.48	6.9	8.0	--	0.11	31.6	1.15
YELL	Bighorn River nr. Kane	Ag.	1	0.13	3.89	3.7	8.4	170	0.60	16.3	2.09
YELL	Bighorn Lake @ Hwy14A	Ag.	1	0.10	2.48	3.8	8.3	190	0.59	33.0	5.31
YELL	Shoshone River	Ag.	0	0.13	5.31	4.2	8.1	240	0.53	11.1	1.83
YELL	Tongue River	Ag.	4	0.06	1.87	3.4	8.5	94	3.05	27.7	8.23
YELL	Yellowstone R. near Sidney	Ag.	1	0.15	4.07	2.9	8.6	110		18.7	2.14
Summary Statistics											
Mean				0.15	16.6	4.15	5.3	55.5	1.87	211	11
Median				0.06	2.28	3.4	7.7	28	0.62	46.3	4
Std. Dev.				0.26	110.4	3.57		83.7	2.39	648	18
Coeff. of Variation				1.73	6.63	0.86		1.51	1.27	3.06	1.64
Minimum				0.01	0.27	0.4	3.3	0.2	0.01	1.85	0.0
Maximum				1.481	1106.7	24	10.09	484	10.851	4517	92
N				104	103	105	104	84	106	106	105

[1] General site categories for the sampling locations within each study basin:

Ag. = agriculture dominant.
A/F = agriculture and forested mix.
Bkg. = Background or reference site.
Mine = Current or abandon mining activity near sampling site.
Urb. = Urban or industrial activity near sampling site.

[2] Percent of each sub-basin classified as wetland areas.

[3] Percent of dry sediment mass lost on ignition (LOI) after firing to 550°C for two hours.

ancillary data (wetland density, pH, DOC and SO_4) than for aqueous MeHg results. This was likely due to the more transient nature of surface water samples compared to sediment, which tends to integrate site conditions over longer periods of time. Of the ancillary measurements, the strongest correlation was between sediment MeHg and percent wetland in the sub-basin (figure 4). Previous studies of MeHg production in boreal ecosystems also concluded that wetland density greatly influenced MeHg production (St. Louis and others, 1994; Hurley and others, 1995).

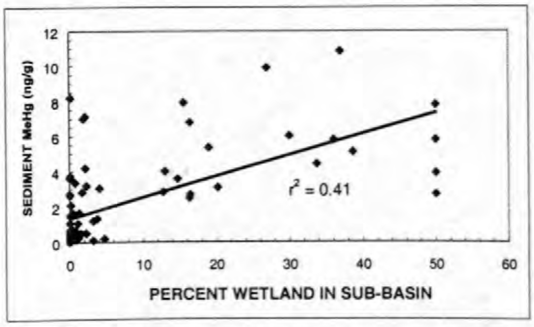

Figure 4. Sediment MeHg versus percent wetland in the sub-basins sampled during this study.

Similar positive correlations observed here suggest that these conclusions are valid over much broader geographical scales and ecosystem types. Sediment MeHg concentration also correlated positvely with LOI ($r^2 = 0.26$), which was used as a surrogate for organic carbon content of sediment. A subset of fifteen samples collected for this study and analyzed for organic carbon concentration showed a strong correlation with LOI ($r^2 = 0.97$). Negative correlations were observed between sediment MeHg and percent dry weight and surface water pH ($r^2 = 0.29$ and 0.15, respectively). The correlation results between MeHg and TOC was lower than expected ($r^2 = 0.18$), given that other studies have observed much stronger relations between these variables (Hurley and others, 1998). A more detailed analyses of the TOC quality from these various study basins may help to unravel the complexities of Hg-carbon interactions. Our observations suggest that mercury methylation is greatest for sub-basins with significant wetland density, organic sediments, and with low surface water pH.

One of the difficulties in analyzing Hg data from such differing ecosystems is the considerable variation in the measurable factors controlling important processes, such as methylation. For example, when we excluded the sites with mining impacts, a strong, positive correlation was observed between sediment MeHg and SO_4. When we included the data from the sulfate-rich mining sites, a weak, negative correlation was observed. At very high sulfate levels, methylation of mercury may not be limited by the availability of sulfate, or methylation can be inhibited by the abundance of sulfide (a by product of sulfate reduction) (Gilmour and others, 1998).

One important question concerning Hg bioaccumulation in aquatic ecosystems is whether Hg load drives methylation. The averaged, LOI-adjusted Hg_T and MeHg data for the 21 study basins suggest that Hg load (as reflected by Hg_T accumulation in sediment) has a logarithmic effect on methylation (figure 5). Methymercury production appears proporational to Hg_T concentrations at low sediment Hg_T levels; but at high Hg_T levels little additional MeHg is produced with additional Hg_T.

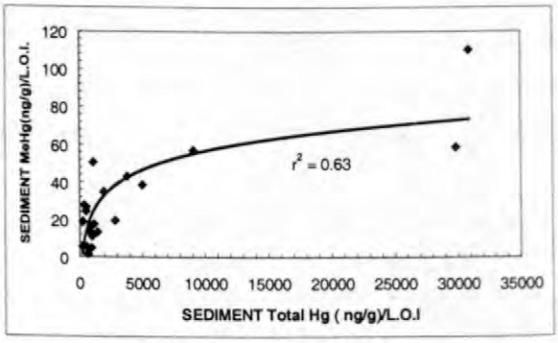

Figure 5. Average normalized (to LOI) sediment MeHg versus Hg_T for the 21 basins sampled for this study.

This finding is consistent with mercury methylation experiments on sediments, where a reduced methylation response to loading was observed when concentrations of $Hg_T > 1,000$ ng/g were used (Rudd and others, 1983). The two data points on the high end of the curve in figure 5 had Hg_T concentrations of about 1,000 ng/g (before normalizing to LOI).

The importance of atmospheric deposition relative other Hg sources within each study basin was assessed by normalizing sediment Hg_T concentrations to estimated current atmospheric deposition rates for each study basin and LOI. In essence, this calculation produces an index of atmospheric Hg accumulation (AHA). High AHA values would be diagnostic of study basins having Hg sources other than atmospheric deposition, and low index values would suggest the atmosphere is the dominant Hg source for the basin. The AHA values for all 21 study basins are shown in table 3. Those study basins with the six highest AHA indices were heavily contaminated with metals from mining. Interestingly, the next highest AHA index value is for the Oahu study basin, which has no known mining contamination sites. One likely Hg source for Hawaii is the Kilauea volcano. Volcanoes are known to emit gaseous Hg vapors; however, quantitative estimates of Hg emissions and their impacts are scarce. Although not a precise estimate, this analysis suggests Hg from Kilauea is depositing on Oahu Island at a rate similar to loading from areas where mining activity is pervasive. After the Oahu study basin, the next highest AHA value was for the Yellowstone study basin. One likely Hg source for this study basin is the numerous hot springs that are known to have high mercury concentrations (Rytuba, 1997). The remaining study basins all had AHA values that were notably lower. Many of these study basins were heavily urbanized, yet the AHA values suggested that the atmosphere was the dominant Hg source for these areas. It should be noted that several of these areas have relatively high Hg_T concentrations in sediment, such as the New England Coastal Basins, but that apparently most of this mercury can be accounted for by high atmospheric deposition rates. Ecosystem effects from pending legislation to reduce atmospheric mercury emissions would likely be most effective in such areas. The Southern Florida study basin, which had the lowest AHA value, provides a calibration point for this index, given that recent studies have demonstrated that atmospheric deposition is the dominant source of mercury to the Everglades (Guentzel and others, 1995).

Methylation efficiency is a critical factor affecting the susceptibility of ecosystems to bioaccumulation. Actual methylation rate estimates would be expensive to perform on a national basis, but the $MeHg/Hg_T$ ratio in sediments and water provides a reasonable predictor of methylation efficiency (Gilmour and others, 1998). Table 3 lists the average MeHg concentration in sediments and the average $MeHg/Hg_T$ ratio for sediment and water for each study basin. Both of these measures identify the New England Coastal Basins, Santee Basin, Long Island and N. J. Coastal Drainages, and Southern Florida as systems having enhanced methylation efficiency. All of these areas have widespread advisories for high Hg concentrations in game fish.

Table 3. Summary data for the 21 study basins.

Site Abbrev.	Hg Atm. Dep. Rate $(\mu/m^2/y)$[1]	AHA Index[2]	Sed. MeHg (ng/g)	$MeHg/Hg_T$ Ratio[3]
ACAD	4	170	0.17	0.05
ALMN	18	80	0.48	0.02
CKIB	1	3,828	1.24	0.03
DELR	25	31	0.77	0.03
GRSL	2	4,533	2.45	0.01
LINJ	27.5	19	6.23	0.06
LTEN	7.5	146	0.65	0.01
MIAM	17	114	0.51	0.03
MOBL	8	126	0.24	0.02
NECB	27.5	182	7.28	0.09
NROK	1.5	20,584	3.52	0.02
NVBR	1	29,817	2.00	0.02
OAHU	1	963	1.10	0.01
SACR	1.5	1,891	0.91	0.01
SANA	2	188	2.30	0.05
SANT	7.5	35	3.60	0.11
SOFL	25	9	5.05	0.10
TRIN	5	114	0.28	0.01
UCOL	1	1,153	1.03	0.04
UIRB	10	110	0.99	0.06
YELL	1	545	1.04	0.04

[1] Estimated from USEPA, 1997.

[2] Average Hg_T concentration observed for each study basin normalized to the atmospheric deposition rate and the average loss on ignition percentage of sediment.

[3] Average value for the $MeHg/Hg_T$ ratio for sediment and water.

All of the study basins were heterogeneous with respect to land cover and use. For the purposes of this analysis, the sub-basins above each of the sampling sites were categorized into one of the five following broad classes: agriculture dominant (Ag); mixed agriculture and forest (A/F); background or reference site for the study basin (Bkg); current or abandoned mining activity near sampling site (Mine); and urban or industrial activity near sampling site (Urb) (table 2). Total and methyl mercury concentrations in sediment and water differ significantly among these broad categories (table 4).

Table 4. Summary data for Hg_T and MeHg by land use/cover category.

Land use/cover (N)[1]	MeHg (ng/L)	Hg_T (ng/L)	MeHg (ng/g)	Hg_T (ng/g)
A/F (11)	0.48	5.59	2.73	34.07
Ag. (30)	0.15	10.76	1.20	73.34
Bkg (21)	0.13	3.43	2.10	104.9
Mine (14)	0.10	84.43	1.89	788.2
Urb. (30)	0.09	3.34	2.07	218.6

[1] Land use/cover definitions are found in table 2, N is the number of sampling sites falling each categories.

Sub-basins with mining operations present had the highest Hg_T concentrations in sediment and water, but MeHg levels were relatively low. Interestingly, sub-basins described as mixed agriculture and forest had the lowest average Hg_T concentration in sediment, yet the highest MeHg levels in sediment and water. Thus, the mixed agriculture and forest land type had the highest methylation efficiency, whereas mining and urban areas had the lowest (figure 6).

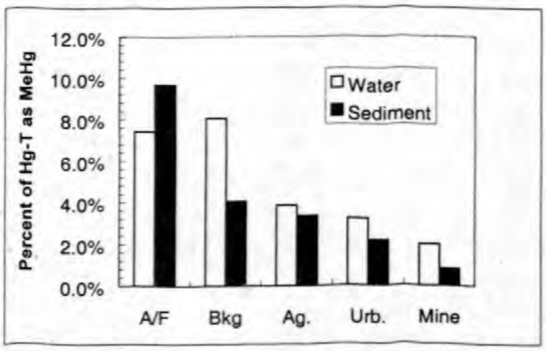

Figure 6. Average, normalized (to LOI) sediment MeHg versus Hg_T for the 21sub-basins sampled for this study.

SUMMARY

The concentrations of Hg_T and MeHg in sediment and water collected at 106 sites from 21 basins across the United States ranged widely. Variability in these data were expected, given the wide array of environmental settings, water chemistry, and Hg loading represented. Wetland density was found to be the most important basin-scale factor controlling MeHg production. By normalizing the sediment Hg_T and MeHg data to LOI, a logarithmic relation between Hg_T and MeHg was revealed. Methylmercury production appears proportional to Hg_T concentrations at low sediment Hg_T levels. At high Hg_T levels ($<1,000$ ng/g), however, little additional MeHg was evidently produced with increasing Hg_T. By normalizing the sediment Hg_T concentrations to LOI and the estimated atmospheric deposition rate for each study basin, a useful index (AHA) for assessing areas where atmospheric deposition is the dominant Hg source was obtained. Surprisingly, this index indicated that a significant Hg source other than atmospheric deposition exists for the Oahu study basin. Volcanic activity was a likely source of mercury in this basin. The New England Coastal Basins, Santee Basin, Long Island and N.J. Coastal Drainage, and Southern Florida showed the greatest methylation efficiency as reflected by the MeHg/Hg_T ratio in sediments. That all of these sites had low AHA indices suggests that pending emission reductions might be especially effective in these areas. Sub-basins characterized as mixed agriculture and forested had the highest methylation efficiency, whereas areas affected by mining were the lowest. This study was designed as a "pilot" effort to test whether multi-media sampling for low-level Hg determinations could be effectively conducted. While the spatial coverage of sampling was good, the site density (106 sites nationally) is probably not adequate for making final conclusions about mercury contamination of aquatic ecosystems across the United States. More detailed sampling, including sampling for seasonal differences, across this same series of study basins is needed to provide a more thorough analysis of what controls Hg methylation, partitioning, and bioaccumulation at national scales.

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Methylmercury in Aquatic Food Webs: Consequences and Management Challenges

By James G. Wiener and David P. Krabbenhoft

ABSTRACT

In the past decade, mercury contamination has prompted steadily increasing numbers of fish-consumption advisories in 40 states, now accounting for more than three-fourths of all such advisories in the Nation. Nearly all of the mercury in fish is methylmercury (MeHg), a highly neurotoxic form that readily crosses biological membranes, can accumulate rapidly in exposed organisms, and can biomagnify to high concentrations in aquatic food webs. Consumption of fish is the primary route of MeHg exposure in humans and in many species of wildlife. Scientific attention to the national mercury problem has been motivated largely by concerns regarding effects on human health, yet wildlife near the top of aquatic food webs are particularly vulnerable to MeHg. Recent work indicates that some fish-eating species, such as the common loon (*Gavia immer*), are being adversely affected in mercury-sensitive ecosystems, in which seemingly small inventories or inputs of total mercury can seriously contaminate aquatic food webs with MeHg. All ecosystems are contaminated to some extent with inorganic mercury from anthropogenic emissions, and actions taken to manage ecosystems can decrease or substantially increase both the net microbial production of MeHg and the subsequent exposure of biota to MeHg. The goal of decreasing biological exposure to MeHg presents major challenges to environmental managers and scientists alike.

INTRODUCTION

Scientific concern about mercury in aquatic ecosystems has stemmed largely from the harmful effects of methylmercury (MeHg) on human health (Clarkson, 1992). Nearly all of the mercury in fish is MeHg, a highly neurotoxic form that biomagnifies to high concentrations in aquatic food webs, and consumption of fish is the primary route of MeHg exposure in humans and most wildlife (Fitzgerald and Clarkson, 1991). The human poisonings caused by localized, industrial mercury pollution of aquatic food webs in Japan in the 1950s and 1960s prompted widespread reductions in direct releases of mercury to surface waters. Such efforts have usually resulted in gradual decreases in mercury levels in fish in affected waters.

More recent surveys have revealed high concentrations of mercury in fish from wetland ecosystems, low-alkalinity lakes, newly flooded reservoirs, and other surface waters lacking direct (on-site) sources of anthropogenic mercury (Ware and others, 1991; Spry and Wiener, 1991; Bodaly and others, 1997; Fitzgerald and others, 1998). Application of trace-metal clean techniques for sampling and analysis of air, rain, and surface water have shown that in certain ecosystems, seemingly small inventories or atmospheric inputs of total mercury can lead to high concentrations of mercury in fish (Fitzgerald and others, 1998). Many surface waters with fish-consumption advisories appear to be lightly contaminated environments in which sufficient inorganic Hg(II) is being converted to MeHg to contaminate aquatic food webs (e.g., Gilmour and others, 1998).

This paper briefly characterizes the Nation's fish-mercury problem and discusses the potential ecological consequences of MeHg in aquatic food webs, focusing largely on risks to fish-eating wildlife. We also briefly consider the implications of mercury contamination for ecosystem management, closing with a comment on the challenges associated with attempts to reduce exposure of biota to MeHg.

FISH-CONSUMPTION ADVISORIES

One response to the fish-mercury problem has been the provision of advice concerning the consumption of fish. Fish-consumption advisories are issued by the states and Native American tribes to protect humans from the health risks of eating contaminated sport fish. Mercury is the major cause of fish-consumption advisories, accounting for 78% of the contaminant-related advisories in the Nation (U.S. Environmental Protection Agency, 1998a). As of December 1997, mercury contamination had prompted 1,782 advisories in 40 states.

Awareness of the mercury problem has grown as more fish populations are surveyed (figure 1). During 1993 through 1997, the total number of mercury-related advisories doubled, and the number of states issuing advisories for mercury increased from 27 to 40. The number of statewide advisories issued for surface waters of a given type (lakes, rivers, or coastal waters) has also increased (figure 1). Eleven states have statewide advisories for mercury in lakes and/or rivers, and five have statewide advisories for coastal waters.

Adherence to fish-consumption advisories can adversely affect certain groups of fish consumers. For example, the effects of mercury pollution on Native American tribes with a historical reliance on subsistence fishing and hunting can be multidimensional, encompassing economic, cultural, social, spiritual, and health effects. For certain tribal groups, the adverse consequences of abandoning subsistence fishing and switching to alternative diets, combined with the associated disruptions in lifestyle, have presented a much greater problem than the direct, clinical effects of exposure to MeHg via consumption of contaminated fish (Wheatley, 1997; Wheatley and others, 1997).

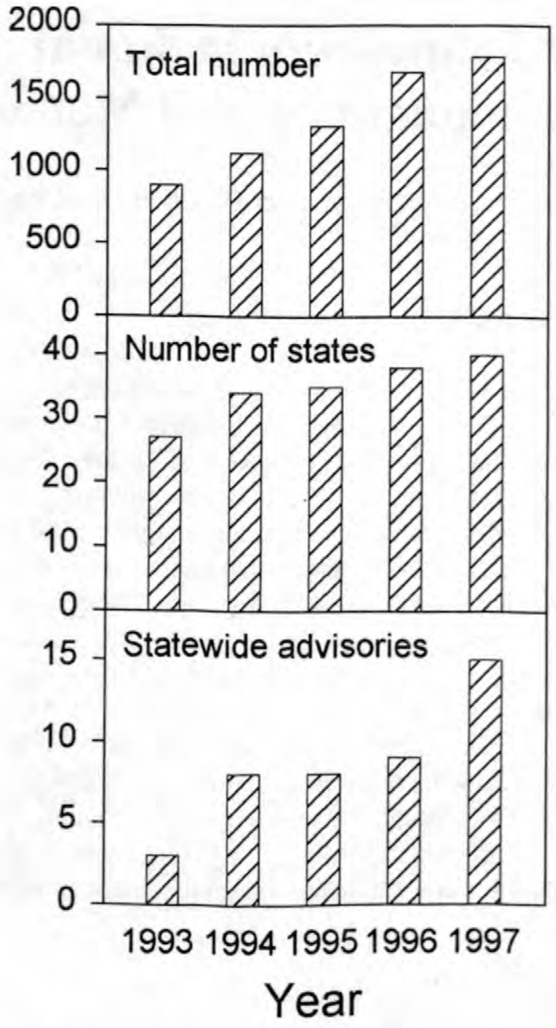

Figure 1. Recent national trends in the issuance of consumption advisories caused by mercury in fish and wildlife (source: U.S. Environmental Protection Agency, 1998a). Shown are total number of advisories for mercury (top panel), numbers of states issuing advisories (center), and number of statewide advisories for surface waters of a given type (bottom).

MERCURY-SENSITIVE ECOSYSTEMS

Many processes and variables influence mercury concentrations in fish (Wiener and Spry, 1996), which accumulate MeHg almost entirely via dietary uptake (Rodgers, 1994). The production of MeHg via the microbial methylation of inorganic Hg(II) in the environment is a key process affecting mercury concentrations in fish and other aquatic biota.

Certain aquatic ecosystems can be classified as mercury sensitive, in that seemingly small inventories or inputs of total mercury can seriously contaminate fish and adversely affect fish-eating wildlife. Given this definition, known mercury-sensitive ecosystems include wetlands, low-alkalinity lakes (acid neutralizing capacity ≤ 60 µeq/L), and surface waters with adjoining terrestrial areas subjected to flooding. A common attribute of these mercury-sensitive ecosystems is their efficient conversion of inorganic Hg(II) to MeHg (Xun and others, 1987; Bodaly and others, 1997; Kelly and others, 1997; Gilmour and others, 1998; Krabbenhoft and others, 1999). In some cases, concentrations of mercury in game fish inhabiting such ecosystems can equal or exceed concentrations in fish from waters heavily contaminated by wastes from industrial point sources, such as chlor-alkali plants (table 1).

Table 1. Elevated mercury concentrations (µg/g wet weight) in axial muscle tissue of game fishes in North American waters. Values shown are based on data reported for northern pike (*Esox lucius*), walleye (*Stizostedion vitreum*), largemouth bass (*Micropterus salmoides*), and smallmouth bass (*Micropterus dolomieu*).

Ecosystem	Hg concentration	
	Range in means	Range in maxima
Waters polluted by chlor-alkali plants[1]	1 - 5	2 - 15
Newly flooded reservoirs[1]	0.7 - 3	2 - 6
South Florida wetlands[2]	0.4 - 1.4	2 - 4
Low-alkalinity lakes[1]	0.5-0.9	1 - 3

[1] From values summarized by Wiener and Spry (1996).
[2] Data for largemouth bass from T. R. Lange (Florida Game and Freshwater Fish Commission, Eustis, FL, USA, personal communication).

EFFECTS ON FISH AND WILDLIFE

Vertebrate organisms have few defenses against MeHg. Methylmercury is efficiently assimilated across the gut, readily crosses internal cellular membranes, distributes rapidly via the blood stream to all internal organs and tissues, and accumulates readily in exposed organisms, exerting its most harmful effects on the central nervous system (Clarkson, 1994). Concentrations of MeHg in fish often exceed those in the surface water in which they live by a factor of 1 million to 10 million. Fish do not effectively excrete MeHg, and the binding of MeHg to proteins in skeletal muscle, the primary storage site, may serve as their primary detoxification mechanism by reducing the exposure of the brain to MeHg (Wiener and Spry, 1996). In birds and mammals, some mercury is excreted slowly in the feces after demethylation in the intestine (Clarkson, 1994). Birds and mammals can also decrease exposure of the central nervous system by sequestering MeHg into growing feathers and hair (Scheuhammer, 1991; Clarkson, 1994).

The developing embryo is the most sensitive life stage of vertebrate organisms (Scheuhammer, 1991; Clarkson, 1994), and reproductive success can be adversely affected by dietary MeHg exposures that cause no apparent symptoms of toxicity in the adult. In birds, for example, the effects of MeHg are most severe in embryos and chicks, and low-level dietary exposures that cause no measurable effect in the adult female can significantly impair egg fertility, hatchling survival, and overall reproductive success (Scheuhammer, 1991). In mammals, the prenatal life stage is most sensitive to MeHg, and all prenatal effects appear to be irreversible (Clarkson and others, 1984). In contrast to the situation for birds and mammals, little is known about the effects of maternally transmitted MeHg on the embryolarval stages of fish (Wiener and Spry, 1996).

It has been widely believed that selenium protects vertebrate organisms against MeHg poisoning. In an experiment with mallards (*Anas platyrhynchos*), however, Heinz and Hoffman (1998) showed that the toxic effects on the developing bird embryo were much greater when selenium and MeHg were added jointly to the maternal diet than when MeHg was added without selenium. In the same experiment, dietary selenium decreased MeHg toxicity in the adult (Heinz and Hoffman, 1998). Thus, selenium does not seem to protect against reproductive effects of MeHg.

Fish

The survival, hatching, and development of the fish embryo can be severely impaired by a small quantity of MeHg or inorganic mercury, but the amount of mercury transferred to the eggs during oogenesis is small (Hammerschmidt and others, 1999). A recent review (Wiener and Spry, 1996) indicated that the principal effect of MeHg on fish populations--if any, at environmentally realistic exposure levels--would be reduced reproductive success resulting from toxicity of maternally derived mercury to embryonic and larval stages. Furthermore, for some fish populations, the margin of safety between harmful and existing mercury exposure levels may be much less for embryo-larval stages than for adults (Wiener and Spry, 1996).

Fish-Eating Wildlife

Piscivorous birds and mammals are particularly vulnerable to MeHg, because of their trophic position near the top of aquatic food webs. Concentrations of mercury in tissues of wild river otter (*Lutra canadensis*) and mink (*Mustela vison*), for example, substantially exceed those in fish from their feeding areas (Kucera, 1983; Wren and others, 1986). Reproduction is the biological endpoint most sensitive to MeHg exposure, given the extreme neurotoxicity of MeHg to the developing embryo (Scheuhammer, 1991; Clarkson, 1994). However, adult birds and mammals subsisting on mercury-contaminated fish can accumulate lethal doses of MeHg (e.g., Wobeser and Swift, 1976; Wren, 1985).

Many species of wildlife consume large amounts of fish, a factor that increases their exposure to MeHg. Rates of fish consumption by the common loon (*Gavia immer*), for example, vastly exceed those for humans, even for persons who consume greater than average quantities of fish (table 2). Chicks of common loons eat more than 50 kg of fish during their first 15 weeks after hatching, whereas older loons consume about 1 kg of fish per day (Barr, 1996). In comparison, the estimated median daily rate of fish consumption by adult humans in the United States is about 42 g in males and 31 g in females, based on the USDA Continuing Surveys of Food Intake by Individuals during 1989-1991 (U.S. Environmental Protection Agency, 1998b).

Barr (1986), who worked in the industrially contaminated English-Wabigoon River system in Canada, demonstrated a strong negative correlation between the successful use of breeding territories by common loons and mercury contamination. Reductions in egg laying and territorial fidelity were associated with mean mercury concentrations of 0.3 μg/g wet weight or greater in the prey of loons (Barr, 1986). Thus, 0.3 μg/g wet weight is an estimated dietary threshold concentration of MeHg causing impaired reproduction in the common loon. In Ontario, an estimated 30% of the lakes examined by Scheuhammer and Blancher (1994) had prey-size fish with mercury concentrations exceeding this threshold level.

Table 2. Relative daily rates of fish consumption by humans and common loons.

Organism	Consumption rate	
	g/d	g/d per kg biomass
Adult female human[1]		
median	31	0.6
95th percentile	110	2.2
Common loon[2]		
chick (first 77 d)	400	220-410
adult	960	190

[1] From USDA Continuing Surveys of Food Intake by Individuals during 1989-1991.

[2] From Barr (1996).

Recent studies suggest that fish-eating wildlife are being adversely affected by dietary MeHg in some mercury-sensitive ecosystems. Common loon chicks reared on low-pH Wisconsin lakes, which contain prey fish with higher mercury concentrations than nearby lakes with high alkalinity, have elevated concentrations of mercury in their blood (Meyer and others, 1998). In addition, the production of chicks is lower in lakes where chicks have elevated mercury concentrations in blood (Meyer and others, 1998). Scheuhammer and others (1998) compared concentrations of mercury in emaciated and healthy common loons in eastern Canada. The mean concentration was several fold greater in the emaciated than in the healthy loons. Moreover, many of the emaciated common loons had mercury concentrations as high or higher than those associated with MeHg poisoning in toxicity studies (Scheuhammer and others, 1998).

Some wildlife species in upper trophic levels of the Florida Everglades, including wading birds (Ogden, 1994) and the Florida panther (Facemire and others, 1995), have declined substantially in recent decades. High exposure to MeHg has been documented in both wading birds (Beyer and others, 1997) and the Florida panther, *Felis concolor coryi* (Facemire and others, 1995) in the Everglades ecosystem. For the Florida panther, the evidence suggests that MeHg-related reproductive failure was a substantial contributor to the population decline (Facemire and others, 1995).

Exposure of fish-eating wildlife to MeHg has probably increased substantially during the past century in response to anthropogenic releases of mercury into the environment. Substantial increases in MeHg in marine food webs in the North Atlantic Ocean, for example, were revealed by analyses of feathers from fish-eating seabirds sampled from 1885 through 1994 (Monteiro and Furness, 1997). The long-term increase in concentration of mercury averaged 1.9% per year in Cory's shearwater (*Calonectris diomedea borealis*) and 4.8% per year in Bulwer's petrel (*Bulweria bulwerii*; figure 2). Monteiro and Furness (1997) determined organic mercury in feathers from museum specimens to avoid problems associated with inorganic mercury contamination of the samples. These increases probably reflect global trends in mercury contamination, rather than trends in contamination from local or regional sources.

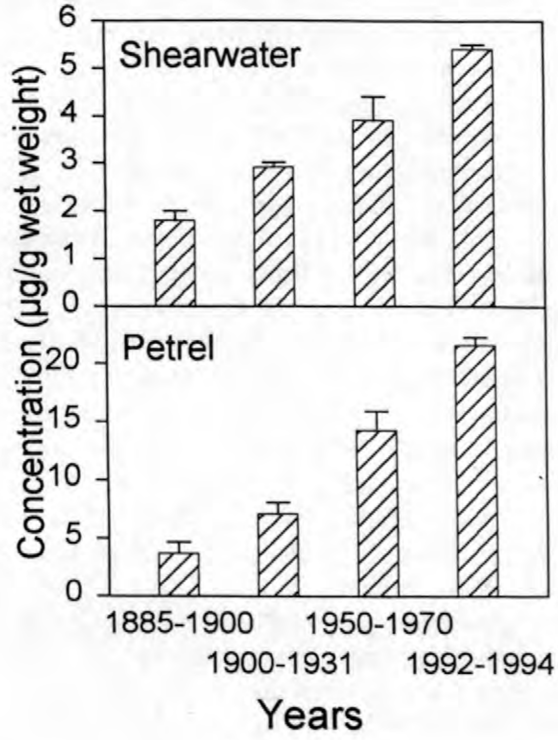

Figure 2. Historical trends of increasing MeHg concentrations (mean ± 1 standard error) in feathers of two species of fish-eating seabirds sampled from the North Atlantic Ocean during 1885-1994 (source: Monteiro and Furness, 1997).

IMPLICATIONS FOR ECOSYSTEM MANAGEMENT

Thus far, little attention has been devoted to ecosystem management as a potential approach for decreasing MeHg exposure. All ecosystems are now contaminated to some extent with inorganic mercury from anthropogenic emissions, because of long-range atmospheric transport and deposition (Fitzgerald and others, 1998). It is evident that ecosystem management actions can either decrease or increase the net microbial methylation of inorganic Hg(II) and the subsequent exposure of biota to MeHg in managed ecosystems.

Alteration of lake chemistry has been used as an ecosystem-management approach for decreasing mercury contamination of fish in certain waters. In low-pH lakes, for example, the high concentrations of MeHg in fish have been attributed partly to within-lake processes, particularly the microbial production of MeHg, that are influenced by pH or associated chemical variables (Winfrey and Rudd, 1990; Gilmour and Henry, 1991). Accordingly, the chemical manipulation of lakes to increase lake water pH is considered a potential management approach for decreasing concentrations of mercury in resident fishes. In Scandinavia, lake and watershed liming have been used to mitigate the effects of surface-water acidification (Appelberg, 1998) and to decrease mercury levels in fish (Andersson and others, 1995).

Some ecosystem-management actions can worsen the MeHg problem by creating environmental conditions that increase the production of MeHg from inorganic Hg(II) present in the managed ecosystem. The flooding of terrestrial habitats during the creation of new reservoirs, for example, greatly increases the microbial methylation of inorganic mercury present in the inundated habitats (Bodaly and others, 1997; Kelly and others, 1997). Increases in MeHg concentrations after flooding, based on a recent review (Bodaly and others, 1997), range from 10 to 20 fold in water and zooplankton, 2 to 3 fold in benthic invertebrates, and 2 to 9 fold in fish. Moreover, mercury concentrations in fish may remain elevated for 20 to 30 years after reservoir creation (Bodaly and others, 1997).

MANAGEMENT AND SCIENTIFIC CHALLENGES

In a toxicological sense, the mercury problem in aquatic ecosystems can be defined quite simply as *exposure to MeHg*. It follows that the challenge to environmental managers is *to decrease exposure to MeHg.*

The identification of effective management responses to the Nation's growing mercury problem has been impeded by a lack of information on the sources, behavior, and effects of mercury in the environment (Krabbenhoft and Wiener, 1999). The mercury problem is characterized by a large geographic scope and by enormous complexity. Integrated science and a firm commitment to action will be needed to resolve critical questions and to guide efforts to decrease MeHg exposure and to reduce the associated risks to humans and wildlife.

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Mercury Contamination: A Nationwide Threat to Our Aquatic Resources, and a Proposed Research Agenda for the U.S. Geological Survey

By David P. Krabbenhoft and James G. Wiener

ABSTRACT

This document describes the national mercury problem, identifies critical data gaps, and proposes the framework for a national mercury investigation. The overall goal of this investigation is to provide scientific information needed by resource managers and environmental planners to identify and evaluate options for reducing exposure of humans and wildlife to this highly toxic metal. Several key questions regarding environmental mercury contamination will be addressed. What are the relative contributions of natural sources and selected human sources to the mercury-contamination problem? What criteria can be used to identify mercury-sensitive ecosystems, in which seemingly small inputs of mercury can cause serious contamination of fish and fish-eating wildlife? What factors and processes control the sensitivity of aquatic ecosystems to mercury? Are there potential ecosystem-management approaches for reducing methylmercury contamination of aquatic biota? What levels of mercury in fish are harmful to fish-eating wildlife? Answers to these questions will provide information critically needed to assess and reduce health and ecological risks of environmental mercury contamination.

INTRODUCTION

Mercury (Hg) is one of the most serious and scientifically challenging contaminant threats to our Nation's aquatic resources. Scientific interest in Hg in aquatic ecosystems has been motivated largely by the health risks of consuming contaminated fish, the primary source of Hg in the human diet. Awareness of the extent of the Hg problem continues to increase as more fish populations are surveyed. For example, the number of Hg-related advisories has grown rapidly, increasing 98% from 1993 (899 advisories) through 1997 (1,782 advisories) (figure 1). Moreover, the number of states issuing advisories for Hg increased from 27 in 1993 to 40 in 1997.

For most ecosystems, atmospheric deposition is the primary Hg source, although there are numerous instances of geologic and anthropogenic point-source contamination problems. Since the industrial revolution, anthropogenic Hg emissions have increased atmospheric Hg levels about three to five fold (Fitzgerald, 1995), and caused corresponding increases in Hg levels in terrestrial and aquatic ecosystems. The rate at which rising atmospheric Hg loads translate into increased concentrations in wildlife, however, has remained difficult to predict due to the complex nature of the aquatic Hg cycle.

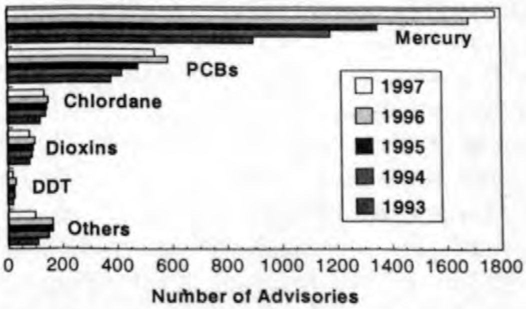

Figure 1. Trends in the number of fish-consumption advisories issued for various pollutants. (source: U.S. Environmental Protection Agency, 1998)

Toxicologically, the most important process affecting Hg in the environment is methylation, the process by which inorganic Hg is converted to methylmercury (MeHg). Methylmercury is highly neurotoxic, damaging to the central nervous system, and the embryos of vertebrate organisms are much more sensitive than adults (Scheuhammer, 1991; Heinz and Hoffman, 1998). In birds, for example, the effects of MeHg are most severe in embryos and chicks. Low-level dietary exposures that cause no measurable effect in the adults can significantly impair egg fertility, survival of newly hatched chicks, and overall reproductive success. Common loon (*Gavia immer*) chicks reared on low-alkalinity Wisconsin lakes, which contain prey fish with higher Hg concentrations than nearby high-alkalinity lakes, have elevated concentrations of Hg in their blood (Meyer and others, 1995; 1998). Moreover, chick productivity in Wisconsin lakes is inversely correlated with blood-Hg concentrations (Meyer and others, 1998). In Ontario, an estimated 30 percent of the lakes examined by Scheuhammer and Blancher (1994) had prey-size fish with Hg levels considered high enough to impair reproduction of common loons.

Many species of wildlife consume large amounts of fish and can obtain harmful doses of MeHg via dietary uptake. Chicks of common loons, for example, consume more than 50 kg of fish during their first 15 weeks after hatching, whereas adults consume about 1 kg of fish per day (Barr, 1996). If fish consumption is expressed as a percentage of the individual consumer's body mass, rates of fish consumption by common loons exceed those of humans (U.S. population) by a factor of 100 or more (table 1). At these consumption rates, fish considered safe for periodic consumption by humans may contain enough MeHg to adversely affect fish-eating wildlife.

This document describes the framework for a national mercury investigation for the U.S. Geological Survey. The goal of the investigation is to provide scientific information needed by resource managers and environmental planners to identify and evaluate options for reducing exposure of humans and wildlife to this highly toxic metal.

Table 1. Relative Rates of Fish Consumption in Common Loons and Humans

	Daily Consumption of Fish	
Organism	Grams per individual	Percent of body mass
Adult female human (U.S.)[1]		
Median	31	0.06
95th percentile	110	0.22
Common loon[2]		
Chick (first 11 weeks)	400	22-41
Adult	960	19

[1] U.S. Department of Agriculture, Continuing Surveys of Food Intake by Individuals during 1989-1991.

[2] Barr, 1996.

A Critical Assessment of the Mercury Problem Facilitated by the USGS

On July 7-9, 1996, the U.S. Geological Survey (USGS) sponsored a multi-agency workshop on Hg in the environment to transfer information, identify data and information gaps, and identify specific areas of investigation where the USGS could provide scientific and technical leadership. Technical working groups at the workshop achieved general consensus on the state of scientific knowledge and critical information gaps concerning Hg sources and transport, biogeochemical cycling processes of Hg, and MeHg bioaccumulation and effects. The conclusions of the technical working groups were as follows:

Sources The USGS should focus on providing critical quantitative information on Hg point sources and atmospheric loading of Hg. A quantitative inventory of the principal man-related and natural Hg sources could be produced with existing databases and some additional studies. The analysis of dated cores would be particularly useful for determining regional and historical trends in Hg deposition patterns on a national scale.

Biogeochemical Cycling The USGS should focus on providing an improved understanding of the processes controlling MeHg production, destruction, and uptake by organisms in a wide range of environments across the United States. Process-oriented studies are needed to explain the large variation in bioavailability of this toxic metal among aquatic ecosystems. Study sites should span gradients in Hg source types (e.g., atmospheric versus point source dominated), as well as climate, geology, hydrology, and trophic structure.

Methylmercury Bioaccumulation and Effects USGS investigations should focus on environmental factors affecting MeHg exposure to top-level consumers and adverse effects on wildlife.

National Mercury Investigation Study Design

A policy-relevant, national research effort on Hg is needed. The U.S. Geological Survey (USGS) has the multidisciplinary expertise, technical capabilities, and nationally distributed workforce to address many critical information gaps concerning the Hg problem. This investigation would include four coordinated, multidisciplinary components: (1) national information inventories of mercury sources; (2) regional assessments to determine patterns (and controlling factors) of mercury contamination in the nation's aquatic ecosystems; (3) ecosystem investigations to determine the processes and factors influencing methylmercury exposure in aquatic ecosystems; and (4) studies to determine the toxicological significance of methylmercury exposure in fish-eating wildlife, with emphasis on reproductive effects.

National Inventories of Mercury Sources and Deposition

Regulatory and management decisions to mitigate Hg exposure through reductions in emissions requires an understanding of the linkages between sources and ecosystem impacts. Until a more refined assessment of atmospheric-Hg loading (natural and anthropogenic) is achieved, however, the efficacy of anthropogenic emissions reductions as a Hg exposure reduction tool is unknown. Currently, the largest uncertainties in the global Hg mass balance are those associated with natural Hg sources.

The first inventory objective is to quantify Hg releases to the environment from natural and anthropogenic sources. Globally, total emissions (natural and anthropogenic) of Hg to the atmosphere range from 6000 to 8000 metric tons per year, with about half to three quarters coming from man's activities (Nriagu and Pacyna, 1988; Fitzgerald, 1995). However, large uncertainties currently exist for these global Hg fluxes. Estimates from natural sources are particularly poorly documented and further research is required to more accurately define the flux from these sources. Emissions from the ocean are thought to contribute about half of the natural Hg flux to the atmosphere (Mason and others, 1994). Other natural sources, in decreasing order of importance, include: erupting volcanoes, soil vapor flux, geothermal system-hot springs, passively degassing volcanoes and fumaroles, and active faults. A concerted effort to quantify the amount of mercury contributed to the global cycle from these geologic sources would greatly reduce the large uncertainties in global mercury fluxes.

A second contribution the USGS can make toward national information inventories is to improve available databases and estimates of man-related mercury emissions from coal combustion. The USGS has a long data record on the mercury content of various coal beds throughout the United States. Although estimates of man-related mercury emissions are probably more accurate than those of natural emissions, additional efforts are needed to reduce the level of uncertainty in these estimates. As the availability of gas and oil resources become more limited, energy production from coal may increase in the United States and elsewhere, which may have a large impact on the atmospheric mercury loading. Mercury content of coal varies substantially, and how this energy resource is utilized may have significant impact on future mercury emissions and contamination levels of aquatic ecosystems.

The last inventory objective is to document current and historical trends in mercury deposition. Dated sediment cores have been used to estimate current and historical mercury deposition rates (Engstrom and Swain, 1997), but an expanded network of sites is needed to map regional or sub-regional differences in deposition rates. Compiling all the existing data on mercury from dated cores and examining this data set for regional and temporal trends could make an immediate contribution. Ice cores provide an alternative to sediments for estimating historical Hg-deposition rates, and give a direct estimate of atmospheric mercury deposition for a particular locality, but in very few locations is ice coring a viable option. Sediment and peat cores, on the other hand, can be taken from many more locations (deep lakes, reservoirs, and ombrotrophic bogs), but are only an indirect estimator of mercury deposition. Combined use of sediment and ice cores could add valuable information on spatial and temporal trends in mercury accumulation patterns at the national scale.

Regional Assessments

The overall goal of the regional assessments is to identify methylmercury hot spots and ecosystem characteristics associated with high methylmercury levels in aquatic biota. This goal would be accomplished by analyzing and comparing existing and new data on Hg and MeHg in biota, sediments, and water. By collecting and analyzing samples of all three media, more can be ascertained about Hg and MeHg partitioning, accumulation, and bioconcentration. Reliable data on MeHg and Hg from multiple media (biota, sediment, and water) are available for few areas, and large areas of the nation lack data to infer existing or potential methylmercury contamination. West of the Mississippi River and excluding California, reliable data on MeHg in aquatic biota, sediment and water are available for only one site, the Carson River basin. In the eastern half of the country, only about five sites have been intensively studied. Moreover, there are many potentially sensitive, aquatic ecosystems for which little or no comprehensive mercury information are available, including black-water coastal streams; dilute, high-elevation lakes; southwestern reservoirs; watersheds downstream of abandoned mercury-contaminated mines; urban rivers and lakes; playa lakes; and arid basins.

We propose a series of regional-to-national scale, multi-media synoptic sampling efforts be conducted through collaborations between this investigation and other USGS programs and other agencies that desire such information. These assessments should be conducted on a national basis, using consistent sampling and analytical methods to ensure comparability of the results. The general sampling design would include ecosystems that represent diverse environmental settings of climate, geology, soils, land use, and land cover. In addition, the selected sites will span gradients in five primary factors that have consistently been shown to affect mercury abundance, methylation, and bioaccumulation: wetland density, Hg-loading rate, and water-chemistry factors of pH, organic carbon, and sulfate. In 1998, a pilot-scale test of this type of study was conducted collaboratively between the Toxics Substances Hydrology program and the National Water Quality Assessment (NAWQA) program (Krabbenhoft and others, 1999). This study showed several important results, including: (1) effective, Hg-clean sampling for sediment, water, and game fish could be carried out nationally by the USGS; (2) wide ranges of Hg and MeHg concentrations were observed in sediment and water, reflecting ranges of source-load impacts; (3) wetland density was the most important basin-scale factor controlling MeHg production; (4) of the basins sampled, those that favored MeHg production were generally in the eastern United States; (5) mining-impacted sites had the highest Hg concentrations in water and sediment, but the lowest methylation-efficiency in sediments (MeHg/Hg ratio); (6) other Hg sources, such as volcanic activity may be important regionally. Although this pilot study included 106 sites in 21 NAWQA study basins, this sampling density was probably not adequate to make final conclusions about mercury contamination of aquatic ecosystems across the United States. We intend to use what was learned from this pilot study to aid the design of the next synoptic sampling effort. New efforts may include examinations of seasonal differences in Hg and MeHg concentrations and partitioning, information on dissolved and particulate phases, increased spatial resolution from selected basins, controls of sulfur biogeochemistry, information on organic carbon quality as well as quantity, and possibly sampling several levels of each food web.

Ecosystem Investigations

The goal of the ecosystem investigations is to determine the biogeochemical processes that control the production, bioaccumulation, and food-web transfer of methylmercury in aquatic ecosystems. Information from the regional assessments will be used to identify sub-regions or a specific category of ecosystems for the concentrated ecosystem investigations. Priority will be given to areas determined to favor MeHg production and bioaccumulation.

Ecosystem studies will be conducted at about six carefully selected field sites that collectively span gradients of relevant environmental characteristics, including mercury loading rate, bioaccumulation of methylmercury, climate, hydrology, geology, water chemistry, and biology. The level of work envisioned for the ecosystem studies would be similar in scope to the USGS mercury study underway in the Everglades (Krabbenhoft, 1996). Key processes to be studied include species transformations, sediment-water partitioning, bioaccumulation, biomagnification, deposition, sedimentation, and evasion (figure 2). Transformation studies will estimate rates of mercury methylation, demethylation, oxidation, and reduction. We anticipate the relative importance of each of these processes (process rates) will vary among the study basins. A goal of this part of the investigation will be to determine the factors controlling these process rates. In addition, important and related biogeochemical investigations, such as studies on sulfur cycling and other diagenetic sedimentary processes, will be critical elements of these site investigations and often provide the key to understanding the factors controlling mercury cycling at a site.

Considerable ancillary information will be needed to support these detailed investigations. Critical ancillary information includes, but may not be limited to, hydrology, land use, sediment transport and deposition, food-web structure, biological productivity, and geochemistry (dissolved organic carbon, pH, temperature, sulfur chemistry, major ions, and other trace elements). During the selection and screening of candidate study sites, preference will be given to sites for which essential historical and environmental information exist, or to sites where such information can be provided by collaborating with ongoing research or monitoring programs. To the extent feasible, the ecosystem investigations will be conducted at sites of greatest interest to the land-management bureaus of the Department of the Interior.

A numerical mercury cycling model such as that described by Hudson and others (1995) will be used to synthesize the information provided by the ecosystem studies. The model will be used to validate the process rate estimates, make comparisons across study sites, and facilitate hypothesis testing. Important questions, such as what effect Hg-emissions reductions will have on MeHg contamination of aquatic biota in upper trophic levels in various ecosystems nationally, could also be addressed with such a model.

Wildlife Exposure and Effects

The goal of the wildlife exposure and effects component of this investigation is to determine the toxicological significance of methylmercury exposure in selected fish-eating wildlife. Because of the very high neurotoxicity of methylmercury to the developing embryo, these studies will place emphasis on examining effects on reproduction, the biological endpoint most likely to be affected by existing levels of methylmercury exposure in piscivorous wildlife (Heinz and Hoffman, 1998). The threshold concentrations of dietary methylmercury that cause reduced reproductive success and adversely affect the health and survival of developing young will also be estimated. Dose-response relations and threshold dietary concentrations for reproductive effects should be estimated via manipulative experiments in the laboratory and

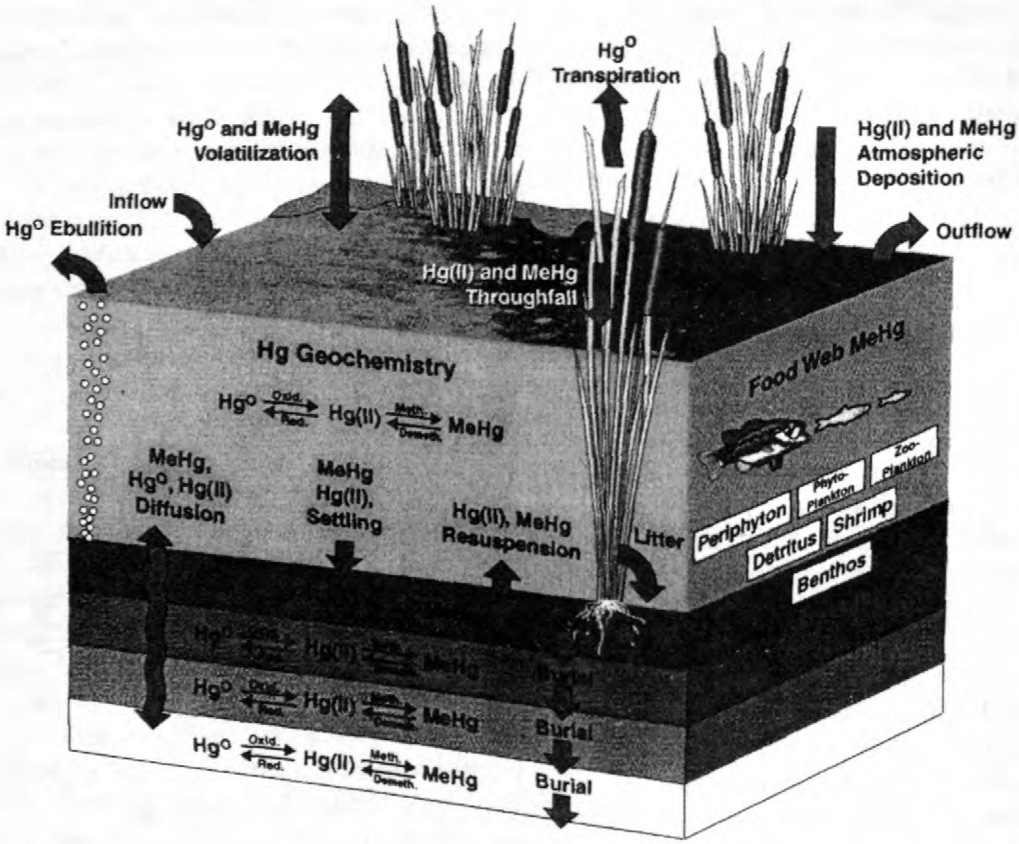

Figure 2. Conceptual model of the mercury cycle and food web for an aquatic ecosystem. Arrows depict various process rates.

field. Laboratory experiments would feature well-defined exposures and control of potentially confounding environmental variables, such as weather, disease, nutrition, and predation. The field experiments would facilitate work with many fish-eating species and analyses of exposure and effects under conditions of varying mercury contamination.

Definition of dietary methylmercury thresholds for reproductive effects should be a primary goal in developing environmental criteria for mercury. Such criteria would give resource managers and regulatory personnel some biologically relevant targets that could be used in environmental decisions regarding mercury. Laboratory and field studies should incorporate a variety of fish-eating and other birds to develop an adequate database for extrapolation to the various groups of birds exposed to methylmercury in natural ecosystems. One of the species recommended for controlled laboratory experiments on the effects of methylmercury on reproductive success is the mallard. Although not a fish eater, the mallard has been used extensively in mercury research and has proven to be a sensitive surrogate for evaluating methylmercury poisoning in other bird species. Large numbers of mallards can be raised and studied to ensure strong statistical defensibility of toxicological results. American kestrels could be used as a surrogate for the effects of contaminants on raptors, such as the bald eagle. A colony of kestrels has been used for many decades at the USGS's Patuxent Wildlife Research Center in studies of reproductive and post-hatching, developmental effects of different classes of contaminants, and the kestrel has proven to be a sensitive species. The Patuxent Center has many years of experience in breeding black-crowned night-herons; the re-establishment of this colony would enable the collection of critical laboratory data on the effects of methylmercury on the reproduction of a fish-eating bird. Other piscivorous birds (adults, chicks, or eggs) to be studied in shorter experiments include double-crested cormorants, common loons, belted kingfishers, and hooded mergansers.

In the laboratory studies, the range of dietary exposures should include environmentally relevant concentrations of methylmercury in prey fish. Reproductive studies should examine endpoints of adult health, fertility, hatching success, growth and survival of young, teratogenic effects, histopathology, and various biomarkers (including nondestructive ones). Mercury concentrations in tissues associated with adverse effects will also be estimated. Other tests will include a comparison of the toxicities of biologically incorporated mercury versus methylmercuric chloride, the interactions of mercury and selenium or other stressors, kinetics, egg injection studies, and behavioral studies.

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Mercury Contamination from Hydraulic Placer-Gold Mining in the Dutch Flat Mining District, California

By Michael P. Hunerlach, James J. Rytuba, and Charles N. Alpers

ABSTRACT

Mercury contamination at historic gold mining sites represents a potential risk to human health and the environment. Elemental mercury (quicksilver) was used extensively for the recovery of gold at both placer and hardrock mines throughout the western United States. In placer mine operations, loss of mercury during gold recovery was reported to be as high as 30 percent. In the Dutch Flat mining district located in the Sierra Nevada region of California, placer mines processed more than 100,000,000 cubic yards of gold-bearing gravel. The placer ore was washed through mercury-charged ground sluices and drainage tunnels from 1857 to about 1900, during which time many thousands of pounds of mercury were released into the environment.

Mine waters sampled in 1998 had total unfiltered mercury concentrations ranging from 40 ng/L (nanograms per liter) to 10,400 ng/L, concentrations of unfiltered methyl mercury ranged from 0.01 ng/L to 1.12 ng/L. Mercury concentrations in sluice-box sediments ranged from 600 µg/g (micrograms per gram) to 26,000 µg/g, which is in excess of applicable hazardous waste criteria (20 µg/g). These concentrations indicate that hundreds to thousands of pounds of mercury may remain at sites affected by hydraulic placer-gold mining. Elevated mercury concentrations have been detected previously in fish and invertebrate tissues downstream of the placer mines. Extensive transport of remobilized placer sediments in the Bear River and other Sierra Nevada watersheds has been well documented. Previous studies in the northwestern Sierra Nevada have shown that the highest average levels of mercury bioaccumulation occur in the Bear and South Fork Yuba River watersheds; this study has demonstrated a positive correlation of mercury bioaccumulation with intensity of hydraulic gravel mining.

INTRODUCTION

Mercury is a potent neurotoxin which has a tendency to biomagnify in the food chain (Krabbenhoft and Rickert, 1995) and is a potential threat to human and ecological health. This research documents previously unrecognized point sources that contain hundreds to thousands of pounds of elemental mercury. Our initial assessment provides information with regard to the specific location of mercury sources in the upper Bear River watershed in the Sierra Nevada region of California (fig.1). Mercury-contaminated watersheds affected by historic placer and hardrock gold mining include extensive public lands managed by the Bureau of Land Management (United States Department of Interior) and the Forest Service (United States Department of Agriculture). The present study is designed to provide a baseline characterization of contaminated areas within the Bear River watershed prior to any remediation efforts. The results of this pilot study may be used to develop a cost-effective, watershed-based approach to addressing regional mercury contamination associated with historic gold mining in the Sierra Nevada.

An abandoned mine in the Dutch Flat mining district, California (fig. 1), which is a highly concentrated point source of mercury impacting the Bear River watershed, was identified as part of the current study. Hydraulic mine tailings are known sources of low concentrations of mercury; however, past studies have failed to locate specific sites with extremely elevated elemental mercury, or *hot spots*. Typically, at streams within deposits of Quaternary age that have elevated mercury, demonstrated point sources can be found, and these hot spots correlate

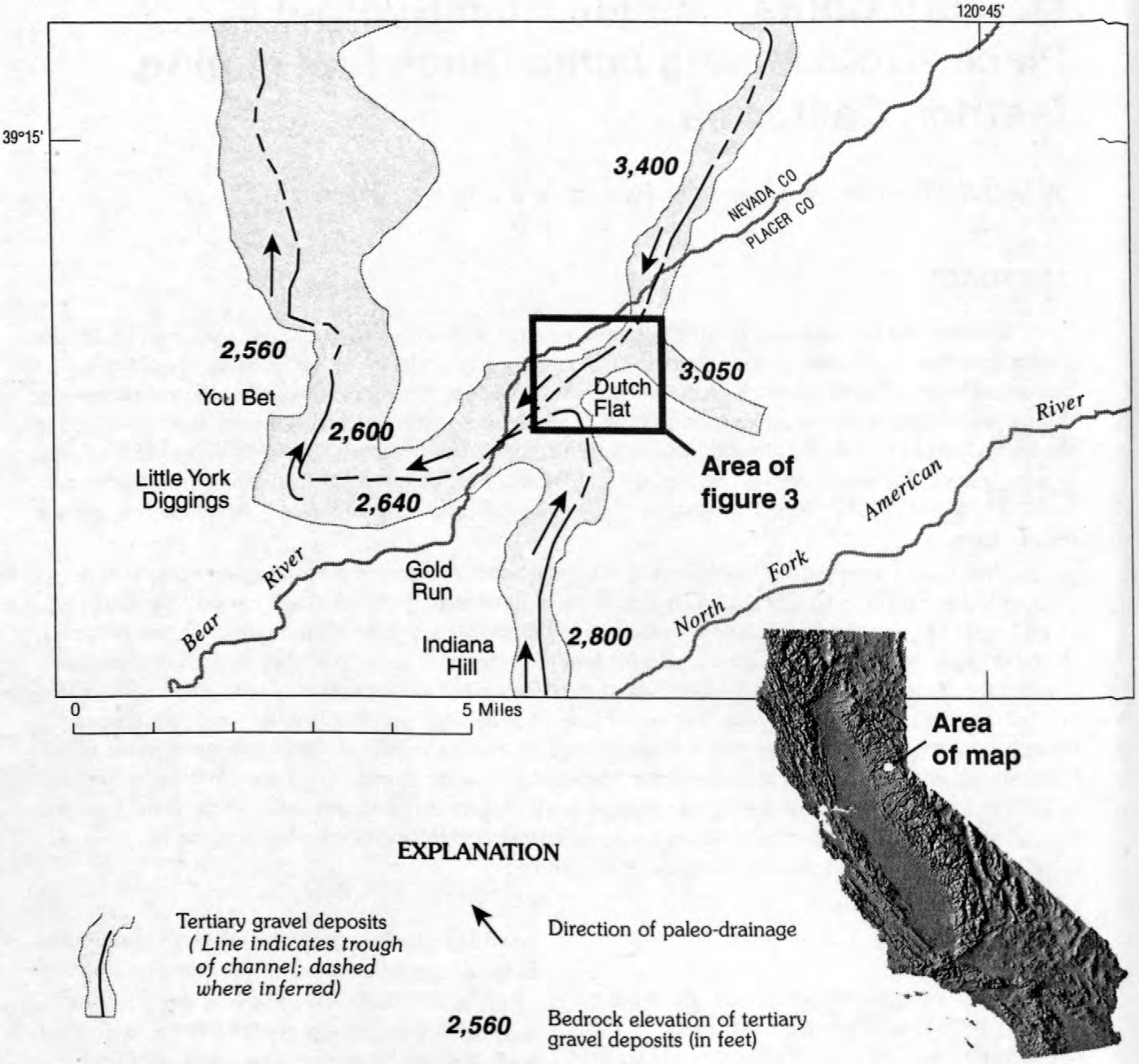

Figure 1. Location map showing trace of Tertiary-age river channels in the ancestral Yuba River (modified from Yeend, 1974), California.

with the location of the river channels of Tertiary age where the extensive gravel deposits were exploited for gold by hydraulic mining and where drainage tunnels, sluice boxes, and underground (drift) gravel mines occur.

There are at least five known sluices that discharged hydraulic mine tailings to the Bear River in the Dutch Flat district. Typical 19th century hydraulic gold mining recovery systems used mercury amalgamation to recover gold. Ground sluices and tunnel sluices from hundreds to thousands of feet in length were charged with hundreds to thousands of pounds of mercury. Some of these sluices remain as well-preserved mining artifacts that are easily accessible and actively visited by local miners who attempt to reclaim gold from the remaining amalgam. This activity can expose large quantities of elemental mercury and associated mercury vapors and may pose human health hazards or environmental hazards to downstream surface waters. Mine tailings and placer sediments at abandoned hydraulic placer-gold mines are abundant and fill numerous drainages, ravines, and benches. The presence of large quantities of elemental mercury associated with these sediments indicates that there is a significant potential risk to surface-water quality.

An extensive regional problem exists in watersheds in the northwestern Sierra Nevada because there are numerous drainage basins where placer-gold mining activities have occurred (Larry Walker Associates, 1997). Information collected for this report will help in evaluating other mercury point sources throughout the many hydraulic gold-mining districts in California and elsewhere in the western United States.

In 1998, the U.S. Geological Survey began a water-quality investigation in the Bear River watershed with the following overall objectives: (1) determine the seasonal variability of mercury loading to the Bear River from tunnel and ground-sluice discharges; (2) determine the distribution of mercury in underground mine workings, hydraulic pits, and sluices by mapping and sampling; (3) assess mercury bioaccumulation in aquatic life; and (4) enhance existing databases with detailed information on the occurrence and speciation of mercury associated with hydraulic mining debris in the Bear River watershed, for use in Geographic Information System analysis and watershed planning.

Purpose and Scope

This report describes a preliminary assessment of the extent of mercury contamination from hydraulic gold mining in the upper Bear River watershed and documents the potential risk to riparian and human health. Data presented include mercury concentrations in water, sediment, and fish tissue; mine discharge measurements; and estimates of total elemental mercury residing in sluice-box sediments. Methyl and total mercury concentrations are reported for selected samples of water (total and filtered) and of sediment to better understand mercury transport and transformation processes.

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HYDRAULIC MINING AND MERCURY USE

Placer gold deposits were the first type of gold discovered and mined on a large scale in California. Vast Tertiary-age gravel deposits from ancestral rivers within the Sierra Nevada gold belt region (fig.1) contained large quantities of gold. In 1852, hydraulic mining technology evolved with the use of water canons to deliver large volumes of water that stripped the ground of all soil, sand, and gravel above bedrock. Water was transported through hundreds of miles of ditches, flumes, and pipes up to 36 inches (in.) diameter under pressure of hundreds of pounds per square inch from over 500 feet (ft) of head, and was discharged through a converging 6-to-9 in. nozzle or *monitor.* Powerful jets of water generated through the monitor were used to dislodge and wash away extensive gravel deposits. Some mines operated several monitors in the same pit simultaneously. Hundreds of millions of cubic yards of sediment and water were directed into sluice boxes to separate and recover gold particles by gravity settling. Hydraulic mining was so popular and effective that it outproduced all other types of mining, even by 1900 when hardrock gold mines had been developed throughout the Mother Lode gold belt.

The capability of mercury to alloy with gold has been well known for more than 2,000 years (Rose and Newman, 1986). Mercury was added to large troughs within the sluice boxes to recover the gold as an amalgam. Because such large volumes of turbulent water flowed through the sluices, much of the finer gold and mercury particles were washed through and out of the sluice before they could settle in the riffles. A modification known as an *undercurrent* was developed to address this loss. Essentially a broad sluice, the undercurrent was set on a shallow grade at the side of, and below, the main sluice. Fine-grained sediment was allowed to

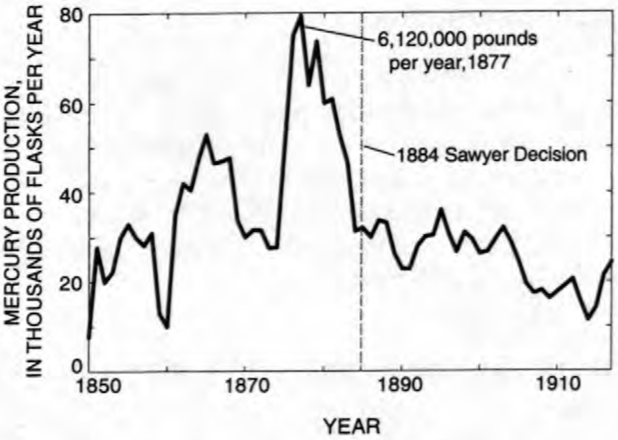

Figure 2. Mercury production from the California Coast Ranges, 1850–1917 (modified from Bradley, 1918). (Prior to 1904, one flask equalled 76.5 pounds; starting in 1904, one flask equals 75 pounds)

drop onto the undercurrent, where gold and amalgam were caught (Averill, 1946). Because this method was so efficient, high profits thus realized from hydraulic operations stimulated mining throughout the Sierra Nevada gold belt region and the western United States.

Most of the mercury used in the amalgamation process was obtained from the Coast Range mercury mineral belt on the west side of California's Central Valley. In 1877, mercury mines in the Coast Range reached a peak production of 6,120,000 pounds (lb) of mercury (Bradley, 1918) (fig. 2). Most of this mercury was used for gold recovery throughout the Sierra Nevada and Klamath–Trinity Mountains in California and elsewhere in the western states.

Mercury was introduced and distributed throughout the entire sluice box. Large troughs built into the sluice held hundreds of pounds of elemental mercury and the entire surface of the undercurrents [as much as 5,000 to 10,000 ft^2 (square feet)] were at times covered with copper plates treated with mercury. Initial charging rates varied at different mines and as a general rule the upper portions of the sluice boxes were most heavily charged with mercury. More than 1,500 lb of elemental mercury were used in a single sluice at the start of each season (Bowie, 1905). As much as 1,300 lb were added every 12 days due to the loss from the pounding and washing of the gravels passing over the liquid mercury. The specific gravity of gravel [2.7 g/cm^3 (grams per cubic centimeter)] is one-fifth that of mercury (13.6 g/cm^3), so the gravel would easily float over the mercury while the gold (19.3 g/cm^3) would sink into the troughs.

Unclassified gravel and boulders that entered the sluices caused the mercury to *flour*, that is, break into minute, dull-coated particles. Flouring was aggravated by agitation or exposure of the mercury to the air, and eventually the entire length of the sluice box would be coated with mercury. Some of the liquid mercury escaped from the sluice box with the tailings and was transported downstream. Some remobilized placer sediments remain close to their source in ravines that drained the hydraulic mines. Bowie (1905) noted that minute globules of quicksilver were reported floating in surface waters as much as 20 miles downstream of mining operations.

It has been estimated by Averill (1946) and others that under the best operating conditions, 10 percent of the mercury used was lost and, under average conditions, the loss of mercury was up to 30 percent. Estimates of mercury usage vary from 0.1 to 0.36 lb/ft^2 (pounds per square foot) of sluice box (Averill, 1946). We estimate that a typical sluice box had an area of 2,400 ft^2 (square feet) and used up to 800 lb of mercury during initial start-up with an additional 100 lb added monthly during its operating season (generally 6 to 8 months depending on water availibility). The annual loss of mercury from a typical sluice was likely to have been several hundred pounds.

HYDROLOGIC SETTING

The Bear River and its tributaries are the primary water resources in the Dutch Flat mining district. Water levels in the Dutch Flat Afterbay fluctuate with the release of water from two hydroelectric powerhouses just upstream of the confluence of the Little Bear River (fig. 3). Both the Bear and Little Bear rivers meander through deeply incised canyons that contain abundant alluvium and terraced placer tailings. Flows into and from the Dutch Flat Afterbay are controlled by the Nevada Irrigation District through a network of forebays, canals, and powerhouse discharges. Flow for the Bear River below the Dutch Flat Afterbay ranged

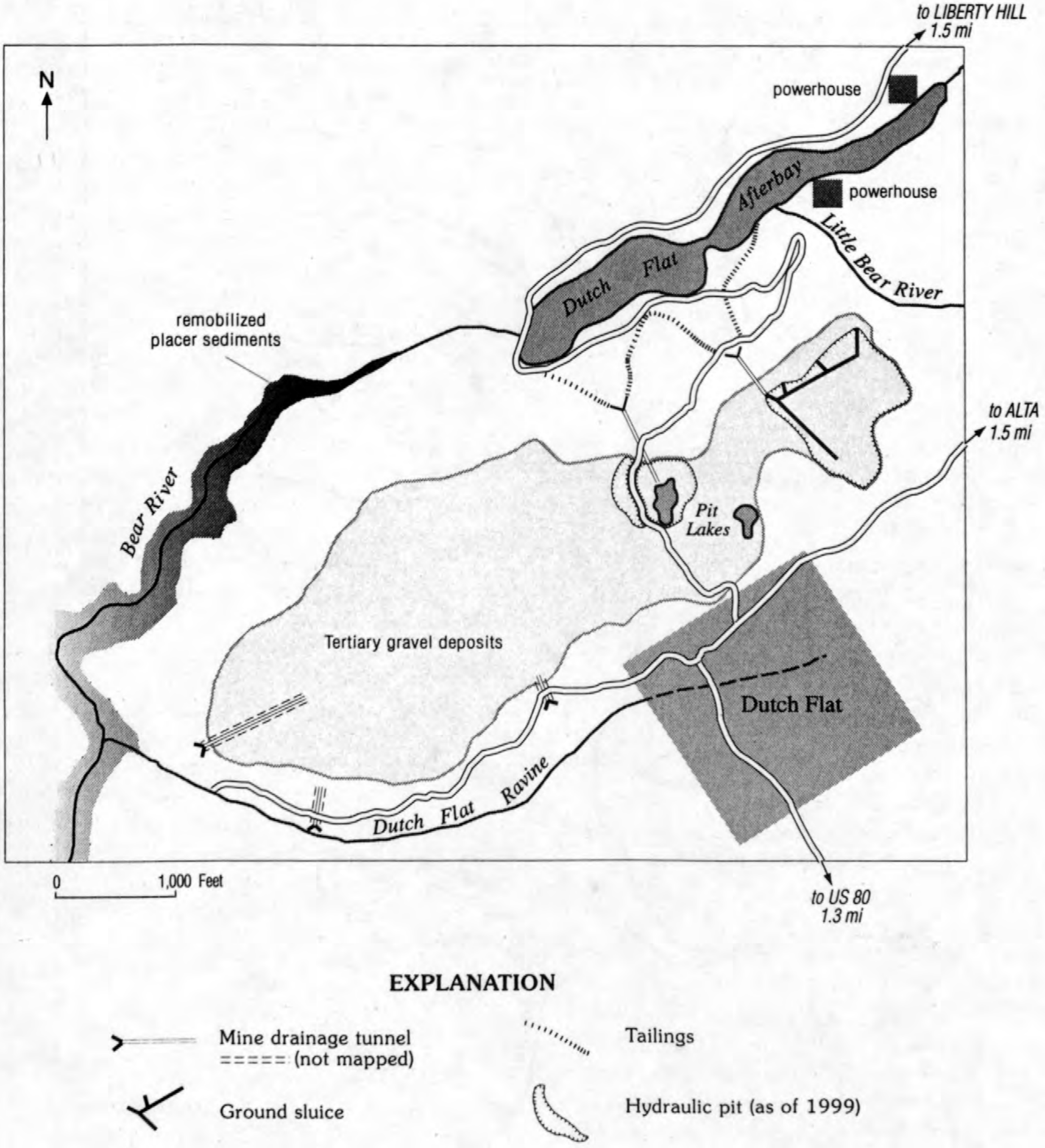

Figure 3. Plan view of Dutch Flat mining district, California.

from 6.9 to 494 ft^3/s (cubic feet per second) for the water year October 1997 to September 1998 (W. Morrow, Nevada Irrigation District, written commun., 1998). The Bear River is tributary to the Feather River, which joins the Sacramento River near Verona and then flows into the Sacramento-San Joaquin Delta and San Francisco Bay (fig. 4).

Geomorphology

Tertiary-age river-channel deposits extend north and south through Nevada and Placer counties of California (fig. 1) (Lindgren, 1911; Yeend, 1974). These quartz-rich, gold-bearing sedimentary channel deposits were part of the large paleodrainage of the Sierra Nevada that was buried

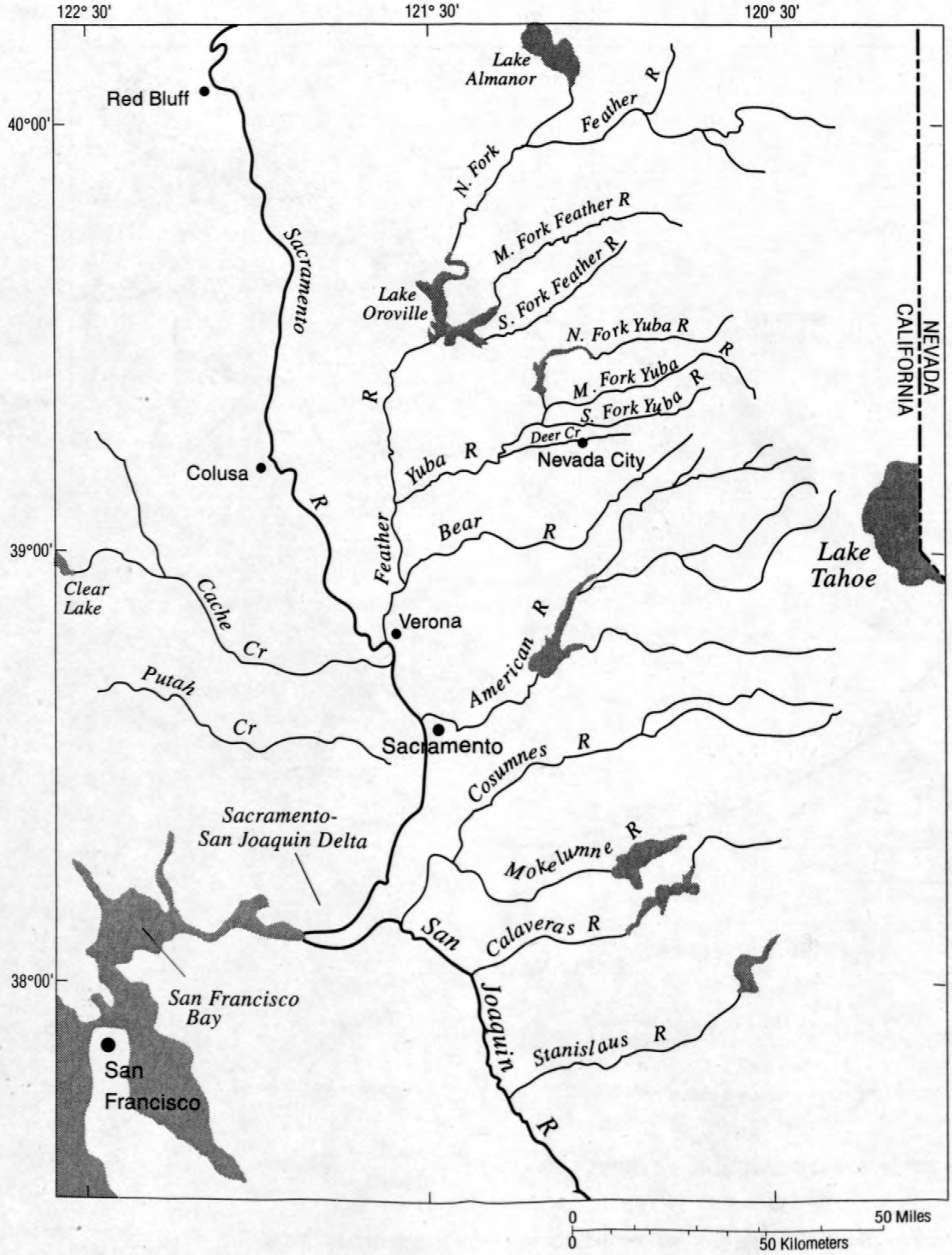

Figure 4. Location map showing selected rivers and reservoirs in the Sacramento River watershed, California.

during the Tertiary by volcanic eruptions and related mudflows. Quaternary-age rivers have cut sharp, V-shaped canyons through the volcanic deposits, exposing cross sections of the Tertiary-age river channels during uplift of the Sierra Nevada. Unexposed portions of the Tertiary-age river channels are covered by volcanic rocks that cap the ridges that divide the rivers of the western slope of the Sierra Nevada.

The Dutch Flat mining district covers about two miles of Tertiary-age river-channel deposits that lie sub-parallel to the present-day Bear River drainage (figs. 1 and 3). The district was one of the largest gold producers in California and was developed along the richest sections of the Tertiary-age channel in Placer County, at the junction where three large segments of the Tertiary-age Yuba River system merged. Remaining unworked gravels in the open pits are semi-circular with vertical banks developed as high as 160 ft. The base of the pits expose bedrock that supports little vegetation except for manzanita bushes and sparse pine trees. Pit lakes locally form in areas where the bedrock forms depressions or was excavated to elevations below the grade of tunnel drainage.

Documentation of hydraulic debris in the Bear River

From 1853 to 1884, unregulated hydraulic mining caused severe aggradation of river channels within the Sierra Nevada with the release of over 1.6 billion yd^3 (cubic yards) of sediment and debris (Gilbert, 1917). Natural drainage carried most of the remobilized gravel to the edge of the Central Valley where it was deposited because gradients in river channels were lower, filling and choking channels. As early as 1867, tailings from placer mines had accumulated to as much as 70-ft thick in the Bear River drainage and had created major problems with flooding of downstream cities and navigation on the Feather and Sacramento rivers (Averill, 1946). After the Sawyer Decision in 1884 (issued by Judge Lorenzo Sawyer against the North Bloomfield Mining Company) hydraulic mining nearly ceased. The Caminetti Act, passed by the U.S. Congress in 1893, allowed mines to operate only if mine operators built approved debris dams.

The Bear River is one of the most environmentally impacted rivers in the Sierra Nevada with more than 254 million yd^3 of gravel and sediment added from hydraulic mining, second only to the much larger Yuba River watershed (Gilbert, 1917). It was estimated that by 1881, more than 105 million yd^3 of gravel had been washed from the mines in the Dutch Flat mining district (U.S Congress, 1881). This figure does not include the deeper gravels washed through the tunnels that were active during the 1880s and 1890s. Drift mining along the gravel–bedrock contact continued after cessation of hydraulic mining with an estimated 30 million yd^3 having been mined in the Dutch Flat district by this method.

We estimate for the period of 1884 through 1901 that more than 50 million yd^3 washed through tunnels in the Dutch Flat district. These sediments entered the Bear River behind a log crib debris dam (since removed, except for bedrock foundation). This dam, jointly used by the Elmore Hill, Nary Red, Polar Star, and Southern Cross mines in Placer County and the Liberty Hill mine in Nevada County, was inundated with debris and sediment that was eventually released down the Bear River when it breached. Much of the coarser material remains along the shoreline and in local ravines whereas finer grained sediments fill wide low-flow sections of the river.

Recent studies (James, 1991) indicate that more than 139 million yd^3 of hydraulic tailings remain stored in the lower Bear River Basin. The sediments released during placer mining in the upper Bear River basin are extensive and their volume is unknown. These sediments are subject to sustained remobilization (James, 1991) which is in contrast with Gilbert's (1917) symmetrical wave model of sediment transport that implied a rapid return of sediment loads to pre-hydraulic mining levels. Recent floods (December 1996 through January 1997) remobilized large quantities of hydraulic mine tailings and sediment in the drainages of the basin, exposing elemental mercury in the stream bed.

MERCURY TRANSPORT AND BIOACCUMULATION

Previous work has documented mercury concentrations as high as 0.33 µg/g (micrograms per gram) in fish tissue (Slotton and others, 1997) and 0.37 µg/g in sediment (Domagalski, 1998) from the Bear River watershed. These compare with background values in uncontaminated areas of less than 0.1 µg/g in fish tissue and 0.06 µg/g in sediments (Porcella and others, 1995; Hornberger and others, 1999). On a watershed scale, we have demonstrated a correlation between mercury bioaccumulation data (Larry Walker Associates, 1997) and volume of gravel hydraulically mined (Gilbert, 1917) (fig. 5). The highest values of bioavailable mercury are found in watersheds that are the most environmentally impacted from hydraulic placer-gold mining.

Previous studies have estimated that substantial amounts of mercury, between 3,300 tons (California Regional Water Quality Control Board–Central Valley Region, 1987) and 10,000 tons (Hornberger and others, 1999), were transported along with remobilized sediment from hydraulic mining to San Francisco Bay. In two San Pablo Bay cores, the isotopic compositions of sediment deposited between 1850 and 1880 (Jaffe and others, 1998) correlate with those found in exposed Tertiary-age gravels at abandoned hydraulic gold mines in the Bear River watershed (Bouse and others, 1996). Mercury concentrations in these core

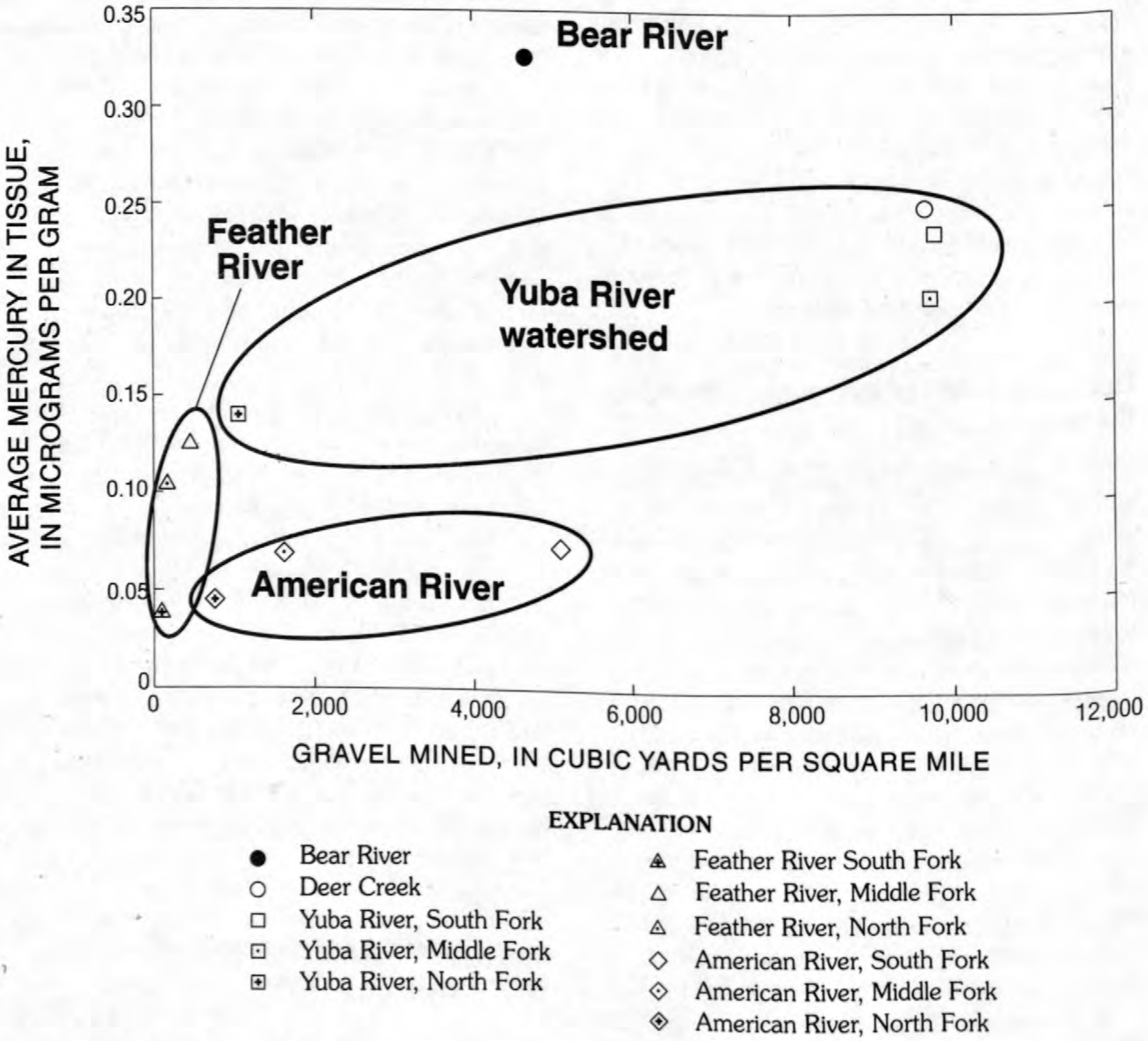

Figure 5. Correlation of yardage mined (normalized to area of drainage basin, in square miles) with average tissue mercury concentration, normalized to an intermediate trophic level (mercury data from Larry Walker Associates, 1997).

sediments range from 0.3 µg/g to 0.5 µg/g. Mercury concentrations as high as 1.2 µg/g have been found in core sediment from Grizzly Bay (Hornberger and others, 1999).

METHODS

Mine drainage waters and sediment were sampled from a historic intact sluice box at an abandoned mine in the Dutch Flat mining district (mine #1) during July and August 1998 and from the portal of another (mine #2) during August 1998. Waters flowing from the portals of these mines were sampled for total and methyl mercury using precleaned bottles provided by Frontier Geosciences Inc. Samples were filtered using an ultraclean 0.45 µm (micrometer) nitrocellulose membrane. Wet gravity separation (that is, panning) was used in the field with a portable balance to estimate the mercury concentrations in the sluice box sediments. Random 1-kg (kilogram) grab samples were weighed, sieved to less than 0.25 in., and panned to separate total recoverable elemental mercury. The mercury was weighed and compared with the initial sample for a gram per kilogram ratio (g/kg). Grab samples were carefully taken from undisturbed top sediments and a specially designed suction tube was used to recover deep sediments at the bedrock contact. Fish collection was done by electrofishing a quarter-mile reach of the Dutch Flat Afterbay (fig. 3). Trout collected from the Dutch Flat Afterbay by USGS personnel were analyzed for total mercury in fillets by the California Department of Fish and Game's laboratory in Moss Landing, California.

RESULTS

Field reconnaissance identified numerous drainage tunnels, bedrock cuts, and ground-sluice remains, all of which contain visible mercury in the Dutch Flat district. In one drainage tunnel an original intact sluice box was identified. Initial results using pan concentration and a portable scale showed as much as 30 g (grams) of elemental mercury from 1 kg of carefully selected sluice-box sediment.

Mercury in mine-drainage waters

Total mercury concentrations in four water samples from mine #1 ranged from 45 to 10,400 ng/L (nanograms per liter) in unfiltered water samples and from 7 to 225 ng/L in filtered water samples. Methyl mercury concentrations ranged from 0.01 to 1.0 ng/L in unfiltered samples. A single sample from mine #2 had 44.7 ng/L unfiltered and 7.4 ng/L filtered total mercury. Unfiltered methyl mercury was 0.01 ng/L in the single sample from mine #2. Limited monitoring data for mine-drainage flows from mine #1 measured with a Parshall measuring flume in April and May 1998 indicated discharge in excess of 50 gallons per minute (R. Humphreys, California State Water Resources Control Board, written commun., 1998)

Mercury in sluice box sediment

Total mercury in sediment samples collected from a sluice box in the Dutch Flat mining district ranged from 1,800 to 15,000 ng/g (nanograms per gram) wet basis, and from 2,400 to 21,000 ng/g dry basis. Methyl mercury in sediment ranged from 0.1 to 0.2 ng/g wet basis, and from 0.2 to 0.3 ng/g dry basis. A sample of white clay precipitate and fine sand from another processing site in the district had 4,270 ng/g wet and 6,710 ng/g dry weight total mercury. Methyl mercury was 0.003 ng/g wet weight and 0.005 ng/g dry weight. Total mercury recovered from panning of sluice box sediment ranged from 0.6 to 26 g/kg. Total mercury concentrations of 0.6, 0.9, and 1.0 g/kg were recovered from top gravels. Total mercury values for the bottom gravels were 16, 18, and 26 g/kg, indicating that the elemental mercury is strongly concentrated near the bedrock contact.

On the basis of observations in the Dutch Flat mining district, a preliminary estimate was made of total mercury in sluice-box sediments. A typical sluice-box has a cross sectional area of 15 ft^2 (5 ft wide and 3 ft high). Assuming that bottom gravels represent about 10 percent of the total sluice-box sediment, and using mercury concentrations for bottom and top sediments determined by panning, each linear foot of sluice box is estimated to contain 3 to 5 lb of mercury. This estimate pertains only to sluice boxes that remain full of sediment.

Ground and tunnel sluice boxes range in length from tens to thousands of feet. Therefore, sluice boxes are likely to contain hundreds to thousands of pounds of mercury in their present condition.

Mercury in fish tissue

The fish collected for mercury analyses were five adult rainbow trout (*Salmo gairdneri*). Total mercury in the fish tissue ranged from 0.1 to 0.2 µg/g (micrograms per gram) on a dry weight basis, or 0.03 to 0.05 µg/g on a wet weight basis.

DISCUSSION

Previous studies identified elevated levels of mercury in the aquatic food web of the Bear River watershed (Larry Walker Associates, 1997), however, identification of point source(s) were lacking. The mercury bioaccumulation problem is pervasive and regional throughout Sierra Nevada streams that are tributary to the Sacramento River, the Sacramento–San Joaquin Delta, and San Francisco Bay (fig. 4). This study has shown a relationship between the intensity of hydraulic gold-mining and degree of mercury bioaccumulation on a watershed scale (Fig. 5). Since the cessation of hydraulic mining, accumulated sediment from hydraulic placer mining has been transported to Sacramento–San Joaquin Delta and San Francisco Bay by sustained remobilization (James,1991). The USGS is working with the Forest Service, the Bureau of Land Management, and the Nevada County Resource Conservation District to develop plans to address mercury occurrence, fate, and transport in the Bear River and South Fork Yuba River watersheds, the areas of the Sierra Nevada that apparently are most environmentally impacted by hydraulic mining (fig. 5).

The extremely high mercury concentrations found in this study in water and sediment suggest that hydraulic placer-gold-mining sluices and drainage tunnels may be important contributors of mercury to the downstream Bay-Delta system and that remobilization of mercury is occurring at specific hot spots on a seasonal basis. Two important conclusions of this paper are that localized point sources of mercury likely exist throughout the entire hydraulic gold mining region, and that methylation of mercury is occuring close to the sources, allowing methyl mercury to enter the food web. These point sources offer the most treatable target areas for investigation of possible remediation projects.

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Techniques for the Collection and Species-Specific Analysis of Low Levels of Mercury in Water, Sediment, and Biota

By Mark L. Olson and John F. DeWild

ABSTRACT

The U.S. Geological Survey has established a laboratory to develop and implement methods for the analysis of low levels of specific species of mercury. This paper outlines sample collection and analysis techniques used to determine species specific mercury concentrations in environmental samples. The laboratory has the capability of determining the concentration of total, methyl, reactive, and dissolved gaseous mercury in surface water, and total and methylmercury in ground water, porewater, sediment, and biota. Until the analytical methods used by the Wisconsin District Mercury Laboratory are approved by the U.S. Geological Survey's Office of Water Quality, the data produced are classified as provisional. The analytical method for total mercury is scheduled for approval by the U.S. Environmental Protection Agency in the spring of 1999. The Wisconsin District Mercury Laboratory will seek approval of the analytical method for methylmercury through the U.S. Geological Survey Office of Water Quality. Currently, results are validated using matrix spikes, blanks, laboratory duplicates, quality-control check samples, and certified reference materials.

INTRODUCTION

Mercury (Hg) has been recognized as an environmental pollutant for several decades. Until recently, scientists have been unable to accurately measure Hg in the parts-per-trillion range, primarily due to sample contamination and lack of instrument sensitivity. The collection of mercury samples requires the use of ultra-clean sampling techniques first published by Patterson and others (1977) for lead (Pb) research, and refined by Gill and Fitzgerald (1985) for Hg. Ultra-clean techniques prevent direct contact between sample media and sampling equipment, field personnel, and any other potential contaminant sources during collection and analysis.

The presence of very high Hg concentrations (≥1.5 micrograms per gram (µg/g) muscle tissue) found in game fish in the Florida Everglades (Ware and others, 1990) initiated the Aquatic Cycling of Mercury in the Everglades (ACME) project in 1995. To provide the analytical support for this project, the U.S. Geological Survey (USGS) office in Middleton, Wisconsin established the Wisconsin District Mercury Laboratory (WDML) to develop and implement methods for the analysis of specific Hg species. Analyses performed by the WDML include total, methyl, reactive, and dissolved gaseous mercury in surface water, and total and methylmercury in ground water, porewater, sediment, and biota.

METHODS AND TECHNIQUES

Equipment Preparation and Sample Collection

Contamination of sampling equipment and sample containers is the largest source of error associated with low-level mercury analysis. To decrease the effect of contamination from equipment and containers, Teflon (any use of trade, product, or firm names is for descriptive purposes and does not imply endorsement by the U.S. Government) is used whenever possible and is subjected to a rigorous cleaning protocol. Teflon containers minimize Hg adsorption to container walls and eliminate gaseous mercury exchange into or out of the container (Horvat and others, 1993). New Teflon equipment is rinsed with tap water, and immersed in an acid bath containing 4 N trace pure hydrochloric acid (HCl), then heated to 65°C for 48 hours. Immediately following removal from the bath, equipment is immersed in fresh reagent-grade water followed by a minimum of three rinses with fresh reagent-grade water. After rinsing, containers are partly filled (25 percent) with 1 percent Omni Trace HCl and capped tightly. The surface of all equipment is then allowed to air dry under a Hg-free class 100 laminar flow-hood. Sampling line and pump-head tubing interior surfaces are dried by purging with Hg-free nitrogen (N_2). Pump-head tubing is cleaned by filling with 50 percent Omni Trace nitric acid (HNO_3) and soaking in a 10 percent HCl bath for a minimum of 7 days. Dry equipment is double bagged in new zip-type plastic bags with the unique identifier and date cleaned written on the outer bag. Subsequent cleaning of containers and equipment requires only a 24 hour period in the acid bath followed by the rinsing procedure outlined above. A minimum of 10 percent of the equipment is tested for total mercury to assure cleanliness. If the equipment is suspect, the batch must be recleaned and retested.

Capsule filters used for field filtering are cleaned by filling with Omni Trace HNO_3 and soaking for 4 days. After four days, the filters are rinsed with 20 filter volumes of fresh reagent-grade water, and refilled with Omni Trace HCl, immersed in a 10 percent HCl bath at room temperature, and allowed to soak for 3 days. Finally, the filters are emptied of the HCl, rinsed with 20 filter volumes of fresh reagent-grade water, filled with reagent-grade water, capped, and double bagged until use. If lab filtering is desired, the unfiltered water samples are shipped on ice via overnight courier to the WDML for vacuum filtering in a clean environment.

Aqueous samples are collected either by means of a peristaltic pump or by grab technique. During sample collection, care is taken to prevent dust or other contaminants (for example, breath from a person with dental amalgam) from entering the sample containers. To help prevent contamination during sample collection, field personnel are required to wear Tyvek suits and arm-length polypropylene gloves.

Aqueous samples collected for total mercury analysis are preserved by acidification to 1 percent (v/v) with Omni Trace HCl. All other samples are preserved by freezing.

Clean Areas

All unbagged equipment is handled with gloved hands and in clean areas. Clean areas are countertops covered by a Teflon overlay within a Hg-free laminar flow-hood. The laminar flow-hoods meet or exceed Federal Standard 209 for class 100 conditions. The laminar flow-hood intakes are covered with gold-coated cheesecloth to remove any mercury vapor prior to particle removal.

TOTAL MERCURY ANALYSIS

Water Samples

Total mercury (Hg_T) analysis is performed by U.S. Environmental Protection Agency (USEPA) Method 1631: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry (CVAFS) with modifications. USEPA Method 1631 is a draft method and should be approved in the spring of 1999. The method consists of three essential parts: Oxidation of Hg species to reactive mercury (Hg^{II}), reduction of Hg^{II} to volatile mercury (Hg^0), and detection of Hg^0 by CVAFS.

Aqueous samples are treated to 1–2 percent (v/v) with 0.2 N Bromine monochloride (BrCl) to oxidize all of the forms of Hg to Hg^{II}. Samples are placed in an oven at 50°C for a minimum of 12 hours to accelerate the oxidation reaction. Oxidation is considered complete if excess BrCl is present after 12 hours as determined by a yellow tint in the sample; therefore, samples must be colorless before BrCl is added. If the BrCl has been consumed, additional BrCl must be added and allowed to react for another 12 hours. Samples with high concentrations of dissolved organic carbon (DOC) are commonly stained a yellow or brown color. These samples are exposed to UV light until all DOC has been oxidized and the sample becomes colorless (Olson and others, 1997).

Prior to analysis, the excess BrCl needs to be reduced with hydroxylamine hydrochloride (NH_2OH-HCl). Failure to reduce the BrCl will result in destruction of the gold traps by the free halogens in the remaining BrCl. Approximately 10 minutes after BrCl reduction, an aliquot is poured into a bubbling flask and 0.5 ml of stannous chloride ($SnCl_2$) is added to reduce the Hg^{II} to volatile mercury or Hg^0. The aliquot is then purged with Hg-free N_2 for 20 minutes. Water vapor and free halogens are removed from the gas-stream by an inline soda-lime trap and the volatile mercury amalgamates onto a gold-coated glass bead trap.

The analytical train consists of the sample trap, an analytical trap, and the detector. The volatile mercury is thermally desorbed from the sample trap to an analytical trap to provide consistent chromatograms. After thermal desorption from the analytical trap, the volatile mercury is carried to a CVAFS with Hg-free Argon (Ar). Peak area is measured and compared to a standard curve for that day, and concentration is determined by the size of the aliquot that was purged.

Data quality objectives (DQO) for precision, accuracy, system control, and background contamination have been established to evaluate analytical results. A summary of DQO for Hg_T analysis is outlined in table 1.

Table 1. Summary of data quality objectives for total mercury analysis for water samples. [ng/L, nanograms per liter; pg, picograms; avg, average; σ, standard deviation; n, number of observations].

Type	DQO	avg	σ	n
QCCS (ng/L)	5.0 ±10%	5.05	0.22	99
% Recovery	90-110%	99.5	7.6	88
DDL (pg)	10	8.7	8.4	95

Precision is evaluated by duplicate analysis of all samples. The analysis is acceptable if the DQO is less than or equal to 10 percent difference. If the percent difference is greater than 10 percent, the sample is analyzed a third time or until a relative standard deviation (RSD) of less than 10 percent between the replicates is achieved.

A minimum of one in every 10 samples is spiked to assess accuracy. Spike recoveries ranging from 90 to 110 percent are acceptable (fig. 1). If the spike recovery fails to meet the DQO, another sample is spiked. If the second spike recovery fails to meet the DQO, all of the samples in that batch will be flagged indicating potential matrix interference.

A quality-control check sample (QCCS) is used to determine statistical control of the system. The QCCS needs to be within 10 percent of the theoretical value to proceed with sample analysis (fig. 2). A QCCS is analyzed at the beginning of the run, at least every tenth sample, and at the end of the run. A National Institute of Standards and Technology (NIST) 3133 certified standard originating from a different source than the calibration standard is used to prepare the QCCS.

Bubbler blanks are used to correct for background contamination and calculate detection limits. A bubbler blank is a pre-purged aliquot of either sample or reagent-grade water and 0.5 ml of $SnCl_2$. A set of bubbler blanks is analyzed at the beginning of the run, at least every ten samples, and at the end of the run. Background contamination is corrected by subtracting the average bubbler-blank peak area from each standard or sample-peak area. An initial detection limit (IDL) is calculated from bubbler blanks analyzed at the beginning of the run. The IDL must be below 5 picograms (pg) (0.04 ng/L based on an aliquot of 125 ml) before proceeding with sample analysis. The daily detection limit (DDL) is determined from the results of all bubbler blanks for the run (fig. 3). Detection limits are 3 times the standard deviation (SD) of the bubbler blanks, expressed as a mass. If a bubbler blank is found to contain more than 25 pg of Hg, the system is out of specified control and data produced on that bubbling flask should be reanalyzed.

A method detection limit of 0.04 ng/L was determined for Hg_T by the WDML according to MDL; 40 CFR 136, Appendix B. When the DDL is less than the MDL, samples that fall at concentrations below the MDL will be reported as less than 0.04 ng/L. When the DDL exceeds the MDL, all sample results that fall below the DDL will be reported as less than the runs DDL.

Presently there are no certified reference materials (CRM) for low-level total mercury analysis of water samples.

Sediment and Biota Samples

Total mercury in solid material is analyzed by placing a homogenized subsample of the material into a Teflon pressure vessel and weighing. Seven milliliters of a 7:3 mixture of HNO_3 and sulfuric acid (H_2SO_4) is then added to the vessel. The vessel is wrench tightened and digested at 125°C for a minimum of 2 hours. The sample is cooled for 1 hour and diluted to 30 ml with a 5% BrCl solution and allowed to oxidize at 50°C for a minimum of 12 hours. The BrCl oxidation is critical because the HNO_3/H_2SO_4 digestion itself is not rigorous enough to convert all the methylmercury to Hg^{II} (N.S. Bloom, Frontier Geosciences Inc. written commun.,1998). An aliquot from the vessel is then reduced with $SnCl_2$ in a bubbling flask and analyzed as described above for Hg_T.

A minimum of 3 digestion blanks are analyzed and used to correct for background contamination and calculate detection limits. The average mass of Hg from the blanks is subtracted from each sample and the daily detection limit is defined as three times the SD of the digestion blanks. If a sample is determined to be less than the detection limit, a larger subsample of the solid must be digested.

In addition to the DQO established for the analysis of water samples, a laboratory split and a CRM is digested and analyzed for every ten samples. The CRM chosen for a given batch is selected to best represent the matrix of interest. The WDML utilizes the following CRMs from the National Research Council (NRC) Canada: DORM-2 or the DOLT-2 for fish tissues, the TORT-2 or the LUTS-1 for biota other than fish, and the BEST-1 for sediments. The WDML DQO for CRM is plus or minus 10 percent of the theoretical value or the reported 95 percent confidence interval whichever is greater. Theoretical values and tolerance limits established by the NRC are outlined in table 2.

Mercury concentrations for sediments are reported on a dry weight basis. Percent dry weight is determined by drying a subsample of material at 105°C overnight. The percent dry weight is calculated by dividing the dry weight by the wet weight and multiplying by 100.

Table 2. Theoretical values and tolerance limits for certified reference materials used for total mercury in sediment and biota analysis. Units are ng/Kg, nanograms per kilogram.

CRM	Theoretical	Tolerance
DORM-2	4.64	±0.26
DOLT-2	2.14	±0.28
TORT-2	0.27	±0.06
LUTS-1	0.0167	±0.0022
BEST-1	0.092	±0.009

METHYLMERCURY ANALYSIS

Water Samples

Methylmercury (MeHg) analysis is performed according to USEPA Method 1630: Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and CVAFS with minor modifications. USEPA 1630 is a draft method yet to be validated or published, therefore, the WDML will seek method approval through the USGS Office of Water Quality.

Direct ethylation of natural waters has been shown to release only "reactive" MeHg, which represents only 5–60 percent of the total MeHg (Horvat and others, 1993). Therefore, samples must first be distilled to eliminate potential interferences. Approximately 100 ml of sample is dispensed into a Teflon reaction vessel. A mixture of potassium chloride (KCl), H_2SO_4, and copper sulfate ($CuSO_4$) is added (Olson and others, 1997). The reaction vessels are then placed in an aluminum block maintained at 121°C until 80–95 percent of the sample has been distilled. The distillates are collected in Teflon receiving vessels housed in a refrigerator.

Ethylation is achieved by transferring the distillate to the bubbling flask, adjusting the pH to

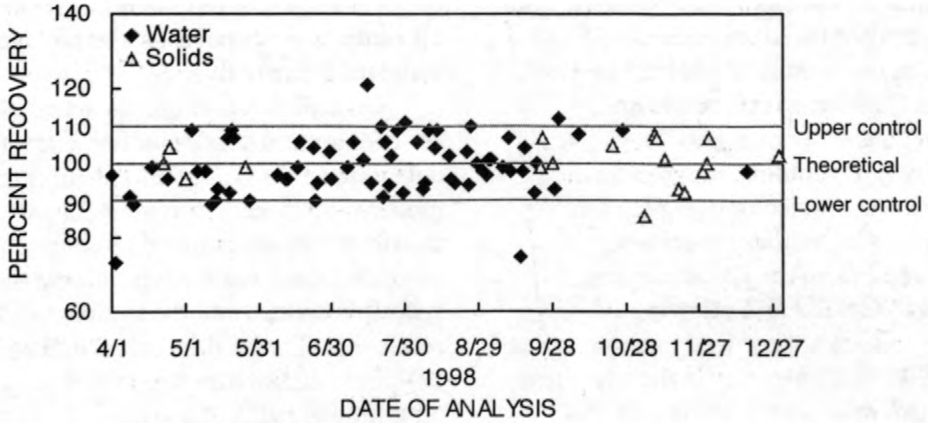

Figure 1. Percent recoveries for analyses of total mercury.

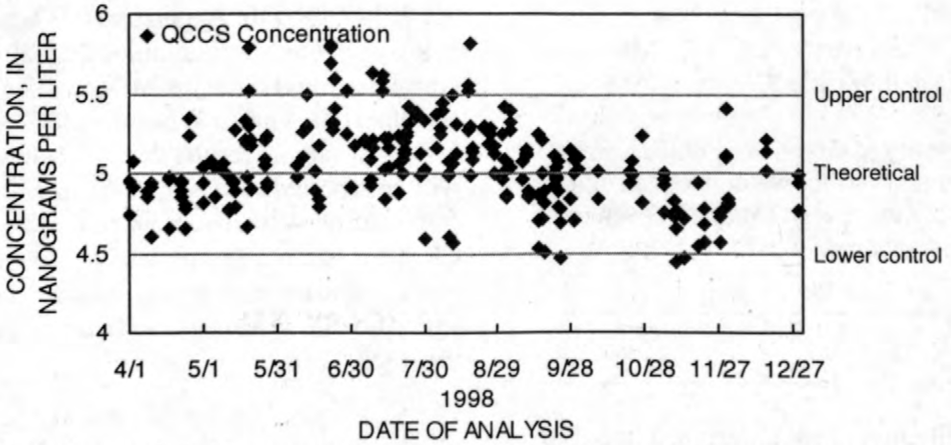

Figure 2. Quality-control check sample concentrations for analyses of total mercury.

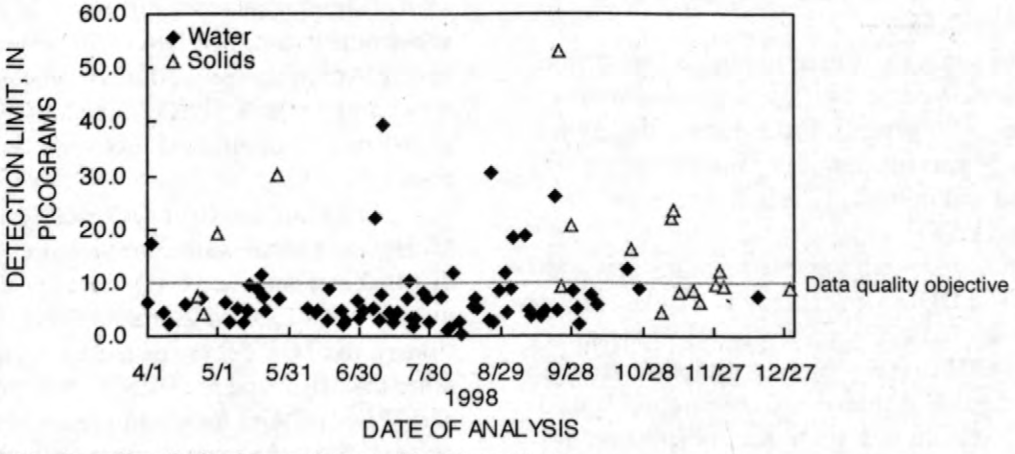

Figure 3. Daily detection limits for analyses of total mercury.

4.7, adding sodium tetraethyl borate (NaTEB) and allowing the mixture to react for 15 minutes. The sample is then purged for 20 minutes with Hg-free N_2. The mercury species are collected onto a Carbotrap. The Carbotrap is placed in an analytical train consisting of a gas chromatography (GC) column, a pyrolytic column and a CVAFS detector using Hg free Ar as the carrier gas. The mercury species are thermally desorbed from the Carbotrap and separated in the GC column. Following separation, the species are reduced in a pyrolytic column to Hg^0. Volatile mercury is then detected by CVAFS where peak area is measured. The peak area is compared to a standard curve and concentration is determined by the size of aliquot that was purged and the percent of sample that was distilled. A summary of DQO for MeHg analysis is outlined in table 3.

Table 3. Summary of data quality objectives for methylmercury analysis for water samples. [pg, picograms; avg, average; σ, standard deviation; n, number of observations].

Type	DQO	avg	σ	n
% Recovery	80-120%	94.7	21.4	67
DDL (pg)	<5	2.2	2.7	77

The distillation block is designed to hold 16 samples. A batch consists of 11 environmental samples, 1 replicate, 1 matrix spike, and 3 distillation blanks.

The replicate is used to evaluate precision. An acceptable percent difference between the two replicates is 20 percent. If the percent difference exceeds 20 percent, samples from that batch are evaluated and potentially reanalyzed based on additional DQO.

Spike recoveries assess accuracy and matrix effects. The DQO for accuracy is a spike recovery between 80 and 120 percent (fig. 4). If the spike recovery fails to meet the DQO, another sample from that batch is spiked and the original spiked sample is reanalyzed. If the second spike recovery fails to meet the DQO, all of the samples in that batch will be reanalyzed or flagged indicating potential matrix interference.

In addition to daily calibration of the instrument, standards are analyzed throughout the analysis run to monitor instrument stability. If a standard differs by more than 10 percent from the original response, the system is recalibrated and all samples analyzed after the last acceptable standard are reanalyzed.

The distillation blanks are used to correct for background contamination and calculate detection limits. Distillation blanks are reagent-grade water, and distillation reagents. If a distillation blank is found to contain more than 15 pg of Hg, the system is considered out of specified control and the sample batch must be redistilled. The DDL is calculated as 3 times the SD of the distillation blanks and may not exceed 5 pg for any batch (fig. 5).

A method detection limit of 0.025 ng/L was determined for MeHg by the WDML according to MDL 40 CFR 136, Appendix B. When the DDL is less than the MDL, samples that fall at concentrations below the MDL will be reported as less than 0.025 ng/L. When the DDL exceeds the MDL, all sample results that fall below the DDL will be reported as less than the runs DDL.

Currently there are no certified standards or CRM for methylmercury analysis in water. The MeHg standard used by the WDML is calibrated against NIST 3133 Hg_T standard to determine a true titer.

Sediment and Biota Samples

A homogenized sediment or biota subsample is weighed into a Teflon reaction vessel. Approximately 50 ml of reagent-grade water and the KCl, H_2SO_4, and $CuSO_4$ mixture is added and the distillation proceeds as detailed above.

In addition to the DQO established for MeHg analysis of water samples, a CRM is distilled and analyzed every batch. The WDML utilizes NRC DORM-2 or the DOLT-2 for fish tissues, the TORT-2 or the LUTS-1 for biota other than fish, and the BEST-1 for sediments. The WDML DQO for CRM is plus or minus 10 percent of the theoretical value or the reported 95 percent confidence interval, whichever is greater. Theoretical values and tolerance limits established by the NRC are outlined in table 4.

Mercury concentrations for sediments are reported on a dry weight basis. Percent dry weight is determined by drying a subsample of material

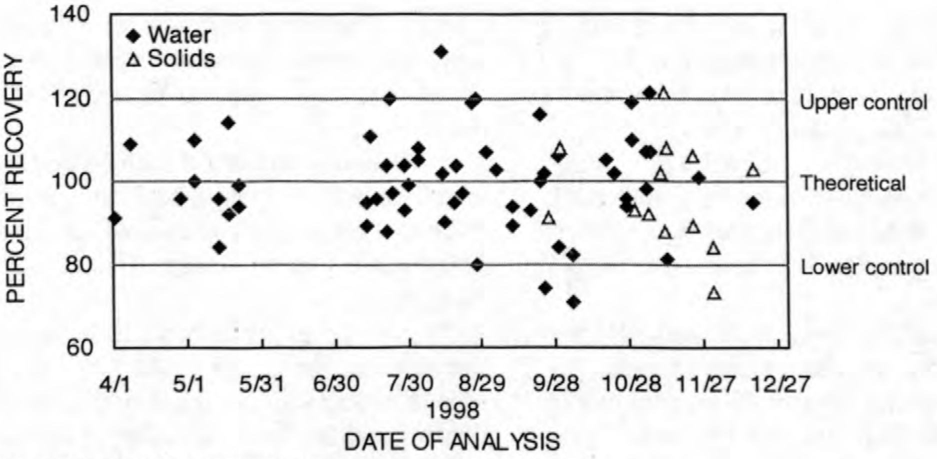

Figure 4. Percent recoveries for analyses of methylmercury.

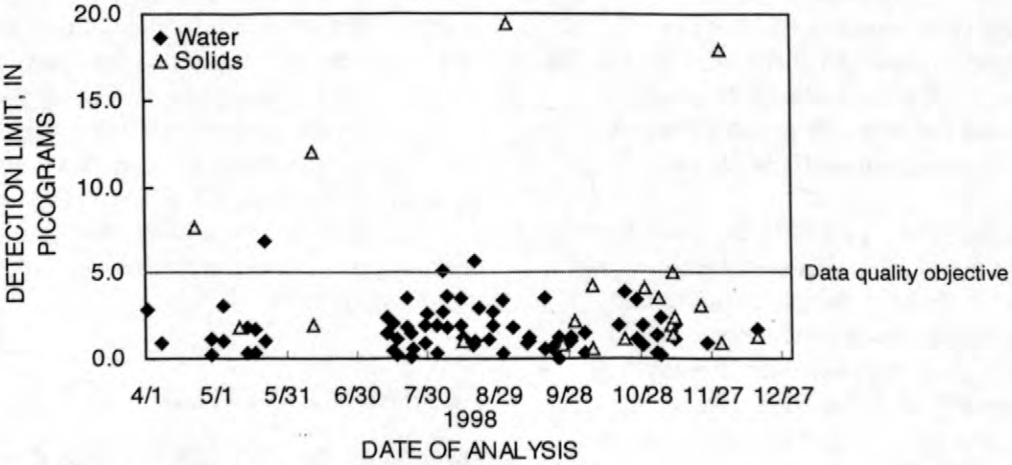

Figure 5. Daily detection limits for analyses of methymercury.

at 105°C overnight. The percent dry weight is calculated by dividing the dry weight by the wet weight and multiplying by 100.

Table 4. Theoretical values and tolerance limits for certified reference materials used for methyl mercury in sediment and biota analysis. Units are ng/Kg, nanograms per liter.

CRM	Theoretical	Tolerance
DORM-2	4.47	±0.32
DOLT-2	0.693	±0.053
TORT-2	0.152	±0.013
LUTS-1	0.0094	±0.0006
BEST-1	0.000162	±0.000052

DISSOLVED GASEOUS AND REACTIVE MERCURY

To increase understanding of the fate and transport of Hg in the environment, the WDML refined techniques for the analysis of dissolved gaseous mercury (DGM) and reactive mercury (RHg). DGM is the measurement of volatile Hg species and RHg is the measurement of easily reducible Hg^{II} in the water column.

Because concentrations of DGM and RHg are generally less than 50 pg/L, a sample volume of 1,700 ml is required to assure sufficient Hg for detection. Unfiltered water is collected into a 2 L Teflon purging vessel. DGM is collected from the sample simply by purging with Hg-free N_2, while the RHg requires the addition of 5 ml of $SnCl_2$ prior to purging, in order to reduce Hg^{II} to Hg^0. The 2 L sample vessel is capped tightly and purged with Hg-free N_2 at a flow rate of 500 ml/min for 60 minutes. The Hg^0 is collected on a gold-coated glass bead trap connected to the outlet of the vessel. The gold trap is sealed and shipped to the WDML for analysis.

Quality assurance (QA) samples are primarily the responsibility of the field personnel. QA samples for DGM and RHg include bubbler blanks, duplicate samples, backup traps and trip blanks.

Bubbler blanks for both DGM and RHg are performed by adding reagents to a prepurged sample and purging for another 60 minutes onto a new gold trap. The DQO for bubbler blanks collected for DGM and RHg are less than 5 pg and less than 10 pg respectively.

A duplicate sample is collected in a separate vessel and purged at the same time as the primary sample. The samples are treated identically and the DQO for precision is a difference of plus or minus10 pg for each parameter.

A backup trap (a gold trap connected to the outlet of the sample trap) is used to trap any Hg not amalgamated to the sample trap. Mercury detected on backup traps should not exceed 5 pg.

Several gold traps not used in sampling, will be shipped from the WDML. These traps are analyzed as trip blanks and evaluated to assess potential contamination of the traps during shipping. Trip blanks should not exceed 5 pg.

After a sample trap has been received by the WDML. The gold trap is placed in the analytical train of the Hg_T system, and treated as a Hg_T sample trap similar to Hg_T techniques. The peak areas measured are compared to calibration standards and concentration is determined by the size of the aliquot that was purged.

An MDL study has not been conducted for either DGM of RHg.

SUMMARY AND CONCLUSIONS

Method performance for Hg species is based on measurements for precision, accuracy, system control, and background contamination. Total mercury performance is based on the analysis of all samples in duplicate, a certified QCCS, spike recoveries, blanks and or DDL's, and CRM analysis for solids. Method performance for methylmercury is based on replicate samples, spike recoveries, check standards, distillation blanks, and CRM analysis for solids.

Reactive and DGM method performance is based on bubbler blanks, replicate samples, backup traps, and trip blanks which are the responsibility of the field personnel collecting the sample.

Low-level Hg methods have yet to be approved by the USGS, USEPA, American Public Health Association, or the American Society for Testing and Materials. Results produced by the WDML are for research purposes only and can be used in any USGS reports if qualified as provisional data with references cited. The total mercury method is scheduled for approval by the USEPA in the spring of 1999. The methylmercury method is in the process of being approved by the USEPA but approval is not expected until sometime in the year 2000. The WDML will be seeking USGS approval of WDML standard operating procedures as a water-quality method in the spring of 1999.

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SECTION C

Occurrence, Distribution, and Fate of Agricultural Chemicals in the Mississippi River Basin

The Mississippi River Basin drains about 41 percent of the United States and contains one of the most productive agricultural regions in the world. It produces the majority of all the corn, soybeans, sorghum, grain, cotton, livestock, and poultry grown in this country. Streamflow from the Mississippi Basin also provides most of the fresh water, nutrients, and sediment that enter the Gulf of Mexico. These inputs help make the Louisiana-Texas Gulf shelf one of the most productive fisheries in the world. The combined economic value of these two major industries has been estimated at more than $100 billion (Malakoff, 1998). Maintaining a highly productive and profitable agricultural economy in the basin requires extensive use of pesticides and chemical fertilizers. It is estimated that more than 100,000 metric tons of pesticides and about 6.5 million metric tons of commercial nitrogen fertilizers are applied to cropland in the basin each year. Most of these chemicals serve their intended purposes, which are to control weed and insect pests, and to increase crop yields. However, a small fraction of some of these chemicals leaks into the environment and may contaminate surface and ground water resources, air, rainwater, and the marine environment. To investigate the occurrence, transport, and fate of agricultural chemicals in the water resources of the Mississippi River Basin, the Toxic Substances Hydrology Program has conducted a number of large-scale nonpoint source studies during the past 10 years. The Midcontinent Herbicide Project, the Mississippi Delta Cotton Pesticides Project, and the Gulf of Mexico Hypoxia Assessment are included in these studies. The overall goal of these studies is to help provide the scientific basis needed to develop agricultural management practices that protect the quality of the region's water resources.

The Midcontinent Herbicide Project began in 1989. Its objectives are to determine the occurrence, transport, and fate of herbicides and herbicide degradates in water resources in the Mississippi Basin. The primary focus of the project has been on herbicides used in the production of corn and soybeans in a 10-State area in the upper Midwest, and in the Mississippi River. During the past 10 years the project has conducted regional-scale studies of herbicides in streams, reservoirs, ground water, rainwater, and air throughout the study area. The project also has supported the development of new analytical methods, including immunoassays for the determination of herbicides, insecticides, and some of their degradates in water and air. The following papers present some of the recent findings from this project.

The Mississippi Delta Cotton Pesticides Project was developed in the mid-1990s to investigate the land use, chemical, and physical processes associated with pesticides used in the production of cotton. Much of the work on this project to date has been done in the Mississippi Delta in collaboration with the Mississippi Embayment Project of the National Water-Quality Assessment Program. However, the study area also includes cotton production areas in Texas, Arizona, and California. Specific objectives of this project are to:

1) Compile and map the current use of pesticides across the cotton belt,
2) Determine which pesticides and degradates enter surface and ground water of the cotton belt and develop models to predict their occurrence,
3) Use knowledge gained from the Midcontinent Herbicide Project to examine the most critical geochemical processes that affect the fate, transport, and toxicity of cotton pesticides in surface and ground water, and
4) Develop analytical methods to determine the occurrence of cotton pesticides and degradates in water resources.

Several of the following papers present the results of field investigations of cotton pesticides and results of analytical methods development.

The Gulf of Mexico Hypoxia Assessment was initiated in 1997 at the request of the White House Office of Science and Technology Policy. The purpose of this science assessment is to determine the causes and consequences of oxygen depletion in the Gulf of Mexico along the Louisiana-Texas shelf. As part of the assessment the USGS was asked to determine the sources and loads of nitrogen, phosphorus, and silica entering the Gulf, and to determine which human activities are most responsible for contributing these nutrients. The following papers present information on the flux of nitrogen in Mississippi River waters, and on sources of nitrogen, including atmospheric deposition, in the basin.

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Nitrogen Flux and Sources in the Mississippi River Basin

by Donald A. Goolsby, William A. Battaglin, Brent T. Aulenbach, and Richard P. Hooper

ABSTRACT

Nitrogen from the Mississippi River Basin is believed to be at least partly responsible for the large zone of oxygen-depleted water that develops in the Gulf of Mexico each summer. Historical data show that concentrations of nitrate in the Mississippi River and some of its tributaries have increased by factors of 2 to more than 5 since the early 1900s. We have used the historical streamflow and concentration data in regression models to estimate the annual flux of nitrogen (N) to the Gulf of Mexico and to determine where the nitrogen originates within the Mississippi Basin. Results show that for 1980-96 the mean annual total N flux to the Gulf of Mexico was 1,568,000 metric tons per year (t/yr). The flux was about 61% nitrate as N, 37% organic N, and 2% ammonium as N. The flux of nitrate to the Gulf has approximately tripled in the last 30 years with most of the increase occurring between 1970 and 1983. The mean annual N flux has changed little since the early 1980s, but large year-to-year variations in N flux occur because of variations in precipitation. During wet years the N flux can increase by 50 percent or more due to flushing of nitrate that has accumulated in the soils and unsaturated zones in the basin. The principal source areas of N are basins in southern Minnesota, Iowa, Illinois, Indiana, and Ohio that drain agricultural land. Basins in this region yield 800 to more than 3,100 kilograms total N per square kilometer per year (kg/km^2/yr) to streams, several times the N yield of basins outside this region. Assuming conservative transport of N, streams draining Iowa and Illinois contribute on average about 35% of the total N discharged by the Mississippi River to the Gulf of Mexico. In years with high precipitation they can contribute a larger percentage.

INTRODUCTION

The Mississippi and Atchafalaya Rivers are the primary riverine sources of freshwater and nutrients discharged to the Gulf of Mexico. The combined mean annual streamflow for the Mississippi and Atchafalaya Rivers since 1980 is nearly 22,000 cubic meters per second and represents about 80 percent of the estimated freshwater discharge from the United States to the Gulf (Dunn, 1996). These two rivers also account for an estimated 90 percent of total nitrogen (N) flux discharged annually to the Gulf (Dunn, 1996). Nitrogen along with other nutrients leads to increased production of algae in the Gulf and has been implicated as a possible cause of hypoxia (dissolved oxygen less than 2 milligrams per liter) in a large zone of bottom water in the Gulf along the Louisiana-Texas coast (Rabalais and others, 1996, 1998). The seasonal decrease in dissolved oxygen develops each year during late spring following inflows of freshwater and nutrients to the Gulf. Hypoxia reaches a maximum in late summer and disappears each fall. Estimates of the size of the hypoxic zone during 1985-1992 averaged about 7,000-9,000 square kilometers (km^2). Following the 1993 Mississippi River flood, the hypoxic zone doubled to about 17,000 km^2, an area twice the size of Chesapeake Bay. In the summers of 1994-97, the hypoxic zone was reported to be as large as or larger than during the summer of 1993 (Rabalais and others, 1998; Rabalais, 1997).

Because of the public concern that has developed over the hypoxia issue, the White House Committee on Environment and Natural Resources (CENR) was asked in 1997 to conduct a scientific assessment of the causes and consequences of Gulf of Mexico hypoxia, and to identify gaps in the understanding of this problem. As part of this assessment, the U.S. Geological Survey was asked to estimate the flux and sources of nutrients (nitrogen, phosphorus, and silica) entering the Gulf of Mexico from the MARB, and to determine which human activities are most significant in contributing these nutrients. This paper presents a summary of the flux and sources of nitrogen entering the Gulf from the MARB.

METHODS

Historical data on streamflow and concentrations of dissolved nitrite plus nitrate as nitrogen (hereafter nitrate) and total N (total organic N plus ammonium plus nitrate) were used in regression models to estimate the daily and annual flux of nitrogen from the Mississippi-Atchafalaya River Basin (MARB) to the Gulf of Mexico. Nitrate concentrations have been measured numerous times each year since 1955, and total N concentrations have been measured since 1975 in the lower Mississippi River at St Francisville, La. To determine the source areas of the nitrogen transported to the Gulf, regression models were also developed for 42 smaller interior basins within the MARB (figure 1). These 42 basins, in aggregate, comprise about 70 percent of the MARB. Nitrate and total N fluxes were estimated for these basins for the period 1980 to 1996.

Regression model structure

Multiple regression models were developed to relate the flux of nitrate and total N to stream discharge. Consistent with many past studies (e.g., Cohn and others, 1992), a seven-parameter model was developed of the form:

$$\ln[\Phi] = \beta_0 + \beta_1 \ln[Q/\overline{Q}] + \beta_2\left(\ln[Q/\overline{Q}]\right)^2 + \beta_3[T-\overline{T}]$$
$$+\beta_4[T-\overline{T}]^2 + \beta_5\sin[2\pi T] + \beta_6\cos[2\pi T] + \varepsilon$$

where:

ln [ ]is the natural logarithm of the argument in brackets;

Φ is the flux of the solute $(C \cdot Q)$;

C is the solute concentration;

Q is the daily average streamflow;

$\overline{Q}$ is a centering term (a constant) to ensure that the linear and quadratic flow terms are independent;

T is time, expressed in decimal years;

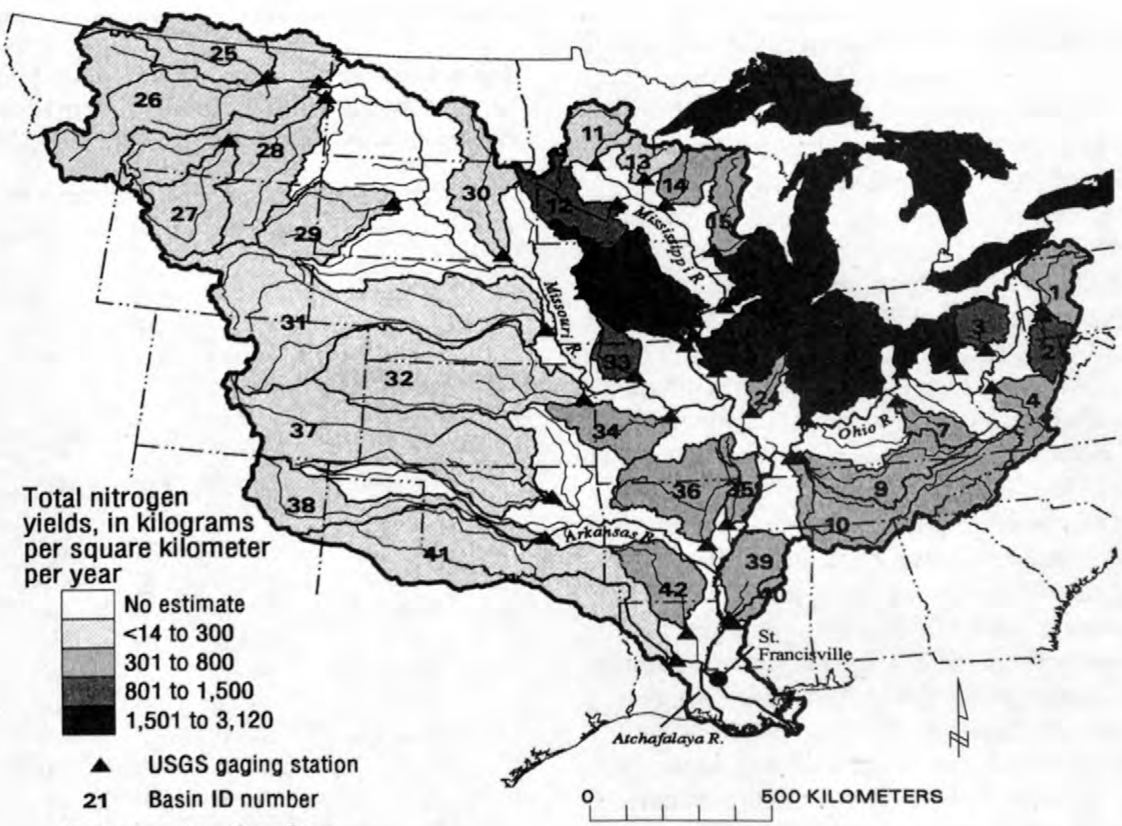
$\overline{T}$ is a centering term (a constant) to ensure that the linear and quadratic time terms are independent;

ε is the error term; and

$\beta_0 \ldots \beta_6$ are the fitted parameters in the multiple regression model.

This model captures the dependence of concentration on discharge, season (the sine and cosine terms), and any long-term trend. Quadratic terms were included to account for curvature that remained after transformation. Model parameters were estimated using the SAS system (SAS Institute Inc., 1990). Standard diagnostics (e.g., plots of observed vs. predicted values, and various residual plots) were calculated and examined for all models. All terms were retained in the models, even if the model parameters were not significant, to simplify calculation of models across all sites and solutes. Daily streamflow was not measured at sites 17 and 21 (fig. 1) and streamflow from a nearby gaging station was used in the regression model to estimate nutrient flux. For six sites (4, 27, 35,36, 39, and 42, fig. 1), continuous measurement of streamflow was not available. For these sites, the annual flux was estimated using the average of the instantaneous discharge measurements made when samples were collected and flow-weighted average nutrient concentrations.

Daily fluxes estimated from the models were summed over time to provide annual and long-term average flux estimates from the 42 basins and to the Gulf of Mexico. Nutrient yields (mass per unit area per unit time) were calculated for each basin by dividing the annual fluxes by the basin drainage area. This normalized the fluxes so that comparisons could be made among basins.



Interior basin sites

1 – ALLEGHENY R, NEW KENSINGTON, PA
2 – MONONGAHELA R, BRADDOCK, PA
3 – MUSKINGUM R, MCCONNELSVILLE, OH
4 – KANAWHA R, WINFIELD, WV
5 – SCIOTO R, HIGBY, OH
6 – G MIAMI R, NEW BALTIMORE, OH
7 – KENTUCKY R, LOCK 2, LOCKPORT, KY
8 – WABASH R, NEW HARMONY, IN
9 – CUMBERLAND R, GRAND RIVERS, KY
10 – TENNESSEE R, RT 60, PADUCAH, KY
11 – MISSISSIPPI R, ROYALTON, MN
12 – MINNESOTA R, JORDAN, MN
13 – ST CROIX R, ST CROIX FALLS, WI
14 – CHIPPEWA R, DURAND, WI
15 – WISCONSIN R, MUSCODA, WI
16 – ROCK R, JOSLIN, IL
17 – CEDAR R, CEDAR FALLS, IA
18 – IOWA R, WAPELLO, IA
19 – SKUNK R, AUGUSTA, IA
20 – RACCOON R, VAN METER, IA
21 – DES MOINES R, KEOSAUQUA, IA
22 – ILLINOIS R, MARSEILLES, IL
23 – ILLINOIS R, VALLEY CITY, IL
24 – KASKASKIA R, VENEDY STA., IL
25 – MILK R, NASHUA, MT
26 – MISSOURI R, CULBERTSON, MT
27 – BIGHORN R, BIGHORN, MT
28 – YELLOWSTONE R, SIDNEY, MT
29 – CHEYENNE R, CHERRY CR, SD
30 – JAMES R, SCOTLAND, SD
31 – PLATTE R, LOUISVILLE, NE
32 – KANSAS R, DESOTO, KS
33 – GRAND R, SUMNER, MO
34 – OSAGE R, ST THOMAS, MO
35 – ST FRANCIS B, RIVERFRONT, AR
36 – WHITE R, CLARENDON, AR
37 – ARKANSAS R, TULSA, OK
38 – CANADIAN R, CALVIN, OK
39 – YAZOO R, REDWOOD, MS
40 – BIG BLACK R, BOVINA, MS
41 – RED R, ALEXANDRIA, LA
42 – OUACHITA R, COLUMBIA, LA

Figure 1. Map of the Mississippi-Atchafalaya River Basin showing the average annual total nitrogen yields from 42 interior basins, 1980

RESULTS AND DISCUSSION

Historical Nitrogen Concentrations

Some of the earliest data available on nitrogen concentrations were published in USGS Water-Supply Paper 194 (Leighton, 1907). This report contains testimony pertaining to a lawsuit heard by the U.S. Supreme Court in 1905 on pollution of the Illinois and Mississippi Rivers by Chicago sewage. It also contains data on hundreds of analyses for nitrate, nitrite, ammonia, and organic nitrogen in samples from the Illinois River basin and Mississippi River in the vicinity of St. Louis, Mo., during 1897-1902. Historical nutrient data are also available from a study conducted by the USGS during 1906-07. In this study water samples were collected each day for about a year from 62 major rivers in the eastern one-half of the United States. These included several sites on the Mississippi River from

Minneapolis, Minn. to New Orleans, La., and sites on rivers in Arkansas, Illinois, Indiana, Iowa, Minnesota, Ohio, and Pennsylvania. The daily samples were composited at about 10-day intervals and analyzed for numerous solutes, including nitrate. Results of these analyses have been published in at least two USGS reports (Dole, 1909; Clarke, 1924). The USGS National Water Information System database provided additional data on nitrate concentrations in the Mississippi River and for several rivers in Iowa for 1944 to1996.

While the historical nitrate concentrations probably do not represent natural background conditions, they do provide a baseline from which changes that have occurred in the past 90-100 years can be determined. A summary of historical nitrate concentrations in a few interior basins in the MARB from the late 1890s to about 1965 are presented in table 1. Mean concentrations for samples collected from these same streams during 1980-96 near where the historical samples were obtained are shown for comparison. These data clearly show that the concentration of nitrate in the Mississippi River and some of its tributaries has increased significantly in the last 100 years. The mean nitrate concentrations in the rivers shown in table 1 have increased by factors of 2 to more than 5.

The longest uninterrupted data set on nitrate concentrations in the MARB is from the lower Mississippi River at St. Francisville, (fig. 1). Numerous samples have been collected at this site each year since1954. From 1954 to 1967 samples were collected each day and composited at 10-day to 30-day intervals for analysis. Compositing was discontinued in late 1967 and all subsequent analyses were on discrete samples collected periodically. The St. Francisville data set has been used extensively by scientists to estimate nitrate flux to the Gulf of Mexico and determine long-term changes in Mississippi River water quality (Turner and Rabalais, 1991; Bratkovich and others, 1994; Dunn, 1996; Rabalais and others, 1996; Goolsby and others, 1997). The long-term pattern in nitrate concentrations at this site is shown in figure 2. The average mean annual nitrate concentration at St. Francisville during 1980-96 was more than double the average concentration during 1955-1970. The average minimum and maximum annual concentrations also more than doubled.

Table 1. Historical and current (1980-96) mean nitrate concentrations in the Mississippi River Basin [n, number os samples; mg/L milligrams per liter].

Basin (fig. 1)	River basin name	Year(s)	n	mg/L
1	Allegheny	1906-07	35	0.16
		1980-96	76	0.65
22	Upper Illinois River	1896-99	Weekly	1.89
		1906-07	36	1.49
		1980-96	175	4.25
23	Lower Illinois River	1897-99	Weekly	1.01
		1906-07	36	0.97
		1980-96	187	4.12
16	Rock River	1906-07	36	0.86
		1980-96	152	3.49
8	Wabash River	1906-07	31	1.44
		1980-96	44	2.55
17	Cedar River	1906-07	37	0.70
		1944-50	175	1.53
		1980-96	83	4.67
20	Raccoon River	1945-47	55	2.93
		1980-96	48	6.67
21	Des Moines River	1906-07	37	0.75
		1955-65	28	3.02
		1980-96	88	4.12
12	Minnesota River	1906-07	30	0.32
		1980-96	122	4.19
3	Muskingum	1906-07	27	0.36
		1980-96	84	1.43
Mississippi River at New Orleans or /St. Francisville		1900-01	9	0.14
		1905-06	52	0.56
		1955-65	308	0.65
		1980-96	182	1.45

Current Nitrogen Concentrations

The two principal forms in which nitrogen occurs in streams of the MARB are nitrate and organic nitrogen (dissolved and particulate). Significant amounts of ammonia also may occur in some stream reaches, particularly downstream

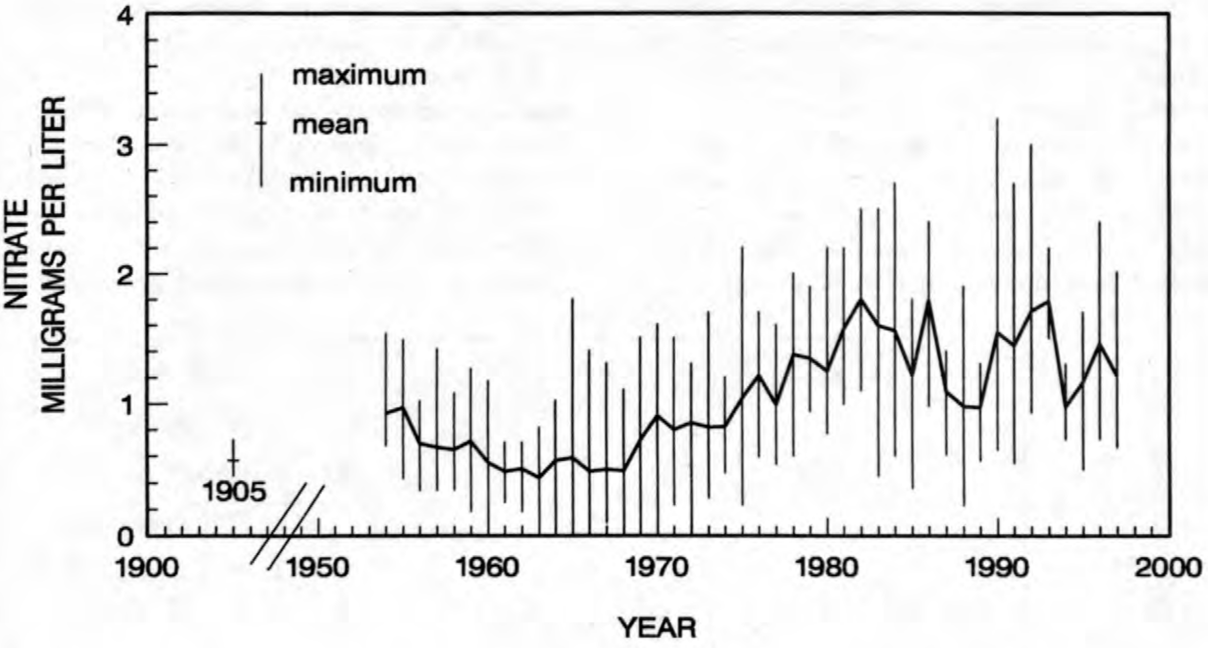


Figure 2. Long-term patterns in nitrate concentrations in the lower Mississippi River at St. Francisville, La.

from sources of human and animal wastes. However, ammonia is quickly transformed to nitrate and concentrations are generally much less than 0.1 mg/L in the lower reaches of the Mississippi River (Antweiler and others, 1995). Trace amounts of nitrite also occur briefly during the oxidation of ammonia to nitrate, which is the end product of the aerobic biochemical oxidation of organic nitrogen and ammonia in soil and water. Nitrate is the most soluble and mobile form of nitrogen and is easily leached by precipitation into ground water, tile drains, and streams.

The distribution of nitrate from 1980-96 at the sites representing the 42 interior basins is shown in the boxplots in figure 3A. As the figure shows, there are two distinctly different groups of basins. Twelve basins have median nitrate concentrations ranging from about 2.5 mg/L to more than 6 mg/L, while the remaining 30 basins have medians of less than 1.5 mg/L. Figure 3A also shows that the maximum nitrate concentration at several of these sites occasionally exceeds the U.S. Environmental Protection Agency drinking water standard of 10 mg/L. The high nitrate concentrations are associated with basins having either a high percentage of land in row crop (corn, soybeans, or sorghum) or a high population density (people per km^2). Basin 20 (Raccoon River, IA) had the highest median nitrate concentration (6.67 mg/L) and the highest percent cropland (74%). Basins 5 and 6 in Ohio and basin 22 in Illinois have the highest population densities (100 to more than 300 people per km^2) and more than 45% of the basin areas are in row crops. The extent of row cropland expressed as a percent of the basin area for the 12 basins with highest median nitrate concentrations ranged from 44 to 74. The percent of row cropland in the remaining 30 basins ranged from 0.1 to 35.

Figure 3B shows the distribution of total organic nitrogen (dissolved and particulate). Median values range from less the 0.5 mg/L to about 1.5 mg/L. The highest concentrations are associated with high population density, high cropland percentage, and/or high suspended-sediment concentrations. Median concentrations of ammonia (not shown in figures) were less than 0.2 mg/L in all 42 basins except the upper Illinois River (basin 22). This basin is dominated by municipal wastes from the Chicago area, and the river water contains significant amounts of ammonia (median is about 0.5 mg/L).

Nitrate concentrations in basins where the supply of nitrate in soils is abundant can vary seasonally over a large range (less than 0.1 mg/L to more than 10 mg/L) in response to climatic and hydrologic conditions. Concentrations tend to be highest in the late winter and spring when streamflow is highest and lowest in the late summer and fall when streamflow is low. This direct relationship between concentration and streamflow indicates that most of the nitrate in these streams is from non-point sources. If the nitrate was predominantly from point sources, concentrations would decrease as streamflow increased due to dilution. Instead nitrate concentrations in streams increase in response to rainfall or snowmelt that leaches nitrate that has accumulated in the soil. Scientific evidence suggests that nitrate levels can build up in soils during dry years from mineralization processes and reduced uptake by crops, and nitrate can be flushed out in larger than normal amounts in

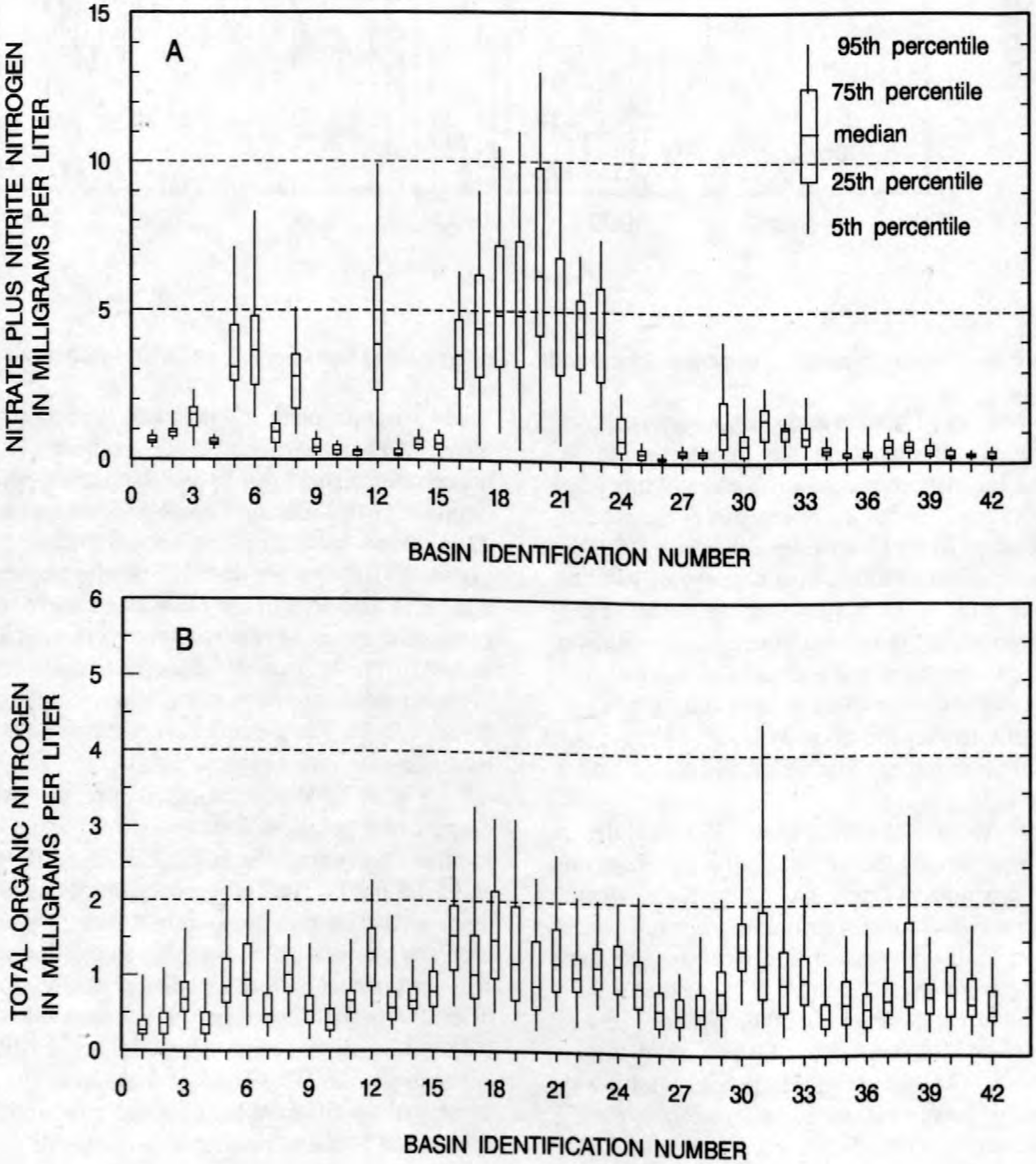


Figure 3. Boxplots showing the distribution of (A) nitrate plus nitrite, and (B) total organic nitrogen concentrations in 42 interior basins for 1980-96

succeeding wet years (Randall and others, 1997). Nitrate can enter streams through agricultural drains, ground-water discharge, and direct runoff. Nitrate concentrations generally decrease in the summer and fall as streamflow and agricultural drainage decrease. Assimilation of nitrate by agricultural crops on the land and aquatic plants in streams also aids in decreasing the nitrate concentrations in streams during summer months.

Agricultural drainage plays a major role in transporting nitrate from cropland to streams in the MARB. More than 50 million acres, mostly cropland, have been drained through use of tiles, ditches, and streams in Illinois, Indiana, Iowa, Ohio, Minnesota, Missouri, and Wisconsin (USDA, 1987). This practice "short-circuits" the flow of water by draining the top of the saturated zone directly into streams, and eventually the Mississippi River. This drainage practice can result in the leaching of large amounts of nitrate that are stored in the soil zone and unsaturated zone. Nitrate concentrations in agricultural drains can be very high -- 20-40 mg/L N or more (Fenelon, 1998; Gentry and others, 1998; Zucker and Brown, 1998; David and others, 1997; Randall and others, 1997).

Flux of Nitrogen to the Gulf of Mexico

The mean annual flux of nitrogen compounds from the MARB to the Gulf of Mexico for 1980-96 is summarized in table 2. This period includes the drought of 1988-89 when fluxes were very low and the flood of 1993 when the flux was very high. The mean annual flux of all forms of nitrogen was 1,568,000 (t/yr). This is within 2% of the flux estimate of 1,597,000 t/yr by Dunn (1996) for 1972-93 and about 14% less than the 1982-87 estimate of 1,824,000 t/yr made by Turner and Rabalais (1991). About three-quarters of the nitrogen flux from the MARB enters the Gulf of Mexico via the Mississippi River channel and the remainder discharges through the Atchafalaya River. However, nearly all of the nitrogen discharging from the Atchafalaya River is diverted into the Atchafalaya from the Mississippi River in northern Louisiana. The total N flux is about 61% nitrate, 2% ammonia and the remaining 37% is dissolved and particulate organic nitrogen. The flux of ammonia and the fraction of organic nitrogen present in dissolved and particulate forms was estimated from concentration data.

Table 2. Mean annual flux of nitrogen from the Mississippi-Atchafalaya River Basin to the Gulf of Mexico, 1980-96.

Nitrogen species	Metric tons	Percent of total	Standard error of estimate	
			Metric tons	% of mean
Nitrate	953,000	61	37,030	3.9
Ammonium	31,000	2	--	--
Dissolved organic N	378,000	24	--	--
Particulate organic N	206,000	13	--	--
Total N	1,568,000	100	58,500	3.7

The annual flux of nitrate to the Gulf increased significantly over the period 1955-96 as shown in figure 4. This increase in flux parallels the increase in concentration shown in figure 2. A Kendall's tau test for trend (Helsel and Hirsch, 1992) shows the increase in nitrate flux to be highly significant ($p < 0.001$), with a trend slope of about 19,000 t/yr. During 1955-70 nitrate flux averaged 328,000 t/yr. However, during 1980-96 the nitrate flux averaged 952,700 t/yr, almost a 3-fold increase. Nearly all of this increase occurred between 1970 and 1983. There is no statistically significant trend, upward or downward, in nitrate from 1980 to 1996, even if the flood year of 1993 is removed.

All of the increase in total N that has occurred since 1972 (figure 4) can be attributed to the increase in nitrate. The large year to year differences in flux are caused by variations in streamflow. The flux of nitrate was relatively low during the drought years of 1988-89 (500,000-600,000 t), but was high (>1,500,000 t) during the flood year of 1993. Nitrate flux was also high during 1979 and during 1982-86 when streamflow was abnormally high.

It should also be noted that streamflow in the MARB generally increased during the 1955-96 period (figure 4) and this could account for part of the increase in nitrate flux. The average

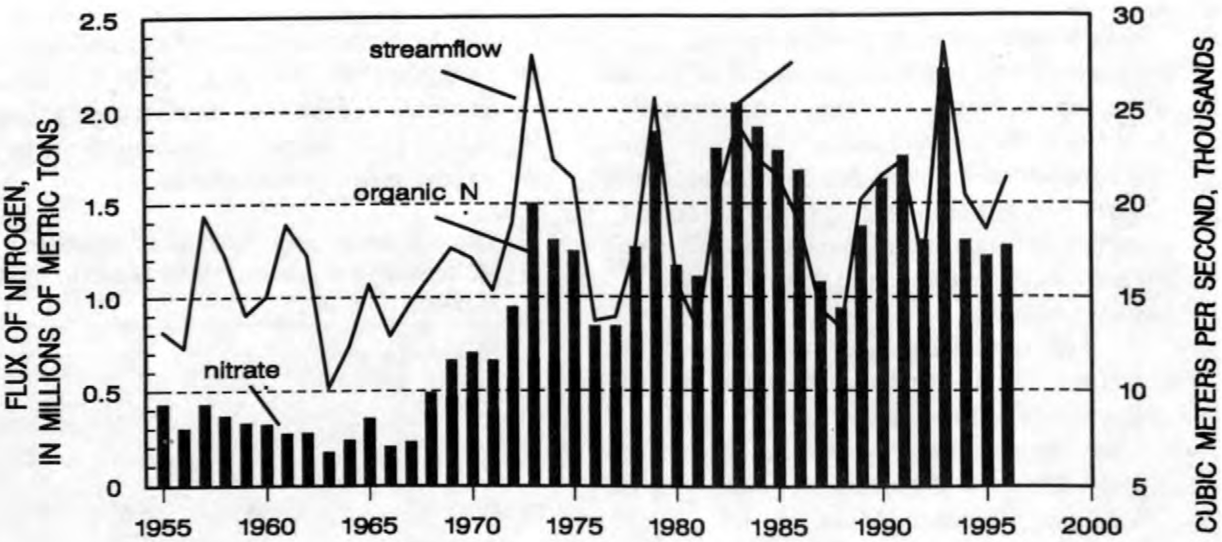


Figure 4. Bar chart of the annual flux of nitrate and organic nitrogen from the Mississippi-Atchafalaya River Basin to the Gulf of Mexico, 1955-96.

annual streamflow was 30% higher during 1980-96 than during 1955-70. A Kendall's tau test on the mean annual streamflow during 1955-96 showed a statistically significant upward trend ($p=0.001$) with a slope of 158 $m^3/s/yr$. The higher streamflow in the later time period could influence nitrate flux in two ways. First, the volume of flow would be larger and more nitrate sould be transported unless concentrations decreased, which does not appear to be the case. Second, the higher precipitation would leach more accumulated nitrate from soils in the basin and would actually cause nitrate concentrations to increase, as was previously discussed. The combination of higher concentrations and higher streamflow in the 1980-96 period would produce significant increases in nitrate flux.

Sources of Nitrogen

The spatial patterns in the average annual total N yields in the MARB are shown graphically in figure 1 for the 42 interior basins. The total N in most of these basins was composed largely of nitrate. Nitrate yields, therefore, have a spatial pattern very similar to that of total N. The distributions of the annual yields of total N for the 42 basins are shown in figure 5 for the 1980-96 period. The highest average annual total N yields ranged from 800 to more than 3,100 $kg/km^2/yr$ and occurred in a band extending from southwestern Minnesota across Iowa, Illinois, Indiana, and Ohio (fig.1). Annual average total N yields of 1,500 to more than 3,100 $kg/km^2/yr$ occurred in the Des Moines, Iowa, and Skunk River basins in Iowa (basins 17-21), the Illinois River basin (22-23), and the Great Miami basin in Ohio (6). Other basins adjacent to these, but not shown in the figures because of insufficient data, may have had similar yields. The maximum annual total N yield from some of these basins exceeded 6,000 $kg/km^2/yr$ (fig. 5). The total N discharged from these five rivers alone accounted for as much as 20% of the nitrogen discharge from the MARB during average years and more than 30% during flood years such as 1993, assuming instream denitrification losses in the lower Mississippi River were not significant. The nitrogen discharged from all streams draining the states of Iowa and Illinois during average years is estimated to accounts for about 35% of the nitrogen discharged to the Gulf. Total N yields were generally 300-800 $kg/km^2/yr$ in basins south of the Ohio River and were generally less than 300 $kg/km^2/yr$ in the Missouri, Arkansas, and lower Mississippi basin. Many of the drier basins in the western part of the MARB had total N yields less than 100 $kg/km^2/yr$.

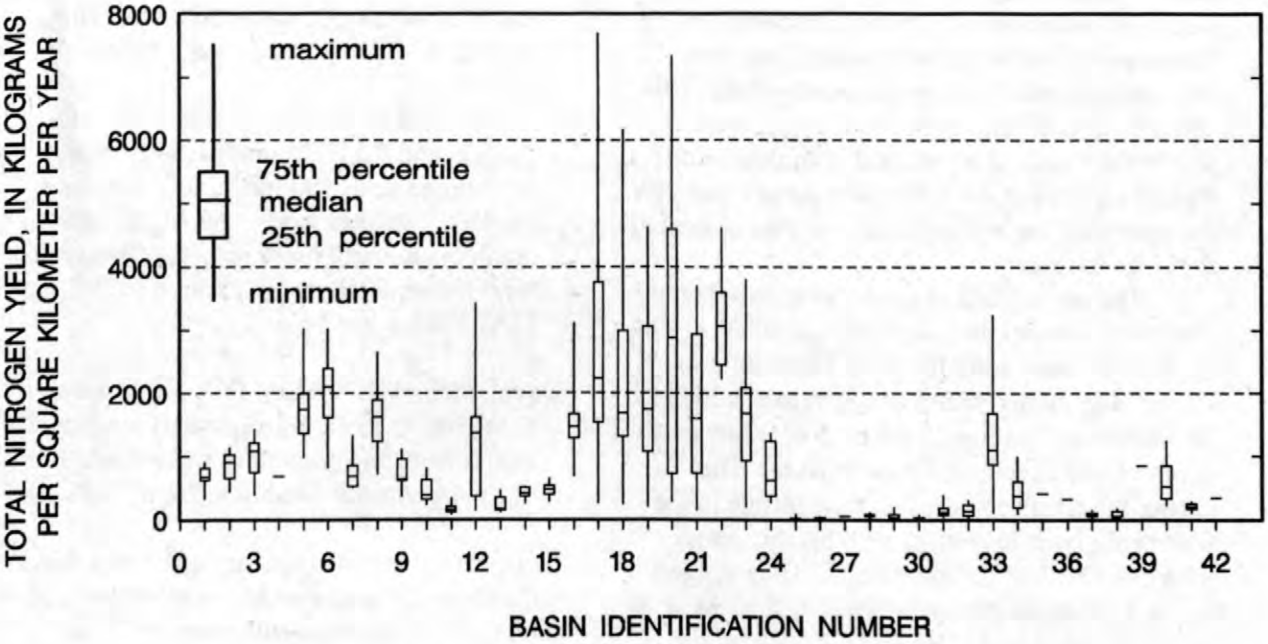


Figure 5. Boxplots showing the distribution of the annual total nitrogen yields for 42 interior basins, 1980-96. Dashes (-) show the median yields for several sites where other statistics could not be computed.

The large range in annual yields of total N shown in figure 5 can be attributed largely to year-to-year variations in precipitation and leaching of nitrogen from nonpoint sources. During dry years, there is little rainfall to transport soluble nitrogen (nitrate) from the soil and unsaturated zone to streams. Under these conditions nitrogen yields were very low, and nitrogen inputs from point sources may dominate in some streams. During periods of high precipitation, nitrate that has accumulated in the soil is flushed into streams via agricultural drains, ground-water discharge, and overland flow; thus, yields are high. Basins with large point source inputs, such as the Upper Illinois River (22) with more than 300 people per km^2 exhibit a different distribution in average annual yields (fig. 5). The minimum annual yield is very high (greater than 2,000 kg/km^2/yr) because of sustained year-round direct inputs of N from point sources. The range in yields is small because point source inputs are not greatly affected by precipitation. Several other basins, with above average population densities, such as the Scioto (5), Great Miami (6), Wabash (8), and the Rock (16) show this same pattern but to a lesser extent.

The nitrogen flux and yield estimates presented in the forgoing discussion represent the amounts of nitrogen delivered to larger rivers, usually the Ohio, Missouri, or Mississippi, and subsequently to the Gulf of Mexico. They do not account for any instream losses such as denitrification or deposition in sediment in reservoirs or flood plains. These processes would not significantly affect our estimates of nitrogen flux from the MARB to the Gulf of Mexico. However, if denitrification is significant in large rivers, this could affect our estimates of the relative contributions from basins within the MARB. The relative contribution of nitrogen to the Gulf by the furthest upstream basins could be overestimated, and the relative contribution by the furthest downstream basins could be underestimated.

Two lines of evidence suggest instream nitrogen losses in large rivers are small. First, the aggregate annual nitrogen yield of the Ohio, lower Missouri, and upper and lower Mississippi basins (826 kg/km^2/yr), an area of 1,668,400 km^2, is similar to the aggregate average annual nitrogen yield of 30 interior basins (880 kg/km^2/yr) that comprise 62% of the area of the large basins. The upper Missouri and Arkansas basins were excluded from this calculation because they contain reservoirs and have low precipitation rates and low nitrogen yields. This small difference in yields, about 6%, is well within the standard errors, and is an indication that no significant denitrification occurs between the outlets of the interior basins and the outlets of the large basins.

The second line of evidence is based on the results of a model that Howarth and others (1996) applied to rivers draining to the North Atlantic Ocean. The model relates nitrogen loss, which is largely denitrification, to the ratio of mean water depth to water residence time of rivers. The deeper the rivers are, the less time nitrate in the water column is in contact with benthic zones where denitrification could occur. They suggest that 5-20% of the nitrogen inputs to streams might be lost through denitrification in larger rivers. The large rivers in the MARB should be near the low end of these denitrification estimates. Short-term removal of nitrogen in algal and plant biomass should be accounted for in the long-term flux estimates if nitrogen is later released in dissolved or particulate forms. The above discussion suggests that most (>90%) of the nitrogen that enters the Ohio, lower Missouri, and Mississippi Rivers is eventually discharged to the Gulf of Mexico. Denitrification may result in significant losses of nitrogen in streams smaller and shallower than those used in this analysis. If this is true, then nitrogen yields in smaller watersheds could be significantly higher than those reported here for the 42 interior basins.

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Occurrence of Sulfonylurea, Sulfonamide, Imidazolinone, and other Herbicides in Midwestern Rivers, Reservoirs, and Ground Water, 1998

By William A. Battaglin, Edward T. Furlong, Mark R. Burkhardt, C. John Peter

ABSTRACT

Sulfonylurea (SU), sulfonamide (SA), and imidazolinone (IMI) herbicides are relatively new classes of chemical compounds that function by inhibiting the action of a plant enzyme, stopping plant growth, and eventually killing the plant. These compounds generally have low mammalian toxicity, but plants demonstrate a wide range in sensitivity to SUs, SAs, and IMIs with over a 10,000-fold difference in observed toxicity levels for some compounds. SUs, SAs, and IMIs are applied either pre- or post-emergence to crops commonly at 1/50th or less of the rate of other herbicides. Little is known about their occurrence, fate, or transport in surface water or ground water in the United States.

To obtain information on the occurrence of SU, SA, and IMI herbicides in the Midwestern United States, 212 water samples were collected from 75 surface-water and 25 ground-water sites in 1998. These samples were analyzed for 16 SU, SA, and IMI herbicides by USGS Methods Research and Development Program staff using high-performance liquid chromatography/mass spectrometry. Samples were also analyzed for 47 pesticides or pesticide degradation products. At least one of the 16 SUs, SAs or IMIs was detected above the method reporting limit (MRL) of 0.01 micrograms per liter (µg/L) in 83% of 130 stream samples. Imazethapyr was detected most frequently (71% of samples) followed by flumetsulam (63% of samples) and nicosulfuron (52% of samples). The sum of SU, SA, and IMI concentrations exceeded 0.5 µg/L in less than 10% of stream samples. At least one SU, SA, or IMI herbicide was detected above the MRL in 24% of 25 ground-water samples and 86% of 7 reservoir samples.

INTRODUCTION

During the last 20 years, low application rate herbicides have been developed that act by inhibiting the action of a key plant enzyme, which stops plant growth and eventually causes plant death. Sulfonylurea (SU), sulfonamide (SA), and imidazolinone (IMI) herbicides are three classes of compounds that share this mode of action (Meister, 1997).

Herbicide use

Crops that can be treated with SU, SA, and IMI herbicides include barley, corn, cotton, durum wheat, rice, canola, peanuts, soybeans, sugar beets, spring wheat, and winter wheat. Some compounds are also approved for use on Conservation Reserve Program acreage and for noncropland weed control.

The amount of cropland treated with SU, SA, and IMI herbicides has nearly tripled since 1990. The total corn, soybean, and wheat acreage on which 9 SUs, 1 SA and 2 IMIs were applied in eleven Midwestern States (Iowa, Illinois, Indiana, Kansas, Kentucky, Minnesota, Missouri, Nebraska, Ohio, South Dakota, and Wisconsin) from 1990 through 1997 is shown in figure 1 (U.S. Department of Agriculture, 1991-98). In 1997, more than 66 million acres were treated with one of the 12 herbicides. For comparison, atrazine, a triazine herbicide, was used on 41 million acres of corn and metolachlor, a chloracetanilide herbicide, was used on 23 million acres of corn and soybeans in the same 11-State area.

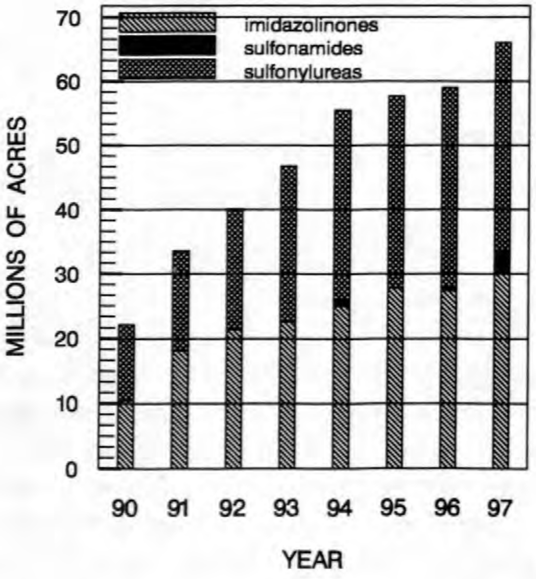

Figure 1. Estimated acres of corn, soybeans, and wheat treated with selected SU, SA, and IMI herbicides, 1990-97, in Midwestern States.

Although applied over comparable areas, SU, SA, and IMI herbicides are frequently applied after crops have emerged, and at low rates (typically less than 25 grams active ingredient/hectare). These application rates are commonly 1/50th or less of the rates for triazine or chloracetanilide herbicides (typically more than 1,200 grams/hectare). Hence, the overall use amount for SU, SA, and IMI herbicides is relatively small. For example, in 1997 in the 11-State area, an estimated 20,300 metric tons (tonnes) of atrazine and 21,500 tonnes of metolachlor were applied to cropland, while the total estimated use of the 9 SUs, 1 SA, and 2 IMIs was only 1,090 tonnes (U.S. Department of Agriculture, 1998).

Herbicide Chemistry

The soil half-life of SUs, SAs, and IMIs generally ranges from 1 to 25 weeks depending on soil pH and temperature. Their water solubilities range from 6 to 40,000 part per million. The water solubility of SUs are dependent on water pH. SUs degrade by chemical hydrolysis and microbial activity. SUs degrade faster in warm, moist, low organic, low pH soils (DuPont, 1998). IMIs degrade by microbial activity and photolysis. IMIs degradate faster in warm, moist, low organic soils (Goetz and others, 1990).

Herbicide Toxicity

SUs, SAs, and IMIs act upon a specific plant enzyme (acetolactate synthase) that is not found in mammals or other animals and are reported to have very low toxicities in animals (Brown, 1990; Meister, 1997). Plants demonstrate a wide range in sensitivity to SUs, SAs, and IMIs (Peterson and others, 1994) with over a 10,000-fold difference in observed toxicity levels for some compounds. EC50 concentrations are measures of compound toxicity. An EC50 is the concentration in water of a compound that causes a 50% reduction in a chosen plant characteristic for which a toxicity endpoint exists. For example, EC50s for algae can be calculated from laboratory tests measuring biomass development in the presence of varying compound concentrations. EC50 values for selected SU, SA, IMI, and other herbicides on 5 aquatic plants are shown on figure 2 (Fahl and others, 1995; U.S. Environmental Protection Agency, 1997; Sabater and Carrasco, 1997; Fairchild and others, 1997; Wei and others, 1998; C. J. Peter, DuPont Agricultural Products, written commun., 1999). The EC50 values plotted are for green algae (Selenastrum capricornutum), duckweed (Lemna gibba), blue-green algae (Anabaena flos-aquae), freshwater algae (Scenedesmus costatum), and freshwater diatom (Navicula pelliculosa). In some cases, EC50 values from more than one test on the same plant species are included. EC50 values for several herbicides range over 3 orders of magnitude. The EC50 data plotted on figure 2 support the hypothesis that a concentration of 0.1 μg/L in water is the baseline for non-target aquatic plant toxicity.

SUs, SAs, and IMIs are active at very low concentrations and can cause a problem with plant vigor in some crop rotations even when only 1 percent or less of the originally applied material remains. Some of these herbicides have demonstrated residual phytotoxicity to rotation crops such as corn, sunflowers, sugar beets, and dry beans (Anderson and Humburg, 1987; Curran and others, 1991). The labels of some of these herbicides restrict the planting of certain rotational crops. Fletcher and others (1993) indicated that spray drift containing SUs at concentrations less than 1 percent of the recommended application rate may adversely impact fruit tree yields. Felsot and others (1996) suggested that the appearance of chlorotic spots on crops in south central Washington is a result of exposure to low levels of SU herbicides in precipitation and not from direct spray drift. However, Obrigawitch and others (1998) questioned the validity of Fletcher's findings and the results of other studies that based their findings on short-term plant-response assessments. Obrigawitch and others (1998) found that a treatment rate of 0.1 gram of the most active SU ingredient per hectare (0.00009 pound per acre) represents a "threshold dose" and would be unlikely to reduce the yields of even the most sensitive non-target plants.

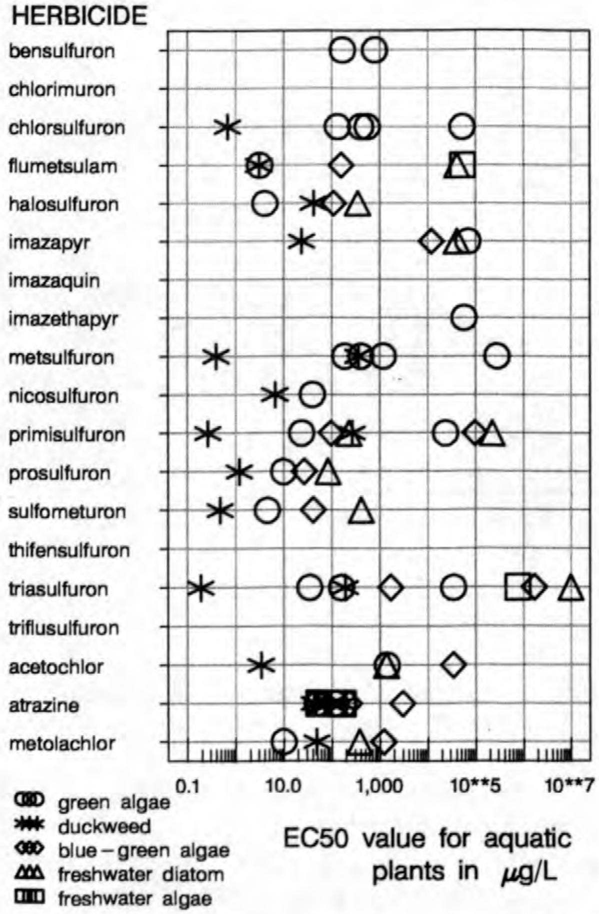

Figure 2. EC50 concentrations in micrograms per liter for 5 aquatic plants for selected SU, SA, IMI, and other herbicides.

Herbicide Occurrence

Detections of SUs, SAs, and IMIs in water collected from environmental settings have been rare and the few reported detections have been at nanogram per liter concentrations (Bergstrom, 1990; Michael and Neary, 1993; D'Ascenzo and others, 1998; Steinheimer and others, 1998; Okamoto and others, 1998). However, several studies indicate that some SUs, SAs, and IMIs herbicides may leach beyond the active root zone and enter ground-water or surface-water systems (Anderson and Humburg, 1987; Bergstrom, 1990; Flury and others, 1995; Veeh and others, 1994). Once in ground water or surface water, some SUs, SAs, and IMIs will tend to persist as the parent compound while others will tend to hydrolyze (Dinelli and others, 1997; Harvey and others, 1985). A study by Afyuni and others, (1997) indicated that between 1.1 and 2.3% of an applied SU was lost in runoff during a simulated rainfall event 24 hours after herbicide application.

Because of their low application rates and low overall use amounts, concentrations of SUs, SAs, and IMIs are expected to be low in most water resources. One can also assume based upon their chemical characteristics, application rates, and acres treated that individual SUs, SAs, and IMIs herbicides would be expected to occur in surface or ground water at 1 to 0.1 percent or less of the concentration of common triazine herbicides. Thus, one could expect to observe SUs, SAs, and IMIs herbicides in Midwestern rivers during post-application runoff events at concentrations ranging from 0.001 to 0.1 μg/L. Further, one could expect maximum concentrations of SUs, SAs, and IMIs herbicides to range from 0.01 to 1.0 μg/L (Battaglin and others, 1998a). In ground water, one would expect SU, SA, and IMI concentrations to seldom exceed 0.01 μg/L.

OBJECTIVES AND METHODS

Purpose and Scope

Currently, little is known about the occurrence, fate, or transport of SUs, SAs, and IMIs in the hydrologic system in the United States. The overall objective of this project is to determine if and at what concentrations selected SUs, SAs, and

IMIs occur in surface- and ground-water resources of the Midwestern United States. Specific objectives include:

- Develop an analytical method for selected SUs, SAs, and IMIs.
- Conduct a reconnaissance to determine the environmental occurrence of SUs, SAs, and IMIs herbicides in surface water and ground water in the Midwestern United States.
- Determine the frequency of detection and concentration distributions of SUs, SAs, and IMIs herbicides relative to those of selected other herbicides in Midwestern surface water and ground water.

A Cooperative Research and Development Agreement (CRADA) between the U.S. Geological Survey (USGS) and DuPont Agricultural Products was developed to accomplish the objectives of this project in a unbiased, yet economical manner (Battaglin and others, 1998b).

The data collected in this study are only adequate to identify the occurrence of selected SU, SA, and IMI herbicides during post-application runoff events in Midwestern streams and in ground water in parts of Iowa and Illinois. The data are not adequate to determine annual mean concentrations of detected herbicides or whether non-detected herbicide are present at other times of the year.

Plan of Study

The study involved collection of 212 samples during a 1998 reconnaissance. Samples were collected from streams, large rivers, reservoir outflows, and wells, sometimes in conjunction with USGS National Stream Quality Accounting Network (NASQAN) (Hooper and others, 1997) and National Water Quality Assessment (NAWQA) (Leahy and Thompson, 1994) activities. All reconnaissance samples were analyzed for 16 SU, SA, and IMI herbicides (table 1) using high performance liquid chromatography coupled with mass spectrometry. This custom analytical method has a method reporting limit (MRL) of 0.01 μg/L for all analytes and is fully described in Furlong and others (1999).

Sampling Sites

Samples were collected from 75 surface-water sites in the Upper Mississippi, Missouri, and Ohio River basins (figure 3). Fifty-two of the surface-water sites have been studied in previous Mid-continent Herbicide Initiative (MHI) investigations (Thurman and others, 1992; Goolsby and others, 1994; Scribner and others, 1998). These sites were selected out of the set of 150 sites sampled in 1989 using a stratified random method (Scribner and others, 1993). It is important to note that the sampling strategy used was not designed to produce an unbiased estimate of herbicide occurrence in all Midwestern streams. Rather the intent was to target higher risk areas while still capturing the variability of the entire population. Samples also were collected at selected NASQAN and NAWQA sites and just downstream from five reservoirs at locations that had been sampled in a previous investigation (Coupe and others, 1995; Scribner and others, 1996).

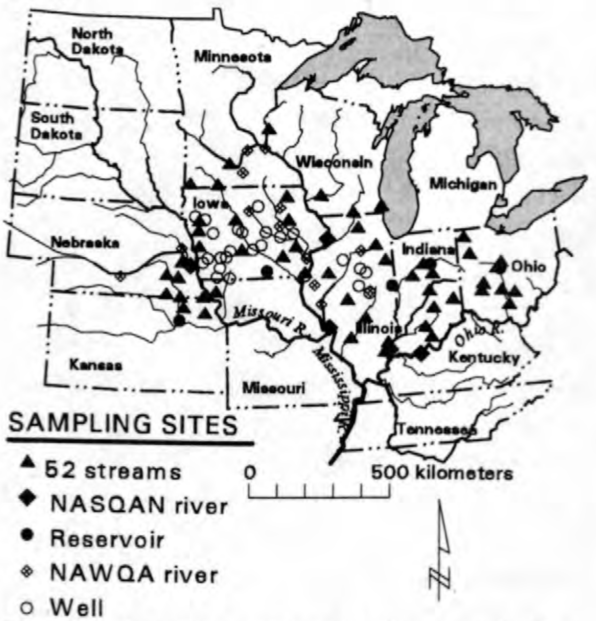

Figure 3. Location and type of sites sampled in 1998 herbicide reconnaissance.

Twenty ground-water samples were collected from a network of municipal wells in Iowa that are part of the Iowa Ground water Monitoring (IGWM) program (Detroy and others, 1988; Kolpin and others, 1997). Wells from this network have been sampled systematically since 1982. The depths to the top of the well screen for the sampled wells, ranged from 6 to 83 meters and most were less than 30 meters. Five samples also were collected from observation wells in the Lower Illinois NAWQA study unit (Warner and Schmidt, 1994). These wells were all less than 8 meters deep.

Sampling Schedule and Procedure

Two samples were collected at each surface-water and reservoir site, and one sample was collected at each ground water site in 1998. The first surface-water samples were collected after pre-emergence herbicides were applied (May or June) and following a precipitation event that produced a significant increase in streamflow. The second surface-water samples were collected after post-emergence herbicides were applied (June or July) again following a precipitation event that produced runoff and an increase in streamflow. The first NASQAN and reservoir samples were collected 2-3 weeks after the first surface-water samples were collected from nearby sites. The second NASQAN and reservoir samples were collected 2-3 weeks after the second surface-water samples were collected from nearby sites. Ground-water samples were collected in June, July, or August.

Samples were collected using protocols that are identical to those used for the collection of samples for low levels of other dissolved organic compounds (Shelton, 1994). The equal-width-increment sampling method was used in all cases except on some large rivers where equal-discharge-increment sampling was used (Edwards and Glysson, 1988).

All equipment was precleaned with a Liquinox/tap-water solution, rinsed with tap water, deionized water, and then methanol, and air dried. All samples were filtered through 0.7-µm pore-size heat-cleaned glass-fiber filters using an aluminum-plate filter holder and a ceramic-piston fluid-metering pump with all Teflon tubing into precleaned 1-liter or 125-ml amber glass bottles. Samples were immediately chilled and shipped on ice from the field to the USGS National Water Quality Laboratory (NWQL) within two days of collection.

Analytical Methods

An analytical method was developed that is an extension and improvement of the high-performance liquid chromatography/mass spectrometry (HPLC/MS) method by Rodriguez and Orescan (1998). Briefly, the method consists of isolation of the analytes of interest from approximately 1 liter of water (precisely measured) using two stacked solid-phase extraction cartridges. After isolation, the second cartridge is dried and the analytes are eluted using acidified acetone. The analytes are then concentrated and solvent exchanged into 1 mL of 10% acetonitrile and 90% water.

HPLC/MS analysis is performed using a Hewlett Packard 1100 series HPLC, coupled to a Hewlett Packard LC/MSD. Electrospray ionization, operated in the positive ion mode, is used to ionize the analytes. Selected-ion monitoring is used to maximize sensitivity. A calibration curve is developed using external standards and the linear range of the method is from 0.005 to 0.5 µg/L. Three ions (1 quantitation, 2 confirmation) are monitored for each compound. For confirmed identification of analytes, the relative ion abundances for the detection must be within 20% of average response for standards, as well as have the correct relative retention time (within 0.1 min). Detected analytes that don't meet one criterion are reported as estimates, as are all detections below the MRL of 0.01 µg/L. Details of this analytical methods are provided elsewhere in this volume by Furlong and others (1999).

In addition, all samples were analyzed for several other classes of pesticides. Samples were analyzed for 41 pesticides and pesticide metabolites by GC/MS with selected-ion monitoring using methods described by Zaugg and others (1995). This method has MRLs that range from 0.001 to 0.018 µg/L.

RESULTS AND DISCUSSION

Sample collection began in May and was completed in August, 1998. Two hundred twelve samples were submitted to Methods Research and Development Program staff at the NWQL. Of these, 177 are from surface-water sites and 29 are from ground water sites (results from 6 samples collected from 2 tile drains in New York State are not discussed here). These numbers include QA samples. One hundred fifty surface-water samples and 25 ground-water samples were submitted to the NWQL for analysis of 47 pesticides or pesticide degradation products.

Occurrence in Surface Water

SU, SA, and IMI Herbicides

Results from 130 stream and river samples are summarized in table 1. At least one of the 16

SU, SA or IMI herbicides was detected in 83% of stream samples. Imazethapyr was the most frequently detected compound (71% of samples) followed by flumetsulam (63%), nicosulfuron (52%), imazaquin and chlorimuron ethyl (32%). Chlorsulfuron, halosulfuron methyl, imazapyr, prosulfuron, sulfometuron methyl, and thifensulfuron methyl were detected in 5 percent or less of samples. The detection frequencies reported are for samples with concentrations at or above the method reporting limit (MRL), currently 0.01 µg/L. Bensulfuron methyl, metsulfuron methyl, primisulfuron methyl, triasulfuron, and triflusulfuron methyl were not detected above the MRL.

The distributions of concentrations of the target analytes in 130 samples are summarized in figure 4. In some cases, estimated concentrations are reported that are below the MRL. These concentrations are not counted as detections above the MRL (numbers to the right of the boxplots in figure 4), but are used in the calculation of summary statistics (the boxplots themselves). The sum of SU, SA, and IMI concentrations exceeded 0.5 ug/L in less than 10% of stream samples.

At least one of the 16 SU, SA or IMI herbicides was detected above the MRL in 6 of 7 (86%) of reservoir samples. Flumetsulam, imazethapyr, imazaquin, and nicosulfuron were each detected in 4 samples. The sum of SU, SA, and IMI concentrations did not exceeded 0.5 ug/L in any reservoir sample.

Other Herbicides

The results of analysis for selected other herbicides in 129 stream or river samples are also included on table 1. Acetochlor, alachlor, atrazine, cyanazine, and metolachlor were all detected in 90% or more of the stream samples. Atrazine had the highest median concentration (3.97 µg/L), followed by metolachlor (1.73 µg/L), and acetochlor (0.411 µg/L). The sum of the 5 other herbicides included in table 1 exceeded 50 µg/L in about 10% of the samples. This sum was expected to be at least 100 times greater than the sum of the SU, SA, and IMI herbicide concentrations.

Table 1. Statistical summary of SU, SA, IMI, herbicide concentrations in 130 samples and selected other herbicide concentrations in 129 water samples from Midwestern streams and rivers, 1998 (in µg/L)

Herbicide	Detections above MRL	median	maximum
	SU, SA, and IMI herbicides		
bensulfuron methyl	0	<0.01	<0.01
chlorimuron ethyl	41	<0.01	0.304
chlorsulfuron	1	<0.01	0.013
flumetsulam	82	0.020	2.11
halosulfuron methyl	7	<0.01	0.067
imazapyr	5	<0.01	0.072
imazaquin	41	<0.01	1.11
imazethapyr	92	0.031	0.689
metsulfuron methyl	0	<0.01	<0.01
nicosulfuron	67	0.010	0.266
primisulfuron methyl	0	<0.01	<0.01
prosulfuron	6	<0.01	0.036
sulfometuron methyl	2	<0.01	0.018
thifensulfuron methyl	1	<0.01	0.015
triasulfuron	0	<0.01	<0.01
triflusulfuron methyl	0	<0.01	<0.01
sum of 16 SUs, SAs, and IMIs	108	0.137	2.23
	Other herbicides		
acetochlor	124	0.411	25.1
alachlor	116	0.045	17.2
atrazine	129	3.97	224.
cyanazine	119	0.326	14.0
metolachlor	129	1.73	143.
sum of 5 other herbicides	129	6.90	385.

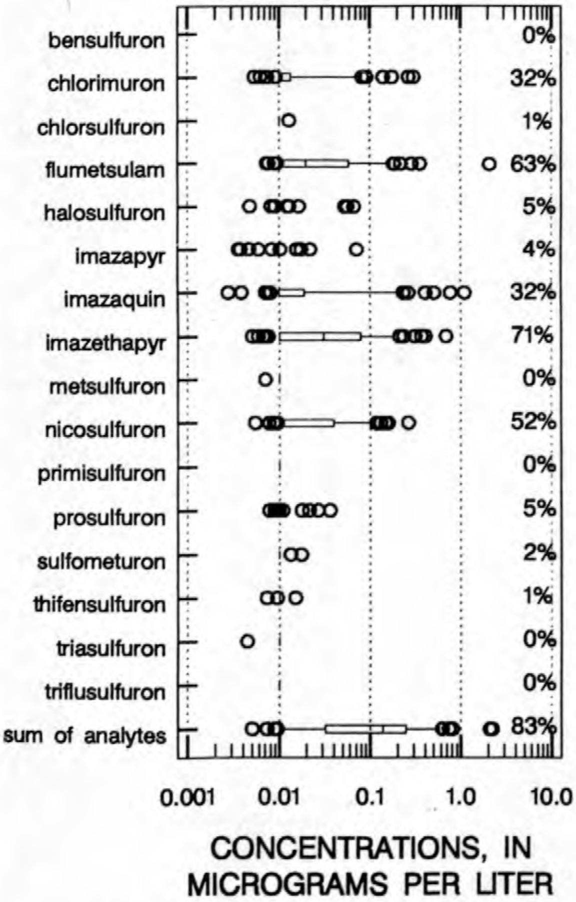

Figure 4. Boxplots of SU, SA, and IMI herbicide concentrations and percent detections above the MRL (0.01 micrograms per liter) in 130 samples from midwestern streams, 1998. The boxes show the 25th, 50th (median) and 75th percentiles, the wiskers extend to the 5th and 95th percentiles, and outliers less than the 5th or greater than the 95th percentiles are shown as circles.

Occurrence in Groundwater

SU, SA, and IMI Herbicides

Results from 25 groundwater samples are summarized in table 2. At least one of the 16 SU's, SA's or IMI's was detected in 24% of groundwater samples. Imazethapyr was the most frequently detected compound (16% of samples) followed by flumetsulam (12%), and nicosulfuron and imazaquin (8%). The sum of SU, SA, and IMI concentrations exceeded 0.01 ug/L in 6 samples.

Other Herbicides

The results of analysis for selected other herbicides are also included on table 2. Atrazine and metolachlor were detected in about one-half of the samples. The sum of the concentrations of the 5 herbicides did not exceeded 1.0 µg/L in any sample. This sum was also expected to be at least 100 times greater than the sum of the SU, SA, and IMI herbicide concentrations.

Concentrations of SUs, SAs, and IMIs Relative to other Herbicides

Because they have similar chemical properties, but much lower application rates, SU, SA, and IMI herbicides were expected to occur at fraction (1/100th or less) of the concentrations of other herbicides such as atrazine (Battaglin and others, 1998). In figures 5a, 5b, and 5c, the concentrations in streams of imazethapyr, flumetsulam, and nicosulfuron, the three most frequently detected target analytes, are plotted versus atrazine concentration. The lines crossing these plots show the 1:10, 1:100, and 1:1,000 ratios of concentration. The data plotted on figure 5 suggest that in about half the samples imazethapyr, flumetsulam, and nicosulfuron occur at 1/100th or less of the concentration of atrazine.

The observed range and maximum concentrations of SU, SA, and IMI herbicide in samples collected from Midwestern streams during post-application runoff events in 1998 was very close to what we expected. The majority of SU, SA, and IMI detections were at concentrations less than 0.1 µg/L. These concentrations are not likely to be toxic to non-target aquatic plants. The maximum concentrations of SU, SA, and IMI herbicide in samples collected from Midwestern ground water in 1998 were slightly higher than expected.

Table 2. Statistical summary of SU, SA, IMI, and selected other herbicide concentrations in 25 samples of Midwestern ground water, 1998 (in µg/L)

Herbicide	Detections above MRL	median	maximum
SU, SA, and IMI herbicides			
bensulfuron methyl	0	<0.01	<0.01
chlorimuron ethyl	0	<0.01	<0.01
chlorsulfuron	0	<0.01	<0.01
flumetsulam	3	<0.01	0.035
halosulfuron methyl	0	<0.01	<0.01
imazapyr	0	<0.01	<0.01
imazaquin	2	<0.01	0.024
imazethapyr	4	<0.01	0.059
metsulfuron methyl	0	<0.01	<0.01
nicosulfuron	2	<0.01	0.016
primisulfuron methyl	0	<0.01	<0.01
prosulfuron	0	<0.01	<0.01
sulfometuron methyl	0	<0.01	<0.01
thifensulfuron	0	<0.01	<0.01
triasulfuron	0	<0.01	<0.01
triflusulfuron methyl	0	<0.01	<0.01
Sum of 16 SUs, SAs, and IMIs	6	<0.01	0.110
Other herbicides			
acetochlor	1	<0.002	0.004
alachlor	1	<0.002	0.016
atrazine	14	0.010	0.410
cyanazine	1	<0.004	0.007
metolachlor	12	<0.002	0.557
sum of 5 other herbicides	14	0.014	0.703

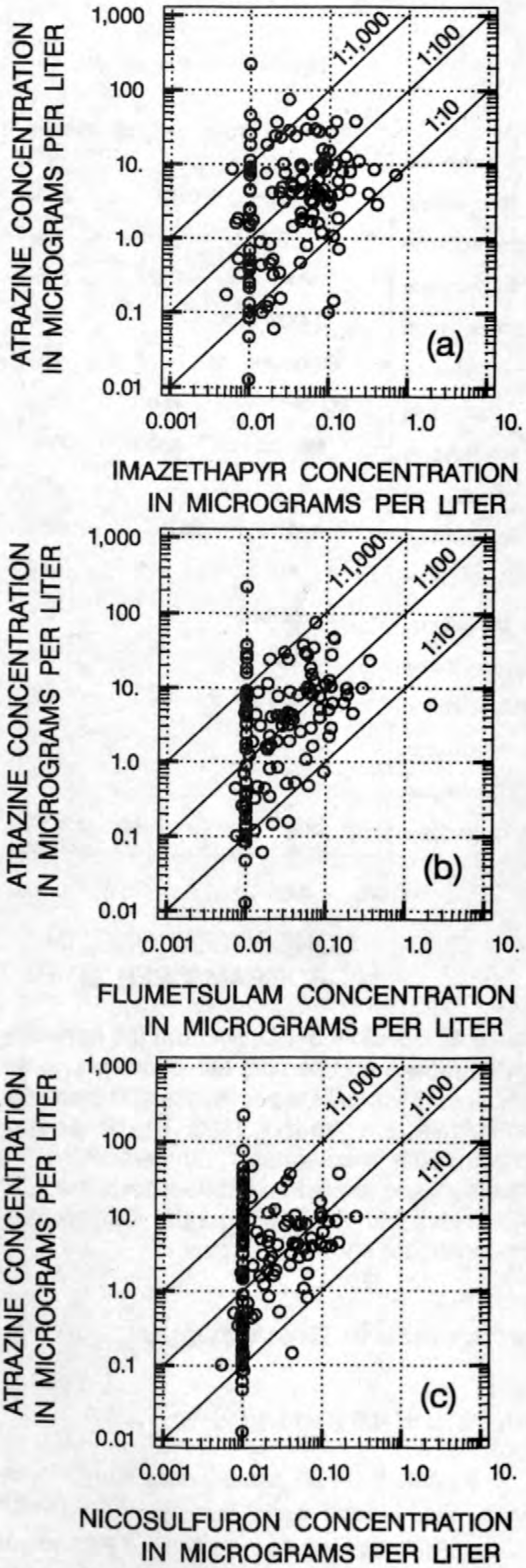

Figure 5. Concentrations of atrazine versus (a) imazethapyr, (b) flumesulam, and (c) nicosulfuron, in 130 samples collected from Midwestern streams in 1998.

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Occurrence of Cotton Herbicides and Insecticides in Playa Lakes of the High Plains of West Texas

By E.M. Thurman, K.C. Bastian, and Tony Mollhagen

ABSTRACT

During the summer of 1997, water samples were collected and analyzed for pesticides from 32 playa lakes of the High Plains that receive drainage from both cotton and corn agriculture in West Texas. The major cotton herbicides detected in the water samples were diuron, fluometuron, metolachlor, norflurazon, and prometryn. Atrazine and propazine, corn and sorghum herbicides, also were routinely detected in samples from the playa lakes. Furthermore, the metabolites of all the herbicides studied were found in the playa-lake samples. In some cases, the concentration of metabolites was equal to or exceeded the concentration of the parent compound. The types of metabolites detected suggested that the parent compounds had been transported to and had undergone degradation in the playa lakes. The types of metabolites and the ratio of metabolites to parent compounds may be useful in indicating the time that the herbicides were transported to the playa lakes. The median concentration of total herbicides was 7.2 micrograms per liter, with the largest total concentrations exceeding 30 micrograms per liter. Organophosphate insecticides were detected in only one water sample. Further work will improve the understanding of the fate of these compounds in the playa lakes area.

INTRODUCTION

Cotton farming may require as much as 7 kg/ha (kilograms per hectare) of herbicides and 5 kg/ha of insecticides annually (Coupe and others, 1998). The intensive application of pesticides often is necessary, especially in the Southern United States, where weed and insect pressures are great. Applications of pesticides to cotton are three to five times greater per hectare than applications to corn and occur more frequently (average of 4.7 annual applications compared to 1.2) (Coupe and others, 1998), yet there have been few regional studies of water quality and pesticide fate in cotton-producing areas of the country. For this reason, the U.S. Geological Survey's (USGS) Toxics Substances Hydrology Program initiated research on the occurrence of cotton pesticides in the aquatic environment with the purpose of determining the extent and magnitude of herbicide and insecticide concentrations in both surface and ground water of the Southern United States.

Since the inception of this water-quality research in 1995, two publications have described the extent of occurrence of cotton pesticides in surface water (Coupe and others, 1998; Thurman and others, 1998). The first publication (Thurman and others, 1998) dealt with the occurrence of cotton pesticides in surface water of the Mississippi Embayment. The work described in this fact sheet indicates that cotton herbicides occur frequently in surface water, with the major compounds, in order of percent detections, being: fluometuron > cyanazine > metolachlor > norflurazon > prometryn. Unfortunately, the phenylurea herbicide, diuron, was not examined in this earlier work. Insecticides were also reported in this study, with the important detections being: dicrotophos > profenofos > methyl parathion > malathion.

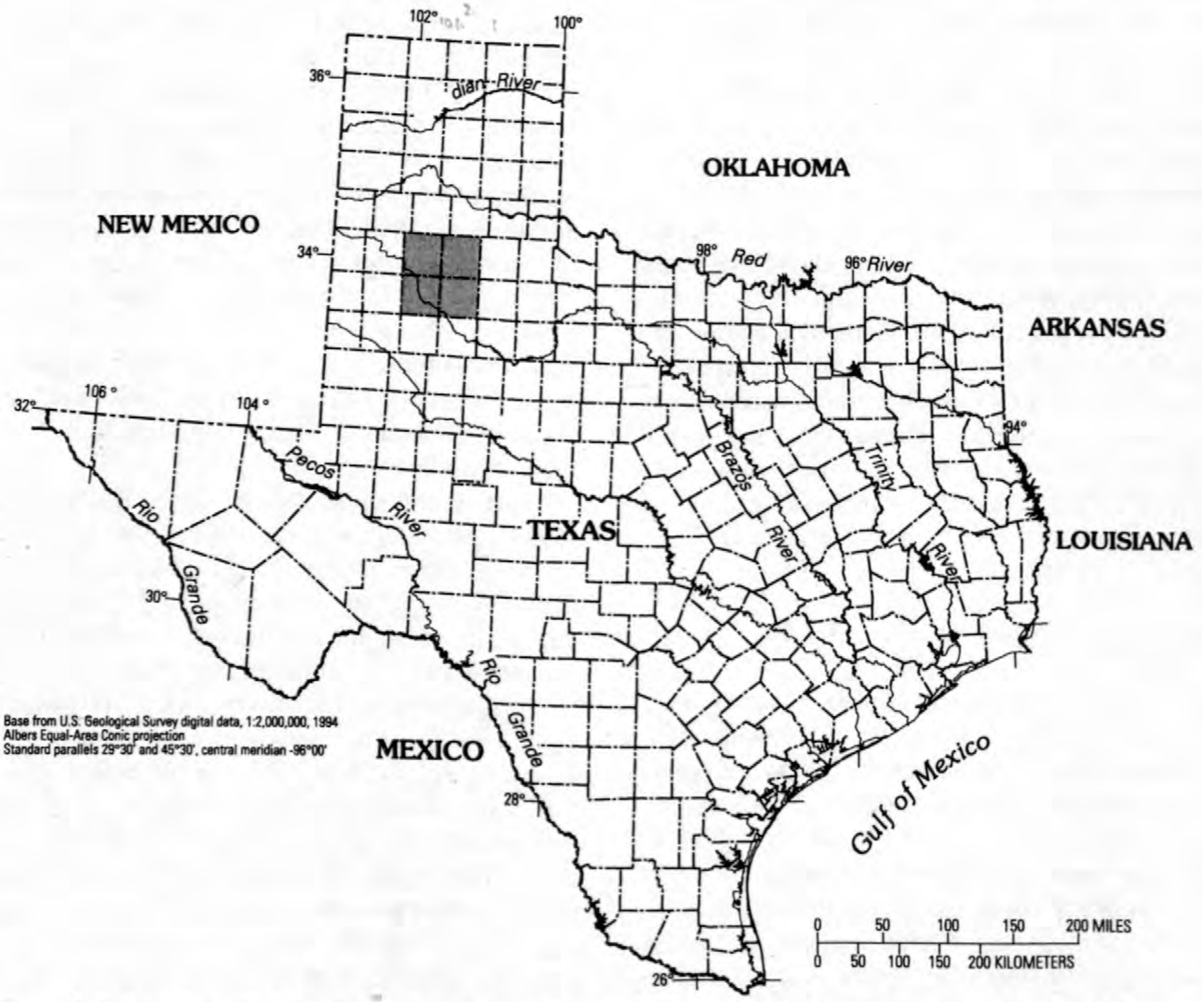
A second publication by Coupe and others (1998) dealt with the usage of herbicides and their occurrence in surface water of the Mississippi Delta. A major finding described in the report was that the distribution and duration of total herbicide concentrations were much different from that found in regional studies of herbicides in the Midwestern United States (Coupe and others, 1998). In the Midwest, the total herbicide concentration in surface water showed a sharp peak during the spring immediately after application of herbicides to crops, followed by a gradual decrease in concentration (Thurman and others, 1991; Thurman and others, 1992). In the Mississippi Delta, the total herbicide concentration in surface water was more sustained, with multiple peaks due to different application times and post-emergent applications of herbicides on cotton and rice (Coupe and others, 1998).

In this paper, the concentrations of herbicides and insecticides in water samples from playa lakes of the West Texas High Plains are reported because playa lakes form the major surface drainage in the area. Playa lakes are small lakes formed from precipitation and runoff in arid regions and are temporary lakes that either evaporate or infiltrate into the soil. Land-surface drainage is to playa lakes after rainfall. Farmers in the High Plains of West Texas grow both dryland (nonirrigated) cotton and irrigated cotton. Water samples were collected from each of 32 playa lakes (fig. 1) on July 22–23, 1997, after pesticide application to cotton in the region.

SAMPLE COLLECTION AND ANALYSIS

Surface-water grab samples were collected at each of the 32 playa lakes. Samples were filtered through 0.70-µm (micrometer) glass-fiber filters and stored on ice until analyzed at the USGS laboratory in Lawrence, KS. Water samples were analyzed for the following parent compounds: atrazine, cyanazine, diuron, fluometuron, metolachlor, norflurazon, prometryn, and propazine.

Materials used in analysis consisted of C-18 cartridges (Waters, Milford, MA) with 350 mg (milligrams) of 40-µm, C-18 bonded silica. Analytical standards were obtained from various sources. Atrazine, cyanazine, 3,4-dichloroaniline (3,4-DCA), deethylatrazine (DEA), deisopropylatrazine (DIA), deisopropylprometryn,



Base from U.S. Geological Survey digital data, 1:2,000,000, 1994
Albers Equal-Area Conic projection
Standard parallels 29°30' and 45°30', central meridian -96°00'

Figure 1. Location of study area where playa lakes were sampled in West Texas, July 22–23, 1997.

dicrotophos, diuron, fluometuron, hydroxyatrazine, metolachlor, norflurazon, prometryn, and propazine were obtained from Supleco (West Chester, PA). Dichloromethylphenylurea (DCMPU) and dichlorophenylurea (DCPU) were obtained from Jennifer Field, Oregon State University (Corvallis, Oregon). Demethylfluometuron (DMFM), trifluoromethylphenylurea (TFMPU), and trifluoromethylaniline (TFMA) were obtained from the U.S. Department of Agriculture, Agriculture Research Service (Stoneville, MS). Demethylnorflurazon was obtained from Sandoz Agro, Inc. (Des Plaines, IL). Metolachlor oxanilic acid was obtained from Novartis (Greensboro, NC). Metolachlor ethane sulfonic acid was synthesized in the USGS laboratory in Lawrence, KS, by Aga and others (1996).

The following gas chromatograph/mass spectrometry (GC/MS) method is also described in Thurman and others (1990). Solid-phase extraction (SPE) was automated on a Waters Millilab workstation (Milford, MA). C-18 Sep-Pak cartridges were conditioned sequentially with 2 mL (milliliters) methanol, 2 mL ethyl acetate, 2 mL methanol, and 3 mL distilled water. Each 123-mL water sample was spiked with a surrogate standard, terbuthylazine (1.23 ng/µL, nanograms per microliter; 100 µL, microliters), and pumped through the cartridge at a rate of 20 mL/min (milliliters per minute) by a robotic probe. Analytes were eluted with ethyl acetate and spiked automatically with phenanthrene d-10 (0.2 ng/µL, 500 µL). The extract was evaporated by a TurboVap (Zymark, Palo Alto, CA) at 45 °C (degrees Celsius) under nitrogen gas to 100 µL.

GC/MS analysis of the eluates was carried out using a Hewlett-Packard model 5890A GC interfaced to a 5970A mass selective detector (MSD) (Palo Alto, CA). One microliter (1 µL) of sample was injected automatically. Separation of the herbicides and insecticides was accomplished with a fused-silica capillary column of 5-percent phenyl methyl silicone (Ultra 2) with a film thickness of 0.33 µm, 12 m (meter) x 0.2 mm (millimeter) inside diameter (Hewlett Packard, Palo Alto, CA). Helium was used as the carrier gas at a flow rate of 1 mL/min and a head pressure of 35 kPa (kilopascals). The column temperature was held at 60 °C for 1 minute ramped at 6 °C per minute to 200 °C, and then ramped at 30 °C per minute to 250 °C, where the temperature was held for 4 minutes. The samples were injected in the splitless mode using an autoinjector at an injector temperature of 180 °C.

The source of the mass spectrometer was held at 280 °C. The emission current was 70 eV (electronvolts). The electron multiplier was set at 400 V (volts) above autotune. The filament and multiplier were turned on after 4 minutes into the analysis. An autotune using perfluorotributylamine was performed daily prior to analysis of samples. The calibration curve was prepared on the basis of the area ratio of the base peaks relative to the response of the 188 (amu) ion of phenanthrene d-10, the internal standard. Confirmation of the compounds was based on the presence of the molecular ion and two confirming ions, a retention-time match within 0.2 percent relative to phenanthrene d-10, and correct area ratios of the confirming ions.

Metolachlor ethane sulfonic acid (ESA) and metolachlor oxanilic acid were analyzed according to the method of Hostetler and Thurman (1999). Briefly, high-performance liquid chromatography (HPLC) is carried out on two in-line analytical columns. First is an octadecyl silica (ODS) 5-µ (micron), 250- x 3-mm column coupled to an ODS 3-µ, 250- x 4.6-mm column. The mobile phase was 60:35:5 of pH 7.0, 25-mM (millimole) phosphate buffer/methanol/acetonitrile with a flow rate of 0.6 mL/min. The instrument consisted of a high-performance liquid chromatograph, Hewlett-Packard 1090 (Hewlett-Packard, Palo Alto, CA), equipped with a diode array detector.

Using the previously described automated SPE procedure, diuron and its metabolites, DCMPU and DCPU, were extracted from water. The ethyl acetate extract was solvent exchanged to methanol by evaporating to approximately 100 µg/L, adding 2 mL methanol, and then evaporated to 40 µL. The final volume of the extract was adjusted to 75 µL by the addition of 35 µL of pH 7.0 phosphate buffer.

The extract then was analyzed by HPLC. The analytical columns used were identical to the previously described columns. The mobile phase consisted of 50 percent methanol and 50 percent, pH 7.0 phosphate buffer, with a gradient ramping to 75 percent methanol. The flow rate was 0.5 mL/min.

The detection and quantitation limits were 0.05 µg/L for all compounds analyzed by GC/MS and 0.2 µg/L for compounds analyzed by HPLC.

All laboratory blanks were free of pesticides or metabolites. The variation of the duplicate samples was within ±5 percent at one standard deviation. The correlation coefficients of the standard curves were 0.998 ± 0.002. Any samples with concentrations greater than 10 µg/L were diluted and re-analyzed.

OCCURRENCE OF HERBICIDES IN PLAYA LAKES

Cotton and (or) corn herbicides were detected in 97 percent of one-time samples from 32 playa lakes in the High Plains of West Texas. The playa-lake samples contained nine herbicides and their metabolites. The herbicides were, in order of percent detections: diuron > prometryn > atrazine > propazine > metolachlor > fluometuron > norflurazon > simazine > cyanazine (fig. 2). Only atrazine and propazine are not used on cotton; however, corn and sorghum are also grown adjacent to the cotton and are the most probable sources of atrazine and propazine in the playa lakes. All of the playa-lake samples contained herbicides, with a median concentration of total herbicide of (parent herbicides and metabolites) of 7.2 µg/L and a range from 0.57 to 33 µg/L.

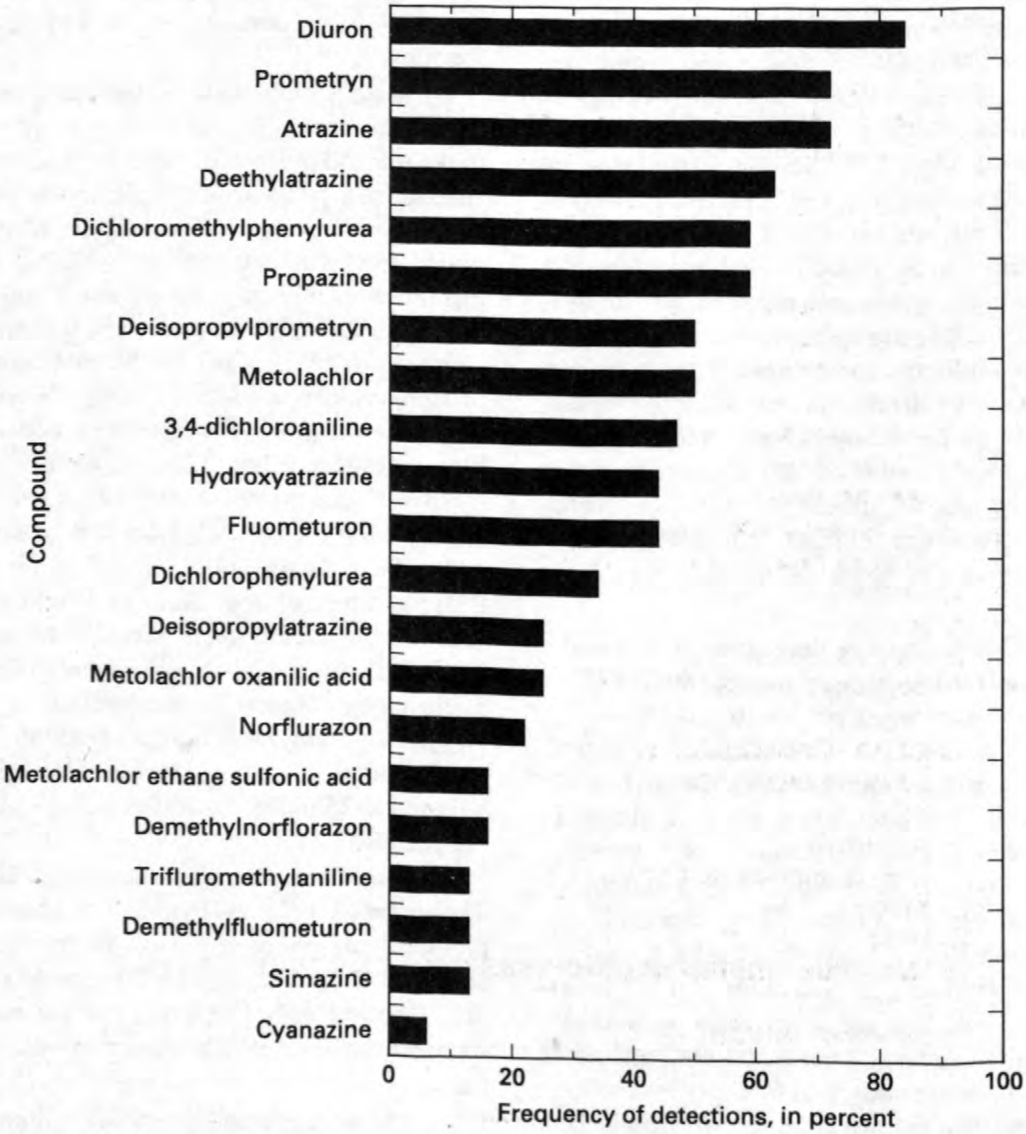


Figure 2. Frequency of detections of herbicides and metabolites in 32 playa-lake samples from High Plains of West Texas.

Figure 3 shows the total concentration of all herbicides and metabolites detected in samples from each playa lake. Metabolites make up a significant proportion of the total herbicide concentration in the lake samples (fig. 3). Only one playa-lake sample (playa lake 19) did not contain metabolites. The median metabolite percentage was 27 percent of the total herbicide present. The highest metabolite percentage was 70.5 percent. The metabolites that were detected (fig. 2) included three metabolites of atrazine (deisopropylatrazine or DIA, deethylatrazine or DEA, and hydroxyatrazine), three metabolites of diuron (dichloromethylphenylurea or DCMPU, dichlorophenylurea or DCPU, and 3,4-dichloroaniline or 3, 4-DCA), two metabolites of fluometuron (demethylfluometuron and trifluoromethylaniline or TFMA), two metabolites of metolachlor (metolachlor ethane sulfonic acid or metolachlor ESA and metolachlor oxanilic acid), one metabolite of norflurazon (demethylnorflurazon), and one metabolite of prometryn (deisopropylprometryn). Of these metabolites, the most frequently detected (fig. 2) were DEA (atrazine), at more than 60 percent detections, followed by DCMPU (diuron) > deisopropylprometryn (prometryn) > 3,4-DCA (diuron) > hydroxyatrazine (atrazine) > DCPU (diuron) > DIA (atrazine) > metolachlor oxanilic acid (metolachlor) >

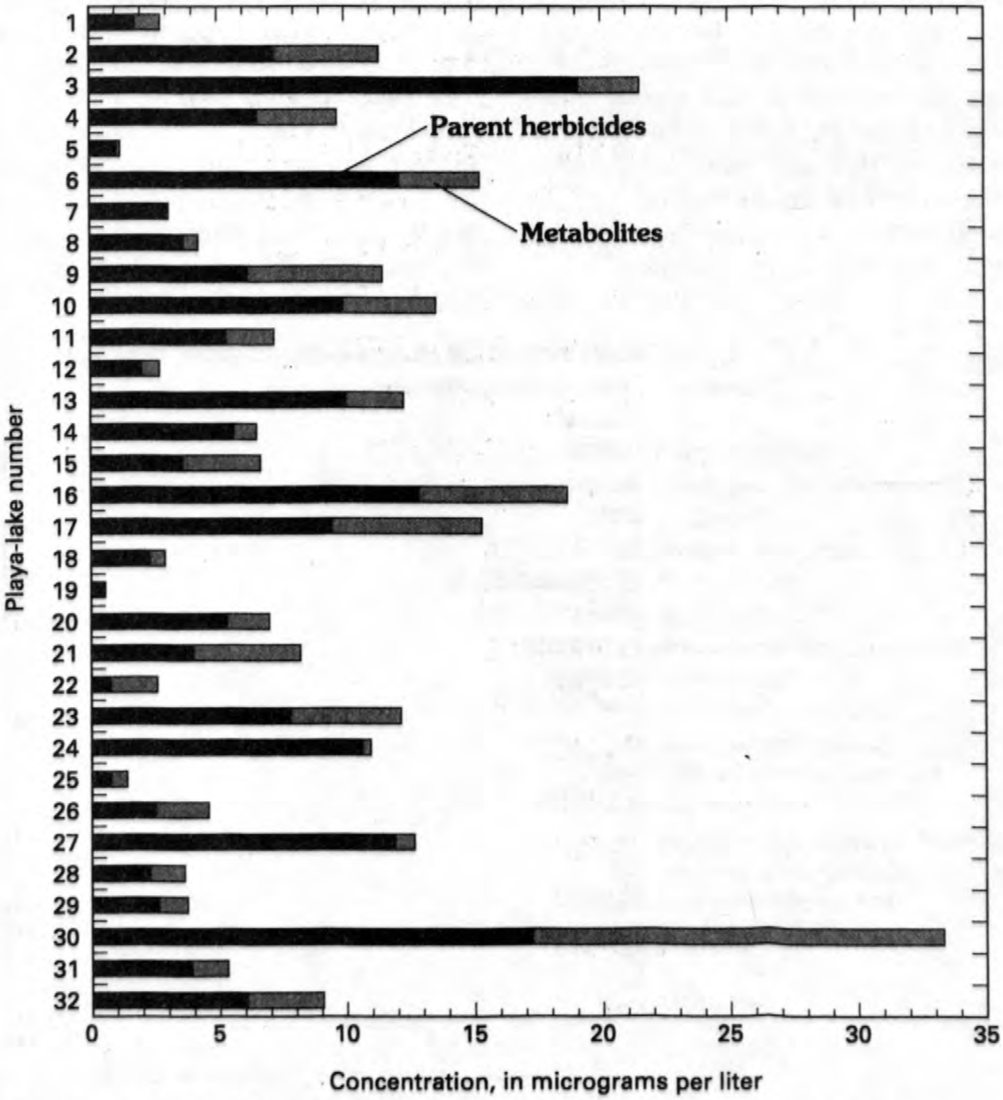


Figure 3. Concentrations of parent herbicides and metabolites in playa-lake samples from High Plains of West Texas.

metolachlor ESA (metolachlor) > demethylnorflorazon (norflurazon) > TFMA > demethylfluometuron (fluometuron).

Figure 4 shows mean concentrations of herbicides and metabolites detected in the playa-lake samples. Compounds with relatively high mean concentrations are diuron (2.7 μg/L), prometryn (1.3 μg/L), and metolachlor (1.0 μg/L). Nondetections were averaged as 0.0 in mean calculations. The frequency of detection (fig. 2) and the mean concentrations (fig. 4) of these three compounds indicate that they are probably the ones most used and readily transported into the playa lakes. All three compounds are used on cotton, and metolachlor also may be used on corn and sorghum. The other cotton herbicide that was detected frequently was fluometuron, which had a mean concentration of 0.5 μg/L. Maps of fluometuron use in 1992 show that it was not used in West Texas (Thurman and others, 1998). However, these maps may be incorrect on the basis of detections of fluometuron in the playa-lake samples. That information is not consistent with the results of the 1997 sampling.

Metabolites of four cotton herbicides (diuron, fluometuron, metolachlor, and prometryn) also were detected in samples from many of these same playa lakes. For example, the metabolites of diuron (DCMPU, DCPU, and 3,4-DCA) were found in 71 percent of the samples with positive detections of diuron. DCMPU was the most frequently detected and was present at the highest concentrations of the three metabolites. The mean concentrations of the three metabolites were 0.45 μg/L for DCMPU, 0.31 μg/L for 3,4-DCA, and 0.2 μg/L for DCPU (fig. 4). Demethylation of the phenylurea herbicides is apparently a common degradation pathway (Mueller and Moorman, 1991), and this pathway also occurs in fluometuron. For example, the detected metabolites of fluometuron were demethylfluometuron and TFMA; one is a demethylated metabolite (DMFM), and the other is trifluomethylaniline and is analogous to the 3,4-DCA metabolite of diuron. Norflurazon also degrades to a demethylated metabolite, demethylnorflurazon. That metabolite was detected in 16 percent of the samples with a mean concentration of 0.17 μg/L. Its concentration and frequency of detection are similar to the parent compound, reflecting the fact that the half-life of norflurazon is relatively short, approximately 45 days in soil (Ahrens, 1994).

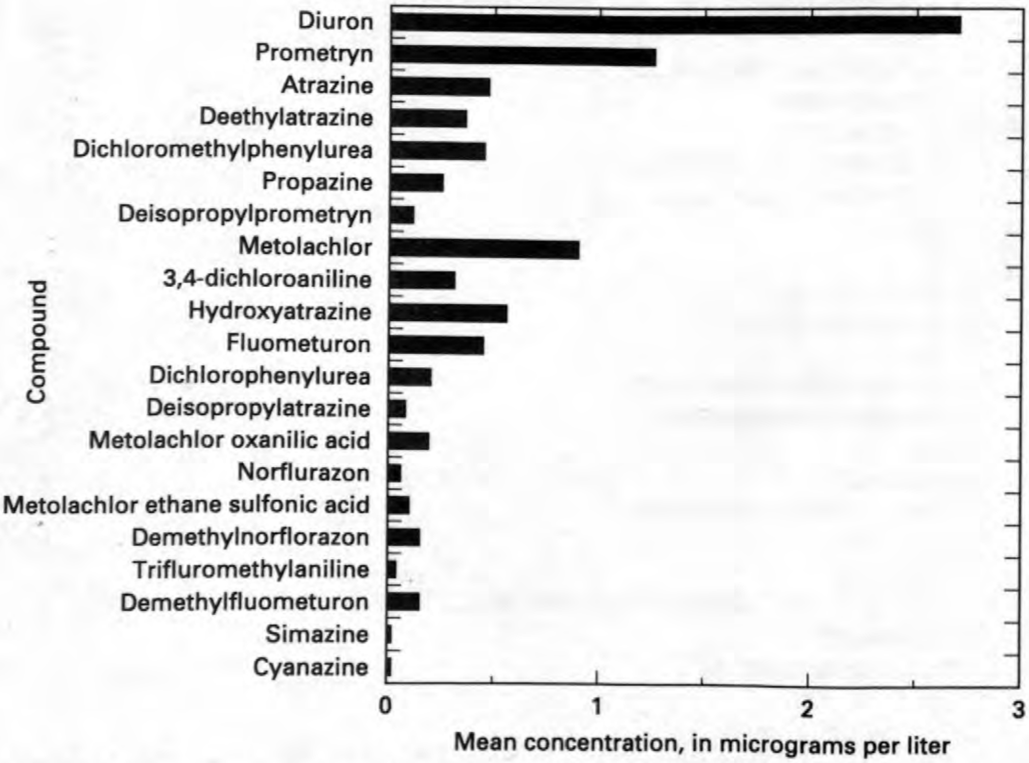


Figure 4. Mean concentrations of herbicides and metabolites in 32 playa-lake samples from High Plains of West Texas.

Metolachlor metabolites included both the ESA and the oxanilic acid of metolachlor. The mean concentration of the oxanilic acid metabolite was 0.19 µg/L as compared to 0.1 µg/L for the ESA metabolite. The fact that oxanilic acid concentration exceeded ESA concentration (true for all samples) suggests that the degradation of the parent metolachlor occurred in the playa lakes rather than in the soil. When metolachlor degrades chiefly in the soil, the major metabolite is the ESA (Field and Thurman, 1996; Thurman and others, 1996; Kalkhoff and others, 1998). Further work is underway to validate this hypothesis.

Another important cotton herbicide frequently detected in the playa-lake samples was prometryn, with a frequency of detections of 72 percent and a mean concentration of 1.3 µg/L. The metabolite of prometryn, deisopropylprometryn, was in nearly every sample that contained prometryn. The metabolite comes from the de-alkylation of the herbicide on the amino side of the molecule, which is a common degradation pathway for triazine herbicides such as prometryn. The relative abundance of the metabolite to the parent compound was 0.1 or about 10 percent of the mass of the parent compound. Prometryn is a resistant triazine with a long half-life of about 60 days or more in the soil (Ahrens, 1994).

The other two triazine herbicides that were detected routinely in the playa-lake samples were atrazine and propazine. Atrazine was detected in 72 percent of the samples with a mean concentration of 0.47 µg/L. Propazine was detected in 59 percent of the samples with a mean concentration of 0.25 µg/L. Atrazine and propazine have one common metabolite, DEA, which was found in 63 percent of the samples with a mean concentration of 0.36 µg/L. Because propazine has a longer soil half-life than atrazine (atrazine is 45–60 days and propazine is at least 60 days), it is thought that atrazine is responsible for the majority of the DEA. Furthermore, atrazine was present at a mean concentration that was about twice as much as propazine. Commonly, propazine is detected at much lower concentrations than atrazine because propazine is no longer used (voluntarily removed from the market in early 1990's by the manufacturer); however, supplies remain, and it is apparent from these data that propazine is still being used in the High Plains of West Texas.

An unusual finding was the presence of hydroxyatrazine in many of the playa-lake samples. It was detected in 44 percent of the samples with a mean concentration of 0.56 µg/L, a value comparable to that of atrazine. This result is unusual compared to the studies that have been carried out in the Corn Belt of the Midwestern United States (Thurman and others, 1991; 1992). Lerch and others (1998) have shown that hydroxyatrazine is a minor component during the growing season compared to the parent compound, atrazine. The more conservative nature of the hydroxyatrazine has been ascribed to the insolubility and sorption of the hydroxyatrazine metabolite to soil by a mixed-mode mechanism (Lerch and others, 1997). One explanation for the higher concentrations of hydroxyatrazine in the playa-lake samples is the more alkaline soils in this part of West Texas, where soil pH is commonly greater than 7. At these high pH values, the mixed-mode mechanism is inactive because the negative log of the acid dissociation constant (pK_a) of hydroxyatrazine is approximately 5. Thus, at a pH that is 2 units higher than the pK_a, the molecule will not be positively charged, which would lessen the effectiveness of the mixed-mode mechanism. For example, the hydroxyatrazine concentration in the water sample from playa lake 30 was 5.4 µg/L, which is one of the highest concentrations for hydroxyatrazine that has been measured (Lerch and others, 1998).

The ratio of metabolites to parent compounds has been suggested as a means of dating the relative age of surface water that contains herbicides. Thurman and Fallon (1996) used the deethylatrazine-to-atrazine ratio to indicate the age of surface water that has received atrazine runoff. Figure 5 shows a comparison of atrazine concentrations to the total concentrations of its metabolites as an indicator of this year's or previous years' application of atrazine. The data show that metabolite concentrations are greater than or equal to atrazine concentrations in many of the playa-lake samples. These data indicate that the water in the lakes contains "old" (degraded) atrazine from previous years' application.

Figure 6 shows a comparison of cotton-herbicide concentrations to the total concentrations of their metabolites in the playa-lake samples. The cotton-herbicide parent compounds exhibit considerably higher concentrations than their metabolites, in spite of the fact that half-lives for the cotton her-

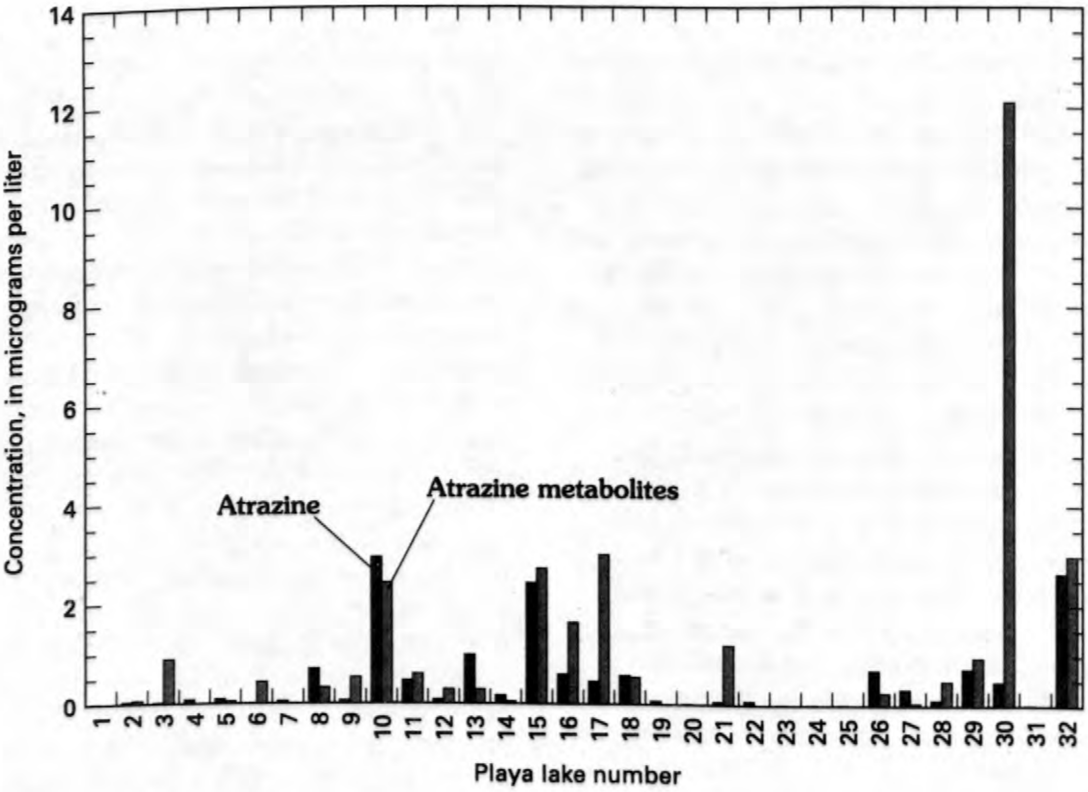


Figure 5. Comparison of atrazine and atrazine metabolite concentrations in playa-lake samples.

bicides are comparable to the half-life for atrazine. Only the sample from playa lake 9 contained about equal parent compound and metabolite concentrations for the herbicides, with a concentration for both of about 6 µg/L. Data from this sampling site suggest that the cotton herbicides may be from previous seasons.

OCCURRENCE OF INSECTICIDES IN PLAYA LAKES

Organophosphate insecticides were detected in only one sample from the 32 playa lakes for a detection frequency of 3 percent. The one detection was dicrotophos. Other organophosphate compounds that were analyzed were azinophos methyl, chlorpyrifos, malathion, methylparathion, and profenfos. Organophosphate insecticide use on cotton in West Texas is extensive, with more than 1.5 million kilograms (Gianessi and Anderson, 1995) applied annually for the State, approximately half of which is used in the High Plains of West Texas. Thus, the amount used is not an explanation for low detection frequency. However, the time of sampling was early summer so that application may not have occurred prior to sampling. A check of application dates for this area suggests that this could be a possible explanation. Another possible explanation is that the half-life of organophosphate insecticides is generally short, less than 10 days. Thus, degradation of the parent compounds also could be an explanation for the low detection frequency. Another sampling of the playa lakes later in the season to provide more information on concentrations in relation to timing of applications is planned for 1999.

CONCLUSIONS

Cotton and (or) corn herbicides were detected in 97 percent of one-time samples from 32 playa lakes in the High Plains of West Texas. The major cotton herbicides detected were diuron, fluometuron, metolachlor, norflurazon, and prometryn. The corn herbicide atrazine and its metabolites also were commonly found in the playa-lake samples.

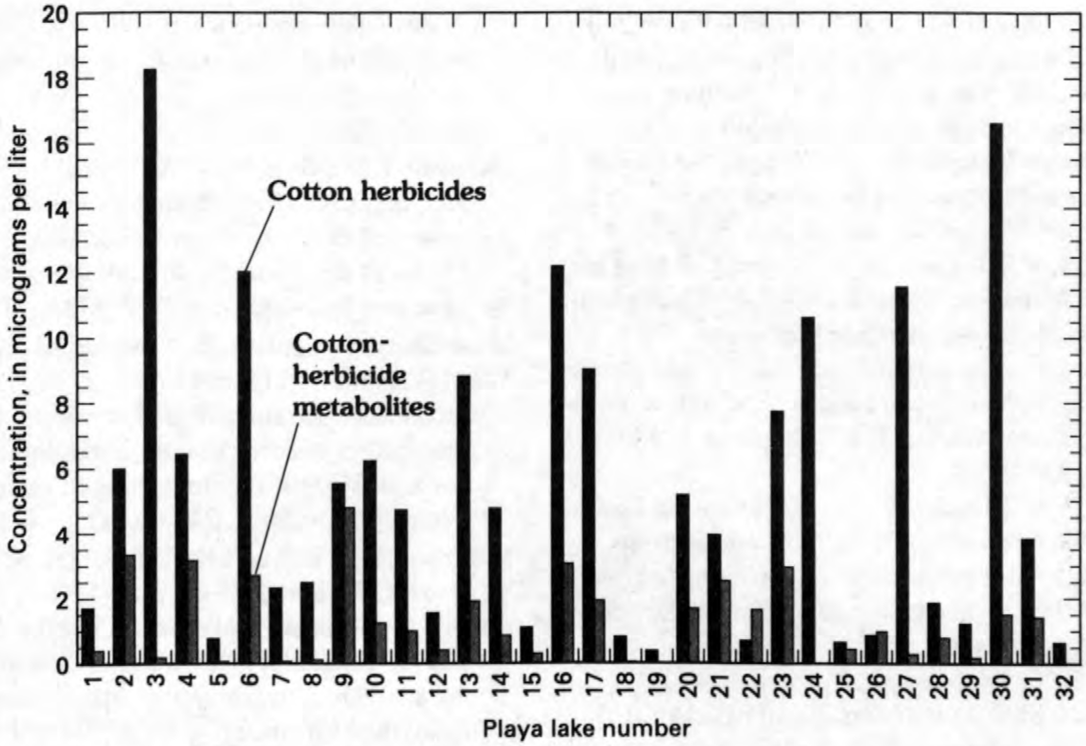


Figure 6. Comparison of cotton herbicide and metabolite concentrations in playa-lake samples.

Relative concentrations of parent herbicides and their metabolites may be used as indicators of recent herbicide runoff to the playa lakes as compared to previous years' applications. The presence of hydroxyatrazine was notably high in concentration relative to many surface waters in the Midwestern United States that have been studied. Organophosphate insecticides were detected in only one sample from the playa lakes.

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Trends in Annual Herbicide Loads from the Mississippi River Basin to the Gulf of Mexico

By Gregory M. Clark and Donald A. Goolsby

ABSTRACT

Analyses of water samples collected from the Mississippi River at Baton Rouge, Louisiana, during 1991-97 indicate that hundreds of metric tons of herbicides and herbicide metabolites are being discharged annually to the Gulf of Mexico. Atrazine, metolachlor, and the ethane-sulfonic acid metabolite of alachlor (alachlor ESA) were the most frequently detected herbicides and, in general, were present in the largest concentrations. Almost 80% of the annual herbicide load to the Gulf of Mexico occurred during the growing season from May through August. The concentrations and loads of alachlor in the Mississippi River decreased dramatically after 1993 in response to decreased use in the basin. In contrast, the concentrations and loads of acetochlor increased after 1994, reflecting its role as a replacement for alachlor. The peak annual herbicide load occurred in 1993, when about 640 metric tons of atrazine, 320 metric tons of cyanazine, 215 metric tons of metolachlor, 53 metric tons of simazine, and 50 metric tons of alachlor were discharged to the Gulf of Mexico. The annual loads of atrazine and cyanazine were generally 1 to 2% of the amount annually applied in the Mississippi River drainage basin; the annual loads of acetochlor, alachlor, and metolachlor were generally less than 1%. Despite a reduction in atrazine use, historical data do not indicate a long-term downward trend in the atrazine load to the Gulf of Mexico. Although a relation (r^2=0.62) exists between the atrazine load and stream discharge during May through August, variations in herbicide use and rainfall patterns within subbasins can have a large effect on herbicide loads in the Mississippi River Basin and probably explain a large part of the annual variation in atrazine load to the Gulf of Mexico.

INTRODUCTION

Current (1999) agricultural practices in the United States rely heavily on pesticides for crop production. Of the more than 500 million kilograms (kg) of pesticides used annually in the United States to control weeds, insects, nematodes, and other pests (Gianessi and Puffer, 1991; Aspelin and others, 1992), about 20% are herbicides applied to field crops in the Mississippi River Basin (fig. 1). Principal herbicides used in the Mississippi River Basin are triazines, such as atrazine and cyanazine, and chloroacetanilides (acetanilides), such as alachlor and metolachlor (Goolsby and Battaglin, 1995). Because most triazine and acetanilide herbicides are water soluble and mobile, they can be transported to streams and ground water. The presence of herbicides in streams is a concern because of potential deleterious effects on water quality. Although most herbicides have low acute toxicity to animals, the potential effects on human health are a concern. For instance, the U.S. Environmental Protection Agency (USEPA) classifies alachlor as a probable human carcinogen, and several other herbicides, including atrazine, cyanazine, metolachlor, and simazine, are classified as possible human carcinogens (Nowell and Resek, 1994). In addition, the effects of long-term, low-level concentrations of herbicides or combinations of herbicides and other organic compounds on aquatic ecosystems are largely unknown (Larson and others, 1997).

A number of studies have documented the presence of herbicides in the Mississippi River and its tributaries (Pereira and Rostad, 1990; Pereira and Hostettler, 1993). Concentrations of herbicides are largest for several weeks to several months following their application to farmlands (Wauchope, 1978; Thurman and others, 1991; Goolsby and Battaglin, 1993). In some small watersheds in the Mississippi River Basin, concentrations of herbicides in streams have been found to exceed 50 micrograms per liter (µg/L) for short periods of time following spring storms (Thurman and others, 1991; Goolsby and Battaglin, 1993). Discharges from small streams transport herbicides into large rivers such as the Mississippi. Although herbicide concentrations in large rivers are generally lower than in many small streams, the cumulative load of herbicides, which eventually discharges to the Gulf of Mexico, can be large.

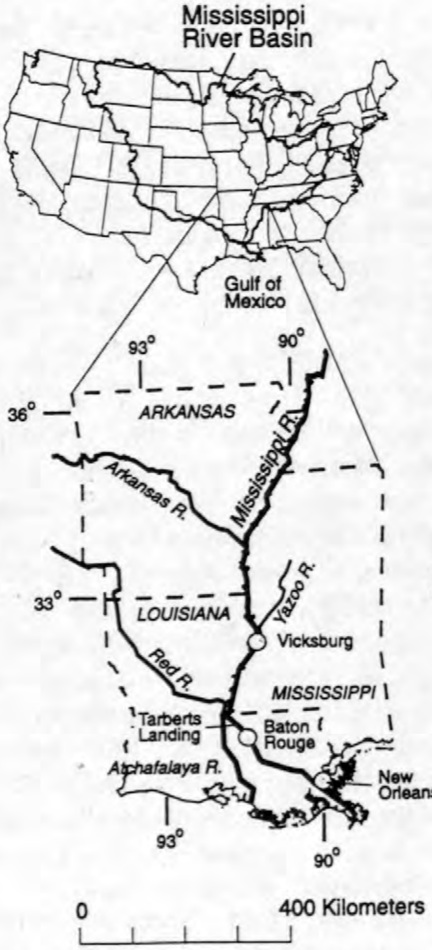

Figure 1. The Mississippi River Basin and sampling sites.

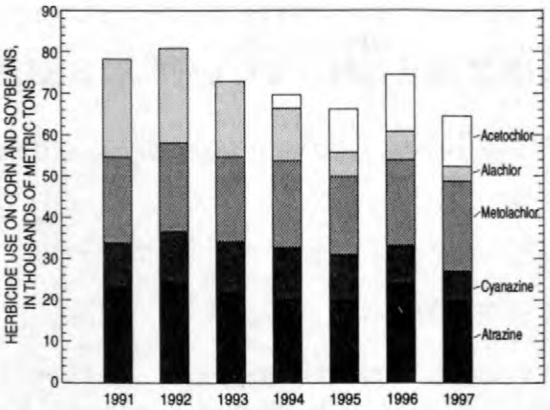

Figure 2. Use of selected herbicides on corn and soybeans in the Mississippi River Basin, 1991-97. (U.S. Department of Agriculture, 1991-97)

During 1991-97, use of five herbicides (acetochlor, alachlor, atrazine, cyanazine, and metolachlor) on corn and soybeans accounted, on average, for about 70% of the annual herbicide use on field crops in the Mississippi River Basin (U.S Department of Agriculture, 1991-97). However, some changes in the quantity and relative use of these herbicides occurred during that time (fig. 2). In 1997, the total amount of acetochlor, alachlor, atrazine, cyanazine, and metolachlor used was about 65,000 metric tons (t), a decrease of about 13,000 t from the amount used in 1991. Although atrazine and metolachlor use remained relatively stable during 1991-97, alachlor use decreased by about 19,000 t, or 85%, and cyanazine use decreased by about 3,100 t, or 30%. The introduction and use of the corn herbicide acetochlor in the United States in 1994 partially offset the decrease in alachlor and cyanazine use and, by 1996, acetochlor was the third most heavily applied herbicide on corn in the Midwestern United States. Because acetochlor has a broader spectrum of weed control than other corn herbicides have, an increase in its use is expected to reduce overall herbicide use in the United States (Capel and others, 1995).

To better understand the occurrence, temporal variability, and load of herbicides in the Mississippi River, the U.S. Geological Survey (USGS) collected herbicide data from the Mississippi River at Baton Rouge, La. (fig.

1), during 1991-97. This paper presents results from the interpretation of these data.

METHODS

Data Collection

A total of 271 water samples were collected from the Mississippi River at Baton Rouge from April 1991 through December 1997. Samples were collected on a weekly to monthly basis and ranged from 17 in 1997 to 60 in 1993. Previous work indicated that dissolved herbicides in the Mississippi River at Baton Rouge are well mixed vertically and laterally at a range of streamflow conditions (Goolsby and others, 1991). Therefore, samples were collected from the upper 6 meters (m) of the water column at the end of a pier extending about 45 m from shore. Samples were collected in glass or Teflon containers, composited in glass or stainless steel containers, and filtered through a nominal 0.7- to 1.0-micrometer pore diameter baked glass-fiber filter into baked glass bottles for shipment to the laboratory. From early March through mid-August 1993, samples were collected by the Jefferson Parish Water Quality Laboratory near New Orleans, La., about 200 kilometers (km) downstream from Baton Rouge. These samples were weekly composites of daily samples collected at two sampling points on opposite banks of the Mississippi River. A review of data collected at comparable times and analyzed by the Jefferson Parish Water Quality Laboratory and USGS laboratories indicated good agreement in analytical results (W.A. Battaglin, USGS, oral communication, 1998).

The U.S. Army Corps of Engineers provided discharge data for the Mississippi River at Tarberts Landing, La., located about 130 km upstream from Baton Rouge (fig. 1). Discharge at Tarberts Landing is similar to the discharge at Baton Rouge. The U.S. Army Corps of Engineers also provided discharge data for water diverted from the Mississippi River to the Atchafalaya River. The sum of the discharge at Tarberts Landing and the discharge diverted to the Atchafalaya River closely represents the total discharge from the Mississippi River to the Gulf of Mexico (Goolsby and others, 1991).

Analytical Procedures

Samples collected during 1991-97 were analyzed at the USGS National Water Quality Laboratory (NWQL) in Arvada, Colo., or the USGS Organic Laboratory in Lawrence, Kans. Herbicides were extracted from samples by solid-phase extraction (SPE) on carbon-18 cartridges (Meyer and others, 1993; Zaugg and others, 1995) and subsequently removed from the cartridges by a small volume of either hexane-isopropanol (NWQL) or ethyl acetate (Kansas Laboratory). The ethane-sulfonic acid metabolite of alachlor (alachlor ESA) was removed by a followup elution of the SPE cartridge with methanol (Aga and others, 1994). Sample extracts were evaporated to a final volume using nitrogen gas and were analyzed by capillary-column gas chromatography/mass spectrometry (GC/MS) with selected-ion monitoring (Thurman and others, 1990; Zaugg and others, 1995). Methanol extracts were analyzed for alachlor ESA using enzyme-linked immunosorbent assay (Aga and others, 1994). Samples collected by the Jefferson Parish Water Quality Laboratory during March through August 1993 were analyzed for herbicides by gas chromatography using USEPA protocols (Goolsby and others, 1993).

Twelve triazine and acetanilide herbicides and four breakdown products, or metabolites, are discussed in this report (table 1). These represent the majority of herbicide usage in the Mississippi River Basin (Gianessi and Puffer, 1991) and are the most frequently detected in the Mississippi River (Periera and Rostad, 1990; Periera and Hostettler, 1993; Goolsby and Battaglin, 1995). Not all of these herbicides or metabolites were analyzed during all 7 years of sampling at Baton Rouge. Alachlor ESA was not included as an analyte until July 1993; analysis for acetochlor and cyanazine amide did not begin until 1994 (table 1). The analytical reporting limits for the samples collected at Baton Rouge were 0.05 µg/L, except for alachlor ESA (0.10 µg/L) and cyanazine (0.05-0.20 µg/L) (table 1).

Table 1. Herbicides and herbicide metabolites analyzed in samples from the Mississippi River at Baton Rouge, Louisiana, April 1991 through December 1997.

[µg/L, micrograms per liter]

Herbicide or Metabolite	Type of herbicide	Number of samples	Years sampled	Analytical reporting limit (µg/L)
Acetochlor	acetanilide	120	1994-97	0.05
Alachlor	acetanilide	271	1991-97	0.05
Alachlor ESA	metabolite	160	1993-97	0.10
Ametryn	triazine	198	1991-97	0.05
Atrazine	triazine	271	1991-97	0.05
Cyanazine	triazine	245	1991-97	0.05-0.20
Cyanazine amide	metabolite	88	1994-97	0.05
Deethylatrazine	metabolite	245	1991-97	0.05
Deisopropylatrazine	metabolite	245	1991-97	0.05
Metolachlor	acetanilide	271	1991-97	0.05
Metribuzin	triazine	243	1991-97	0.05
Prometon	triazine	199	1991-97	0.05
Prometryn	triazine	199	1991-97	0.05
Propazine	triazine	199	1991-97	0.05
Simazine	triazine	271	1991-97	0.05
Terbutryn	triazine	115	1993-97	0.05

Load Calculations

Linear interpolation was used to estimate the herbicide load in the Mississippi River at Baton Rouge. Herbicide concentrations on nonsampling days were estimated by interpolating between concentrations measured on sampling days. Measured or interpolated daily concentrations were multiplied by the mean daily discharge to estimate a daily load. Daily loads were summed to estimate a total load over a specified period of time. For herbicide concentrations less than reporting or method detection limits (censored data), values of one-tenth the limit were used for load estimates. Sensitivity analysis indicated that using the reporting limit or method detection limit instead of one-tenth the limit resulted in differences of less than 10% in the annual herbicide loads. For the most heavily used herbicides, the differences in load estimates were substantially less than 10% because of the high frequency of detection and generally larger concentrations.

RESULTS AND DISCUSSION

Herbicide Occurrence and Concentrations

The total herbicide concentration (sum of the herbicides listed in table 1) in the Mississippi River at Baton Rouge during 1991-97 varied seasonally; concentrations were largest during May through August (fig. 3). This seasonal pattern has been noted in many streams in the Mississippi River Basin and has been termed the "spring flush" (Thurman and others, 1991).

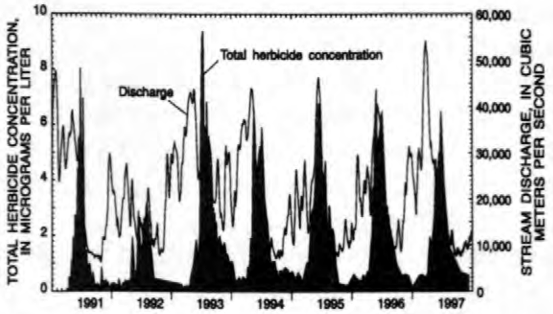

Figure 3. Stream discharge and total herbicide concentration in the Mississippi River at Baton Rouge, Louisiana, April 1991 through December 1997. [The total herbicide concentration represents the sum of the herbicides and metabolites listed in table 1. All herbicides and metabolites, however, were not sampled during all years (see table 1)]

At Baton Rouge, peak herbicide concentrations in the Mississippi River generally followed peak discharge, which typically occurred in late winter or early spring. Although individual herbicide concentrations in some small tributaries to the Mississippi River have been reported to exceed 50 µg/L, no compound exceeded 5 µg/L in the Mississippi River at Baton Rouge during 1991-97, and the total herbicide concentration did not exceed 10 µg/L (fig. 3). Smaller, more drawn-out peak herbicide concentrations in the Mississippi River, compared with those in smaller streams, are attributable to the integrating effect of the Mississippi River, which receives input from many smaller streams (Goolsby and Battaglin, 1995). These smaller streams drain areas of variable land use and crop groups and deliver herbicides to the Mississippi River at different times during the growing season. Peak herbicide concentrations at Baton Rouge also are attenuated by the presence of upstream reservoirs. Data collected at outlets of 76 midwestern reservoirs indicate that reservoirs collect and store the spring flush of herbicides and subsequently deliver smaller concentrations downstream over longer periods of time (Battaglin and Goolsby, 1998; Stamer and others, 1999).

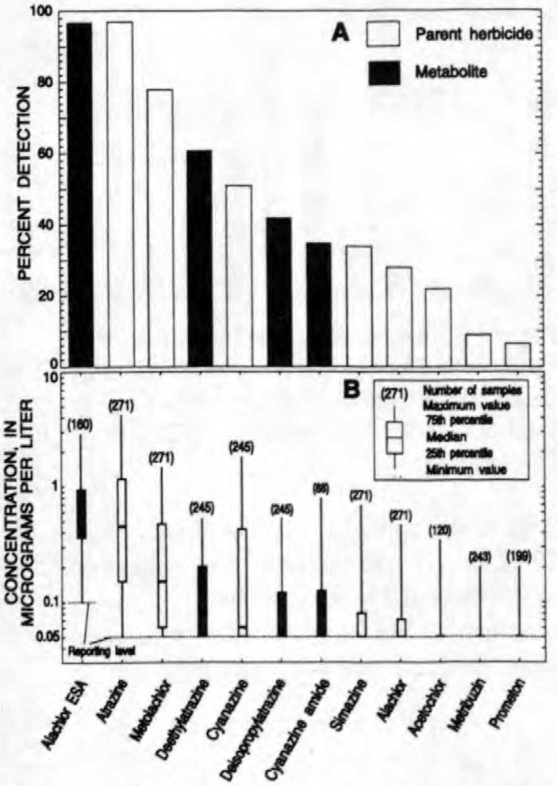

Figure 4. Percent detection and concentrations of herbicides and herbicide metabolites in samples from the Mississippi River at Baton Rouge, Louisiana, April 1991 through December 1997. (The herbicides ametryn, prometryn, propazine, and terbutryn were detected in less than 2% of samples)

Of the seven most frequently detected compounds in the Mississippi River at Baton Rouge, four were herbicide metabolites (fig. 4A). Individual herbicides and metabolites detected in more than 50% of the samples were alachlor ESA, atrazine, metolachlor, deethylatrazine, and cyanazine. In general, concentrations of these five herbicides and metabolites also were larger than concentrations of the other herbicides analyzed (fig. 4B). No herbicide or metabolite was detected at a concentration exceeding 5 µg/L. The concentration of atrazine exceeded the maximum contaminant level (MCL) of 3 µg/L (USEPA, 1995) in 11 of 271 samples (fewer than 5%). Cyanazine concentrations exceeded the health advisory (HA) of 1 µg/L in 15 of 245 samples (6%). Alachlor and simazine concentrations did not exceed their MCLs of 2 and 4 µg/L, respectively (USEPA, 1995). However, because MCLs and HAs are based on average concentrations in public water supplies over a specified time period, one or more exceedances of the specified value does not necessarily indicate noncompliance. None of the average annual concentrations of the herbicides examined in this study exceeded MCLs or HAs.

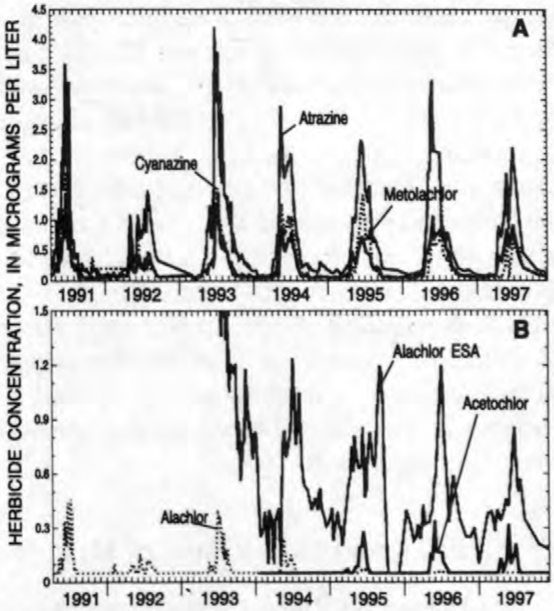

Figure 5. Temporal variation in concentrations of selected herbicides in samples from the Mississippi River at Baton Rouge, Louisiana, April 1991 through December 1997.

Concentrations of atrazine, metolachlor, and cyanazine in the Mississippi River generally peaked simultaneously in late May and early June, then decreased by the end of August (fig. 5A). Acetochlor was first detected in the Mississippi River at Baton Rouge in 1995 (fig. 5B), a year after its introduction for use in the

United States. The increase in the concentrations of acetochlor coincided with a decrease in the concentrations of alachlor (fig. 5B). However, the alachlor metabolite alachlor ESA was detected in nearly all of the samples collected during 1995 through 1997 (fig. 4A). Because it has undergone dechlorination, alachlor ESA is less toxic than the parent compound (Aga and others, 1994) but is more mobile and persistent in the soil and water environment (Thurman and others, 1996). Numerous studies in the Midwest have documented widespread occurrence of alachlor ESA in ground water, whereas alachlor has been detected infrequently (Holden and others, 1992; Baker and others, 1993; Kolpin and others, 1997; Kalkhoff and others, 1998). Because ground-water discharge has been identified as a primary source of herbicides to the Mississippi River during base-flow conditions (Periera and Hostettler, 1993), it also might be responsible for the continued presence of alachlor ESA in the river. The presence of alachlor ESA also has been documented in midwestern reservoirs well past the end of the growing season and at larger concentrations than those of the parent compound (Thurman and others, 1996). Thus, the continued presence of alachlor ESA in the Mississippi River during 1995-97 might be attributable to a number of factors. These include degradation of current-year applications of alachlor in upstream parts of the Mississippi River Basin and residual compounds applied during prior years and released through ground-water and reservoir discharge.

Herbicide Load to the Gulf of Mexico

During 1991-97, the annual load of herbicides from the Mississippi River Basin to the Gulf of Mexico ranged from about 450 t in 1992 to 1,920 t in 1993 (fig. 6A), when extensive flooding occurred in the upper Mississippi and Missouri River Basins. During 1993, about 640 t of atrazine, 320 t of cyanazine, 215 t of metolachlor, 53 t of simazine, and 50 t of alachlor were discharged to the Gulf of Mexico. Not all of the compounds listed in table 1 were analyzed during all years from 1991 through 1997. Nearly 80% of the annual herbicide load was discharged during May through August (fig. 6B). The monthly load of herbicides was largest during June, when an average of about 300 t, or 25% of the annual total, was discharged. In June 1995, nearly 650 t of herbicides was discharged to the Gulf of Mexico, the largest single monthly load of herbicides in the Mississippi River during 7 years of sampling.

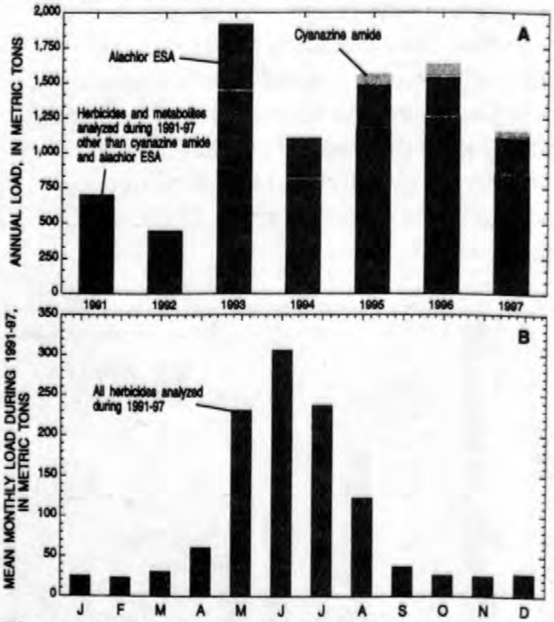

Figure 6. Annual and monthly loads of herbicides discharged from the Mississippi River Basin to the Gulf of Mexico, 1991-97. (The 1993 load of alachlor ESA represents July through December only)

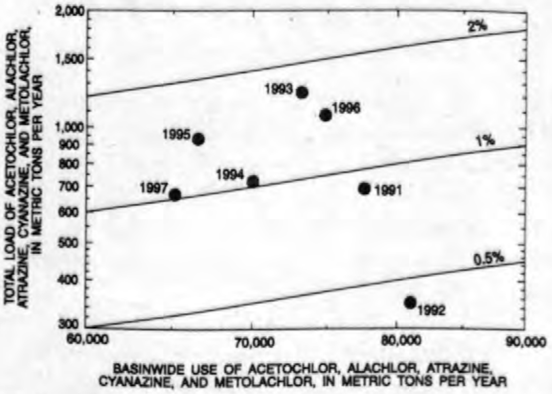

Figure 7. Relation of total annual load and use for five herbicides discharged from the Mississippi River Basin to the Gulf of Mexico, 1991-97. (Lines are annual load as a constant percentage of annual use)

The total loads of acetochlor, alachlor, atrazine, cyanazine, and metolachlor as a percentage of total basinwide use varied during 1991-97 (fig. 7). Use of these five herbicides was largest in 1992, when more than 81,000 t was applied to crops in the Mississippi River Basin. However, the total load of these five herbicides in 1992 was only about 350 t, less than 0.5% of the use total. Conversely, in 1993 the use total was about 73,000 t, but the total load was about 1,200 t, or about 1.6% of the use.

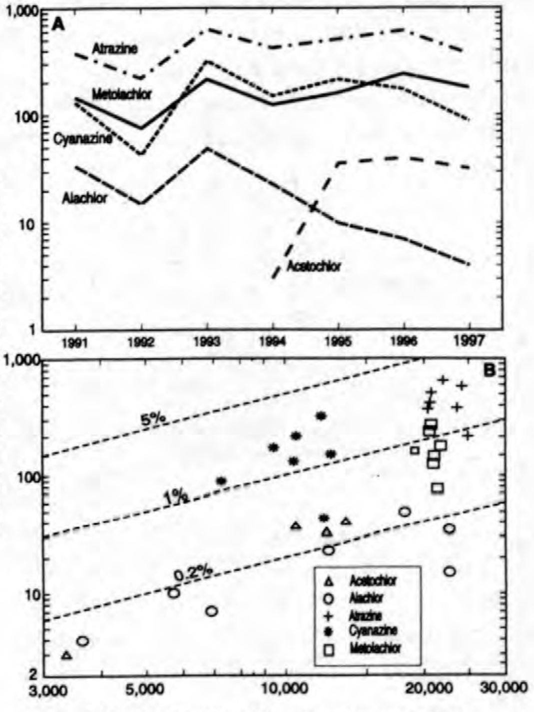

Figure 8. Annual loads and relation of total annual load and use for five herbicides discharged from the Mississippi River Basin to the Gulf of Mexico, 1991-97. (Lines in B are annual load as a constant percentage of annual use)

Of the herbicides analyzed, atrazine accounted for the largest proportion of the annual load to the Gulf of Mexico and ranged from about 220 t in 1992 to about 640 t in 1993 (fig. 8A). During 1991-97, the average annual load of atrazine represented about 2% of its basinwide use. Only in 1992 (0.9%) was the annual load of atrazine less than 1.5% of annual use (fig. 8B). If the loads of atrazine metabolites (deethylatrazine and deisopropylatrazine) were included with the load of atrazine, the average annual load would represent about 3% of the annual basinwide use of atrazine. The annual load of cyanazine as a percentage of use was similar to that of atrazine (fig. 8B), averaging about 1.5% of basinwide use during 1991 through 1997. Only in 1992 did the cyanazine load represent less than 1% of the annual use. During 1995 through 1997, the combined load of cyanazine and cyanazine amide (the primary metabolite of cyanazine), on average, accounted for about 2.4% of the cyanazine used in the Mississippi River Basin.

Of the acetanilide herbicides, metolachlor accounted for the largest proportion of the annual load to the Gulf of Mexico and ranged from 76 t in 1992 to 240 t in 1996 (fig. 8A). The load of acetochlor increased from less than 5 t in 1994 to 40 t in 1996 in response to its increased basinwide use, whereas the load of alachlor decreased from 49 t in 1993 to less than 5 t in 1997. The annual load of acetanilide herbicides, in general, represented a smaller percentage of the total basinwide use than the loads of atrazine and cyanazine (fig. 8B). The average annual loads of metolachlor, acetochlor, and alachlor, all acetanilide herbicides, represented 0.8, 0.3, and 0.15% of their basinwide use, respectively.

Long-Term Atrazine Load

Atrazine data for the Mississippi River at Vicksburg, Miss., from 1975 to 1990, in combination with the data collected for this study during 1991-97, provide information about long-term trends in the atrazine load to the Gulf of Mexico. Starting in 1975, in response to concerns about the city of New Orleans' drinking-water supply, Ciba-Geigy Corporation established a monitoring program to evaluate the presence of atrazine in the Mississippi River (Ciba-Geigy Corporation, 1992). Width-integrated samples were collected from the Mississippi River at Vicksburg 20 to 50 times per year and analyzed by gas chromatography with confirmation by GC/MS. Daily discharge data for the Mississippi River at Vicksburg were provided by the U.S. Army Corps of Engineers and were assumed to be comparable to discharge in the Mississippi River at Baton Rouge plus the discharge diverted to the Atchafalaya River.

Annual use estimates of herbicides in the Mississippi River Basin indicate that atrazine use has declined substantially since the mid-1970's and early 1980's (Gianessi, 1992). In 1976, about 38,000 t of atrazine was applied in the Mississippi River Basin. Applications declined to about 32,000 t in 1982 and to 20,000-25,000 t during 1991-97. However, estimates of the annual load of atrazine to the Gulf of Mexico during 1975-97 do not reflect the reduction in its annual basinwide use over the same period (fig. 9). During 1975-80 and 1987-92 (excluding 1990, when atrazine data at Vicksburg were unavailable), the atrazine load to the Gulf of Mexico was, in general, less than the 22-year average of about 370 t (fig. 9). In contrast, the annual atrazine load during 1981-86 and 1993-96 exceeded the long-term average.

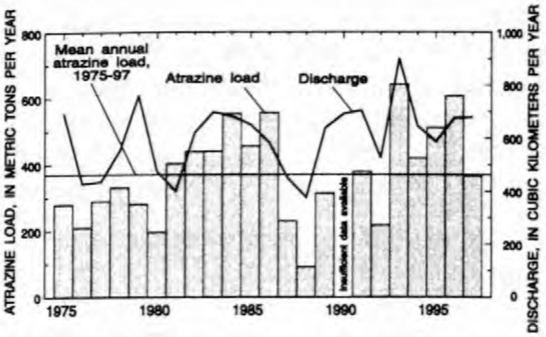

Figure 9. Annual discharge and atrazine load from the Mississippi River Basin to the Gulf of Mexico, 1975-97.

Annual variations in stream discharge during the growing season appear to be a better predictor of atrazine load in the Mississippi River than does annual atrazine use in the basin. Regression of the atrazine load with stream discharge during May through August explains a large part (r^2=0.62) of the annual variation (fig. 10A). However, on the basis of the regression residuals, a long-term downward trend in atrazine load from 1975 to 1997 is not apparent as a result of the decrease in basinwide atrazine use (fig. 10B). An apparent increase in the annual May through August 1975-97 discharge in the Mississippi River (fig. 10C) might be responsible for offsetting the basinwide decrease in atrazine use during the same period. Numerous factors influence the load of atrazine in the Mississippi River, and available data are insufficient to observe gradual trends in atrazine load to the Gulf of Mexico. Variations in herbicide application rates and storm patterns within subbasins can have a large effect on the herbicide load in the Mississippi River and probably explain a large part of the annual variation in atrazine load to the Gulf of Mexico. Subbasins contribute variable amounts of herbicides to the Mississippi River each year, depending on farming practices and climatic conditions. Long-term data from individual subbasins are needed to explain long-term variability in the loads of herbicides to the Gulf of Mexico.

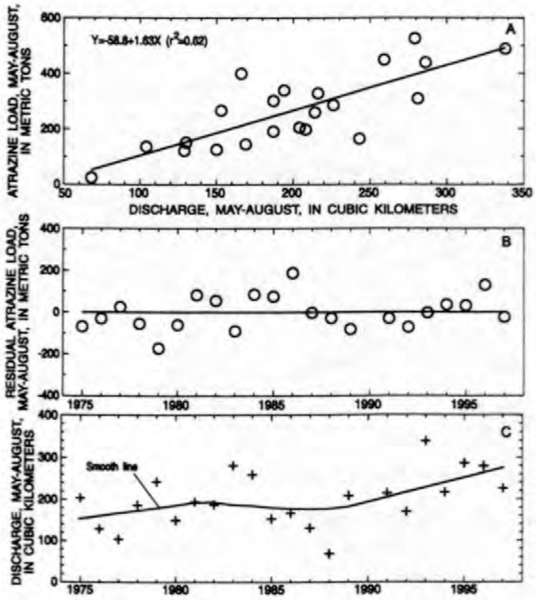

Figure 10. Relation of discharge and atrazine load, May-August, in the Mississippi River at Vicksburg, Mississippi, and Baton Rouge, Louisiana, 1975-97. (1975-89 data are for Vicksburg; 1991-97 data are for Baton Rouge)

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Finding Minimal Herbicide Concentrations in Ground Water? Try Looking for the Degradates

By Dana W. Kolpin, E. Michael Thurman, and S. Michael Linhart

ABSTRACT

Extensive research has been conducted regarding the occurrence of herbicides in the hydrologic system, their fate, and their effects on human health and the environment. Few studies, however, have considered the degradates of herbicides. In this study of Iowa ground water, herbicide degradates were frequently detected. In fact, herbicide degradates were 8 of the 10 most frequently detected compounds. The total residue concentration (sum of parent compound plus degradates) was substantially greater than the concentration of just the parent compound for every herbicide examined.

The frequencies of herbicide detection and concentrations varied substantially among the major aquifer types sampled. These differences, however, were much more pronounced when herbicide degradates were included. Aquifer types having the most rapid recharge rates (alluvial and bedrock/karst region aquifers) were those most likely to contain detectable concentrations of herbicide compounds.

Two indirect estimates of ground-water age (depth of well completion and dissolved-oxygen concentration) were used to separate the sampled wells into general vulnerability classes (low, intermediate, and high). The results show that the frequencies of herbicide detection and concentrations varied substantially among the vulnerability classes regardless of whether or not herbicide degradates were considered. Nevertheless, when herbicide degradates were included, the frequency of herbicide compound detection within the highest vulnerability class approached 90 percent, and the median total herbicide residue concentration increased over an order of magnitude to 2 micrograms per liter. The results of this study indicated that obtaining data on herbicide degradates is critical in attempting to understand not only the fate of a specific herbicide but also its overall effect on human health and the environment.

INTRODUCTION

Extensive research has been conducted regarding the occurrence of herbicides in the hydrologic system (Holden and others,1992; Walls and others, 1996), their fate (Bintein and Devillers, 1996; Kruger and others, 1997), and their effects on human health (Carbonell and others, 1995; Bain and LeBlanc,1996; Ribas and others, 1997) and the environment (Longley and Sotherton, 1997; Pratt and others, 1997; Carder and Hoagland, 1998; Howe and others, 1998). However, the understanding of the total consequences from herbicide use are limited by the fact that most investigations have focused on the active ingredient (parent compound) and did not consider their transformation products (degradates).

Complete mineralization of most parent herbicide compounds has not been established (Stamper and others, 1997). However, relatively stable and persistent degradates can be formed during the transformation of many herbicides (Coats,1993). These degradates can, in some cases, be more toxic than their parent compounds (La Clair and others, 1998; Belfroid and others, 1998).

This paper describes the results of a study to determine the relative transport of selected herbicide degradates to ground water compared to that of their parent compounds. Results from the sampling of 131 municipal wells from the major aquifer types in Iowa during 1995-98 are presented (fig. 1). The degradates analyzed include acetochlor ethanesulfonic acid (acetochlor ESA), acetochlor oxanilic acid (acetochlor OA), alachlor oxanilic acid (alachlor OA), deethylatrazine (DEA), deisopropylatrazine (DIA), cyanazine amide, hydroxyatrazine, metolachlor ethanesulfonic acid (metolachlor ESA), and metolachlor oxanilic acid (metolachlor OA). This research is an extension of the multi-agency Iowa Ground Water Monitoring Program (IGWM) (Detroy 1985; Kolpin, Sneck-Fahrer, and others, 1997).

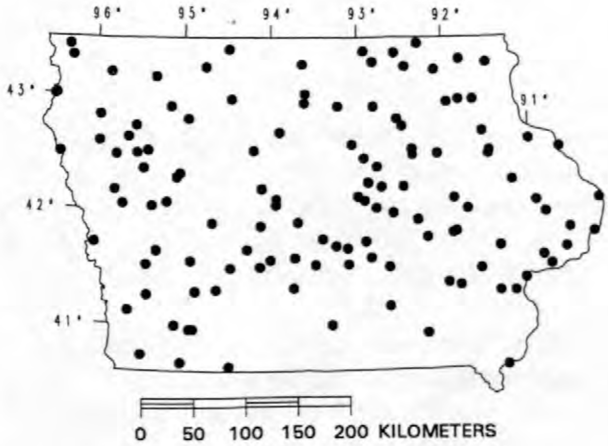

Figure 1. Location of wells sampled in Iowa during 1995-98.

MATERIALS AND METHODS

The 131 wells sampled were randomly selected within each major aquifer type (alluvial, glacial drift, bedrock/karst region, and bedrock/nonkarst region) for the IGWM from a population of over 2000 Iowa municipal wells. The 328 water samples collected from these 131 wells represent the annual sample collection carried out for the IGWM during 1995-98. The sampling protocol for this study was identical to that reported in previous discussion of these data (Kolpin, Kalkhoff, and others, 1997; Kolpin, Thurman, and Linhart, 1998). All wells were pumped a minimum of 30 minutes prior to obtaining measurements of dissolved oxygen, pH, specific conductance, and water temperature. Once the values for the above parameters stabilized, the water samples were collected. Water samples were filtered through a 0.7 micrometer glass-fiber filter into amber baked-glass bottles and immediately chilled.

All water samples were sent to the U.S. Geological Survey, Organic Research Laboratory in Lawrence, Kansas, to determine concentrations of 13 herbicides and 10 herbicide degradates (table 1). The 13 parent compounds and 3 of the triazine degradation products (table 1) were analyzed from 125 milliliter (mL) water samples by gas chromatography / mass spectrometry (GC/MS) following solid-phase extraction on C_{18} cartridges (Thurman and others, 1990; Meyer and others, 1993). The analytical reporting limit for this method was 0.05 microgram per liter (μg/L) for all target compounds. The other seven herbicide degradates (table 1) were analyzed from 125-mL water samples by high-performance liquid chromatography (HPLC) with diode-array detection following solid-phase extraction on C_{18} cartridges (Hostetler and Thurman, 1999). Complete separation of all analytes was achieved using this method. The analytical reporting limit for all target compounds was 0.2 μg/L for this method. Confirmation was achieved for alachlor OA, acetochlor OA, metolachlor OA, and metolachlor ESA by negative ion spray mass spectrometry (Ferrer and others, 1997).

The variation in the total number of samples collected per well for this study (from 1 to 4 samples) would have created a spatial bias in the dataset if left unmodified.Thus, average concentrations for each of the 23 compounds were calculated from all water samples collected from each well.

RESULTS AND DISCUSSION

The most important finding of this study was that herbicide degradates were detected frequently. In fact, herbicide degradates were 8 of the 10 most frequently detected compounds (fig. 2). Atrazine was the only herbicide in which the parent compound was found more frequently than any of its degradates analyzed, perhaps because of the greater environmental persistence of atrazine compared to the other parent compounds under investigation (Wauchope and others, 1992; Stamper and others, 1997). However, the total residue concentration (sum of parent compound plus degradates analyzed) was substantially greater than the concentration of just the parent compound for every herbicide examined (fig. 3). Whereas previous investigations of herbicides generally found concentrations of the parent compounds to be

Table 1. Herbicides and herbicide degradation products analyzed.

[MCL, maximum contaminant limit; HA, health advisory limit; GC/MS, gas chromatography/mass spectrometry; HPLC, high performance liquid chromatography]

Common name	Chemical name	Use or origin	Method of analysis	MCL or HA
acetochlor	2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide	herbicide	GC/MS	--
acetochlor ethansufonic acid (acetochlor ESA)	2-[(2-ethyl-6-methylphenyl)(ethoxymethyl)amino]-2-oxoethanesulfonic acid	herbicide degradate (acetochlor)	HPLC	--
acetochlor oxanilic acid (acetochlor OA)	2-[(2-ethyl-6-methylphenyl)(ethoxymethyl)amino]-2-oxoacetic acid	herbicide degradate (acetochlor)	HPLC	--
alachlor	2-Chloro-2'-6'-diethyl-*N*-(methoxymethyl)-acetanilide	herbicide	GC/MS	2
alachlor ethanesulfonic acid (alachlor ESA)	2-[(2,6-diethylphenyl)(methoxymethyl)amino]-2-oxoethanesulfonic acid	herbicide degradate (alachlor)	HPLC	--
alachlor oxanilic acid (alachlor OA)	2-[(2,6-diethylphenyl)(methoxymethyl)amino]-2-oxoacetic acid	herbicide degradate (alachlor)	HPLC	--
ametryn	2-(ethylamino)-4-isopropylamino-6-methyl-thio-s-triazine	herbicide	GC/MS	2000
atrazine	2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine	herbicide	GC/MS	3
deethylatrazine (DEA)	2-amino-4-chloro-6-(isopropylamino)-s-triazine	herbicide degradate (atrazine, propazine)	GC/MS	--
deisopropylatrazine (DIA)	2-amino-4-chloro-6-(ethylamino)-s-triazine	herbicide degradate (atrazine, cyanazine, simazine)	GC/MS	--
hydroxyatrazine (HA)	2-hydroxy-4-(ethylamino)-6-(isopropylamino)-*s*-triazine	herbicide degradate (atrazine)	HPLC	--
Cyanazine	2-[[4-chloro-6-(ethylamino)-1,3,5-triazin-2-yl]amino]-2-methylpropionitrile	herbicide	GC/MS	1
Cyanazine amide	2-chloro-4-(1-carbamoyl-1-methyl-ethylamino)-6-ethylamino-s-triazine	herbicide degradate (cyanazine)	GC/MS	--
metolachlor	2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide	herbicide	GC/MS	100
metolachlor ethanesfonic acid (metolachlor ESA)	2-[(2-ethyl-6-methylphenyl)(2-methoxy-1-methylethyl)amino]-2-oxoethanesulfonic acid	herbicide degradate (metolachlor)	HPLC	--
metolachlor oxanilic acid (metolachlor OA)	2-[(2-ethyl-6-methylphenyl)(2-methoxy-1-methylethyl)amino]-2-oxoacetic acid	herbicide degradate (metolachlor)	HPLC	--
metribuzin	4-Amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one	herbicide	GC/MS	200
prometon	2,4-bis(isopropylamino)-6-methyoxy-s-triazine	herbicide	GC/MS	100
prometryn	2,4-bis(isopropylamino)-6-(methylthio)-s-triazine	herbicide	GC/MS	--
propachlor	2-chloro-*N*-isopropylacetanilide	herbicide	GC/MS	90
propazine	2-chloro-4,6-bis(isopropylamino)-s-triazine	herbicide	GC/MS	10
simazine	2-chloro-4,6-bis(ethylamino)-s-triazine	herbicide	GC/MS	4
terbutryn	2-tert-butylamino-4-ethylamino-6-methylthio-s-triazine	herbicide	GC/MS	--

less than 0.5 µg/L in ground water (Holden and others, 1992; Kolpin and others, 1994; Kolpin, Barbash, and Gilliom, 1998), it was common for the total residue concentrations for this study to exceed this concentration (fig. 3).

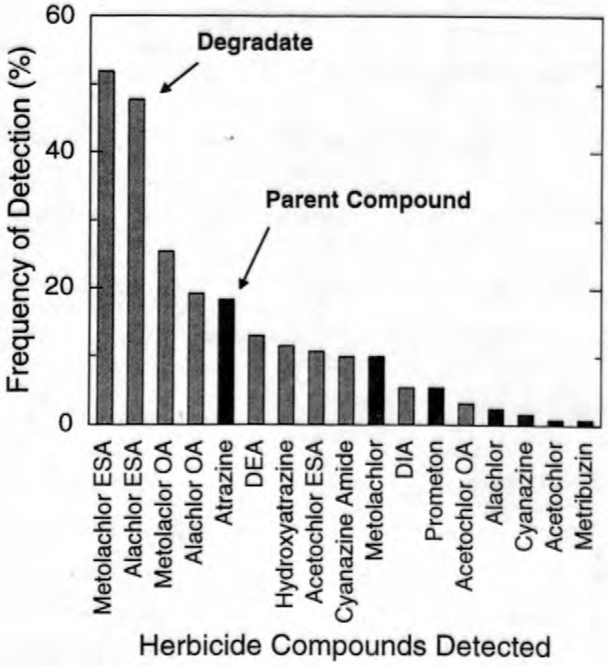

Figure 2. Frequency of detection for selected herbicide compounds (adjusted to a common detection threshold of 0.20 µg/L). See table 1 for compound abbreviation definitions.

Although no analyte concentrations exceeded their respective U.S. Environmental Protection Agency (USEPA) maximum contaminant levels or health advisory levels for drinking water (table 1), over half of the herbicide compounds under investigation had no such levels established. Furthermore, these drinking-water criteria only apply to individual compounds and do not consider the effects of more than one herbicide compound. Studies have shown that some combinations of compounds may exhibit additive or synergistic toxic effects (Marinovich and others, 1996; Thompson, 1996; Pape-Lindstrom and Lydy, 1997). The presence of multiple compounds in ground water was common during this study, particularly when herbicide degradates were included. An average of two herbicides was found in each ground-water sample in which a herbicide parent compound was detected. As many as 6 parent compounds were detected in a single sample; the

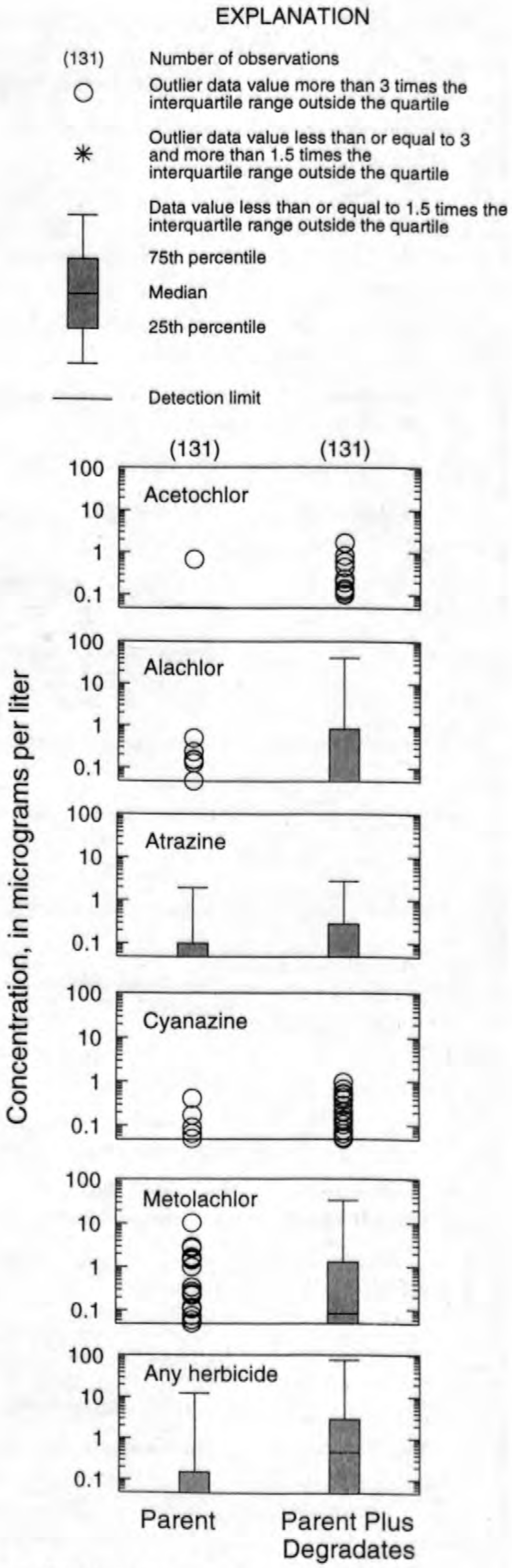

Figure 3. Distributions of concentrations of herbicide parent compounds and parent compound plus degradates.

maximum total herbicide concentration exceeded 12 µg/L (fig. 4). When herbicide degradates were considered, the number of compounds detected and total concentrations greatly increase. An average of five herbicide compounds was found in each ground-water sample in which either a herbicide parent compound or one of its degradates was detected. As many as 15 herbicide compounds were detected in a single sample; the maximum total herbicide residue concentration exceeded 78 µg/L (fig. 4).

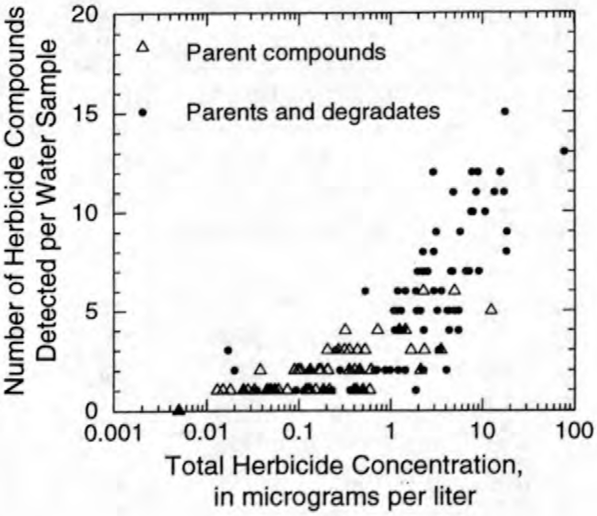

Figure 4. Relation between total herbicide residue concentration (parent plus degradates) and number of herbicide compounds detected per water sample.

Although herbicides were commonly detected in ground water across Iowa during this study, their frequencies of detection and concentrations varied substantially among the major aquifer types sampled (fig. 5). These differences were much more pronounced when herbicide degradates were included (fig. 5). Aquifer types having the most rapid recharge rates (alluvial and bedrock/karst region aquifers) were those most likely to contain detectable concentrations of herbicide compounds, indicating that ground-water age could be an important factor in explaining these variations in herbicide contamination.

Although no direct measures of ground-water age were obtained for this study, two indirect estimates of age were available -- dissolved-oxygen concentrations and well depth. These two factors can be used as general indicators of water age because oxygen tends to be consumed through biotic and abiotic processes as water travels from zones of recharge to greater depths, and well depth provides a general indication of distance from the recharge zone (Kolpin, Kalkhoff, and others, 1997). The detection and concentration of herbicides for this study were significantly related to these indirect estimates of ground-water age (fig. 6). In general, these indicators of younger ground water corresponded to more frequent detections and higher concentrations of herbicide compounds.

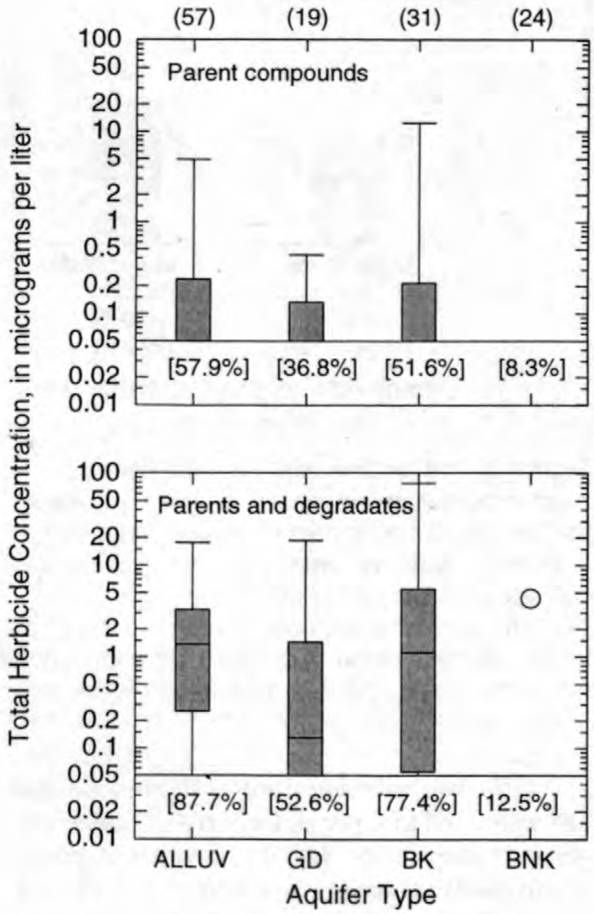

Figure 5. Total herbicide concentration by aquifer type (ALLUV = alluvial, GD = glacial drift, BK = bedrock/karst region, BNK = bedrock/nonkarst region). Numbers in brackets are the frequency of herbicide detection for that aquifer type. An explanation of a boxplot is provided in figure 3.

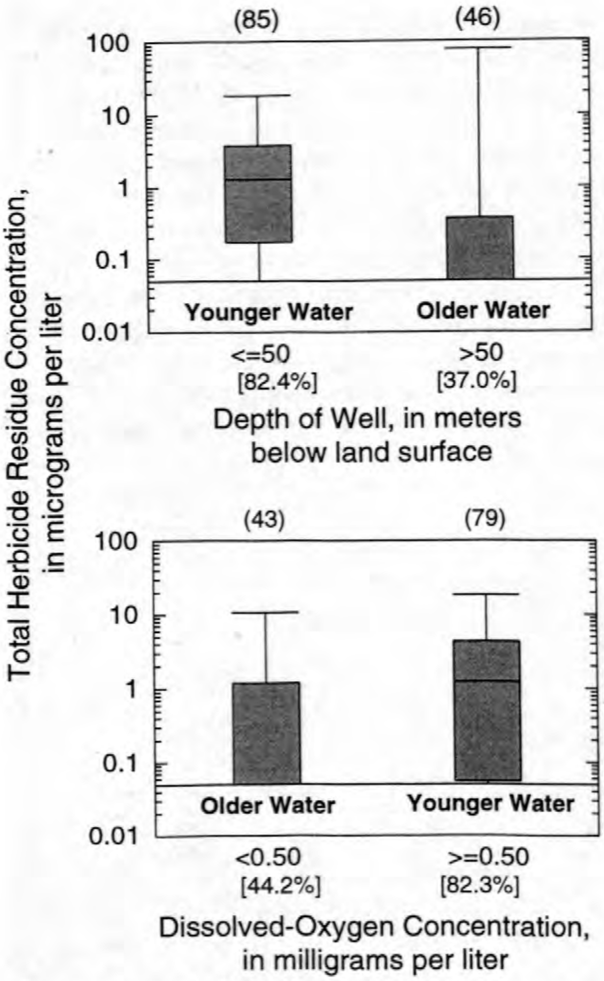

Figure 6. The relation between total herbicide residue concentration (parent plus degradates) and the two indirect estimates of ground-water age. Numbers in brackets are the frequency of herbicide compound detection for that group. Dissolved-oxygen concentrations could not be measured in water from nine of the municipal wells sampled. An explanation of a boxplot is provided in figure 3.

The two indirect estimates of ground-water age were used to separate the sampled wells into general vulnerability classes (low = well depth >50 m and dissolved-oxygen concentration < 0.5 mg/L; intermediate = well depth >50 m and dissolved-oxygen concentration ≥0.05 mg/L, or well depth ≤50 m and dissolved-oxygen concentration <0.5 mg/L; and high = well depth ≤50 m and dissolved-oxygen concentration ≥0.5 mg/L). The results show that the frequencies of detection and concentrations varied substantially among the vulnerability classes regardless of whether or not herbicide degradates were considered (fig. 7). Nevertheless, when herbicide degradates were included, the frequency of herbicide compound detection within the highest vulnerability class approached 90% and the median total herbicide residue concentration increased over an order of magnitude to 2 µg/L (Figure 7).

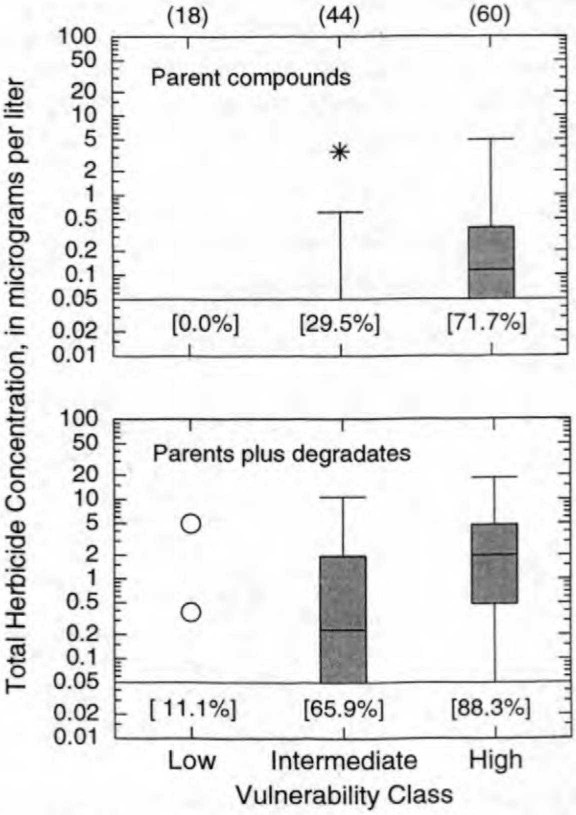

Figure 7. The relation between total herbicide concentration and vulnerability class (Low = well depth >50 m and dissolved-oxygen concentration <0.5 mg/L; Intermediate = well depth >50 m and dissolved-oxygen concentration ≥0.5 mg/L, or well depth ≤50 m and dissolved-oxygen concentration <0.5 mg/L; High = well depth ≤50 m and dissolved-oxygen concentration ≥0.5 mg/L). Numbers in brackets are the frequency of herbicide detection for that group.The nine wells where dissolved-oxygen data were not available were not used for this analysis. An explanation of a boxplot is provided in figure 3.

This study documents that obtaining data on herbicide degradates is critical in attempting to understand not only the fate of a specific herbicide, but also its overall effect on human health and the environment.

ACKNOWLEDGEMENTS

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Pesticides in the Atmosphere of the Mississippi River Valley, Part I — Rain

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ABSTRACT

Weekly composite rainfall samples were collected in three paired urban and agricultural regions of the midwestern United States and along the Mississippi River during April–September 1995. The paired sampling sites were located in Mississippi, Iowa, and Minnesota. A background site, removed from dense urban and agriculture areas, is located near Lake Superior in Michigan. Herbicides were the predominant type of pesticide detected at every site. Each sample was analyzed for 47 compounds and 23 of 26 herbicides, 13 of 18 insecticides, and 3 of 3 related transformation products were detected in one or more samples at each paired site. The detection frequency of herbicides and insecticides were nearly equivalent at the paired Iowa and Minnesota sites. In Mississippi, herbicides were detected more frequently at the agricultural site and insecticides were detected more frequently at the urban site. The highest total wet depositional amounts (μg pesticide/m^2 per season) occurred at the agricultural sites in Mississippi (1,980 μg/m^2) and Iowa (490 μg/m^2) and at the urban site in Iowa (696 μg/m^2). Herbicides accounted for the majority of the wet depositional loading at the Iowa and Minnesota sites, but methyl parathion (1,740 μg/m^2) was the dominant compound contributing to the total loading at the agricultural site in Mississippi. Atrazine, CIAT (a transformation product of atrazine), and dacthal were detected most frequently (76, 53, and 53 percent, respectively) at the background site indicating their propensity for long-range atmospheric transport.

INTRODUCTION

More than 500 million kg (kilograms) of pesticides are used each year in the United States to control many different types of weeds, insects, and other pests in a wide variety of agricultural and urban settings (Aspelin, 1994). The highest density of agricultural activity and harvested cropland in the United States is in the upper Midwest and along the lower Mississippi River. A wide variety of herbicides and insecticides are used on many of the diverse crops grown in this region. Although increased pesticide use has resulted in increased crop production, concerns about the potential adverse effects of pesticides and pesticide transformation products on the environment and human health have grown steadily.

A wide variety of pesticides have been detected in the atmosphere throughout the world (Grover, and others, in press; Majewski and Capel, 1995). Pesticides become airborne through volatilization and wind erosion of particles both during and after the application process. Volatilization is a continuous process and can be a major dissipative route for many pesticides (Glotfelty, 1978; Majewski, 1991; Seiber and Woodrow, 1995). Once airborne, the pesticide can be carried by wind and deposited in unintended areas by dry (gas and particle) and wet [fog and precipitation (rain and snow)] depositional processes. These deposited residues can revolatilize, re-enter the atmosphere, and be transported and redeposited downwind repeatedly until they are transformed or accumulate, usually in areas of cooler climate (Risebrough, 1990;

Wania and Mackay, 1996). This same process also can occur for the products from abiotic or biotic transformations of pesticides. For some persistent compounds, this deposition and revolatilization process can continue for decades.

A six-month study was conducted that was designed to characterize the atmospheric occurrence, temporal patterns, transport, and deposition of a variety of pesticides used in agricultural and urban environments in three geographically different regions of the Mississippi River Valley. Weekly composite air (gas and particle phase) and precipitation (rain) samples were collected.

Two principal study objectives were 1) to document the occurrence and detection frequency of a wide variety of herbicides, insecticides, and selected transformation products in the various atmospheric matrices (gas phase, particle phase, and precipitation) at urban and agricultural areas over one growing season, and 2) to compare the types of pesticides detected at three geographically different areas of the Mississippi River Valley. The results of the study in relation to the precipitation data are reported here.

Sampling Sites

Each monitoring site was chosen to be within or near an existing NAWQA (U.S. Geological Survey National Water Quality Assessment program) study unit. The urban and suburban sampling sites (fig. 1) for this study were located at Jackson, Miss. (Mississippi Embayment NAWQA study unit), Iowa City, Iowa (Eastern Iowa Basins NAWQA study unit), and Minneapolis, Minn. (Upper Mississippi River Basin NAWQA study unit). The agricultural sampling sites were located near Rolling Fork, Miss. [sampler located about 0.5 km (kilometer) from cotton field], at the Cedar Rapids, Iowa, airport [sampler located about 15 m (meters) from corn field], and at the U.S. Department of Agriculture's MSEA (Management Systems Evaluation Area) near Princeton, Minn., (sampler located about 300 m from MSEA fields). A background site removed from large urban and agriculture areas was located at Eagle Harbor, Mich. This site is part of the IADN (Integrated Atmospheric Deposition Network) for Lake Superior.

Sampling began during the first week of April and continued through September, 1995. The precipitation samples were collected using a modified automatic wet–dry precipitation collector. Modifications to the collector included replacing the plastic collection bucket with a 31-cm (centimeter) diameter, Teflon coated, metal funnel connected by Teflon tubing to a 3-L (liter) glass carboy inside a small refrigeration unit located beneath the deposition collector. Teflon sheeting also lined the cover of the collector, which was placed over the funnel during dry periods. Each sample was a composite of the precipitation events that occurred during a one week period. If more than one liter of liquid was collected during a sampling period, the total volume was recorded and a 1-L sub-sample was taken for analysis. If less than 75 ml (milliliter) of rainwater was collected at the end of the 1-week sampling period, it was left in the container and the sampling period was extended for another week. If at the end of a 2-week period there was not sufficient rainwater for an appropriate extraction, the liquid was discarded, the carboy cleaned, and a new collection period was begun.

Each sample was extracted using C-18 solid-phase extraction followed by selected ion GC/MS analysis for 47 herbicides, insecticides, and selected transformation products (Zaugg, and others, 1995). The concentration of each pesticide compound in µg/L (microgram per liter) was divided by the actual rainfall amount in cm (centimeter) to obtain the total depositional flux [µg/m^2 (microgram per square meter) per sample and per growing season]. This was done to facilitate the comparison between each sampling period and site.

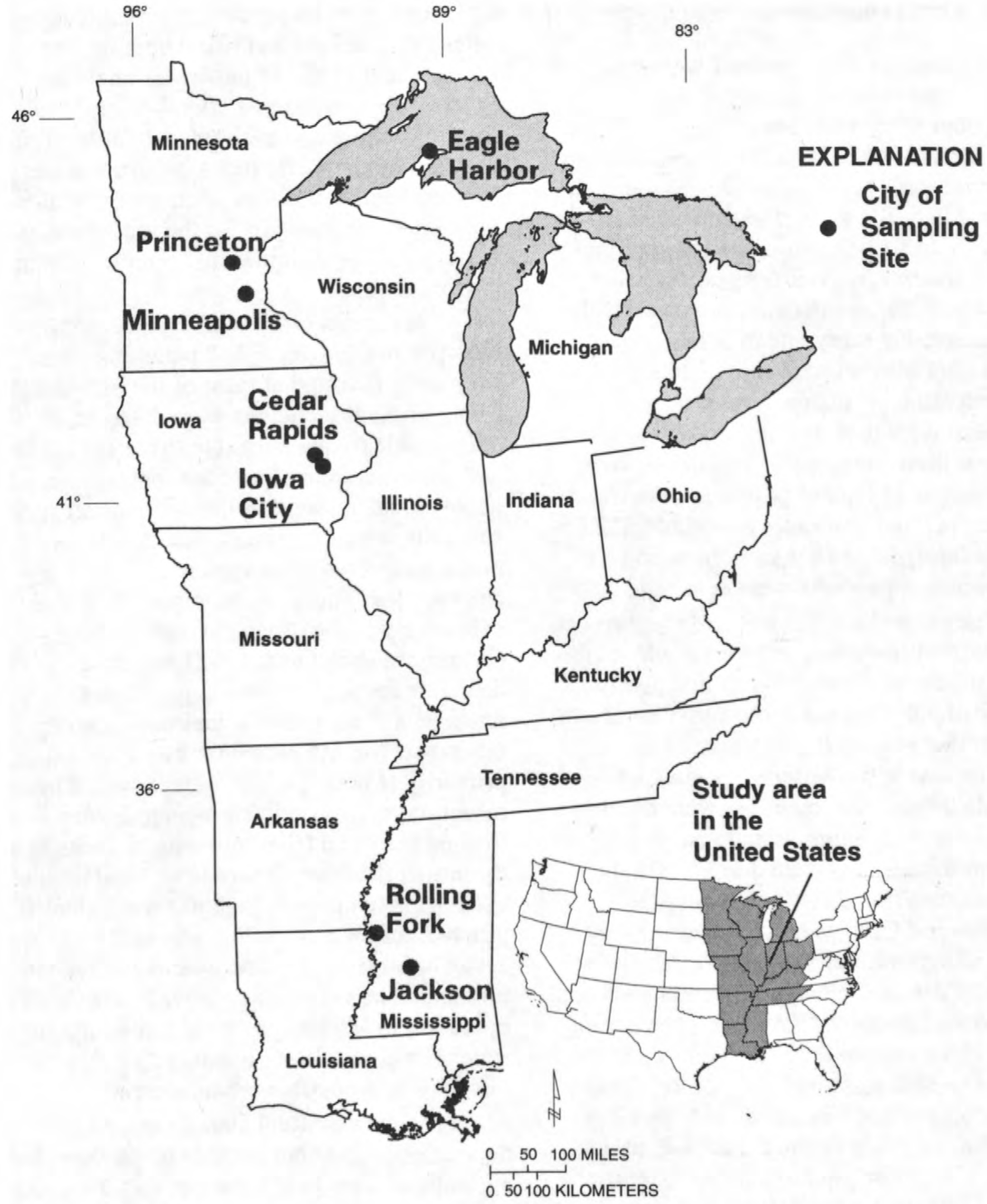

Figure 1. Sampling locations: urban (Jackson, Iowa City, Minneapolis), agricultural (Rolling Fork, Cedar Rapids, Princeton), and background (Eagle Harbor) sites.

Results and Discussion

An average of 18 samples were collected at each location — 16 at each of the Mississippi sites; 18 at Iowa City, Iowa; 20 at Cedar Rapids, Iowa; 20 at Minneapolis, Minn.; 17 at Princeton, Minn.; and 17 at Eagle Harbor, Mich. Six herbicides [alachlor, atrazine, CIAT (2-chloro-4-isopropylamino-6-amino-s-triazine, a transformation product of atrazine), cyanazine, dacthal, and metolachlor] were detected at every site in at least one sample. Six others (acetochlor, EPTC, pendimethalin, prometon, simazine, and trifluralin) were detected at six of the seven sites in at least one sample. Herbicides were the predominant type of pesticide detected at every site. Of the pesticides analyzed for, 23 of 26 herbicides, 13 of 18 insecticides, and 3 of 3 transformation products were detected.

The general trend in pesticide occurrence and detection frequency in rain samples at the three paired sampling sites was that nearly every pesticide detected at the agricultural site also was detected at the urban site. The exception was at the Mississippi sites where herbicide detection frequencies were dominate in the agricultural samples and insecticide detection frequencies were dominate in the urban samples (fig. 2 A). The same nine herbicides and CIAT were detected at every urban and agricultural site (fig. 2). The most frequently detected classes of herbicides were the triazines (atrazine, CIAT, and cyanazine) and the chloroacetanilides (acetochlor, alachlor, metolachlor, and propachlor). Both the triazine and chloroacetanilide herbicides are used extensively in the production of corn, soybeans, sorghum, and to a lesser extent, cotton. Their state-wide use in Iowa and Minnesota range from 1 to 4 million kg each annually with an order of magnitude less used in Mississippi (Gianessi and Anderson, 1995). In Mississippe, the rice herbicides, molinate, propanil, and triobencarb were among the most frequently detected pesticides at both the agricultural and urban sites

Atrazine, CIAT, and dacthal were the most frequently detected compounds (76, 53, and 53 percent, respectively) at Eagle Harbor, Mich., the background site. These findings indicate that of the 47 pesticides analyzed for in this study, these three are relatively stable in the atmosphere and are subject to at least regional-atmospheric transport. Atmospheric transport and deposition often are the major or only input mechanisms for the movement of these types of pesticides into remote, pristine environments.

Five insecticides (carbaryl, carbofuran, chlorpyrifos, diazinon, and parathion) were frequently detected at most of the paired sites. Many of the insecticides were detected at comparable frequencies at both the agricultural and urban sampling locations, but the depositional amounts at the agricultural sites generally were higher with the exception of malathion, carbaryl, diazinon, and chlorpyrifos. These four compounds are used extensively in the urban environment (Whitmore and others, 1992) and the elevated detection frequencies and depositional amounts are, most likely, an indication of urban use. Methyl parathion was detected primarily at both the Mississippi sites. The detections were continual from mid-May at Rolling Fork and from mid-June at Jackson to the end of the study. There is no legal urban use for methyl parathion, but it was detected nearly as often at the urban site as the agricultural site. This compound was recently implicated in an illegal housing fumigation operation in Mississippi and other southern states (Hileman, 1996; Hileman, 1997) and this may be a possible urban source.

The greatest total amount of pesticide deposition (μg/m^2 per season) occurred at the agricultural sites in Mississippi and Iowa and the urban site in Iowa (fig. 3). Herbicides were the most significant pesticide type contributing to the depositional loading by precipitation in Iowa, Minnesota, and, with the exception of methyl parathion at Rolling Forks, Mississippi. Methyl parathion (1,740 μg/m^2) which is used primarily in the production of cotton, but also to a lesser extent on corn, rice, and soybeans, was the single greatest contributor to the wet depositional loading at Rolling Fork. In

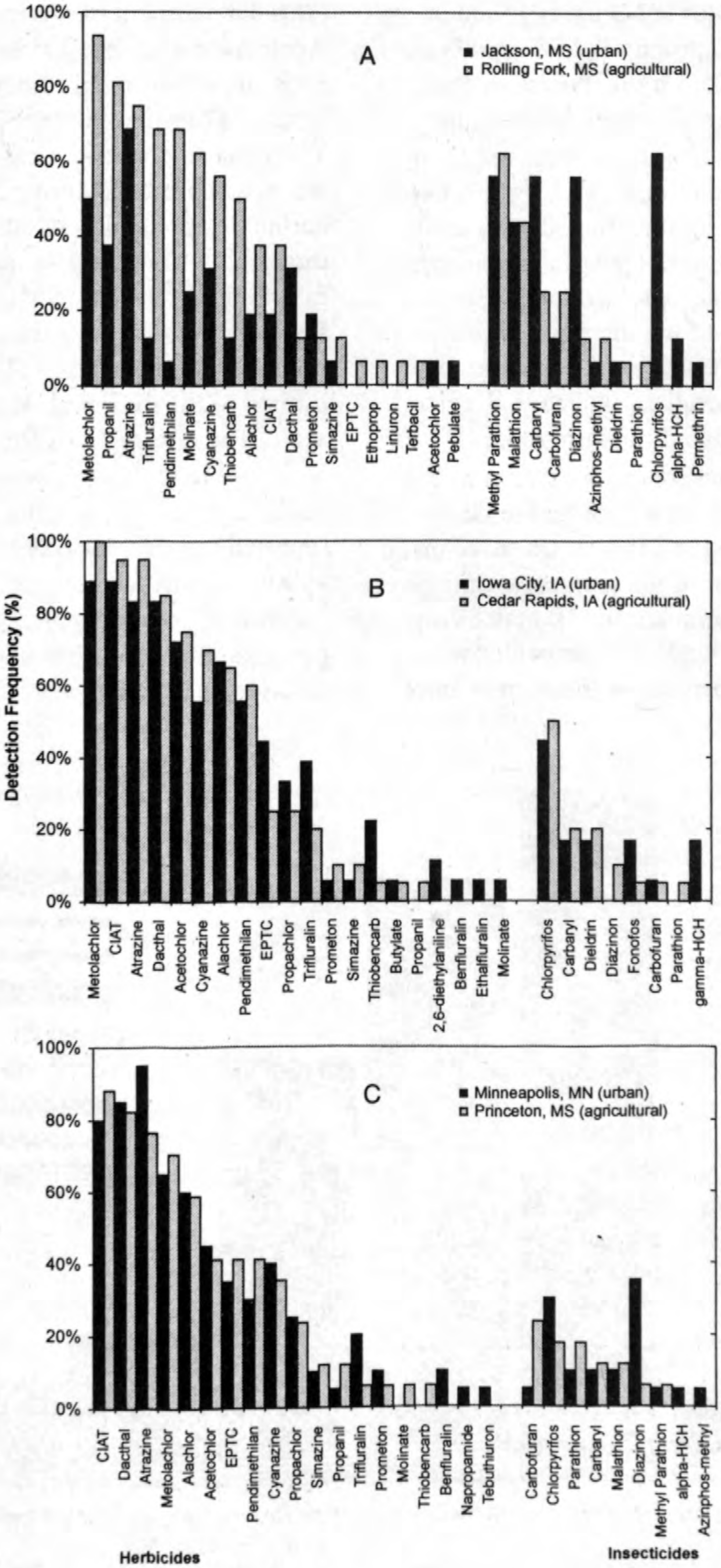

Figure 2. The frequency of detection (%) for each pesticide detected at the A) Mississippi sites, B) Iowa sites, and C) Minnesota sites.

addition, metolachlor (81.3 μg/m^2) used on corn and soybeans, propanil (25.8 μg/m^2) used on rice, atrazine (25.6 μg/m^2) used on corn, trifluralin (22.1 μg/m^2) and pendimethalin (20.4 μg/m^2) used on corn, cotton, sorghum, and soybean, and molinate (12.2 μg/m^2) used on used on rice, were the other significant contributors to the wet depositional loading at the Rolling Fork site. The occurrence of atrazine is a result of the increasing corn production in the Mississippi delta region (Mississippi Cooperative Extension Service, 1995; U.S. Department of Agriculture, 1996).

The first widespread application in the United States of the new corn herbicide acetochlor occurred in 1994. It was used on an estimated 7 percent of the total corn acreage using 1994 data compiled for 10 major crop producing states. For 1995, acetochlor was used on about 20 percent of the corn acreage (U.S. Department of Agriculture, 1996). Acetochlor was detected in rain samples at each site in Iowa and Minnesota from the initiation of sampling during April–July, 1995. The greatest concentration and detection frequency occurred during periods of extensive herbicide application in late April and throughout May. Lack of acetochlor detections in samples collected in Mississippi and at Eagle Harbor suggests that the atmospheric transport and deposition of this compound is limited to the local and, at most, a regional scale. The observations for acetochlor were comparable to those observed for alachlor in this study and were similar to those previously reported for alachlor (Glotfelty, and others, 1990; Goolsby, and others, 1997). Acetochlor accounted for more than 35 percent of the total pesticide flux (500–700 μg/m^2) for the 1995 growing season at the Iowa sites (fig. 3).

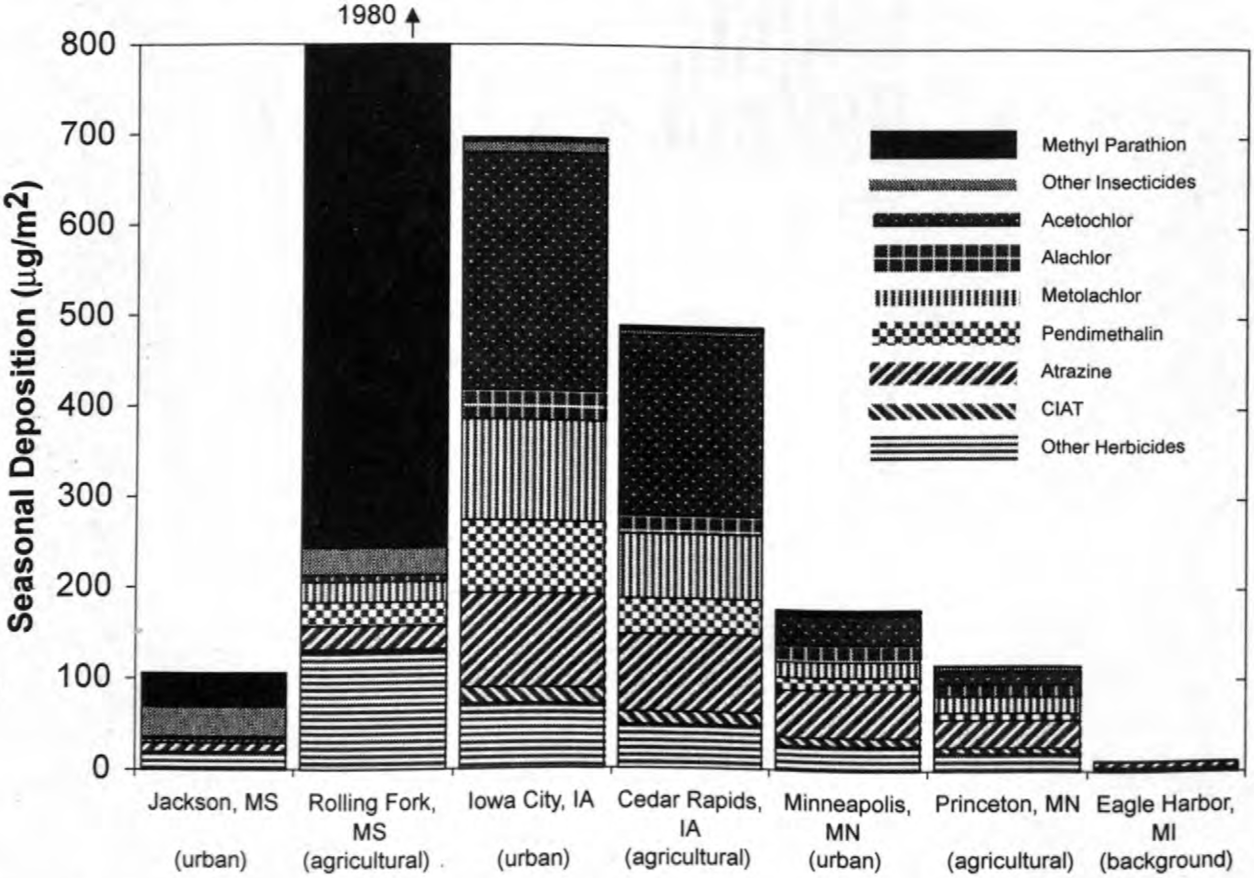

Figure 3. Total deposition by rain for those pesticides detected at the sample collection locations from April through mid-September, 1995.

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Pesticides in the Atmosphere of the Mississippi River Valley, Part II – Air

By William T. Foreman, Michael S. Majewski, Donald A. Goolsby, Frank W. Wiebe, and Richard H. Coupe

ABSTRACT

Weekly composite air samples were collected from early April through mid-September 1995 at three paired urban and agricultural sites along the Mississippi River region of the Midwestern United States. The paired sampling sites were located in Mississippi, Iowa, and Minnesota. A background site, removed from dense urban and agricultural areas, was on the shore of Lake Superior in Michigan. Each sample was analyzed for 49 compounds; of these, 21 of 26 herbicides, 13 of 19 insecticides, and 4 of 4 related transformation products were detected during the study, with most pesticides detected in more than one sample. The maximum number of pesticides detected in an air sample was 18; this sample was from the Iowa City urban site in mid-June. Herbicides were the predominant type of pesticide detected at every site. The detection frequencies of most herbicides generally were comparable at the paired Iowa and Minnesota sites. In Mississippi, herbicides generally were detected more frequently at the agricultural site. The insecticides chlorpyrifos, diazinon, and carbaryl, which are used in agricultural and nonagricultural setttings, were detected more frequently in urban sites than agricultural sites in Mississippi and Iowa. Methyl parathion was detected in 70 percent of the samples from Rolling Fork, Miss., and at the highest concentration (62 nanograms per cubic meter of air) of any insecticide measured in the study. At the background site, dacthal (100 percent), atrazine (35 percent), cyanazine (22 percent), and the (primarily atrazine) triazine transformation products CIAT (35 percent) and CEAT (17 percent) were detected most frequently, suggesting their potential for long-range atmospheric transport. Estimates of the pesticides' particle and gas distribution provided insight into the primary mechanisms of pesticide transport and deposition.

INTRODUCTION

Of the environmental compartments—air, water, soil, sediment, and biota—the atmosphere represents the largest (for gas-phase contaminants) and most mobile compartment into which a chemical contaminant might be directly released or subsequently move into, undergo transport, and, in some cases, accumulate (Mackay and others, 1997). Pesticides applied in agricultural and nonagricultural settings enter the atrmosphere by volatilization and by wind erosion of particles (soil, vegetation, formulation powders) on which the pesticide is sorbed. Pesticide losses to the atmosphere typically are large during application, but losses continue after the application process. Volatilization is a continuous process and can be a major dissipative route for many pesticides (Glotfelty, 1978; Majewski, 1991; Seiber and Woodrow, 1995).

Once airborne, the scale of transport through the atmosphere will depend on a variety of meteorological conditions and pesticide physical/chemical properties. Atmospheric removal processes include photochemically driven reaction and physical depositional mechanisms. Pesticides that are readily susceptible to photochemical reactions (for example, trifluralin; Woodrow and others, 1978; Mongar and Miller, 1988) have short (minutes to to several hours) atmospheric residence times and usually are transported short distances from the source. Pesticides that are less susceptible to these chemical removal processes typically have atmospheric chemical lifetimes of a day or considerably longer (for example, lindane and DDT; Mackay and others, 1997), and, thus, can be transported greater distances (regional or long-range scales).

The atmospheric transport and fate of a pesticide also depends on whether it exists as a gas, sorbed to atmospheric particles, or both. This particle/gas distribution is largely dependent on the pesticide's vapor pressure. For some pesticides, this distribution changes substantially as atmospheric conditions fluctuate, especially temperature and, to a less extent, concentration and type of airborne particles (Bidleman, 1988). The distance that a pesticide can be carried by wind will depend on this distribution. Pesticides in the gas phase are removed by dissolution into precipitation and surface water, and sorption onto soil and vegetation. Particle-bound pesticides are washed out of the atmosphere by precipitation or removed by dry-particle deposition to surfaces (water, soil, vegetation). These deposited residues can reenter the atmosphere by subsequent revolatilization or wind erosion, and be transported and redeposited downwind repeatedly until they are transformed or accumulate, usually in areas with cooler climates (Risebrough, 1990; Wania and Mackay, 1996). This same process also can occur for the products from abiotic or biotic transformations of pesticides. For some persistent compounds (for example, 4,4'-DDT and its primary degradation products 4,4'-DDD and 4,4'-DDE), this deposition and revolatilization process can continue for decades and lead to global contaminant distribution.

A variety of pesticides has been detected in the atmosphere throughout the world, but many of these studies have focused on older generation organochlorine insecticides, most of which are banned in many countries (Majewski and Capel, 1995; Grover and others, in press). Several recent studies have focused on current-use pesticides (for example, Baker and others, 1996; Aston and Seiber, 1997; McConnell and others, 1998). Nevertheless, reports of current-use pesticides in precipitation, and, especially, in air remain sparse in comparison to studies in other matrices, in part because of low ambient air concentrations and a lack of regulatory focus on the atmosphere.

To address the lack of information on pesticides in air, the U.S. Geological Survey (USGS) conducted a 6-month study to characterize the atmospheric presence, temporal patterns, transport, and deposition of a variety of pesticides used in 1995 in agricultural and urban environments in three geographically different regions of the Mississippi River Valley. Weekly composite air (gas and particle phase) and precipitation (rain) samples were collected.

Two principal study objectives were (1) to document the presence and detection frequency of a wide variety of herbicides, insecticides, and selected transformation products in three atmospheric matrices (gas phase, particle phase, and precipitation) at urban and agricultural areas over one growing season, and (2) to compare the types of pesticides detected among three geographically different areas of the Mississippi River Valley region. This region was chosen because the highest density of agricultural activity and harvested cropland in the United States is in the upper Midwest and along the lower Mississippi River (see Majewski and others, 1998, and references therein). A variety of herbicides and insecticides are used on many of the diverse crops grown in this region (U.S. Department of Agriculture, 1996). The Midwestern United States also was the focus of two previous atmospheric-related studies by the USGS (Goolsby and others, 1997; Majewski and others, 1998). Selected air results are reported in this paper. A companion paper describes results for the precipitation samples (Majewski and others, this volume).

EXPERIMENTAL

Field Sampling

Weekly composite air samples were collected from early April through mid-September 1995 at seven locations across the Midwestern United States (fig. 1). Sampling sites at Jackson, Miss. (at Interstate 55 and McDowell Road); Iowa City, Iowa (downtown); and Minneapolis, Minn. (near Lake Harriet) represented urban areas. Sites near Rolling Fork,

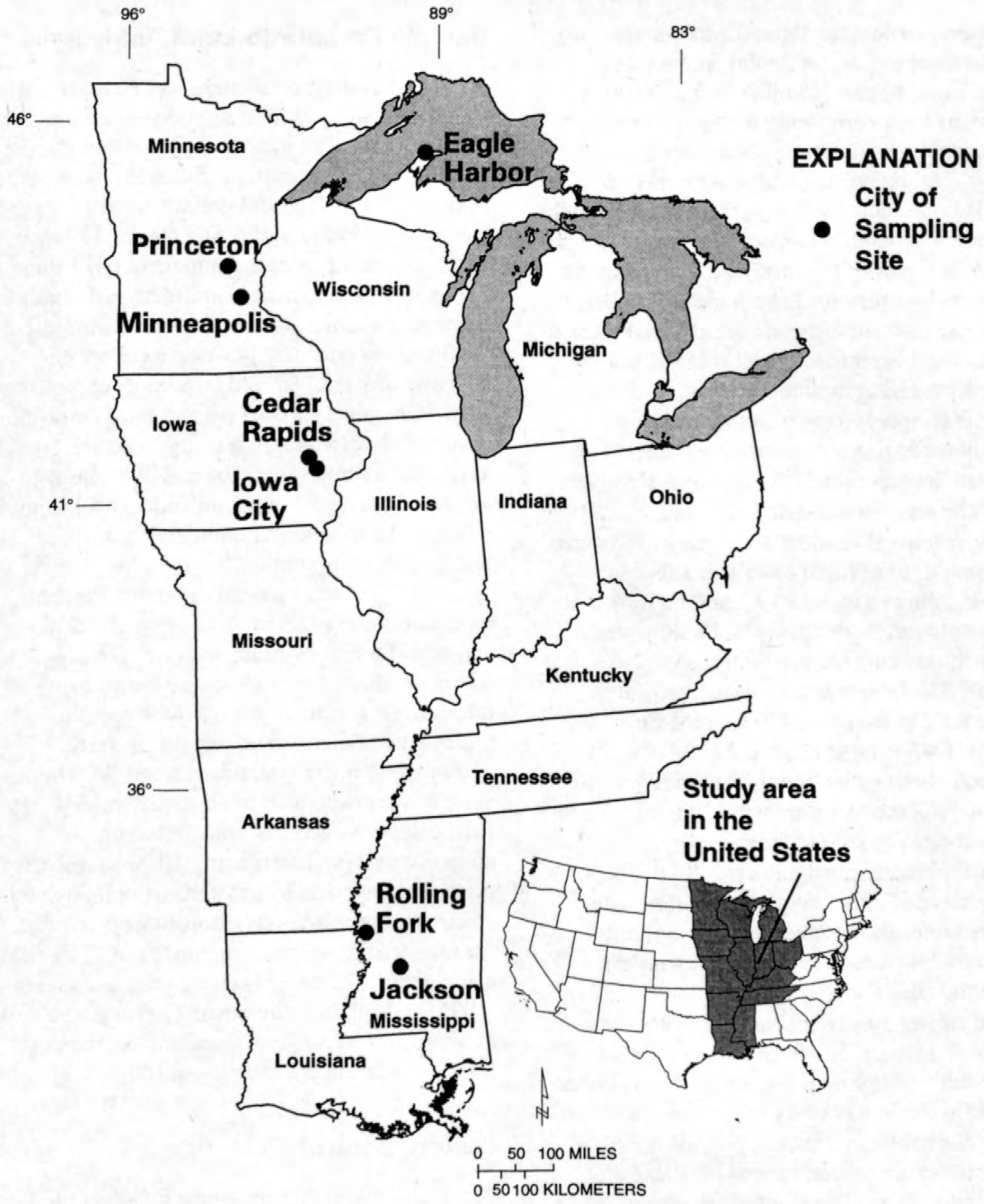

Figure 1. Sampling locations: urban (Jackson, Iowa City, Minneapolis), agricultural (Rolling Fork, Cedar Rapids, Princeton), and background (Eagle Harbor) sites.

Miss. [sampler located about 0.5 kilometer (km) from surrounding cotton fields]; at the Cedar Rapids, Iowa, airport [sampler located about 15 meters (m) from corn field]; and at the U.S. Department of Agriculture's Management Systems Evaluation Area (MSEA) near Princeton, Minn. [sampler located 300 m from MSEA fields] represented agricultural areas. The Eagle Harbor, Mich., site is part of the Integrated Atmospheric Deposition Network for Lake Superior (Hillery and others, 1998) and is considered a background site because it is removed from areas of heavy urbanization and agriculture.

Air samples were collected using a conventional high-volume sampler (Graesby-Anderson, Incorporated*) (Thrane and Mikalsen, 1981). The sampler consisted of a 20.3 × 27.9-centimeter (cm) glass-fiber filter (GFF, Whatman, Incorporated EPM2000) to collect airborne particles, followed by two 7.6-cm long by 8.6-cm diameter polyurethane foam (PUF) plugs that were positioned in series to collect gas-phase pesticide. The GFF was precleaned by heat treating for 2 hours (h) at 450 degrees Celsius (°C). The GFF is rated to have 99.999 percent retention efficiency for sodium chloride particles having a mass median diameter of 0.6 microns at a 5 centimeter per second face velocity (Whatman, Incorporated, undated). PUF plugs were precleaned by sequential extraction with water, acetone, and hexane, and vacuum dried.

Samples were collected by drawing air through the GFF and PUF plugs at about 1 cubic meter of air per minute (m^3/min) initially for a continuous 4 h each day from 10 a.m. to 2 p.m. By mid-June, sampling times at all sites had been modified to 5 minutes every hour to avoid missing diurnal variations and provide more representative air concentrations (Wallace and Hites, 1996). Typical weeklong air volumes were about 850 m^3.

Sample Preparation and Analysis

The custom air method was based in part on the method used for rain samples to facilitate data interpretation. The method used for rain samples was designed to determine multiple classes of pesticides in surface and ground water (Zaugg and others, 1995; Lindley and others, 1996). Briefly, each air sample component (GFF and two PUF plugs) was extracted and analyzed separately to provide estimates of particle/gas distributions and to assess pesticide gas-phase collection efficiency by the PUF plugs. Sample components were extracted with 36.5 percent by volume ethyl acetate in hexane for 16 h using a Soxhlet apparatus. Extracts were dried with sodium sulfate and reduced to 0.5 milliliter (mL) using a Kuderna-Danish concentrator followed by nitrogen gas evaporation.

Extracts were passed through a Pasteur pipet column containing 0.75 gram of fully activated Florisil overlain with 1 cm of powdered sodium sulfate. Pesticides were eluted using 4 mL of ethyl acetate into a test tube containing 0.1 mL of a perdeuterated polycyclic aromatic hydrocarbon internal standard solution. The extract was evaporated to about 150 microliters (µL) using nitrogen gas, transferred to autosampler vial inserts using a 100-µL toluene rinse, and analyzed by gas chromatography with electron-impact mass spectrometry operated in the selected-ion-monitoring mode (GC/MS–SIM) using conditions described in Zaugg and others (1995) and Lindley and others (1996). Extracted PUF plugs were vacuum dried and reused in at least one subsequent sample collection.

Quality Control

Quality-control measures included the addition of surrogate compounds to each sample prior to extraction to monitor laboratory sample preparation and analysis. Field air blanks were obtained at each site on rotation throughout the study and typically consisted of a GFF plus two PUF plugs that were briefly placed in the air sampler to simulate field handling. PUF plugs used for blanks included reused cleaned PUF from previous field samples.

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* Use of firm, brand, or trade names in this article is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey.

Laboratory spike samples were prepared for each of the 22 sets of analyzed air samples by fortifying 100 nanograms of the 49 compounds onto a PUF plug prior to extraction. In addition to the estimates of gas-phase collection efficiencies for PUF plugs from field samples, three spike collection-efficiency tests were performed to monitor the migration of pesticides through the two PUF plugs. These tests were carried out by spiking GFFs with the pesticides and drawing ambient air through the sampler at about 1 m^3/min under the following volume/average temperature conditions: 310 m^3/17 °C, 850 m^3/16.1 °C, and 1,730 m^3/24.5 °C. Some quality-control results are provided in this paper.

RESULTS AND DISCUSSION

Detection frequencies of pesticides in air samples are dependent on the following:

- Pesticide presence in air during the collection period in either the gas or particle phase, or both, in sufficient concentration to be detected by the GC/MS–SIM method.
- Adequate efficiency by the PUF plugs to collect at least a substantial portion, if not all, of the gas-phase pesticide during the collection period.
- Chemical stability of the pesticide once collected on the GFF or PUF.
- Adequate recovery during the sample preparation and analysis steps.

Butylate, 2,6-diethylaniline, EPTC, and molinate had poor collection efficiency on PUF at the 850 m^3 and greater air volumes used in this study. Pebulate and phorate also were poorly collected by the PUF and were not detected in any samples. For these six compounds, detection frequencies might be biased substantially low. Azinphos-methyl, carbaryl, carbofuran, and terbacil are susceptible to thermal instability during GC/MS–SIM analysis (Zaugg and others, 1995), a condition that can lead to high or low bias during quantitation. Of these, the insecticides were completely collected by the two PUF plugs for the air volumes used in this study. Mean laboratory spike recoveries are provided in table 1 and ranged from 37 ± 28 percent for tebuthiuron (which, along with prometon, were incompletely eluted from the Florisil cartridge) to 140 ± 56 percent for carbaryl, with an overall mean recovery of 92 ± 20 percent for all compounds. Estimates of method reporting levels were provided in Majewski and others (1998), and range from about 0.006 ng/m^3 for atrazine to 0.1 ng/m^3 for prometon for an 850 m^3 air volume. Estimated concentrations below the reporting level were used if all GC/MS–SIM qualifying information were obtained (Zaugg and others, 1995).

For the 25-week study, the following week-long air samples were collected at each site: 24 at Jackson, Miss.; 21 at Rolling Fork, Miss.; 19 at each Iowa site; 22 at Minneapolis, Minn.; and 23 each at Princeton, Minn. and Eagle Harbor, Mich. Equipment or power failures reduced the number of samples to less than 25.

Frequencies of detection in air at paired urban and agricultural sites are shown in figure 2 and are grouped into herbicides and insecticides. The bar graphs are ordered from left to right beginning with the highest number of herbicide or insecticide detections at the agricultural location for each paired site. Detection frequencies at the Eagle Harbor, Mich., background site are shown in figure 3.

Each sample was analyzed for 49 compounds (table 1). Including all sites and samples, 21 of 26 herbicides, 13 of 19 insecticides, and 4 of 4 related transformation products were detected, with most pesticides detected in more than one sample (figs. 2 and 3). The maximum number of pesticides detected was 18 in an air sample taken at the Iowa City urban site from June 13 to 20.

The herbicide trifluralin was the most commonly detected pesticide measured in air samples at six sites, with greater than 80 percent detections at two sites and 100 percent detection at four sites. Nearly all detections of trifluralin were on the PUF sorbent only, indicating nearly exclusive gas-phase transport (table 1). Trifluralin can be applied to more than 40 different crop types (Meister, 1997). Application of this volatile herbicide to multiple crop types likely accounts for its presence in air over the entire 1995 growing season. However, atmospheric transport of trifluralin beyond the local, to at most regional, scale was not evident because it was detected in

Table 1. Mean and standard deviation of recovery for 22 laboratory spike samples, and apparent phase in which the pesticide was detected for air samples collected April to September 1995. Gas phase noted in boldface type when apparent from field results

[±, plus or minus; G, gas phase; **G**, gas phase dominant; P, particle phase; nd, not detected; --, irrelevant]

Herbicides	Mean spike recovery (percent)	Apparent phase distribution	Insecticides	Mean spike recovery (percent)	Apparent phase distribution
Chloroacetanilides			*Organophosphates*		
Acetochlor	98 ± 12	G P	Azinphos-methyl[6]	76 ± 51	G P
Alachlor	102 ± 12	G P	Chlorpyrifos	95 ± 12	**G** P
2,6-Diethylaniline[1,2]	72 ± 9	G P	Diazinon	97 ± 12	G P
Metolachlor	102 ± 11	G P	Dimethoate	100 ± 33	nd
Propachlor	103 ± 21	G P	Disulfoton	66 ± 44	nd
Dinitroaniline			Ethoprop[2]	91 ± 11	G P
Benfluralin	66 ± 16	**G** P	Fonofos	88 ± 12	G
Ethalfluralin	76 ± 23	nd	Malathion	107 ± 16	G P
Trifluralin	71 ± 17	**G** P	Methyl parathion	96 ± 19	**G** P
Thiocarbamates			Parathion	110 ± 23	nd
Butylate[2]	89 ± 14	**G** P	Phorate[2]	73 ± 23	nd
EPTC[2]	90 ± 21	**G** P	Terbufos	82 ± 22	nd
Pebulate[2]	86 ± 11	**G** P	*Carbamates*		
Triallate	90 ± 13	nd	Carbaryl[6]	140 ± 56	G P
Thiobencarb	100 ± 11	**G** P	Carbofuran[6]	135 ± 37	G P
Triazines			*Organochlorines*		
Atrazine	97 ± 12	G P	alpha-HCH	86 ± 17	**G** P
CEAT[3]	107 ± 8	G P	Lindane	100 ± 26	**G**
CIAT[4]	107 ± 48	G P	4,4'-DDE[7]	82 ± 12	**G** P
Cyanazine	112 ± 21	G P	Dieldrin	80 ± 14	**G**
Metribuzin	97 ± 13	G P	cis-Permethrin	99 ± 19	G P
Prometon[5]	53 ± 34	nd	*Fungicide*		
Simazine	96 ± 11	G P	Propargite I & II	86 ± 22	nd
Miscellaneous					
Dacthal	99 ± 14	**G** P	*Surrogates*		
Linuron	90 ± 32	nd	$^{2}H_6$-Diazinon	103 ± 21	--
Molinate[2]	91 ± 14	**G** P	$^{2}H_6$-alpha-HCH	90 ± 12	--
Napropamide	93 ± 15	nd	Terbutylazine	94 ± 14	--
Pendimethilan	66 ± 19	**G** P			
Pronamide	93 ± 12	P			
Propanil	109 ± 19	G P			
Tebuthiuron[5]	37 ± 28	G			
Terbacil[6]	108 ± 27	G			

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[1] Alachlor degradation product.

[2] Gas-phase compound is poorly collected by two PUF plugs at air volumes used in this study.

[3] CEAT, 2-chloro-4-ethylamino-6-amino-*s*-triazine, a degradation product of atrazine, cyanazine, simazine, and terbuthylazine.

[4] CIAT, 2-chloro-4-isopropylamino-6-amino-*s*-triazine, a degradation product of atrazine and propazine.

[5] Variably low recovery during extract preparation.

[6] Estimated quantitation because of thermal instability during gas chromatography.

[7] DDT degradation product. DDT not determined in this method.

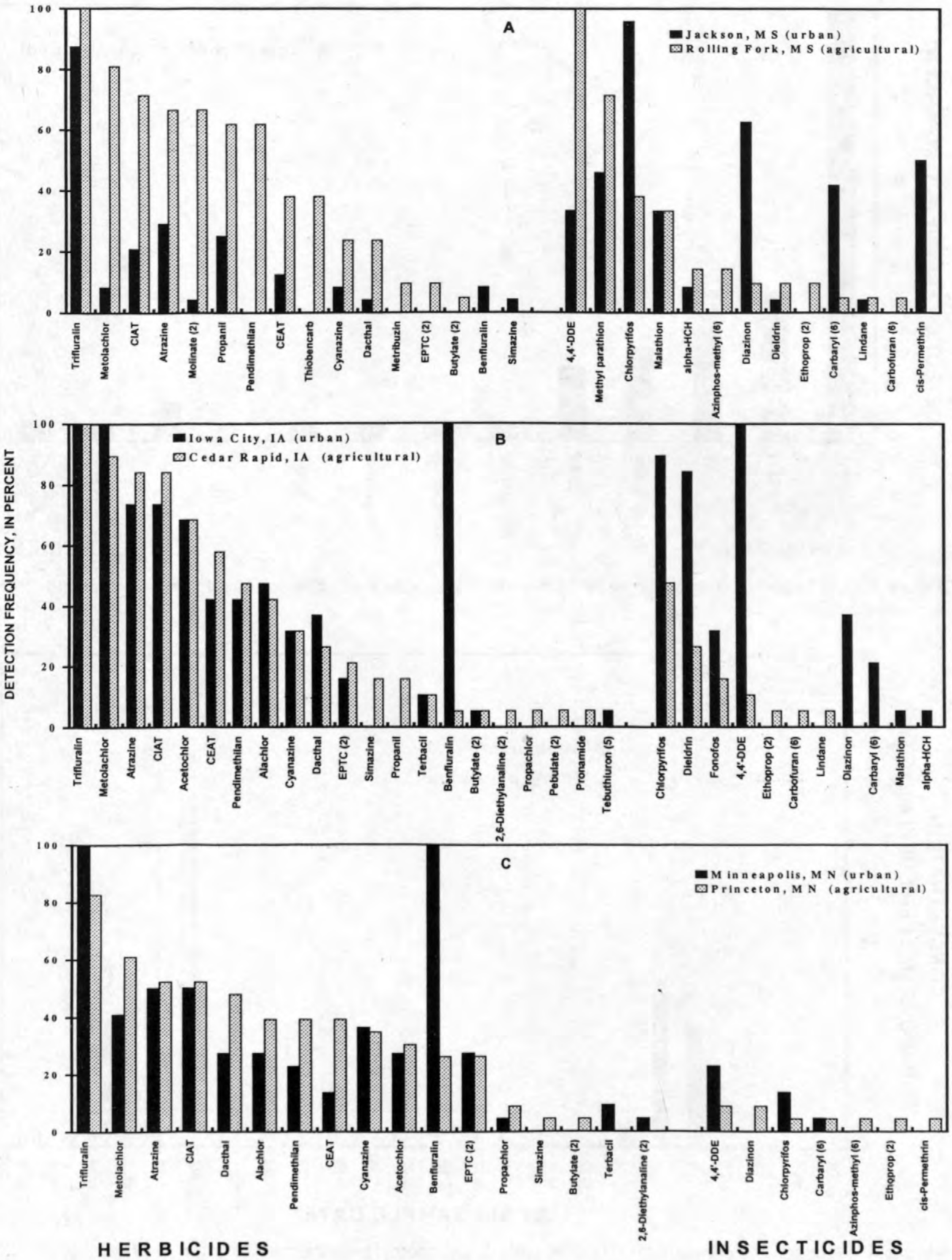

Figure 2. The frequency of detection for herbicides and insecticides in air at the (A) Mississippi, (B) Iowa, and (C) Minnesota sites. Footnotes (2), (5), and (6) are explained in table 1.

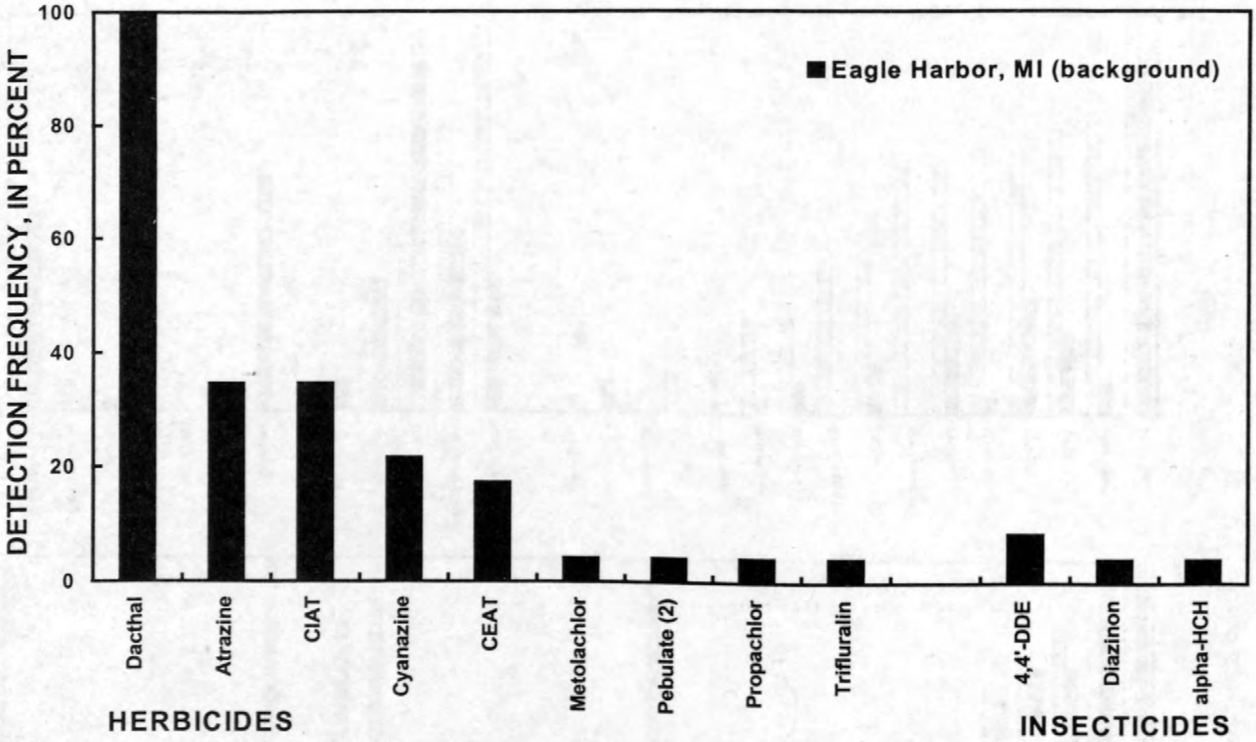

Figure. 3. The frequency of detection for herbicides and insecticides in air at Eagle Harbor, Mich. Footnote (2) explained in table 1.

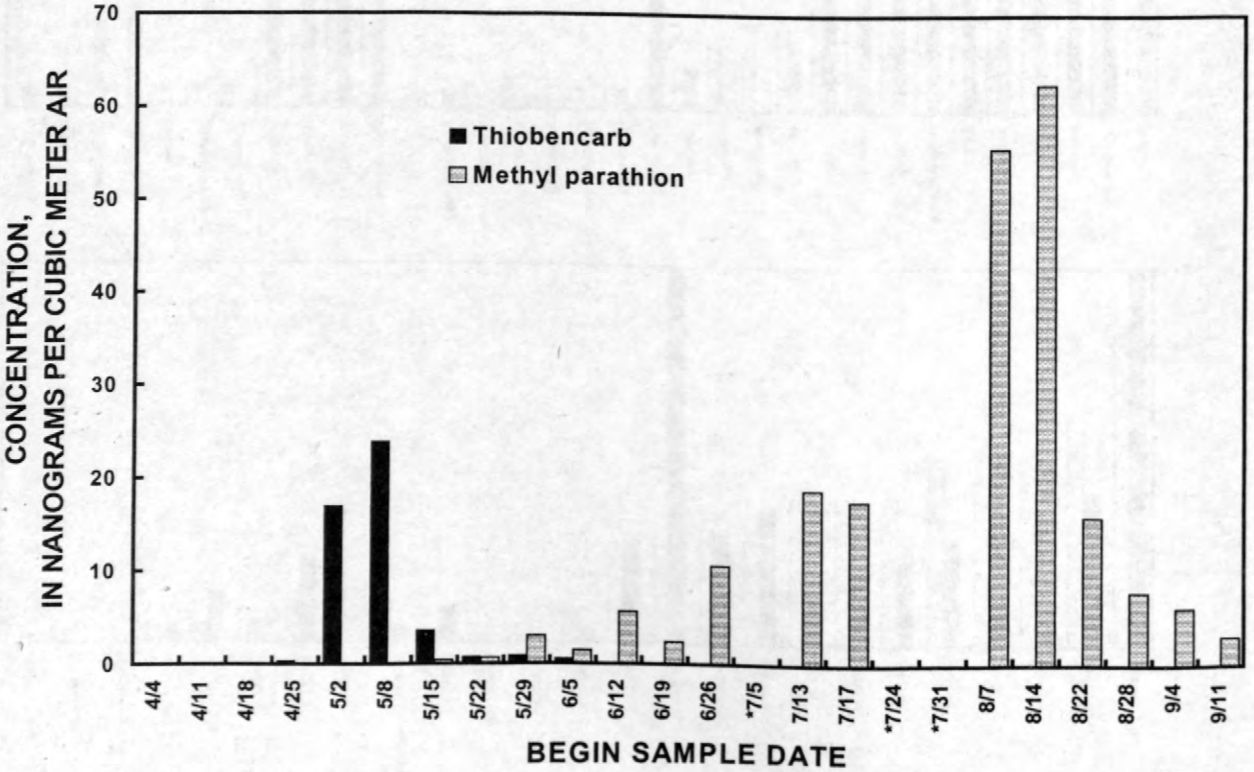

Figure 4. Atmospheric concentration of thiobencarb and methyl parathion at Rolling Fork, Miss., site throughout the study period in 1995. No air sample collected for dates with asterisk.

only one air sample (and no rain samples) from Eagle Harbor (fig. 3). Trifluralin's apparent short atmospheric lifetime also was indicated from its reduced detection frequency in rain samples collected at the other six sites (mean 28 percent; range 5 to 69 percent) (Majewski and others, this volume). These observations are not unexpected in light of the reported rapid gas-phase and aqueous-suspension direct photolysis and photooxidation reaction rates for trifluralin (Leitis and Crosby, 1974; Woodrow and others, 1978; Mongar and Miller, 1988).

Herbicides applied primarily for corn and soybean production were detected often and in nearly equivalent frequencies at agricultural and urban sites in Iowa and Minnesota. Several of these herbicides (for example, atrazine, metolachlor, pendimethalin) also were commonly observed at the agricultural site in Mississippi.

Acetochlor, metolachlor, atrazine, and the triazine degradation product 2-chloro-4-isopropylamino-6-amino-*s*-triazine (CIAT, a degradation product of atrazine and propazine) were detected in more than 68 percent of the air samples at both Iowa sites. These compounds were detected in the gas- and particle-phase in air samples collected near agricultural fields (including the Iowa City site, which is about 2.5 km from the nearest fields). However, at the Jackson, Minneapolis, and Eagle Harbor sites that are more distant from fields (about 10 km or more), these compounds were predominantly or exclusively detected (if observed at all) on the GFF (the operationally defined particle phase). These findings suggest either preferred sorption of the volatilized herbicide to airborne particles during transport or a selective dissipation (by either chemical or physical processes) of the gas-phase component, or both. The presence of atrazine and cyanazine, and possibly dacthal, CIAT, and 2-chloro-4-ethylamino-6-amino-*s*-triazine (CEAT, a degradation product of atrazine, cyanazine, simazine, and terbuthylazine) at Eagle Harbor (fig. 3) suggests their protential for long-range transport. Atrazine's ability to undergo long-range transport also has been reported by Glotfelty and others (1990), Cromwell and Thurman (1996), Goolsby and others (1997), and Rawn and others (1998).

The herbicide benfluralin was detected in 100 percent of the air samples from Iowa City and Minneapolis (fig. 2). This herbicide is applied preemergent to control annual grasses and broadleaf weeds in, for example, alfalfa and various forage crops (Meister, 1997). However, about twice as much benfluralin reportedly is used in noncrop setttings (Gianessi and Puffer, 1991), primarily on established turf, including golf courses and lawns in urban and suburban locations.

The insecticides chlorpyrifos, diazinon, and carbaryl are applied to a wide variety of agricultural crops. In addition, they are used extensively in nonagricultural settings (Gianessi and Puffer, 1991), which likely accounts for the more frequent detections at the urban sites than agricultural sites in Mississippi and Iowa. For example, all three are used in lawn applications, with chlorpyrifos and diazinon commonly applied to control fire ants. Chlorpyrifos and permethrin are used in termite control, which also might contribute to their higher detection frequency in Jackson than in Rolling Fork. Although applied subterranean, pesticides applied for termite control are lost to indoor and outdoor air (Dearth and Hites, 1991).

Methyl parathion was detected in 70 percent of the samples from Rolling Fork, Miss. It existed almost exclusively in the gas phase and exhibited the highest concentration — 62 nanograms per cubic meter of air (ng/m^3) — of any insecticide measured in the study. Methyl parathion is applied extensively in cotton production and represents the highest-use pesticide applied in Mississippi and Louisiana, with about 1 million kilograms applied in each State in 1995 (U.S. Department of Agriculture, 1996). Because it is designed to control insects on the growing cotton plant, methyl parathion is applied later in the growing season relative to typical herbicide applications, with the earliest low-level detections in air (and rain) in mid-May and increasing to a maximum concentration in air in mid-August (fig. 4). By contrast, rice herbicides, another important category of herbicide applied in the Mississippi delta region, are applied earlier in the growing season, and were detected in air from mid-April (0.12 ng/m^3 for 4/18 sample) until early June for thiobencarb (fig. 4) and until mid-July for propanil and molinate. In comparison to thiobencarb, about twice as much molinate and four times as much propanil are used on rice in Mississippi (Coupe and others, 1998a). Molinate and propanil also are suggested more often than thiobencarb for postemergent and midseason applications to rice (Mississippi Cooperative Extension Service, 1995). A summary of the air and rain concentrations of pesticides detected at the Mississippi sites is provided in Coupe and others (1998b).

The primary aerobic degradation product 4,4'-DDE of the insecticide 4,4'-DDT was observed in 100 percent of air samples from Rolling Fork and Iowa City, and was detected in at least two samples at every other sampling site. Neither DDT nor the anaerobic degradation product 4,4'-DDD were included as compounds in the air and rain methods. Detection of DDE was not unexpected, and higher detection frequencies at the other sampling sites likely would have resulted if the more responsive electron-capture detector had been used. DDE concentrations at Rolling Fork ranged from 0.13 to 1.1 ng/m^3 and averaged 0.62 ng/m^3. This average is only about 60 percent less than the average measured range (1.4 to 1.8 ng/m^3) observed in the region 23 years earlier by Kutz and others (1976). This highly persistent DDT degradation product, which has been classified as a global pollutant because of its detection in remote areas of the earth (Bidleman and others, 1989; Iwata and others, 1993), continues to cycle between the soil and air more than two decades after DDT use was banned in 1972 in the United States.

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Routine Determination of Sulfonylurea, Imidazolinone, and Sulfonamide Herbicides at Nanogram-Per-Liter Concentrations by Solid-Phase Extraction and Liquid Chromatography/Mass Spectrometry

By Edward T. Furlong, Mark R. Burkhardt, Paul M. Gates, Stephen L.Werner, and William A. Battaglin

ABSTRACT

Sulfonylurea (SU), imidazolinone (IMI), and sulfonamide (SA) herbicides are new classes of low-application-rate herbicides increasingly used by farmers. Some of these herbicides affect both weed and crop species at low dosages and must be carefully used. Less is known about the effect of these compounds on noncrop plant species, but a concentration of 100 ng/L (nanograms per liter) in water has been proposed as the threshold for possible plant toxicity for most of these herbicides. Hence, analytical methods must be capable of detecting SUs, IMSs, and SAs at concentrations less than 100 ng/L in ambient water samples. The authors developed a two-cartridge, solid-phase extraction method for isolating 12 SU, 3 IMI, and 1 SA herbicides by using high-performance liquid chromatography/ electrospray ionization–mass spectrometry (HPLC/ESI–MS) to identify and quantify these herbicides to 10 ng/L. This method was used to analyze 196 surface- and ground-water samples collected from May to August 1998 throughout the Midwestern United States, and more than 100 quality-assurance and quality-control samples.

During the 16 weeks of the study, the HPLC/ESI-MS maintained excellent calibration linearity across the calibration range of 5 to 500 ng/L, with correlation coefficients of 0.9975 or greater. Continuing calibration verification standards at 100-ng/L concentration were analyzed throughout the study, and the mean measured concentrations for individual herbicides ranged from 93 to 101 ng/L. Mean recovery of herbicides from 27 reagent water samples spiked at 50 and 100 ng/L ranged from 39 to 92 percent and averaged 73 percent. The standard deviation of recoveries ranged from 14 to 26 percent and averaged 20 percent. This variability reflects multiple instruments, operators, and the use of automated and manual sample preparation. Spiked environmental water samples had similar recoveries, although for some herbicides, the sample matrix enhanced recoveries by as much as 200 percent above the spiked concentration. This matrix enhancement was sample and compound dependent. Concentrations of herbicides in unspiked duplicate environmental samples were typically within 25 percent of each other. The results demonstrate the usefulness of HPLC/ESI-MS for determining low-application-rate herbicides at ambient concentrations.

INTRODUCTION

Sulfonylurea (SU), imidazolinone (IMI), and sulfonamide (SA) herbicides are new classes of herbicides that function by inhibiting the action of acetolactate synthase or acetohydroxyacid synthase, key enzymes in the biosynthesis of amino acids in plants. These herbicide classes are applied at rates that are typically much lower than those used for the triazine or acetanilide herbicides that are most commonly used in agriculture. In addition, SUs, IMIs, and SAs are much less toxic to mammals and other animals (Beyer and others, 1987; Meister, 1997). As a result, these herbicides are gaining in popularity among farmers. Crops that are treated with SUs, IMIs, and SAs include barley, corn, cotton, durum wheat, peanuts, rice, soybeans, spring wheat, and winter wheat.

The range in observed toxicity of SUs, IMIs, and SAs to crop and noncrop plants varies by a factor of greater than 1,000. Some of these herbicides are active on crop species at low dosages. Field studies indicate that some yield is lost in the most sensitive crop species when these herbicides are applied or present at between 1/100th and 1/500th of the typical application rate of 0.5 to 0.1 g (gram) active ingredient per ha (hectare)(Fletcher and others, 1993; Obrigawitch and others, 1998). Little is known about the effect of these compounds on noncrop plant species, but a concentration of 100 ng/L in water has been proposed as the concentration below which possible plant toxicity is minimized (Michael Barrett, U.S. Environmental Protection Agency, oral commun., 1997).

It may be difficult to detect SUs, IMIs, and SAs in many hydrologic settings because of their low application rates and low overall use amounts. Detections of SUs, IMIs, and SAs in samples collected from surface and ground water have been reported at nanogram-per-liter concentrations (Bergstrom, 1990; Michael and Neary, 1993). Commonly used analytical techniques might not have adequate sensitivity or selectivity to unambiguously detect and quantify SU, IMI, and SA herbicides, particularly in the presence of coextracted matrix interferences. Most of the SU, IMI, and SA herbicides are highly polar, water soluble, and labile, limiting their analysis by gas chromatography or gas chromatography/mass spectrometry. High-performance liquid chromatography (HPLC) has been successfully used to separate SU, IMI, and SA herbicide mixtures, but standard optical detection methods have lacked sensitivity, particularly in the presence of the ultraviolet-absorbing dissolved organic carbon present in natural-water samples.

Several investigators have used coupled HPLC/electrospray ionization-mass spectrometry (HPLC/ESI-MS) to separate, detect, and quantify SU, IMI, and SA herbicides in soils (D'Ascenzo and others, 1998a; Marek and Koskinen, 1996; Powley and de Bernard, 1998; Stout and others, 1998; Stout and others, 1997), plant materials (Chivanov and others, 1997; Stout and others, 1996a; Stout and others, 1996b), and water (Bossi and others, 1996; D'Ascenzo and others, 1998b; Di Corcia and others, 1997; Koppen and Spliid, 1998; Krynitsky, 1997; Rodriguez and Orescan, 1998). These studies have demonstrated that HPLC/ESI-MS is well suited to the determination of SU, IMI, and SA herbicides because these compounds are efficiently ionized under electrospray conditions. However, these studies were limited in the number of compounds or the types of water samples analyzed. To date (1999), routine application of HPLC/ESI-MS has not been demonstrated, particularly for surface- and ground-water samples collected from a range of hydrologic conditions and water chemistries.

In this paper the authors present an improved two-cartridge, solid-phase extraction (SPE) method for isolation of 12 SU, 3 IMI, and 1 SA herbicides (table 1), using HPLC/ESI-MS for detection, identification, and quantitation. This method was used to determine the concentrations of the 16 SU, IMI, and SA herbicides in about 200 surface-and ground-water samples collected throughout the Midwestern United States. More than 100 quality-assurance and quality-control (QA/QC) samples were concurrently analyzed. By using this data set, the authors demonstrate that SPE and HPLC/ESI-MS can be used to routinely identify and quantify SU, IMI, and SA herbicides at concentrations less than 10 ng/L in water samples of varying chemical composition.

Table 1. Common names, herbicide classes, CAS numbers, and trade names of the sixteen herbicides measured in this study (data from Meister, 1997)
[CAS, Chemical Abstracts Service]

Common name	Herbicide class	CAS number	Trade name[1]
Bensulfuron methyl	sulfonylurea	83055-99-6	Londax
Chlorimuron ethyl	sulfonylurea	90982-32-4	Classic
Chlorsulfuron	sulfonylurea	64902-72-3	Glean, Telar
Flumetsulam	sulfonamide	98967-40-9	Broadstrike, Preside, Scorpion
Halosulfuron methyl	sulfonylurea	100784-20-1	Battalion, Manage, Permit, Sempra
Imazapyr	imidazolinone	81334-34-1	Arsenal, Chopper
Imazaquin	imidazolinone	81335-37-7	Scepter, Detail
Imazethapyr	imidazolinone	81335-77-5	Pursuit
Metsulfuron methyl	sulfonylurea	74223-64-6	Allie, Ally, Escort, Quit, Reform
Nicosulfuron	sulfonylurea	111991-09-4	Accent
Primisulfuron methyl	sulfonylurea	86209-51-0	Beacon, Tell
Prosulfuron	sulfonylurea	94125-34-5	Peak
Sulfometuron methyl	sulfonylurea	74222-97-2	Oust
Thifensulfuron methyl	sulfonylurea	79277-27-3	Pinnacle
Triasulfuron	sulfonylurea	82097-50-5	Amber
Triflusulfuron methyl	sulfonylurea	126535-15-7	Upbeet

[1] Use of brand, firm, and trade names in this article is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey

EXPERIMENTAL

More than 212 surface- and ground-water samples were collected from a range of surface- and ground-water sites throughout the Midwestern United States. Most surface-water sites were sampled twice and ground-water sites were sampled once. The sampling design and trends in SU, IMI, and SA herbicide concentrations, as well as trends for triazine and acetanilide herbicides, are described in a separate paper in this volume (Battaglin and others, 1999).

All samples were filtered through a 0.7-um (micrometer) nominal pore size glass fiber filter and collected in Teflon-lined, screw capped 1-L amber glass bottles. Two 1-L bottles were collected for each sample. The bottles were baked at 425°C (degrees Celsius) for 8 hours prior to use to minimize the potential for organic contamination. After filtration and collection, the samples were shipped on ice by overnight express and kept at 2 to 4°C in the dark until extraction.

Solid-Phase Extraction Preparation

Ultrapure (distilled-in-glass or equivalent) reagent water and solvents were used in the development of the method and in the determination of SU, IMI, and SA herbicides. For each sample extraction, two SPE cartridges were stacked in series. The first cartridge was a 6-mL (milliliter) barrel, 500-mg (milligram) bed strong anion exchange resin cartridge (Jones Chromatography, Inc., Lakewood, Colorado). This cartridge removed much of the dissolved organic carbon (DOC) from the sample; for most environmental samples, the strong anion exchange resin bed was colored with DOC. The IMIs were retained on the strong anion exchange cartridge, while the SUs and SAs were not retained. The second cartridge was a 6-mL barrel, 1-g bed, RP-102 (Applied Separations, Allentown, Pennsylvania) styrene-divinyl benzene polymeric SPE cartridge. The RP-102 cartridge retained the SUs, IMIs, and any SAs that were not retained on the strong anion exchange cartridge. Prior to analysis, the combined cartridge stack was conditioned by passing 60 mL of 1 percent acetic acid in acetone and 40 mL of 1 percent acetic acid in water through the cartridges. The stack was not allowed to dry after conditioning.

Sample Extraction

The pH of the samples was measured prior to sample extraction, and the pH was adjusted to 3 or less by addition of acetic acid. The volume of acetic acid used ranged from 10 to 18 mL. The samples were then extracted using a Zymark AutoTrace SPE Workstation (Zymark Corporation, Hopkinton, Massachusetts). The sample extraction flowrate through the stacked SPE cartridges was 10 mL/min (milliliters per minute). The stack was then dried in-line with nitrogen for 1 minute to remove excess water. A 1.2-mL aliquot of methanol was then passed through each stack to remove interstitial water trapped in the SPE material. The stack was then dried in-line with nitrogen for 45 minutes to remove as much residual water as possible. Three 4-mL aliquots of 1 percent acetic acid in acetone were used to elute the compounds from the stacked SPE cartridges. The flowrate for elution was 3 mL/min. The 12-mL final volume was collected in graduated polyethylene test tubes.

Sample Concentration

The eluted sample extracts were placed in a Zymark TurboEvap (Zymark Corporation, Hopkinton, Massachusetts) solvent reduction workstation for solvent removal. The nitrogen gas pressure was kept at 10 pounds per square inch and the water bath temperature maintained at 38°C. Sample reduction to just dryness took about 60 minutes because all residual water was not removed in the previous drying steps. A 1-mL aliquot of acetonitrile was then added to each sample extract. The sample extract was dried again. An additional 1-mL aliquot of acetonitrile was again added and the sample dried for a third time. These two drying steps were used to ensure the removal of acetic acid. The sample extracts were then reconstituted with 100 µL (microliters) acetonitrile, followed by 900 µL of water in the polyethylene test tubes. The sample extracts were then filtered through a 0.2-µm Teflon membrane syringe filter to remove any particulate material prior to analysis. The filtered extracts were put into amber, graduated 2-mL sample vials (Hewlett Packard, Palo Alto, California). The sample vials were capped and kept at 2 to 4°C until analysis.

Sample Analysis

The sample extracts were placed in the autosampler of a Hewlett Packard Series 1100 HPLC/MS system (Hewlett Packard, Palo Alto, California). The HPLC and MS operational characteristics are listed in table 2. A Metasil Basic 3-µm pore size 2x150 mm (millimeter) analytical HPLC column and a Metasil Basic 3-µm pore size 2x10 mm (Metachem Technologies, Inc, Torrance, California) guard column were used for HPLC separation. Acetonitrile and a 10-mM (millimolar) ammonium formate/formic acid buffer, pH 3.7 in water, were used as the two components to the HPLC gradient. The solvent compositions used for the HPLC gradient in this analysis are listed in table 3.

Full-scan electrospray ionization mass spectra were collected for each compound using a Hewlett Packard Series 1100 LC/MSD. The instrument was tuned for positive ions using horse skeletal muscle myoglobin. Ionization conditions were optimized so that at least three ions were formed for each herbicide. The relative abundances of the three ions were used to confirm identification, and the sum of the three ion signals was used for maximum detectability and quantitation. The selected ions monitored for each compound and the fragmentor voltages required to generate those ions are listed in table 4.

SU, IMI, and SA detections were verified in two ways. First, the chromatographic retention time observed for a peak in a sample chromatogram was compared to the retention of the compound observed in a standard chromatogram (fig.1). The qualitative identification was then confirmed by comparing the relative abundances of the three ions measured in the sample peak to known relative abundances of a library reference spectrum

Table 2. High-performance liquid chromatography (HPLC) and mass spectrometry (MS) operating conditions for the combined analysis of sulfonylurea, imidizolinone, and sulfonamide herbicides

HPLC Operating Conditions	
Flowrate	0.2 milliliter per minute
Autosampler temperature	8 degrees Celsius
Analysis time	45 minutes
Column temperature	30 degrees Celsius
Injection volume	50 microliters

MS Operating Conditions	
Ionization mode	electrospray
Polarity	positive
Electron multiplier voltage	2,748
Sheath (drying) gas volume	10 liters per minute
Nebulizer pressure	55 pounds per square inch
Gas temperature	325 degrees Celsius
Spray chamber capillary voltage	4,000 volts

Table 3. High-performance liquid chromatography binary gradient program for the combined analysis of sulfonylurea, imidizolinone, and sulfonamide herbicides

Time, in minutes	Acetonitrile, in percent	Aqueous buffer, in percent
0.50	20	80
1.75	35	65
10.0	40	60
13.0	50	50
20.0	55	45
23.0	90	10
27.0	90	10
30.0	20	80
45.0	20	80

Table 4. Selected-ion monitoring (SIM) program for the combined analysis of sulfonylurea, imidizolinone, and sulfonamide herbicides

Compound	Ions monitored	Fragmentor voltage for SIM analysis
Imazapyr	217, 234, 262	95
Imazethapyr	177, 245, 290	115
Flumetsulam	129, 262, 326	95
Nicosulfuron	182, 213, 411	95
Imazaquin	199, 267, 312	95
Thifensulfuron methyl	167, 270, 388	80
Metsulfuron methyl	167, 264, 382	80
Chlorsulfuron	141, 167, 358	85
Sulfometuron methyl	150, 199, 365	85
Triasulfuron methyl	141, 167, 402	85
Bensulfuron methyl	149, 182, 411	90
Halosulfuron methyl	182, 403, 435	85
Prosulfuron	141, 167, 420	85
Chlorimuron ethyl	186, 213, 415	85
Triflusulfuron methyl	264, 461, 493	90
Primisulfuron methyl	199, 254, 469	90

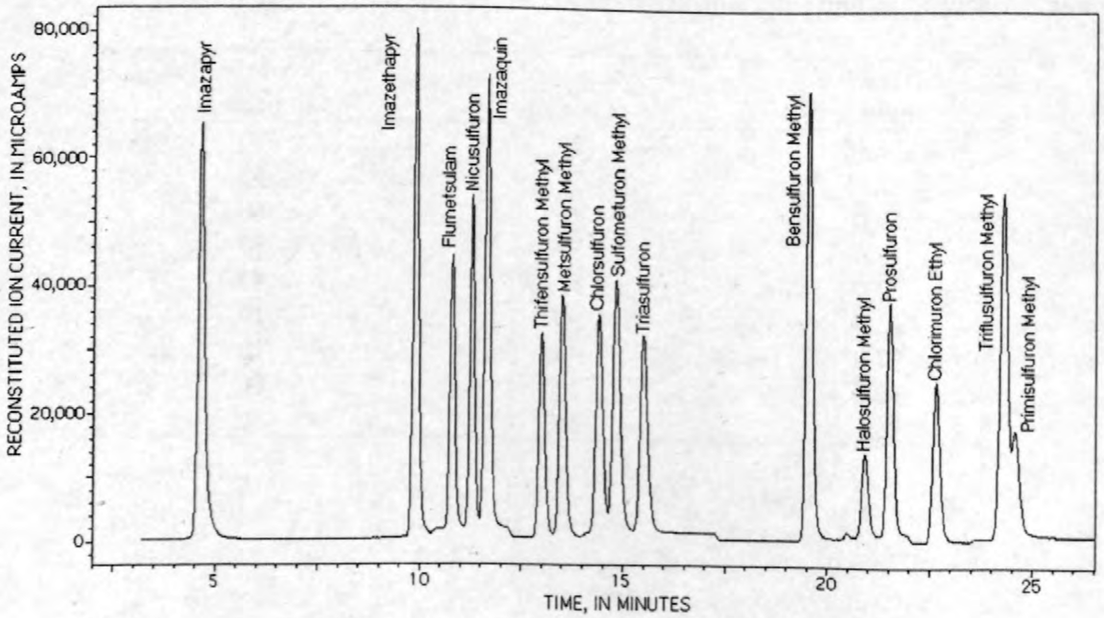

Figure 1. Chromatogram of a 100-ng/L (nanogram-per-liter; equivalent to 5 ng on-column) 16 component sulfonylurea-imidazolinone-sulfonamide standard using high-performance liquid chromatography/electrospray ionization–mass spectrometry with selected-ion monitoring.

produced from a pure standard. This comparison, however, might not be conclusive if unknown compounds interfere with the sample peak, either partially or completely. If comparison of the unknown peak spectrum with the library reference spectra yields dissimilar spectral curves, then the presence of the compound was notconfirmed, and so it was not identified. If a chromatographically resolvable peak was identified, with all three ions present, but one ion's relative abundance varied by more than 20 percent from the standard, then the compound concentration was qualified as an estimate. All detections less than 10 ng/L were qualified as estimates.

Concentrations were quantified by comparing the sum of the three integrated peak areas from an environmental sample to the sum of integrated peak areas from a calibration line. A five-point external calibration curve for a concentration range of 5 to 500 ng/L was produced for each compound. Calibration lines were linear and had correlation coefficients of 0.9975 or greater.

Samples were extracted and isolated in sets of twelve: ten environmental samples, a method blank, and a method spike in which a blank water sample was amended with all 16 compounds to make a concentration of 50 or 100 ng/L per compound. In addition, duplicate environmental samples were analyzed for quality-control purposes. Thirty-four duplicate samples were amended with a solution of all 16 compounds to 50 or 100 ng/L. These matrix spikes were analyzed in the same set as the unspiked environmental sample. Thirty-two samples were analyzed in duplicate without amendment. These replicates were analyzed within and between sets to estimate method variability over time.

A set of five different ca produced from a pure standard. This comparison, however, might not be conclusive if libration standards was analyzed with each set of processed samples. In addition, continuing calibration verification samples and instrumental blank samples were interspersed between the environmental samples and after the calibration standards to verify that the instrument remained within calibration limits, and that cross- contamination between injections did not occur.

RESULTS AND DISCUSSION

Recoveries for this method are shown in figure 2. Reagent water and South Platte surface-water samples were spiked at 100 ng/L and analyzed in quadruplicate. Recoveries ranged from 56 to 86 percent in reagent water, averaging 76 percent. The variation in recovery for all 16 compounds averaged 6 percent. Recoveries are more variable in surface water, ranging from 46 to 163 percent and averaging 93 percent. The variation in recovery for all 16 compounds averages 7 percent. Enhanced recoveries were consistently observed for South Platte surface water (and also observed in ground water), particularly for thifensulfuron methyl, metsulfuron methyl, triasulfuron methyl, and bensulfuron methyl. The observed matrix-enhanced recoveries ranged as high as 300 percent for a of water-soluble salts and DOC.sample fortified at 100 µ/L. After minor modification of the method by introduction of a solvent-exchange step, the mean matrix enhancement was less than 200 percent, likely the result of improved removal of water-soluble salts and DOC.

The samples were analyzed in 27 separate sets, and instrumental and method QA/QC samples were analyzed as part of each set. Table 5 lists data for long-term calibration stability, as indicated by the performance of 75 continuing calibration verification samples analyzed over the course of the study. The continuing calibration verification sample-equivalent concentration for each compound was set at 100 ng/L. The mean measured concentration for individual SU, IMI, and SA herbicides ranged from 93 to 101 ng/L. The mean standard deviation for all SU, IMI, and SA herbicides measured in continuing calibration verification samples throughout the study was 6 ng/L. These data, along with linear calibration

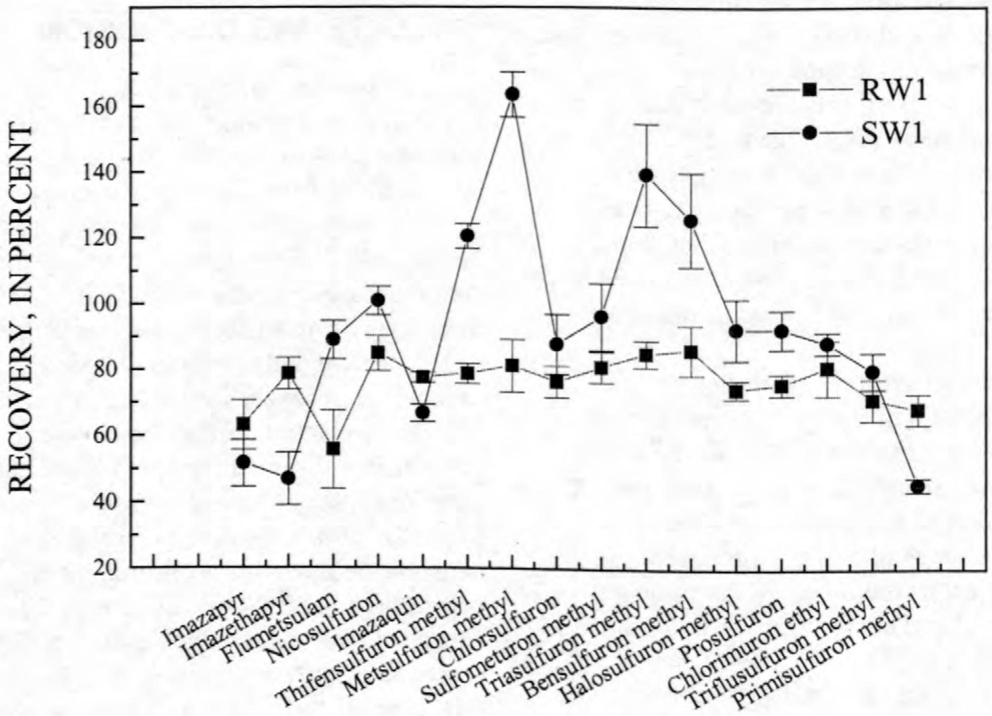

Figure 2. Recoveries of 16 herbicides from reagent water (RW1) and South Platte River water (SW1) fortified at 100ng/L (nanograms per liter).

coefficients of 0.9975 or greater, indicate that instrument performance was highly stable throughout the course of the study.

Long-term method performance is indicated by the recovery calculated from the results of 27 set spikes, one analyzed with each set during the study. The recoveries for all individual SU, IMI, and SA herbicides are shown in figure 3. Mean recoveries for all 16 herbicides from 27 reagent water set spike samples, fortified at 50 and 100 ng/L, ranged from 39 to 92 percent and averaged 73 percent. The standard deviation of recoveries ranged from 14 to 26 percent and averaged 20 percent. This variability reflects multiple instruments, operators, and the use of automated and manual sample preparation. Chlorsulfuron, halosulfuron methyl, prosulfuron, triflusulfuron methyl, and primisulfuron methyl had the lowest and most variable recoveries.

Table 5. Continuing calibration performance during the course of the study. The concentration statistics were calculated from determinations of 75 continuing calibration verification samples
[All concentrations in nanograms per liter]

Compound	Compound mean	Compound median	Compound standard deviation	Minimum concentration	Maximum concentration
Imazapyr	100	100	6.6	85	128
Imazethapyr	100	101	6.4	84	117
Flumetsulam	96	96	5.2	84	114
Nicosulfuron	95	96	4.3	83	111
Imazaquin	101	101	6.6	85	126
Thifensulfuron methyl	93	93	7.0	60	120
Metsulfuron methyl	94	94	5.8	81	117
Chlorsulfuron	95	95	6.3	80	122
Sulfometuron methyl	96	96	5.9	80	113
Triasulfuron methyl	93	93	5.1	79	108
Bensulfuron methyl	93	94	5.3	78	106
Halosulfuron methyl	94	94	6.9	81	122
Prosulfuron	94	95	6.6	71	121
Chlorimuron ethyl	94	94	5.4	79	110
Triflusulfuron methyl	93	94	6.2	76	114
Primisulfuron methyl	96	96	6.6	81	118
Set mean concentration	95	96	6.0	79	117
Set median concentration	95	95	6.3	80	117
Set standard deviation	2.7	2.7	.8	6.2	6.3
Minimum concentration	93	93	4.3	60	106
Maximum concentration	101	101	7.0	85	128

Thirty-one unspiked duplicate samples were analyzed to estimate method precision at ambient herbicide concentrations and in the presence of interferences. These duplicates were analyzed within a single sample set (intraset replicates) or in two separate sets (interset) replicates. Intra- and interset replicates were combined for data analysis. Box plots of the distributions of relative percentage differences calculated for the five herbicides detected in unspiked replicates more than three times are shown in figure 4. Except for chlorimuron ethyl, concentrations of herbicides in unspiked duplicate environmental samples were typically within 25 percent of each other.

In addition to unspiked replicates, 28 randomly selected sample duplicates were spiked at 100 ng/L and analyzed. This spike determined the magnitude of possible matrix enhancement during the study. These results are shown in figure 5.

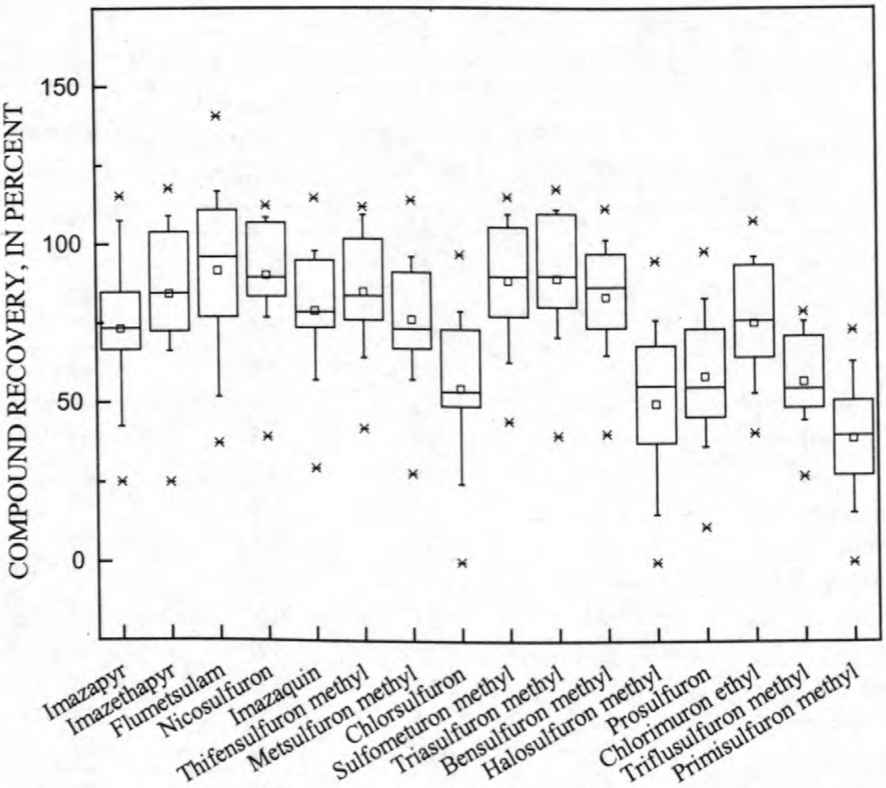

Figure 3. Box plots of recoveries of sulfonylurea, imidazolinone, and sulfonamide herbicides from 27 reagent water samples processed and analyzed from September 1998 to January 1999. Reagent water samples were spiked at 50 or 100 ng/L (nanograms per liter). In this plot, hinges indicate median recoveries, gates indicate 25th and 75th percentiles, and whiskers indicate 5th and 95th percentiles. The small ▫ indicates the compound mean recovery, and the small x indicates outlier recoveries.

The box plots (fig. 5) indicate that there was measurable matrix enhancement of recovery. This enhancement was variable and seemed to affect most of the herbicides measured. However, the results suggest that matrix enhancement would result in overestimation by no more than a factor of two, and more likely compound recoveries would be enhanced by a factor of 1.5 or less. Given other aspects of analysis and sample collection variability, and the typically low concentrations measured in environmental samples, this level of matrix enhancement is unlikely to alter the interpretation of surface- or ground-water data.

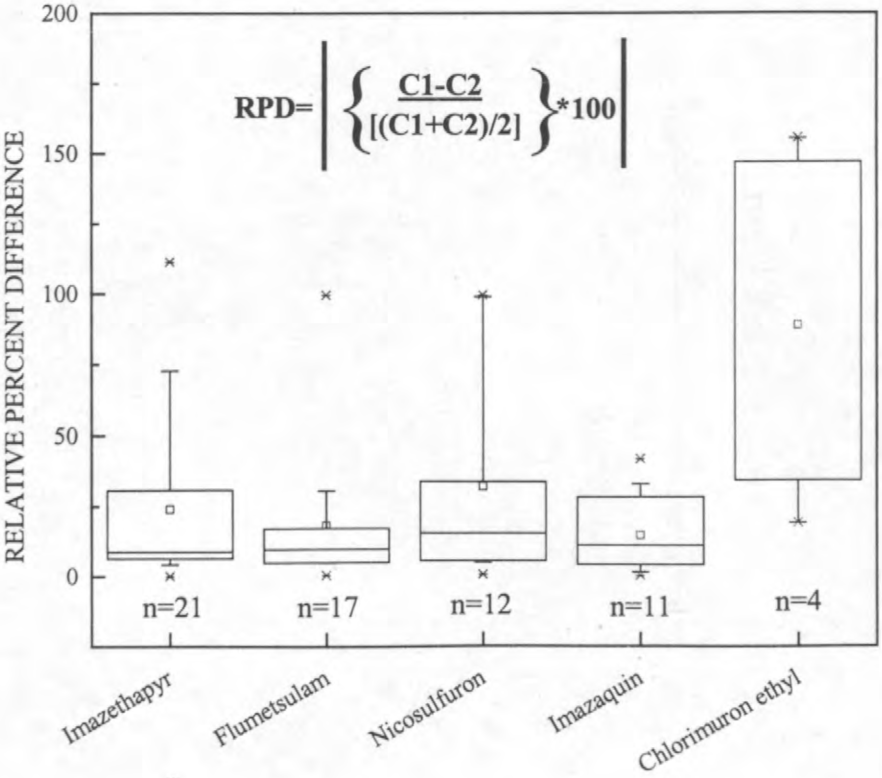

Figure 4. Relative percent differences (RPD) between unspiked duplicate natural-water samples. In this plot, hinges indicate median recoveries, gates indicate 25th and 75th percentiles, and whiskers indicate 5th and 95th percentiles. The small ▫ indicates the compound mean recovery, and the small x indicates outlier recoveries. Thirty-one total samples were analyzed (n=number of samples with detections in both duplicates; C1 and C2 refer to the concentrations of the first and second duplicate, respectively).

A summary of field results from the analysis of 196 surface- and ground-water samples is presented in Battaglin and others (1999). The selected herbicides were detected at concentrations greater than the reporting limit in 83 percent of stream samples. In surface-water samples, the three most frequently detected herbicides were imazethapyr (71 percent), flumetsulam (63 percent), and nicosulfuron (52 percent). Average concentrations of these five herbicides ranged from 11 to 92 ng/L. Six other herbicides were measured in 2 to 24 percent of samples. The data suggest that several of the sixteen selected compounds are commonly detected in surface-water samples of the Midwestern United States, and that the average concentrations are less than the 100-ng/L level of concern. Battaglin and others (1999) provide a detailed statistical analysis of the field results.

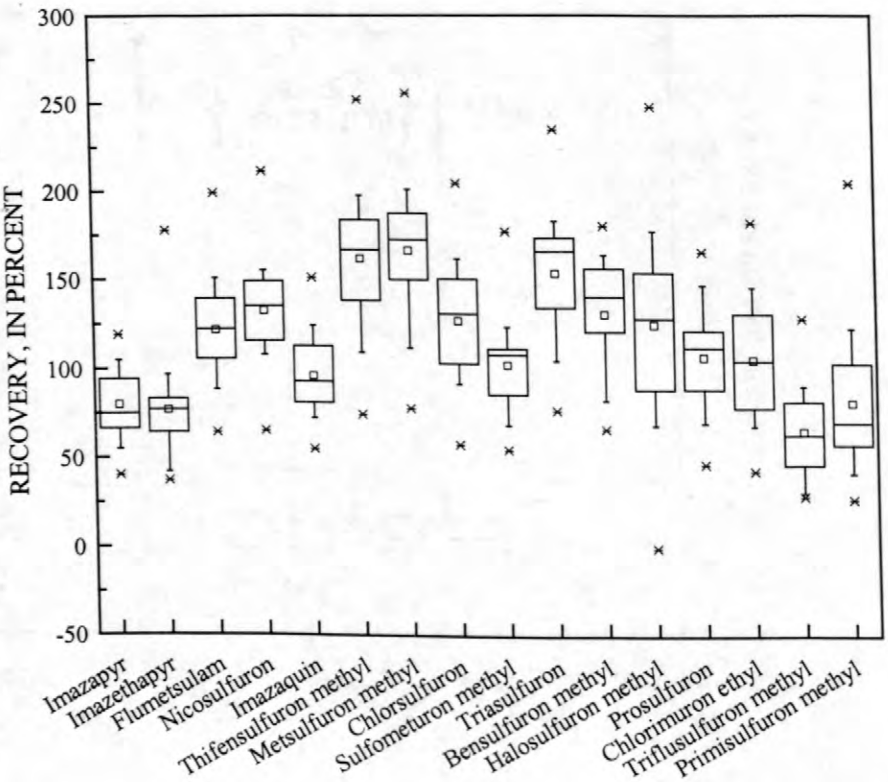

Figure 5. Recovery of herbicides added to duplicate surface- and ground-water samples at a concentration of 100 ng/L (nanograms per liter). Recoveries corrected for ambient water herbicide concentrations. In this plot, hinges indicate median recoveries, gates indicate 25th and 75th percentiles, and whiskers indicate 5th and 95th percentiles. The small ▫ indicates the compound mean recovery, and the small x indicates outlier recoveries.

In this study, the authors have demonstrated the usefulness of HPLC/MS for the routine determination of SU, IMI, and SA herbicides by using a tandem SPE isolation coupled to HPLC/ESI–MS. The analytical technologies used in this method are critical to adequate assessment of the presence and distribution of low-application-rate herbicide residues in surface and ground water. The long-term QA/QC data developed as part of this study show that HPLC/ESI–MS is a robust technique that can be used for the routine determination of herbicides at concentrations at or less than 10 ng/L. Extensive QA/QC data collection and interpretation are required to verify the performance of this method and to assess the magnitude of sample matrix enhancement of concentrations.

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Herbicides and Herbicide Degradates in Shallow Ground Water and the Cedar River near a Municipal Well Field, Cedar Rapids, Iowa

By Robert A. Boyd

ABSTRACT

Water samples were collected near a Cedar Rapids, Iowa municipal well field from June 1998 to August 1998 and analyzed for selected triazine and acetanilide herbicides and degradates. The purpose of the study was to evaluate the occurrence of herbicides and herbicide degradates following springtime application of herbicides to upstream cropland. The well field is in an alluvial aquifer adjacent to the Cedar River. Parent herbicide concentrations generally were greatest in June and decreased in July and August. Atrazine was most frequently detected and occurred at the greatest concentrations; acetochlor, cyanazine, and metolachlor also were detected, but at lesser concentrations than atrazine. Triazine degradate concentrations were relatively small (<0.50 micrograms per liter) and generally decreased from June to August. Although the rate of ground-water movement is relatively fast (about 1 meter per day) in the alluvial aquifer near the Cedar River, deethylatrazine (DEA) to atrazine ratios in ground-water samples collected near the Cedar River indicate that atrazine and DEA probably are gradually transported into the alluvial aquifer from the Cedar River. Deisopropylatrazine (DIA) to DEA ratios in water samples indicate most DIA in the Cedar River and alluvial aquifer is produced by atrazine degradation, although some could be from cyanazine degradation. Acetanilide degradates were detected more frequently and at greater concentrations than their corresponding parent herbicides. Ethanesulfonic-acid (ESA) degradates comprised at least 80 percent of the total acetanilide-degradate concentrations in samples collected from the Cedar River and alluvial aquifer in June, July, and August; oxanilic-acid degradates comprised less than 20 percent of the total concentrations. ESA-degradate concentrations generally were smallest in June and greater in July and August. Acetanilide-degradate concentrations in ground water adjacent to the Cedar River indicate acetanilide degradates are transported into the alluvial aquifer in a manner similar to that indicated for atrazine and DEA.

INTRODUCTION

Agriculture in the United States is most intense in the Midwest, where corn and soybeans are major row crops. Synthetic organic herbicides are applied to cropland to control a variety of broadleaf weeds and annual grasses. About two-thirds of the estimated 218 million kilograms of herbicides used for agriculture in the United States is applied to cropland in the Midwest (Barbash and Resek, 1996).

Triazine and acetanilide compounds are commonly used pre-emergent herbicides. Larson and others (1997) indicate that triazine and acetanilide compounds account for about one-half of the total herbicides used annually for agriculture in the United States. Triazine compounds, such as atrazine and cyanazine, typically are applied to corn. Acetanilide compounds, such as acetochlor, alachlor, and metolachlor, typically are applied to corn and soybeans (Larson and others, 1997).

Triazine and acetanilide herbicides are moderately to highly soluble in water and have relatively low soil-sorption coefficients. Because of these properties, herbicides applied to cropland can be transported to shallow ground water by infiltration or to surface waters by storm-water runoff. Goolsby and Battaglin (1993) indicated that a relatively small fraction of the total amount of herbicides applied to cropland are transported to streams (less than 4 percent of triazines and less than 2 percent of acetanilides). Herbicides that

Table 1. Targeted constituents for chemical analyses of water samples collected from the alluvial aquifer and Cedar River, Cedar Rapids, Iowa, June-August, 1998.

[ESA, ethanesulfonic acid; OA, oxanilic acid]

Constiuent	Chemical name
	Parent compounds
Acetochlor	2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide
Alachlor	2-chloro-2'-6'-diethyl-N-(methoxymethyl)-acetanilide
Ametryn	2-(ethylamino)-4-isopropylamino-6-methyl-thio-s-triazine
Atrazine	2-chloro-4-ethylamino-6-isopropylamino-s-triazine
Cyanazine	2-[[4-chloro-6-(ethylamino)-1,3,5-triazin-2-yl]-amino]-2-methylpropionitrile
Metolachlor	2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide
Metribuzin	4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one
Prometon	2,4-bis(isopropylamino)-6-methoxy-s-triazine
Prometryn	2,4-bis(isopropylamino)-6-methylthio)-s-triazine
Propachlor	2-chloro-N-isopropylacetanilide
Propazine	2-chloro-4,6-bis(isopropylamino)-s-triazine
Simazine	2-chloro-4,6-bis(ethylamino)-s-triazine
Terbutryn	2-tert-butylamino-4-ethylamino-6-methylthio-s-triazine
	Degradate compounds
Acetochlor ESA	2-[(2-ethyl-6-methylphenyl)(ethoxymethyl)-amino]-2-oxoethanesulfonic acid
Acetochlor OA	2-[(2-ethyl-6-methylphenyl)(ethoxymethyl)-amino]-2-oxoacetic acid
Alachlor ESA	2-[(2,6-diethylphenyl)(methoxymethyl)amino]-2-oxoethanesulfonic acid
Alachlor OA	2-[(2,6-diethylphenyl)(methoxymethyl)amino]-2-oxoacetic acid
Cyanazine-amide	2-chloro-4-(1-carbamoyl-1-methyl-ethylamino)-6-ethylamino-s-triazine
Deethylatrazine	2-amino-4-chloro-6-(isopropylamino)-s-triazine
Deisopropylatrazine	2-amino-4-chloro-6-(ethylamino)-s-triazine
Hydroxy-atrazine	2-hydroxy-4-(ethylamino)-6-(isopropylamino)-s-triazine
Metolachlor ESA	2-[(2-ethyl-6-methylphenyl)(2-methoxy-1-methylethyl)amino]-2-oxoethanesulfonic acid
Metolachlor OA	2-[(2-ethyl-6-methylphenyl)(2-methoxy-1-methylethyl)amino]-2-oxoacetic acid

runoff to streams present a concern because many municpal-water supplies in the Midwest are obtained from surface water or from shallow aquifers in which infiltration from surface waters can affect ground-water quality. Herbicide compounds have been associated with a variety of human-health and environmental problems including cancer, genetic diseases, reproductive disorders, and deformities. The U.S. Environmental Protection Agency (1996) has established a Maximum Contaminant Level in public drinking-water supplies for alachlor (2 micrograms per liter [µg/L], atrazine (3 µg/L), and simazine (4 µg/L).

A number of compounds that are intermediate degradation products (degradates) have been isolated and detected in the environment. Triazine and acetanilide degradates primarily are produced by biological processes, but chemical reactions such as hydrolysis, oxidation and reduction, and photolysis also can produce degradates. Larson and others (1997) indicated that acetanilides generally degrade faster than triazines. Degradates typically are more mobile in the environment than parent compounds. Recent studies, such as those by Thurman and others (1996), Kolpin and others (1996) and Kalkhoff and others (1998), have detected degradates in surface-water and ground-water samples at greater frequency and concentrations than the parent compounds. Data are lacking on toxicity and long-term human health and environmental effects associated with degradate compounds.

The City of Cedar Rapids, Iowa obtains its municipal water supply from four well fields along the Cedar River. The wells are completed in the Cedar River alluvium, an alluvial aquifer adjacent to the Cedar River. The City of Cedar Rapids and the U.S. Geological Survey (USGS) are conducting a cooperative study of the ground-water-flow system and water-quality near the well fields. The purpose of this paper is to evaluate the occurrence of herbicides and herbicide degradates in the alluvial aquifer near the City's Seminole Well Field following springtime application of herbicides to upstream cropland. Analytical results from water samples collected from June 1998 to August 1998 were used in the study.

Description of Study Area

Cedar Rapids is in east-central Iowa (fig. 1) and has a population of about 110,000. The city is located along a flood plain of the Cedar River; the flood plain ranges in width from about 350 to 1,000 meters (m) near the study area. Upland topography is characterized by rolling hills of low relief. The upland area near the well field is used for rural housing. Land use surrounding the well field consists of a municipal park and riparian wetlands along the Cedar River. Upstream land use in the Cedar River basin is over 80 percent agricultural.

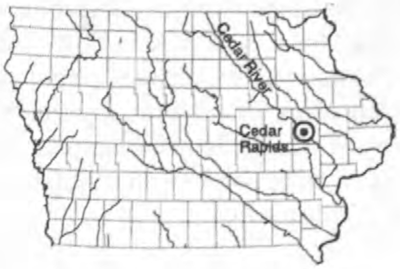

Figure 1. Location of Cedar Rapids, Iowa

Corn and soybeans are the major crops. Livestock raised in the area include beef and dairy cattle, hogs, and sheep. Iowa has a typical continental, subhumid climate, with relatively large seasonal variations in temperature (typically ranging between 40°C in summer to -30°C in winter). The average annual precipitation is about 84 centimeters (cm), with the greatest amounts occurring from May to August (Squillace and others, 1996).

Hydrogeology

Geologic units near the well field consist of an unconsolidated surficial layer of glacial till, loess, and the Cedar River alluvium (alluvial aquifer), underlain by carbonate bedrock of Devonian and Silurian age (bedrock aquifer). The glacial till and loess form upland areas that bound the alluvial aquifer. The alluvial aquifer ranges in thickness from about 2 to 30 m near the well field. The alluvial aquifer consists of a sequence of coarse sand and gravel at the base, grading upward to fine sand, silt, and clay near the surface. The sand and gravel consist of carbonate, shale, and ferro-magnesium-rich rock fragments. The bedrock aquifer has a maximum thickness of about 210 m near the well field. The bedrock aquifer primarily consists of limestone and dolomite, but some inter-bedded shale and chert also occur. Joints and fractures are common in the bedrock aquifer (Hansen, 1970, Wahl and Bunker, 1986).

The alluvial aquifer is recharged by infiltration from the Cedar River induced by pumping of municipal wells, infiltration of precipitation, and flow from adjacent hydrogeologic units. In areas influenced by the pumping of municipal wells, the water-table gradient is from the Cedar River to the well field; in areas outside the influence of municipal pumping, the water-table gradient generally is toward the Cedar River (Hansen, 1970, Wahl and Bunker, 1986). Results from a regional ground-water-flow model constructed by Schulmeyer and Schnoebelen (1998) indicated that about 74 percent of water pumped from the alluvial aquifer is from the Cedar River, about 21 percent of the water is from adjacent and underlying hydrogeologic units, and about 5 percent of the water is from infiltrating precipitation. The bedrock aquifer is recharged by downward leakage from the alluvial aquifer and by infiltrating precipitation on outcrop areas distant from the study area. The bedrock aquifer is not used as a municipal water supply but is used for private residential and industrial water supplies.

Previous Studies

Herbicides have been most frequently detected in samples collected from the Cedar River and alluvial aquifer between mid-spring and mid-summer (Schulmeyer and Schnoebelen, 1998, Squillace and others, 1996). Atrazine is most frequently detected, but other herbicides such as acetochlor, alachlor, cyanazine, and metolachlor

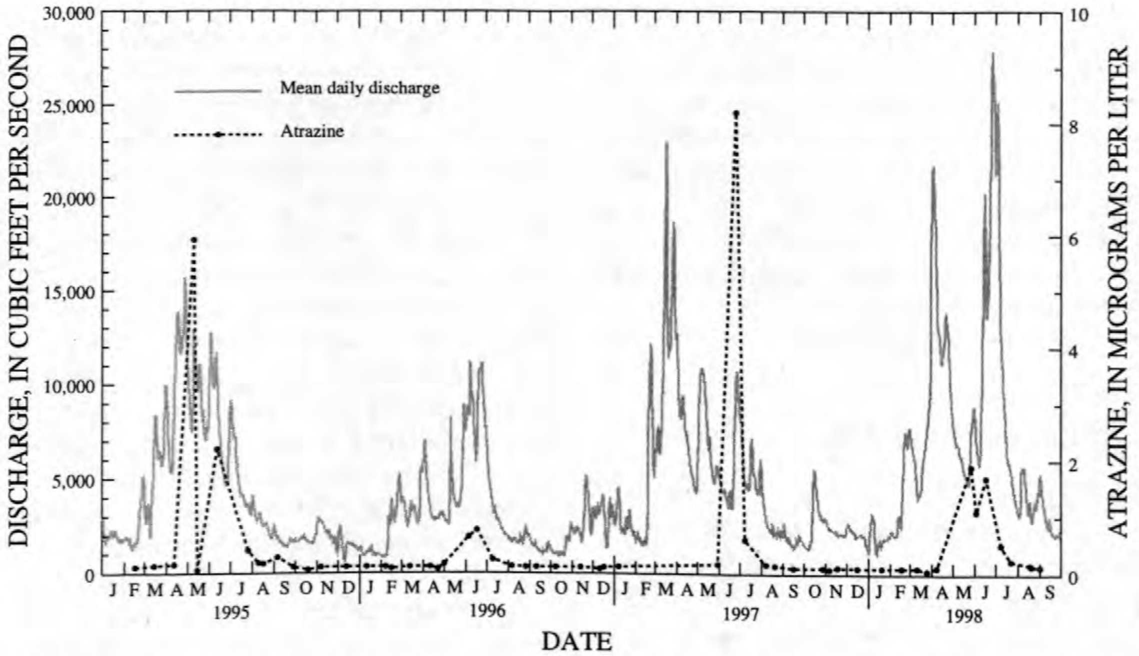

Figure 2. Mean daily discharge and atrazine concentrations in the Cedar River near Cedar Rapids, Iowa, 1995-1998.

also have been detected. Atrazine typically is detected at greater concentrations than the other herbicides. Figure 2 shows atrazine concentrations in the Cedar River near Cedar Rapids from 1995 to 1998 (Schnoebelen and Schulmeyer, 1997, Tom Noth, City of Cedar Rapids Water Department, written commun., July 1998, and unpublished data maintained by the USGS). The seasonal increase in atrazine concentrations during the spring and summer corresponds to the timing of herbicide applications to cropland and the amount of surface runoff resulting from rain storms. The same compounds detected in the Cedar River have been detected in samples collected from the alluvial aquifer, but typically at smaller concentrations than the samples collected from the Cedar River (Schnoebelen and Schulmeyer, 1997 and unpublished data maintained by the USGS).

Recharge from precipitation is a potential source for herbicides in the alluvial aquifer. Goolsby and others (1997) reported that triazine and acetanilide herbicides are frequently detected (with maximum concentrations detected from May to July) at relatively small concentrations in rainfall samples collected from National Atmospheric Deposition Program/National Trends Network sites. Ninety-nine percent of atrazine, alachlor, metolachlor, and cyanazine concentrations were less than or equal to 1 µg/L. Herbicides detected in the alluvial aquifer near the well field are most likely from the Cedar River because most of the water pumped from the alluvial aquifer is from induced infiltration from the river (Schulmeyer and Schnoebelen, 1998). In addition, herbicide concentrations in recharge from precipitation likely would be reduced by sorption onto surface soils and uptake by vegetation.

METHODS

Water samples were collected from 5.1-cm outer-diameter observation wells and 0.36-cm outer-diameter observation wells (see figure 3 for locations). The 5.1-cm diameter wells were installed using hollow-stem auger drilling techniques, completed with polyvinyl-chloride casing and slotted screen, and screened across a 76-cm interval above the bottom of the well. The 5.1-cm diameter wells at CRM-16, CRM-18, and CRM-19 were completed to a depth of about 13 m; the 5.1-cm diameter wells at CRM-15 and CRM-17 were completed to a depth of about 6 m. The 0.36-cm diameter wells were installed with a portable hammer-drill by advancing a stainless-

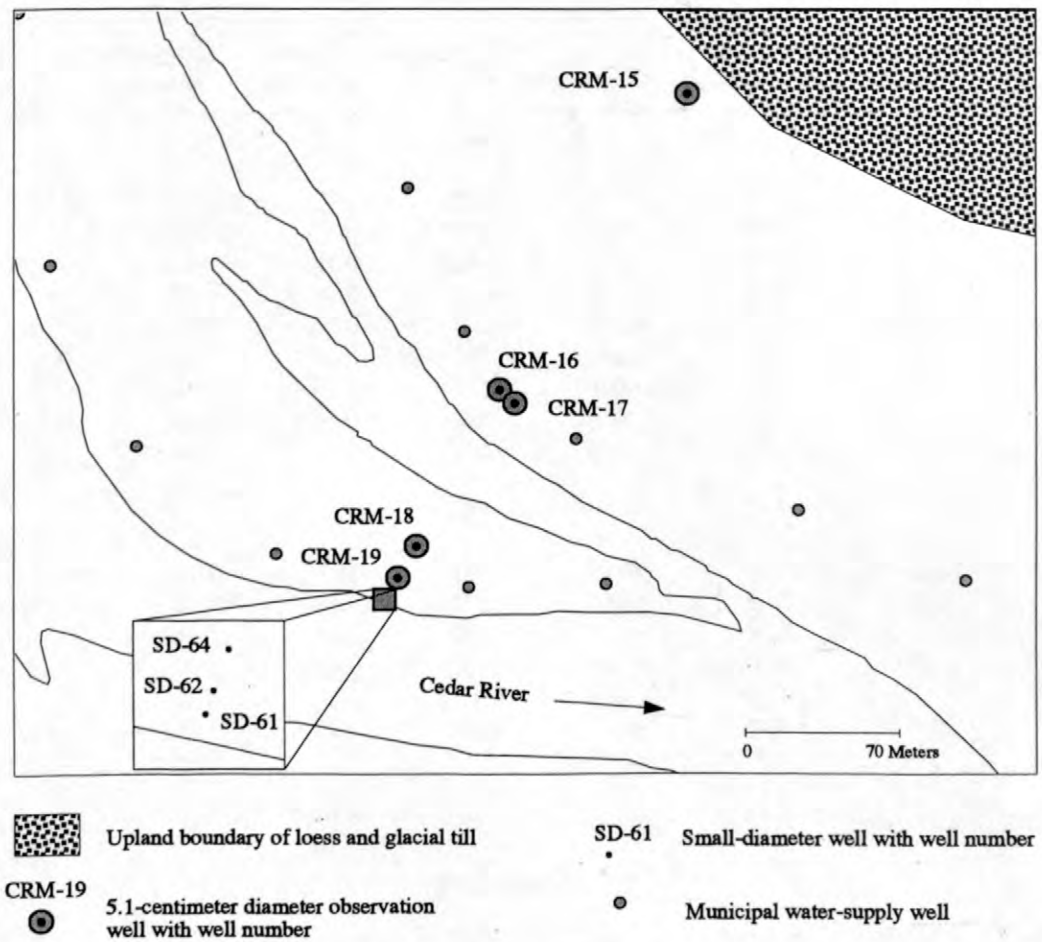

Figure 3. Observation wells in the Seminole Well Field sampled for selected herbicides and degradates, Cedar Rapids, Iowa, June-August, 1998.

steel sandpoint screen (7.6-cm long) attached to Teflon tubing (0.36-cm outer diameter) inside a 3.2-cm outer-diameter borehole. A total of five 0.36-cm diameter wells were installed at about 1-m intervals on the bank of the Cedar River (SD-60 was closest to the river and SD-64 was farthest from the river). During this study, only three of the 0.36-cm diameter wells were used (SD-61, SD-62, and SD-64). SD-61 was completed to a depth of about 3.8 m, SD-63 was completed to a depth of about 4.9 m, and SD-64 was completed to a depth of about 6.7 m.

Prior to collecting samples, each observation well was pumped to remove about three borehole volumes of water. Water samples were collected from 5.1-cm diameter wells with a submersible pump and from 0.36-cm diamter wells with a peristaltic pump. Samples were filtered through a 0.7-micrometer, baked, glass-fiber filter. A set of three baked, amber glass, 125-milliliter bottles was collected from each observation well. Samples were shipped chilled to the USGS Organic Geochemistry Research Laboratory in Lawrence, Kansas. Samples were analyzed using gas chromatography/mass spectrometry (GC/MS) for the 13 parent herbicides listed in table 1 and three triazine degradates (cyanazine-amide, deethylatrazine [DEA], and deisopropylatrazine [DIA]). The minimum reporting limit for GC/MS analyses was 0.05 µg/L for all constituents. Samples were analyzed with high-performance liquid chromatography (HPLC) for the six acetanilide degradates

Table 2. Concentrations of selected herbicides and triazine degradates detected in samples collected from the alluvial aquifer and Cedar River, Cedar Rapids, Iowa, June-August, 1998.

[All concentrations in micrograms per liter.]

Site name (figure 3)	Acetochlor	Atrazine	Cyanazine	Cyanazine-amide	Deethyl-atrazine	Deisopropyl-atrazine	Metolachlor
June 1998							
CRM-15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
CRM-16	<0.05	0.05	<0.05	<0.05	0.07	<0.05	<0.05
CRM-17	<0.05	0.51	0.05	0.08	0.18	0.07	0.10
CRM-18	<0.05	0.06	<0.05	<0.05	0.08	0.05	<0.05
CRM-19	<0.05	0.22	<0.05	<0.05	0.14	0.08	0.11
SD-64	0.07	0.87	<0.05	0.19	0.21	0.10	0.14
SD-62	<0.05	0.95	0.09	0.23	0.22	0.12	0.11
SD-61	0.17	2.71	0.16	0.31	0.44	0.26	0.33
Cedar River	0.16	1.10	<0.05	<0.05	0.21	0.12	0.17
July 1998							
CRM-15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
CRM-16	<0.05	0.08	<0.05	<0.05	0.16	<0.05	<0.05
CRM-17	<0.05	0.54	<0.05	0.08	0.18	0.06	0.19
CRM-18	<0.05	0.09	<0.05	<0.05	0.13	0.05	0.18
CRM-19	0.09	1.39	0.07	0.30	<0.05	0.12	0.23
SD-64	<0.05	0.48	<0.05	<0.05	0.14	<0.05	0.08
SD-62	<0.05	1.01	<0.05	<0.05	0.24	0.09	0.16
SD-61	<0.05	0.71	<0.05	<0.05	0.20	0.06	0.07
Cedar River	<0.05	0.23	<0.05	<0.05	0.15	0.08	0.05
August 1998							
CRM-15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
CRM-16	<0.05	0.07	<0.05	<0.05	0.13	<0.05	<0.05
CRM-17	<0.05	0.59	<0.05	<0.05	0.17	<0.05	0.06
CRM-18	<0.05	0.12	<0.05	<0.05	0.14	0.06	0.13
CRM-19	<0.05	<0.05	<0.05	<0.05	0.22	0.11	0.15
SD-64	<0.05	0.38	<0.05	<0.05	0.08	<0.05	0.08
SD-62	<0.05	0.72	<0.05	<0.05	0.17	0.06	0.08
SD-61	<0.05	0.29	<0.05	<0.05	0.13	0.05	<0.05
Cedar River	<0.05	0.14	<0.05	<0.05	0.13	0.05	<0.05

listed in table 1 and hydroxy-atrazine. The minimum reporting limit for HPLC analyses was 0.20 µg/L for all constituents.

HERBICIDES AND DEGRADATES IN THE ALLUVIAL AQUIFER AND CEDAR RIVER

Analytical results are summarized in table 2 and table 3. Table 2 lists concentrations of selected herbicides and triazine degradates detected in water samples collected from the alluvial aquifer and Cedar River from June to August 1998. Table 3 lists the concentrations of acetanilide degradates and hydroxy-atrazine detected in water samples collected from the alluvial aquifer and Cedar River from June to August 1998. Results for some compounds listed in table 1 are not summarized because reported concentrations were all less than the minimum reporting limit.

Table 3. Concentrations of acetanilide degradates and hydroxy-atrazine detected in water samples collected from the alluvial aquifer and Cedar River, Cedar Rapids, Iowa, June-August, 1998.

[All concentrations in micrograms per liter; ESA, ethanesulfonic acid; OA, oxanilic acid.]

Site name (figure 3)	Acetochlor ESA	Acetochlor OA	Alachlor ESA	Alachlor OA	Hydroxy-atrazine	Metolachlor ESA	Metolachlor OA
				June 1998			
CRM-15	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
CRM-16	0.39	0.20	1.02	<0.20	<0.20	2.27	0.45
CRM-17	1.26	0.85	1.62	<0.20	<0.20	4.83	1.22
CRM-18	0.71	<0.20	1.06	<0.20	<0.20	3.09	0.77
CRM-19	0.99	0.66	1.39	<0.20	<0.20	4.24	0.78
SD-64	1.72	1.04	1.43	<0.20	<0.20	5.27	1.20
SD-62	1.98	1.18	1.31	<0.20	<0.20	5.16	1.19
SD-61	2.06	1.66	1.32	<0.20	0.22	5.42	1.14
Cedar River	1.47	0.64	1.38	<0.20	<0.20	5.45	0.92
				July 1998			
CRM-15	<0.20	<0.20	<0.20	<0.20	<0.20	0.38	<0.20
CRM-16	0.85	0.28	0.69	<0.20	<0.20	3.92	0.76
CRM-17	1.37	0.69	1.73	0.29	<0.20	4.51	1.25
CRM-18	1.14	<0.20	2.19	<0.20	<0.20	5.64	0.89
CRM-19	2.06	1.17	2.06	<0.20	<0.20	6.36	1.17
SD-64	2.48	1.34	1.97	0.25	<0.20	5.64	1.40
SD-62	3.32	1.75	2.20	0.31	<0.20	6.51	1.72
SD-61	2.90	1.08	2.36	0.20	<0.20	6.84	4.25
Cedar River	1.08	0.29	1.96	<0.20	<0.20	4.25	0.62
				August 1998			
CRM-15	<0.20	<0.20	<0.20	<0.20	<0.20	0.37	<0.20
CRM-16	1.10	0.35	1.21	<0.20	<0.20	3.81	0.82
CRM-17	1.48	0.61	1.28	<0.20	<0.20	4.14	0.87
CRM-18	1.39	<0.20	2.50	<0.20	<0.20	6.49	0.81
CRM-19	1.58	0.20	2.35	<0.20	<0.20	5.48	0.69
SD-64	1.07	<0.20	2.15	<0.20	<0.20	4.75	0.59
SD-62	2.49	0.67	2.23	0.29	<0.20	6.47	1.04
SD-61	1.55	0.26	2.24	<0.20	<0.20	5.85	0.88
Cedar River	0.90	<0.20	2.13	<0.20	<0.20	4.15	0.54

Triazines

Atrazine was detected more frequently and at greater concentrations (<0.05 to 2.71 μg/L) than any other parent compound in samples collected during the study. Atrazine was detected in every sample except the three collected from CRM-15, the site representative of conditions least influenced by induced infiltration from the Cedar River, and one sample collected from CRM-19 in August. Cyanazine was detected in four samples collected from the alluvial aquifer at relatively small concentrations (<0.05 to 0.16 μg/L). Cyanazine was not detected in samples collected from the Cedar River for this study, but cyanazine was detected in samples independently collected from the Cedar River by the City of Cedar Rapids on May 27 and June 17 at concentrations of 0.40 μg/L and 0.34 μg/L, respectively (Tom Noth, City of Cedar

Table 4. Ratios of selected triazine compounds in water samples collected from the alluvial aquifer and Cedar River, Cedar Rapids, Iowa, June-August, 1998.

[DEA, deethylatrazine; DIA, deisopropylatrazine; --, ratio not calculated because at least one compound was not detected above the minimum reporting limit.]

Site name	DEA/atrazine			DIA/DEA		
(figure 3)	June	July	August	June	July	August
CRM-15	--	--	--	--	--	--
CRM-16	1.4	2.0	1.9	--	--	--
CRM-17	0.35	0.33	0.29	0.39	0.33	--
CRM-18	1.3	1.4	1.2	0.62	0.38	0.43
CRM-19	0.64	--	--	0.57	--	0.50
SD-64	0.24	0.29	0.21	0.48	--	--
SD-62	0.23	0.24	0.24	0.54	0.38	0.35
SD-61	0.16	0.28	0.45	0.59	0.30	0.38
Cedar River	0.19	0.65	0.93	0.57	0.53	0.38

Rapids Water Department, written commun., July 1998).

Triazine degradates detected in samples included cyanazine-amide, DEA, DIA, and hydroxy-atrazine. DEA and DIA were the most frequently detected triazine degradates. Triazine degradate concentrations were relatively small, ranging from less than the minimum reporting limit to a maximum DEA concentration of 0.44 µg/L.

Thurman and others (1994) used the DEA to atrazine ratio (DAR) in surface-water samples collected from Midwestern streams and rivers to relate parent herbicides and degradate concentrations to the period of herbicide applications. Thurman and others (1994) indicated that DAR values in runoff from cropland on which atrazine was applied inccrease with time after the initial herbicide application. Thurman and others (1994) reported DAR values less than 0.05 in runoff from field plots shortly after atrazine application and DAR values between 0.5 to 0.7 about 60 days after the atrazine application. The DAR values in samples collected from the Cedar River for this study (table 4) increased from June (0.19) to August (0.93). This is consistent with the results of Thurman and others (1994) for DAR values in runoff from cropland following the application of atrazine.

The DAR also is useful to evaluate atrazine transport in water infiltrating from the Cedar River into the alluvial aquifer because the DAR is not affected by dilution resulting from mixing. Schulmeyer (1995) and Schulmeyer and Schnoebelen (1998) estimated the rate of infiltration from the river into the alluvial aquifer to be about 1 m per day. Because the infiltration rate is relatively rapid, DAR values in shallow ground water near the river (SD-61, SD-62, and SD-64) were expected to increase with DAR values in the river from June to August. SD-61, the site closest to the river, was the only site with DAR values that increased with DAR values in the river from June to August. DAR values in SD-62 and SD-64, located about 1 m and 3 m from SD-61, remained relatively constant (0.21 to 0.29) from June to August. Squillace and others (1996) studied the movement of herbicides between the Cedar River and alluvial aquifer in an area of the Cedar River alluvium (about 16 kilometers downstream of Cedar Rapids) that is not influenced by pumping of municipal supply wells. Their results indicated that herbicides enter the alluvial aquifer with bank-storage water during periods of high river stage, but are released as bank-storage water discharges to the river during periods of lower river stage. Infiltration induced by pumping of municipal wells during this study may have prevented the release of bank-storage water during periods of lower river stage, so atrazine and DEA remained in bank-storage water (rather than discharged back into the river) and were transported into the alluvial aquifer with induced infiltration from the river. As indicated by the DAR values from this study, atrazine and DEA likely are gradually transported into the alluvial aquifer away from

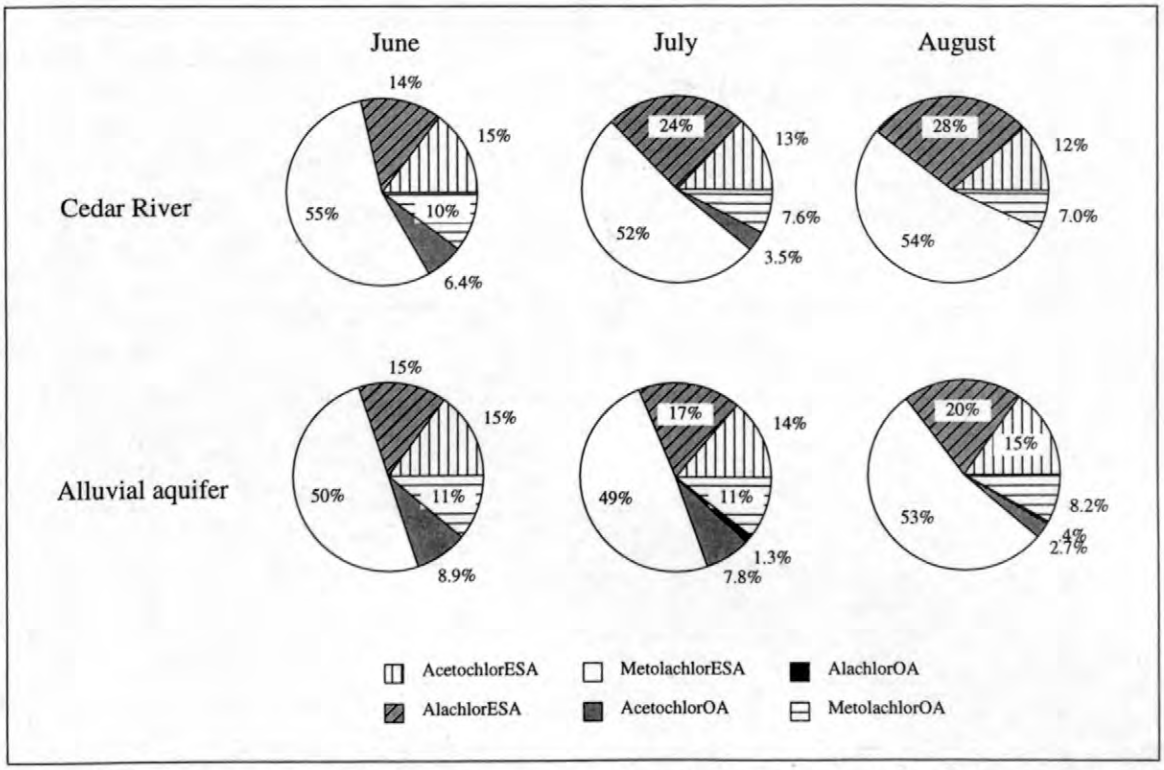

Figure 4. Distribution of acetanilide degradates in samples collected from the Cedar River and alluvial aquifer , Cedar Rapids, Iowa, June-August, 1998.

bank-storage water. DAR values in samples from deeper observation wells (CRM-16, CRM-18, and CRM-19) were larger than DAR values in samples from shallower wells. Recharge requires greater time to flow to deeper zones of the alluvial aquifer, and atrazine entering the alluvial aquifer at the time of recharge probably degrades during transport.

Atrazine, cyanazine, and simazine degradation can produce DIA. DIA detected in samples during this study likely resulted from atrazine and cyanazine degradation because simazine is not used in significant quantities in the Midwest. Thurman and others (1994) used DIA/DEA ratios in samples collected from field-plot runoff studies to estimate amounts of DIA produced by the degradation of atrazine and cyanazine. Thurman and others (1994) reported DIA/DEA ratios of 0.4±0.1 when atrazine was the only triazine applied to field plots; DIA/DEA ratios of 0.6±0.1 were reported when both atrazine and cyanazine were applied. DIA/DEA ratios in samples collected from the Cedar River and alluvial aquifer (table 4) in June were between 0.39 and 0.62, indicating that some DIA could have resulted from cyanazine degradtion. DIA/DEA ratios calculated for 9 of 10 samples collected in July and August were less than 0.5, indicating that most DIA resulted from atrazine degradation.

Acetanilides

Acetochlor and metolachlor were the only parent acetanilide compounds detected in water samples collected during the study; alachlor was not detected in any samples. Acetochlor (<0.05 to 0.17 μg/L) and metolachlor (<0.05 to 0.33 μg/L) were detected at relatively small concentrations. Metolachlor was detected in more samples than acetochlor and was detected in June, July, and August; acetochlor only was detected in samples collected in June and July.

Acetanilide degradates generally were detected at greater concentrations than their respective parent compounds (table 2 and table 3). At least one acetanilide degradate compound was detected in every sample collected during the study except the sample collected from CRM-15 in June. Shallow ground water near CRM-15 most likely is not influenced by induced infiltration from the Cedar River; recharge is primarily from infiltrating precipitation and leakage across adjacent and

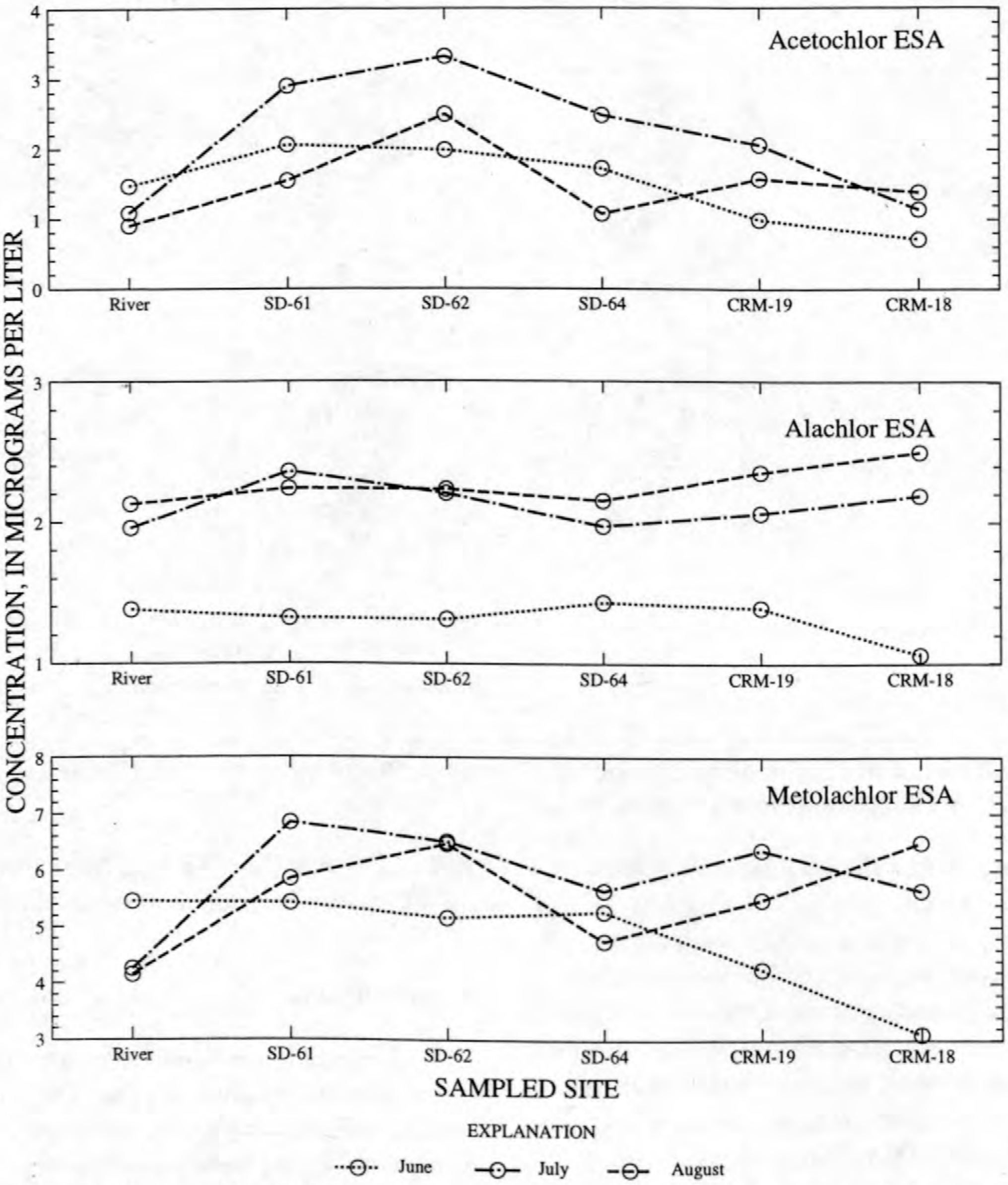

Figure 5. Ethanesulfonic-acid (ESA) degradate concentrations in samples from the alluvial aquifer and Cedar River, Cedar Rapids, Iowa, June-August, 1998.

underlying hydrogeologic units. Metolachlor ESA detected in ground-water samples from CRM-15 in July and August likely was from surface water that inundated the area near CRM-15 during a localized flooding event produced by unseasonably high stages in the Cedar River in early July.

Figure 4 shows the ratio of each acetanilide degradate to the total acetanilide degradate concentration in each round of samples collected from the Cedar River and alluvial aquifer. ESA degradates were detected at greater frequency and at greater concentrations than OA degradates. ESA-degradate concentrations represented at least 80 percent of the total acetanilide-degradate concentration in each round of samples collected during the study. Kalkhoff and others (1998) noted similar results for ground-water and surface-water samples collected from eastern Iowa. Thurman and others (1996) and Aga and others (1996) indicated that acetanilide degradates are produced by dechlorination of parent compounds by glutathione conjugate reactions in plants, algae, and microorganisms. Eykholt and Davenport (1998) indicated acetanilide degradates also can be produced by abiotic reductive dechlorination in the presence of iron metal. Water-quality data collected near the wells sampled for this study show that the shallow ground water is moderately reducing, likely has significant biological activity, and that minerals within the aquifer matrix contain iron. Even though conditions in the ground water are conducive to reductive dechlorination, results from this study do not indicate significant degradation of parent compounds because ratios of individual degradates to total degradate concentrations are similar in samples collected from the Cedar River and alluvial aquifer.

Figure 5 shows ESA-degradate concentrations in samples collected from observation wells near the Cedar River. ESA-degradate concentrations generally were smallest in June and greater in July and August. The differences in ESA-degradate concentrations between June and later in the summer probably result from the trend in the rate of formation of degradates from parent herbicides in cropland soils within the drainage basin. Aga and others (1996) observed the temporal variation of metolachlor and metolachlor ESA in soil samples collected from a field plot. Metolachlor decreased exponentially after application, with a half-life of about 25 days. Metolachlor ESA concentrations rapidly increased during the first five weeks after application, gradually increased to a maximum during the next 7 to 8 weeks, and then steadily decreased. Although data from field studies are lacking, similar trends probably occur for other acetanilide herbicides and corresponding ESA degradates. Most acetanilide-degradate concentrations in ground water at wells SD-61 and SD-62 were greater than concentrations in the Cedar River. Acetanilide-degradate concentrations in shallow ground water probably reflect concentrations in the river prior to sampling, assuming acetanilide degradates are transported in a manner similar to that indicated for atrazine and DEA.

SUMMARY

Triazine and acetanilide herbicides were detected in water samples collected from June to August 1998 from the alluvial aquifer and Cedar River near a Cedar Rapids, Iowa municipal well field. Atrazine was the most frequently detected compound and was detected at the greatest concentrations. Acetochlor, cyanazine, and metolachlor also were detected, but at smaller concentrations than atrazine. Concentrations generally decreased from June to August.

Triazine degradates were detected at relatively small concentrations in water samples collected during the study. Concentrations generally decreased from June to August. DEA and DIA were detected more frequently than cyanazine-amide or hydroxy-atrazine. DAR values in samples from the Cedar River were consistent with degradation characteristics of atrazine from runoff from cropland on which atrazine was applied. Atrazine and DEA in the Cedar River probably are transported into the alluvial aquifer with infiltration induced by pumping of municipal wells, based on DAR values of samples collected during the study. DAR values in samples from observation wells in deeper zones of the alluvial aquifer were greater than those in shallower wells probably because of atrazine degradation during transport to the deeper zones of the alluvial aquifer. The DIA to DEA ratio in samples indicated that most DIA is produced by atrazine degradation, although some DIA might be produced by cyanazine degradation in June.

Acetanilide degradates were detected at greater frequency and at greater concentrations than their corresponding parent compounds. ESA degradates were detected more frequntly and at greater concentrations than OA degrates. ESA degradates represented at least 80 percent of the total acetanilide-degradate concentration from each sampling date; OA degradates represented 20 percent or less of the total acetanilide degradate concentration. The composition of degradates detected in samples from the Ceadr River was similar to the composition detected in samples from the alluvial aquifer, indicating that relatively little degradation occurs during transport from the river into the alluvial aquifer. Acetanilide-degradate concentrations in ground water adjacent to the Cedar River suggest that acetanilide degradates are transported into the alluvial aquifer in a manner similar to that indicated for atrazine and DEA.

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Occurrence of Pesticides in Rain and Air in Urban and Agricultural Areas of Mississippi, April-September 1995

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ABSTRACT

In April 1995, the U.S. Geological Survey began a study to determine the occurrence and temporal distribution of 49 pesticides and pesticide metabolites in air and rain samples from an urban and an agricultural sampling site in Mississippi. The study was a joint effort between the National Water-Quality Assessment and the Toxics Substances Programs and was part of a larger study examining the occurrence and temporal distribution of pesticides in air and rain in the Mississippi River Basin. Concurrent high-volume air and wet-only deposition samples were collected weekly. The air samplers consisted of a glass-fiber filter to collect particles and tandem polyurethane foam plugs to collect gas-phase pesticides. Every rain and air sample collected from the urban and agricultural sites had detectable levels of multiple pesticides. The magnitude of the total concentration was 5 to 10 times higher at the agricultural site as compared to the urban site. The pesticide with the highest concentration in rain at both sites was methyl parathion. The pesticide with the highest concentration in the air samples from the agricultural site was also methyl parathion, but from the urban site the highest concentration was diazinon followed closely by chlorpyrifos. A metabolite of *p,p*'-DDT, *p,p*'- DDE, was detected in every sample from the agricultural site and in more than half of the air samples from the urban site more than two decades since DDT was banned from use in the United States.

INTRODUCTION

Pesticides are widely used in the United States to protect crops from pests, to reduce crop yield loss, and to increase the comfort and safety of citizens. Although the use of pesticides has resulted in increased crop production and other benefits, there is concern about the ultimate fate of pesticides. Pesticides have the potential to contaminate the hydrologic cycle when they move from their point of application. One potential path for off-site movement is through the atmosphere. Small amounts of pesticides can be transported long distances through the atmosphere and deposited into aquatic and terrestrial ecosystems far from their point of use (Majewski and Capel, 1995). Atmospheric transport can occur in the gas phase through volatilization or in the particulate phase when attached to dust particles, or a combination of both depending on the pesticide's physical and chemical properties. After introduction into the atmosphere, pesticides can be degraded, transported, and redeposited. Deposition can be either wet such as with rain or snow, or dry such as gaseous sorption and particle fallout.

There have been several studies that have examined the movement of pesticides in the atmosphere, and an excellent review of many of the major studies is in Majewski and Capel (1995). In Mississippi, there have been a limited number of studies on the transport of pesticides in the atmosphere. Many of these studies have dealt with the volatilization of pesticides after application (Hollingsworth, 1980; Willis and others, 1980; Harper and others, 1983; and Willis and others, 1983). Hollingsworth (1980), examined volatilization of trifluralin after incorporation, the other studies examined toxaphene or DDT or both. Arthur and others (1976) collected weekly air samples and analyzed them for a suite of pesticides, most of which have since been discontinued in the

United States. There have been a few national studies that have included agricultural and urban sites in Mississippi, (Tabor, 1965; Stanley and others, 1971; and Kutz and others, 1976), but these studies focused on pesticides that were then in use, most of which are no longer used in the United States.

In June 1994, Majewski and others (1998) collected air samples during a cruise up the Mississippi River from New Orleans, Louisiana to St. Paul Minnesota. This was a precursor to the current study and used the same equipment and analytical techniques. Their results indicated that the occurrence and atmospheric concentration of the observed pesticides were most closely related to their use within 40 kilometers of the river. Additionally, some pesticides heavily used in urban areas such as chlorpyrifos, diazinon, and malathion had their highest concentration near urban areas. There have been no other studies on pesticides in the atmosphere in Mississippi in recent years. The purpose of this paper is to present the results of a study of pesticides in rain and air from April 12 to September 19, 1995, collected from an urban and an agricultural setting in Mississippi by the U.S. Geological Survey (USGS).

Sampling Sites

The urban sampling site is located in Hinds County, Mississippi, in a residential neighborhood of the south Jackson, metropolitan area. The site was chosen to represent urban air and is several kilometers from the nearest agricultural field.

The agricultural sampling site is in the center of a catfish pond complex near the town of Rolling Fork in Sharkey County, Mississippi. This area is in the Mississippi River Alluvial Plain and is one of the most intensively farmed areas in the United States. The major crops were, soybean, cotton, corn, and rice. The site location was selected to minimize the influence of direct application of pesticides to nearby fields. The nearest agricultural field was approximately a kilometer away.

Sampling Procedures

Weekly samples of wet-only deposition were collected by using a modified Aerochem Metric Precipitation Collector (any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government). This collector is equipped with a moisture sensor that triggers the lid of the collection bucket to open when rain begins and to close when the rain ends. The collector was modified by installing a Teflon-coated funnel in the collection bucket and attaching a Teflon tube from the funnel through the bottom of the bucket into the top of a small refrigerator and into a glass bottle. The inside of the refrigerator was maintained at 4°C.

Samples were collected weekly, if there had been enough precipitation. Samples were transported to the USGS office, and a 1-L aliquot was withdrawn and passed through a C-18 solid phase extraction cartridge for isolation of the compounds of interest. The cartridge was then sent to the National Water Quality Laboratory (NWQL) in Arvada, Colo. Samples were eluted from the cartridges with solvent and analyzed for 47 pesticides and pesticide degradates by gas/chromatography mass/spectrometry (GC/MS) using selected ion monitoring (Zaugg and others, 1995).

The last rain sample from the urban site was collected during the week of August 15-22 and the last rain sample for the agricultural site was the week of August 29 through September 5. During the week of April 19-26, more than 20 centimeters (cm) of rain fell at both sites. The sample bottles were designed to hold about 13 cm of rain. At the agricultural site, the sample bottle was replaced on April 22; however, the urban site could not be accessed, and the sample bottled overflowed. In all, there was sufficient rainfall for 16 weekly samples from the urban site and 15 weekly samples and 1 midweek sample at the agricultural site out of a possible 24 weekly samples.

The air samplers consisted of a baked glass-fiber filter (GFF), 21.6 cm x 27.9 cm, to collect particles and tandem polyurethane foam (PUF) plugs, 8.9 cm diameter x 7.6 cm, to collect gas-phase pesticides. The air was pulled through the GFF and then through the PUFs at about 1 cubic meter (m^3) per minute. The PUFs were mounted in tandem and analyzed separately to estimate the efficiency of the two PUFs for the collection of gas-phase pesticides. If a pesticide was detected on the first PUF and not on the second, it was assumed that the gas-phase pesticide was completely collected by the first PUF. If, however, there was an equal or larger amount of the pesticide on the second PUF, as on the first, then it was assumed that extraction of the pesticide by the PUFs was not complete and the concentrations derived from the PUFs must be considered a minimum. Diazinon, molinate, and trifluralin had concentrations on the second PUF equal to or more than on the first PUF. These concentrations are considered minimums; the actual concentrations were higher. As a quality assurance measure the collection efficiencies were evaluated from a spiked sample and are reported in Majewski and others (1998). The collection efficiencies for most compounds were excellent except for those noted on table 1.

At the beginning of the study (April 12, 1995) the air sampler was programmed to sample air continuously for 4 hours during the day; later (May 5, 1995), this was changed to 5 minutes out of every hour to better represent average air concentrations. The GFF and the PUFs were replaced after 7 days. The GFFs were analyzed separately to provide an estimate of the particle and distribution of the pesticides. The last air samples at both sites were collected for the week of September 12-19. At the agricultural site, equipment failures prevented the collection of samples for the weeks of July 5-12, July 25-August 1, and August 1-8.

The GFFs and PUFs were sent on ice to the NWQL where they were extracted with 36.5 percent ethyl acetate in hexane for 16 hours using a soxhlet apparatus. The extract was dried using sodium sulfate and reduced to 0.5 mL using a Kuderna-Danish concentrator followed by nitrogen gas evaporation. The extract was passed through a Pastuer pipet column containing 0.75 g of fully activated florisil overlain with 1 cm of powdered sodium sulfate. Pesticides were eluted using 4 mL of ethyl acetate into a test tube containing 0.1 mL of a perdeuterated polycyclic aromatic hydrocarbon internal standard solution. The extract was evaporated to about 150 mL using nitrogen gas, transferred to autosampler vial inserts using a 100 mL toluene rinse, and analyzed by the same GC/MS procedure used for the rain samples.

RESULTS OF ANALYSES

The pesticides for which the rain and air samples were analyzed are listed in table 1. The agricultural pesticide-use rankings for Mississippi are also listed along with possible urban use. Descriptive statistics for occurrence and concentrations of several of the frequently detected pesticides are listed in table 2.

Rain Samples

Twenty-five of 47 measured pesticides were detected at least once in rain samples from the urban site (table 1). Methyl parathion was measured in the highest concentration. Methyl parathion is an insecticide that is used very heavily on row crops in Mississippi, but it is not registered for use in urban areas. Five pesticides (4 insecticides and 1 herbicide) were measured in more than 50 percent of the rain samples from the urban site: carbaryl, chlorpyrifos, diazinon, methyl parathion, and atrazine. No pesticide concentration exceeded 0.5 microgram per liter (µg/L).

Twenty-six of 47 measured pesticides were detected at least once in rain from the agricultural site (table 1). The pesticide measured in the highest concentration was methyl parathion. Eight pesticides (1 insecticide and 7 herbicides) were detected in more than 50 percent of the rain samples from the agricultural site; methyl parathion, atrazine, cyanazine, metolachlor, molinate, pendimethalin, propanil, and trifluralin. There were three pesticides measured at concentrations higher that 0.5 µg/L; they were atrazine (0.83 µg/L), methyl parathion (8.6 and 22.9 µg/L), and propanil (1.8 µg/L).

Air

Twenty-one of 47 measured pesticides were detected in air (GFF and PUFs) from the urban site (table 1). The most frequently detected pesticide was chlorpyrifos, followed by trifluralin and diazinon. Four pesticides (3 insecticides and 1 herbicide) were detected in more than 50 percent of the samples; chlorpyrifos, diazinon, *cis*-permethrin, and trifluralin. Methyl parathion was measured in 11 of the 24 samples.

Twenty-seven of 47 pesticides were detected in air from the agricultural site (table 1). The most frequently detected pesticides were trifluralin and *p,p'*-DDE, a metabolite of DDT; they were detected in every sample. Four other pesticides were also detected in more than 50 percent of the air samples: atrazine, methyl parathion, molinate, and propanil.

OCCURRENCE OF PESTICIDES

Previous studies of pesticides in the atmosphere have indicated that the highest concentrations typically are seasonal and correspond to local use, usually originating within tens of kilometers of the collection point, and that there is a component related to long-range transport, usually only identifiable before or after use and planting season. Because sampling occurred during the growing season, the concentrations reported here are probably related to local use. This would indicate that a component of the pesticides in the air at the urban site would be from agriculture, as there is intensive agriculture within a 100-kilometer radius of Jackson, Mississippi.

Pesticide use for agricultural purposes is well documented; however, urban pesticide use, which includes consumer applications in and around the home and professional application in industrial settings, golf courses, parks, cemeteries, roadways, and railroads, is not well documented. Therefore, comparisons of the occurrence of pesticides in the atmosphere as the occurrence relates to local use, while practical for the agricultural sites, is more difficult for an urban setting. In general, the distribution of the detected pesticides in rain and air, within the urban and agricultural data in this study is quite different. The concentrations of pesticides in rain and air, in general, are higher at the agricultural site than at the urban site, and the types of pesticides detected reflect their local use, although in the case of the urban site there were some agricultural pesticides detected. In urban rain and air, the insecticides carbaryl, chlorpyrifos, and diazinon were detected more frequently than at the agricultural sites. These insecticides are used heavily in the South for fire ant and termite control; their use in agricultural settings is limited. [Note: Although chlorpyrifos is used heavily in agricultural settings in other States, its use in Mississippi has been limited since 1993, because of concerns about residues detected in farm-raised catfish (R. McCarty, Bur. of Plant Industry, written commn., 1997).]

Pesticides in Rain

The total pesticide concentrations in rain for samples collected at the urban and agricultural site are shown in figure 1. The stacked bars show concentrations in μg/L for atrazine, carbaryl, methyl parathion, propanil, and other. The other category is and aggregation of those pesticides infrequently detected. The total pesticide concentrations were 5 to 10 times higher at the agricultural site, reflecting the heavy use of agricultural chemicals on local crops. The pesticides making up a large proportion of the total concentrations in rain at the urban site were atrazine, carbaryl, methyl parathion, and propanil. Because methyl parathion and propanil do not have any legal urban uses, it is assumed that these pesticides were transported from agricultural areas. Methyl parathion and propanil, respectively, are the first and sixth heaviest used pesticides in Mississippi. The total pesticide concentrations in rain at the agricultural site are dominated by atrazine, methyl parathion, and propanil, with some metolachlor and molinate. In two rain samples (weeks beginning June 27 and August 1), the concentrations of methyl parathion, 22.9 and 8.6 μg/L, were very high compared to the concentrations of other pesticides in rain. The highest concentrations of methyl parathion in air, 55.6 and 62.5 nanograms per cubic meter (ng/m^3), occurred during the weeks of August 8 and August 15, respectively, corresponding to weeks with little or no rain. The week of the

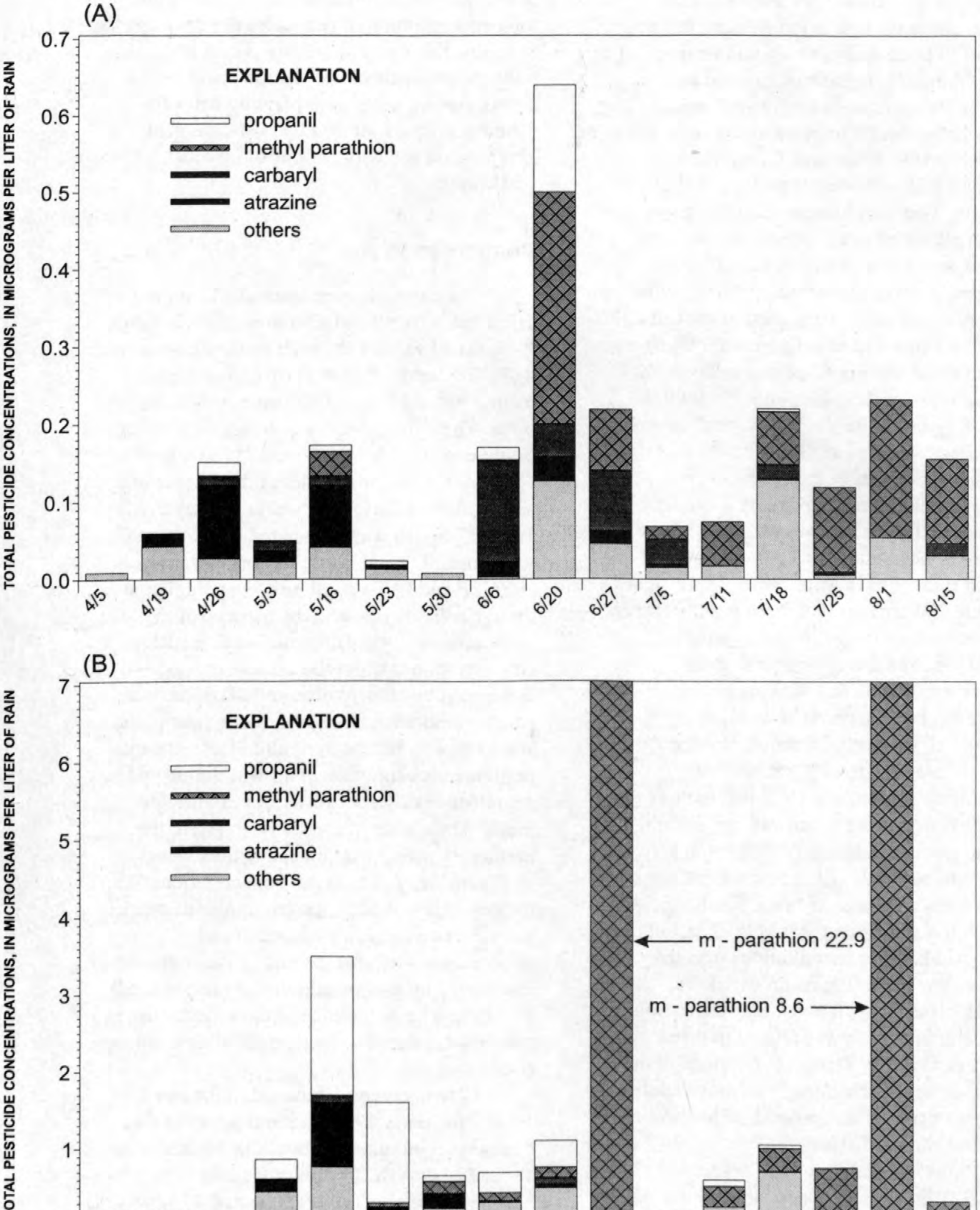

Figure 1. Pesticide concentrations in rain samples collected from an (A) urban and (B) agricultural site in Mississippi, April - September, 1995.

highest concentration in rain (June 27, 22.9 µg/L), the concentration in the air sample was 10.8 ng/m^3. The data in table 2 indicate that methyl parathion is present in rain and air. Methyl parathion must be easily scavenged from the air by raindrops but will persist in the atmosphere without rain and, therefore, is available to be transported from the point of application. This is consistent with the presence of methyl parathion at the urban site.

In a paired study that looked at the differences in triazine concentrations (atrazine, cyanazine, simazine, terbutylazine) between a rural site and an urban site, Chevreuil (1996) noted that there was no difference in diversity and abundances of these herbicides in bulk deposition (rain and particulate phases) between the two sites. This was attributed to the fact that the urban site, located in Paris, France, is relatively small and surrounded by an area of intense agriculture. The concentrations in the French study were similar to those found at the urban and rural sites in Mississippi. From figure 1 and table 2, it appears that there is a difference in concentrations of atrazine and cyanazine between the urban and agricultural site, although there are too few data above the reporting level to determine if this is a statistically significant difference. However, when examining the total pesticide concentrations in rain, it is clear that there is a difference between the urban and agricultural sites. Nations and Hallberg (1992) noted a difference in pesticide concentrations between an urban and a rural site in Iowa. The herbicides were detected as frequently at both sites, but the rural site had higher concentrations than the urban site. The urban site had most of the insecticide detections (fonofos, malathion, and methyl parathion); this was related to urban lawn and garden use. The concentrations of the corn and soybean herbicides in the Iowa study were higher than those measured at the agricultural site in Mississippi.

Nations and Hallberg (1992) and Chevreuil (1996) noted an annual cycle for the triazines: a rapid rise of the concentrations corresponding with spring planting and a decrease to a minimum by the end of summer. Although a similar cycle was noted in this study in Mississippi for the triazine herbicides, the total concentration of pesticides does not appear to follow this cycle as closely. There are multiple pesticide concentration peaks corresponding to varying planting dates for different crops, followed by post-emergent applications and applications of insecticides for pest control.

Pesticides in Air

The pesticide concentrations in air at the urban and agricultural sites are shown in figure 2. The stacked bars show air concentrations in ng/m^3 for carbaryl, chlorpyrifos, diazinon, methyl parathion, pendimethalin, trifluralin, and other. The other category is an aggregation of pesticides infrequently detected. The other category for the air samples at the agricultural sites collected during the weeks of May 3, May 10, and May 16 was dominated by thiobencarb and propanil. Total pesticide concentrations in air (GFF and PUFs combined) were higher at the agricultural site, and the makeup of the total concentrations was different. Total pesticide concentration in air at the urban site was dominated by chlorpyrifos and diazinon, with smaller amounts of carbaryl, methyl parathion, and trifluralin. At the agricultural site, the total pesticide concentration in air was dominated by a number of different pesticides at different times. At the start of the study, in April, the herbicides pendimethalin and trifluralin made up the majority of the total concentrations. At the beginning of May, the two major pesticides were the rice herbicides propanil and thiobencarb. Towards the end of the study, the insecticide methyl parathion was the dominant pesticide. The occurrence of these pesticides in the air was related to local application times on cotton and rice.

In two studies conducted in the late 1960's and early 1970,s air was sampled for methyl parathion near Stoneville, Mississippi, about 70 kilometers north of Rolling Fork. Stanley and others (1971) collected 24-hour samples during 1967 and 1968. Most of the samples were collected during July through October, the high use period for methyl parathion. The range of concentrations of

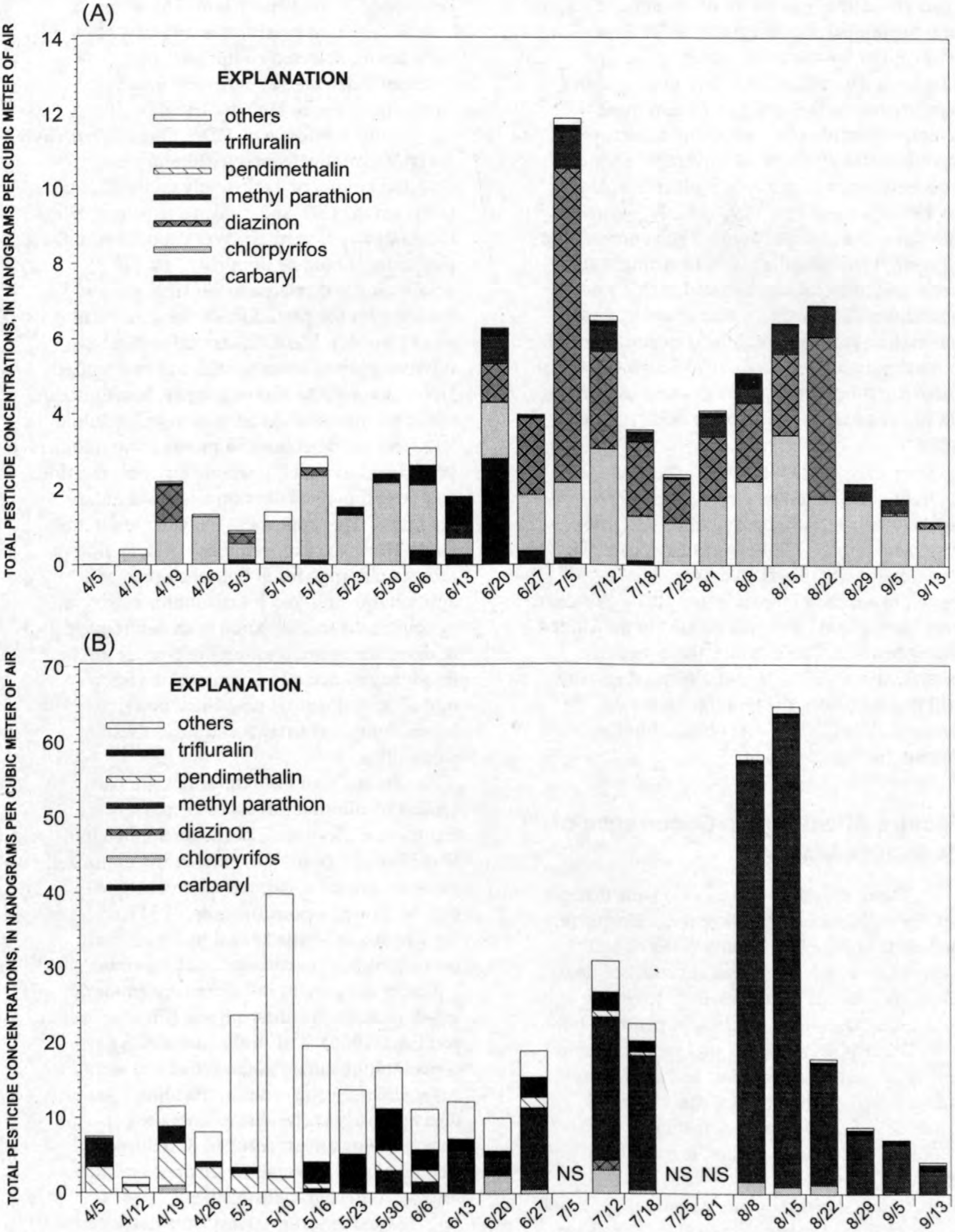

Figure 2. Pesticide concentrations in air samples collected from an (A) urban and (B) Agricultural site in Mississippi, April - September, 1995 (NS, no sample).

methyl parathion in air for the months of August and September was from 20.6 to 71.0 ng/m^3. The results for this study compare well with Stanley's data collected nearly three decades ago. Arthur and others, (1976) presented average monthly concentrations of methyl parathion for 1972-74. The average monthly concentrations of methyl parathion for August of 1972-74 were 217, 129, and 341, ng/m^3 for the three years, respectively. The concentrations of methyl parathion in air from Arthur's study are higher than those presented in this study. The authors for Arthur's study noted some anomalous results in that the concentrations of methyl parathion in air in 1973 was much higher than in 1972 overall, although there had been a 38 percent reduction in its use from 1972 to 1973.

Stanley and others (1971) detected *p,p'*-DDE in concentrations ranging from 2.6 to 7.1 ng/m^3 during April through September 1967. The range of *p,p'*-DDE concentrations at the agricultural site in this study was from 0.13 - 1.1 ng/m^3, lower than Stanley's, but still significant considering that DDT was banned in the United States in 1972. These results indicate that a persistent *p,p'*-DDT degradation product was still measurable in the air more than two decades after DDT use was banned in the United States.

Factors Affecting the Occurrence of Pesticides in Rain

There are numerous mechanisms that can deliver organic compounds to the atmosphere, such as volatilization, wind erosion of soil particles to which pesticides are attached, and direct spraying of the compound to the atmosphere during pesticide application. Once in the atmosphere, a compound will distribute among the aqueous, gaseous, and particulate phases based on the physical and chemical properties of the compound, including water solubility and vapor pressure, and on the conditions of the atmosphere such as temperature, moisture content, and the type and concentration of particulate matter. The phase distribution of the compound strongly affects the behavior, transport, and ultimate fate of the compound in the atmosphere. The water solubility, vapor pressure, and Henry's law constant for selected compounds that were frequently detected at either the urban or agricultural site or both are listed in table 3.

Chlorpyrifos, *p,p'*-DDE, diazinon, methyl parathion, molinate, and trifluralin were detected largely or exclusively on the PUF and rarely on the GFF and, thus, were primarily in the gaseous phase in air. Wet deposition of these pesticides should be dominated by gas scavenging and related to the Henry's law constant for the pesticide. Of these pesticides, chlorpyrifos, *p,p'*-DDE, and trifluralin have relatively lower water solubilities and higher Henry's constants. Consequently, less (gaseous) pesticide mass should be scavenged, resulting in less frequent detections in rain relative to other pesticides having comparable air concentrations and detection levels in rain but lower Henry's constants. This appears to be the case for *p,p'*-DDE. However, the frequency of detection in rain for chlorpyrifos at both the urban and agricultural sites and for trifluralin at the agricultural site, compared with the frequency of detection in air, was not different from the frequency of detection in rain of diazinon, molinate, and methyl parathion, pesticides with lower Henry's constants and higher water solubilities.

Reduced air concentrations are partly caused by dilution effects as air parcels are transported away from pesticide application sites. Further, pesticides such as trifluralin and molinate are susceptible to photochemical degradation reactions (Grover, 1991), the rates for which can be stimulated by increased concentrations of oxidants, such as ozone, that typically are present in higher concentrations in urban versus rural atmospheres (Finlayson-Pitts and Pitts, 1986). Trifluralin and molinate concentrations in air at the urban site were approximately one order of magnitude lower than at the agricultural site. These lower concentrations in air resulted in reduced frequencies of detectable rain concentrations relative to the agricultural site.

Atrazine and propanil were detected in substantial concentrations in both the gaseous and particulate phases in air at the agricultural site. These pesticides have relatively high water solubilities and low Henry's constants. Therefore, scavenging of these pesticides by rain from both sources is important. Detection of these pesticides in rain was more frequent than in air at both sites.

SUMMARY AND CONCLUSIONS

Every rain and air sample collected from an urban and an agricultural site in Mississippi during April-September 1995 had detectable levels of multiple pesticides. The magnitude of the total concentration was 5 to 10 times higher at the agricultural site as compared to the urban site. The pesticide with the highest concentrations in rain at both sites was methyl parathion. Methyl parathion was also the pesticide in the highest concentration in air from the agricultural site, but at the urban site, the pesticide in the highest concentration in air was diazinon followed closely by chlorpyrifos. A metabolite of *p,p'*-DDT, *p,p'*-DDE, occurred in all of the air samples from the agricultural site more than two decades since DDT was banned in the United States. The occurrence of pesticides in rain and air at the agricultural site was related to the timing of application and local use. The occurrence of pesticides in urban rain and air for which there are no legal uses in an urban area was related to transport through the atmosphere from areas of heavy agricultural use.

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Table 1. Pesticide detections in rain and air from agricultural and urban sites in Mississippi, April – September 1995

[h, herbicide; X, detected; ND, not detected; m, metabolite; NR, not reported; i, insecticide; NA, not applicable; dc, discontinued]

Pesticide	Urban detections		Agricultural detections		Rankings of agricultural use in Mississippi[1]	Urban use (1990)[2]
	Rain	Air	Rain	Air		
Acetochlor (h)	X[3]	ND[4]	ND	ND	NR	NR
Alachlor (h)	X	ND	X	ND	20[5]	NR
alpha-HCH (i)	X	X[4]	ND	X	NA	NR
Atrazine (h)	X	X	X	X	18	yes
Benfluralin (h)	ND	X	ND	ND	NR	NR
Butylate (h)	ND	ND	ND	X[3]	75	NR
Carbaryl (i)	X	X	X	X[3]	37	yes
Carbofuran (i)	X	ND	X	X[3]	38	NR
Chlorpyrifos (i)	X	X	X	X	30	yes
CIAT[6] (m)	X	X	X	X	NA	NA
Cyanazine (h)	X	X	X	X	9	NR
DCPA (h)	X	X[3]	X	X	94	yes
p, p'- DDE (m)	ND	X	ND	X	NA	NA
Diazinon (i)	X	X	X	X	92	yes
Dieldrin (i)	X[3]	X[3]	X	X	dc	dc
2,6-Diethylaniline (m)	ND	ND	ND	X	NA	NA
Dimethoate (i)	NA[7]	ND[4]	NA	ND	56	yes
CEAT[8] (m)	NA[7]	X[4]	NA	X	NA	NA
Disulfoton (i)	ND	ND[9]	ND	ND	34	yes
EPTC (h)	ND	ND[9]	X[3]	X	65	yes
Ethalfluralin (h)	ND	ND[9]	ND	ND	49	NR
Ethoprop (i)	ND	ND	X[3]	X	96	NR
Fonofos (i)	ND	ND	ND	ND	NR	NR

Table 1. Pesticide detections in rain and air from agricultural and urban sites in Mississippi, April – September 1995--Continued

[h, herbicide; X, detected; ND, not detected; m, metabolite; NR, not reported; I, insecticide; NA, not applicable; dc, discontinued]

Pesticide	Urban detections		Agricultural detections		Rankings of agricultural use in Mississippi[1]	Urban use (1990)[2]
	Rain	Air	Rain	Air		
Lindane (i)	ND	X[3]	ND	X	102	NR
Linuron (h)	ND	ND	X[3]	ND	44	NR
Malathion (i)	X	X[9]	X	X	21	yes
Methyl Azinphos (i)	X	ND[4]	X	X	47	yes
Methyl Parathion (i)	X	X	X	X	1	NR
Metolachlor (h)	X	X[9]	X	X	12	NR
Metribuzin (h)	ND	ND	ND	X	27	NR
Molinate (h)	X	X[3]	X	X	23	NR
Napropamide (h)	ND	ND	ND	ND	101	NR
Parathion (i)	ND	ND	X[3]	ND	NR	NR
Pebulate (h)	X[3]	ND[9]	ND	ND	NR	NR
Pendimethalin (h)	X[3]	ND	X	X	13	yes
cis-Permethrin (i)	X[3]	X	ND	ND	77	yes
Phorate (i)	ND	ND	ND	ND	62	NR
Prometon (h)	X	NA[10]	X	NA	NR	yes
Pronamide (h)	ND	ND	ND	ND	NR	NR
Propachlor (h)	ND	ND	ND	ND	NR	NR
Propanil (h)	X	X	X	X	6	NR
Propargite I & II (i)	ND	ND[9]	ND	ND	NR	NR
Simazine (h)	X[3]	X	X	ND	87	yes
Tebuthiuron (h)	ND	NA[10]	ND	NA	NR	NR
Terbacil (h)	ND	ND[9]	X[3]	ND	NR	NR
Terbufos (i)	ND	ND	ND	ND	54	NR
Thiobencarb (h)	X	ND	X	X	31	NR
Triallate (h)	ND	ND[4]	ND	ND	NR	NR
Trifluralin (h)	X	X	X	X	3	yes

[1] From Gianessi and Puffer, 1991, 1992a, 1992b

[2] From Majewski and Capel, 1995

[3] Detected once

[4] Method performance data are not available

[5] Gaps in rankings due to pesticides not included in this study

[6] CIAT, chloroisopropylaminotriazine

[7] Analyzed for in air only

[8] CEAT, chloroethylaminotriazine

[9] Recovery of spiked sample was less than 60 percent from Majewski and others, 1998.

[10] Analyzed for in rain only

Table 2. Statistics on selected pesticides in rain and air
[rain units,micrograms per liter; gas and particulate units, nanograms per cubic meter; #, number of samples; %, percent of sample detections; max, maximum concentrations; med, median concentration; nd, not determined]

Pesticide	Phase	Urban				Agriculture			
		#	max	med	%	#	max	med	%
Atrazine	rain	16	0.096	0.006	69	16	0.83	0.02	75
	gas	24	nd	nd	0	21	2.6	nd	42
	particulate	24	0.019	nd	29	21	0.42	0.058	67
Chlorpyrifos	rain	16	0.009	0.005	63	16	0.04	<0.005	38
	gas	24	3.5	1.5	96	21	3.1	nd	38
	particulate	24	nd	nd	0	21	nd	nd	0
Cyanazine	rain	16	0.074	<0.013	31	16	0.32	0.008	56
	gas	24	0.61	nd	8	21	0.25	nd	5
	particulate	24	nd	nd	0	21	0.39	nd	24
Diazinon	rain	16	0.019	0.005	56	16	0.013	<0.008	13
	gas	24	8.4	0.14	50	21	1.4	nd	10
	particulate	24	0.2	nd	25	21	nd	nd	0
Methyl Parathion	rain	16	0.3	0.024	56	16	22.9	0.12	69
	gas	24	0.99	nd	46	21	62	2.5	71
	particulate	24	nd	nd	0	21	0.4	nd	29
Molinate	rain	16	0.025	<0.004	25	16	0.37	0.026	63
	gas	24	0.44	nd	4	21	3.4	0.076	62
	particulate	24	nd	nd	0	21	0.089	nd	5
Propanil	rain	16	0.14	<0.016	38	16	1.8	0.036	81
	gas	24	0.24	nd	13	21	7.6	0.37	57
	particulate	24	0.043	nd	21	21	4.3	0.54	62
p,p'-DDE	rain	16	<.006	<0.006	0	16	<.006	<.006	0
	gas	24	0.19	nd	33	21	1.1	0.67	100
	particulate	24	nd	nd	0	21	0.019	0.01	52
Trifluralin	rain	16	0.01	<0.002	13	16	0.024	0.007	69
	gas	24	0.76	0.028	88	21	5.5	0.81	100
	particulate	24	nd	nd	0	21	0.013	nd	5

Table 3. Water solubility, vapor pressure, and Henry's law constant (between 20 and 25°C) for selected compounds
[mole/m ^{3}, mole per cubic meter; Pa, pascal]

Compound	Subcooled liquid		Henry's law constant Pa m^3/mole
	Water solubility (mole/m^3)	Vapor pressure (Pa)	
Atrazine	4.48E+00	1.29E-03	2.87E-04
Chlorpyrifos	1.25E-03	2.19E-03	1.75E+00
Cyanazine	1.85E+01	5.21E-06	2.82E-07
Diazinon	1.25E-01	8.00E-03	6.41E-02
Methyl parathion	1.27E-01	2.67E-03	2.11E-02
Molinate	4.70E+00	7.46E-01	1.59E-01
Propanil	6.50E+00	2.36E-02	3.64E-03
p,p'-DDE	5.48E-04	4.36E-03	7.95E+00
Trifluralin	2.44E-03	9.84E-03	4.03E+00

Changes in Herbicide Concentrations in Midwestern Streams in Relation to Changes in Use, 1989–98

By Elisabeth A. Scribner, William A. Battaglin, Donald A. Goolsby, and E.M. Thurman

ABSTRACT

Water samples were collected from Midwestern streams in 1994–95 and 1998 as part of a study to help determine if changes in herbicide use resulted in changes in herbicide concentrations since a previous reconnaissance study in 1989–90. Sites were sampled during the first significant runoff period after the application of preemergent herbicides in 1989–90, 1994–95, and 1998. Samples were analyzed for selected herbicides, two atrazine metabolites, three cyanazine metabolites, and one alachlor metabolite. In the Midwestern United States, alachlor use was much greater in 1989 than in 1995, whereas acetochlor was not used in 1989 but was commonly used in 1995. The use of atrazine, cyanazine, and metolachlor was about the same in 1989 and 1995. The median concentrations of atrazine, alachlor, cyanazine, and metolachlor were substantially higher in 1989–90 than in 1994–95 or 1998. The median acetochlor concentration was higher in 1998 than in 1994 or 1995.

INTRODUCTION

During 1989, a reconnaissance study of 147 streams in 10 Midwestern States (as shown in fig. 1*A*) was conducted to determine the geographic and seasonal distribution of herbicides and nutrients (Scribner and others, 1993). Sites were selected to ensure geographic distribution and regional-scale interpretation of the data. Results from the 1989 study showed that large amounts of alachlor, atrazine, cyanazine, and metolachlor were flushed into streams during the first post-application runoff (Thurman and others, 1991; Goolsby and Battaglin, 1995).

A follow-up study was conducted in 1990 because of increased concern about the findings of high post-application concentrations of herbicides in 1989. On the basis of a ranking of 1989 concentrations from highest to lowest values, 50 sampling sites were selected using a stratified random approach. The distribution of major herbicide concentrations detected in these streams was essentially the same in 1989 and 1990 for both the pre- and the post-application samples. Results further indicated that the flush of herbicides following application is an annual occurrence (Goolsby and others, 1991). At three additional sites, automatic samplers were operated in 1990 to determine the temporal distribution of herbicides in several Midwestern streams (Scribner and others, 1994). These sites were also sampled in 1989. Thus, the 53 sites selected for this study had been sampled previously during post-application runoff in both 1989 and 1990.

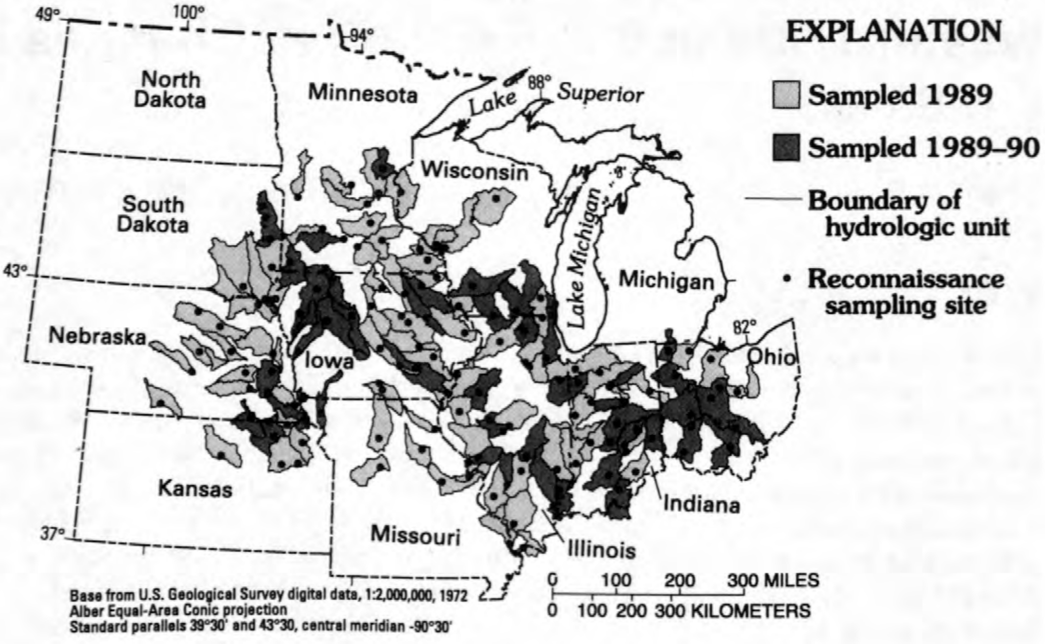
In 1994 and 1995, post-application runoff samples were collected at 53 of the sites sampled in 1989–90. In 1998, post-application runoff samples were collected at 52 of the 53 sites sampled in 1994–95 (fig. 1*B*). These samples were collected to help determine if changes in the application rates recommended by the manufacturers of atrazine had resulted in a reduction of atrazine concentrations in early summer (post-application) runoff (Goolsby and others, 1994; Scribner and others, 1998). The samples also were used to help determine if concentrations of other major herbicides had changed since 1989–90 and if acetochlor (a herbicide used only since 1994) would be detected.

METHODS

Sample Collection

In 1994, sites were sampled twice, but in 1995 and 1998, sites were sampled only once. All water samples were collected using a depth-

A. **1989–90**



B. **1994–95 and 1998**

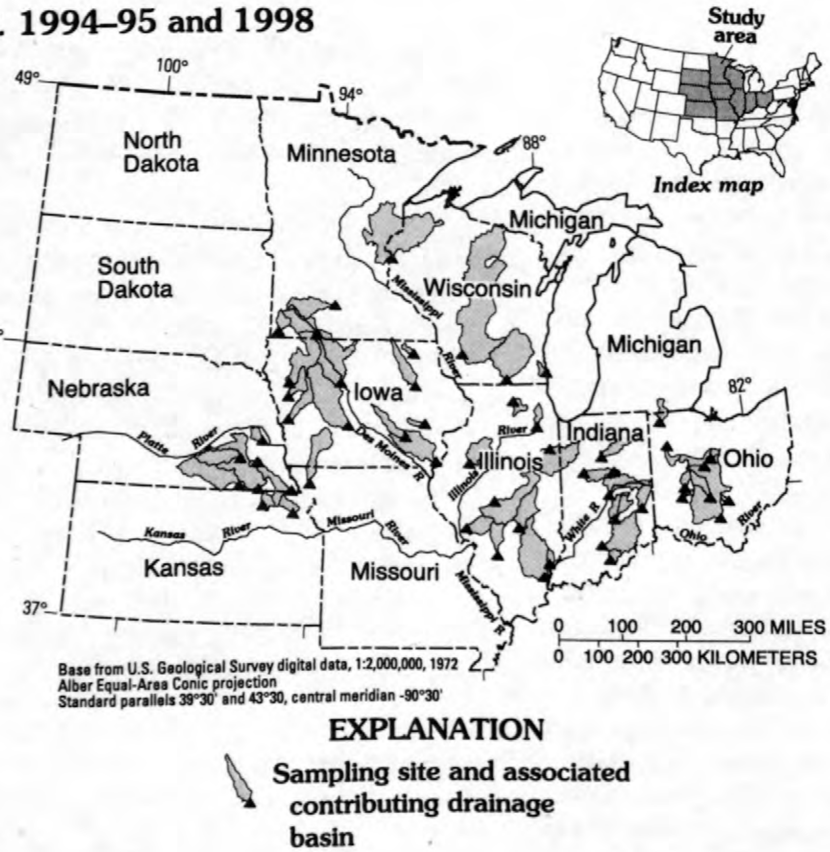


Figure 1. Location of study areas, stream sites, and associated contributing drainage areas sampled in Midwestern United States, *(A)* 1989–90 and *(B)* 1994–95 and 1998.

integrating technique at three or more locations across each stream (Ward and Harr, 1990). Herbicide samples were withdrawn from the compositing container and filtered through a 0.70-µm (micrometer) glass-fiber filter using a peristaltic pump. Measurements were made onsite for specific conductance and pH. Filters were leached with about 200 mL (milliliters) of sample prior to filtration of herbicide samples. Samples were collected, where possible, after the first significant post-application rainfall when a subsequent rise in stream level was occurring.

Laboratory Methods

Herbicide samples were sent to the U.S. Geological Survey (USGS) laboratory in Lawrence, Kansas. In 1989–90, samples were analyzed for 11 herbicides and two atrazine metabolites by gas chromatography/mass spectrometry (GC/MS). In 1994–95 and 1998, samples were analyzed for 13 herbicides, two atrazine metabolites, and three cyanazine metabolites by GC/MS. Solvents used for analyzing samples included pesticide-grade methanol and ethyl acetate. Deionized water was charcoal filtered and glass distilled prior to use. Internal quantitative standard solutions were prepared in methanol and phenanthrene-d10. GC/MS procedures were completed as described by Thurman and others (1990) and Meyer and others (1993).

In 1989–90 and 1994–95, samples were analyzed for alachlor ethanesulfonic acid (ESA) by solid-phase extraction (SPE) and enzyme-linked immunosorbent assay (ELISA) by the method described in Aga and others (1994). The SPE procedure was automated with a Waters Millilab (Milford, MA) workstation for extraction of the analyte. The C_{18} (18 carbon chain) Sep-Pak Plus cartridges (Waters, Milford, MA) were preconditioned sequentially with 2 mL methanol, 6 mL ethyl acetate, 2 mL methanol, and 2 mL distilled water. Each 100-mL water sample was passed through a cartridge at a flow rate of 20 mL/min (milliliters per minute). The cartridge was eluted first with 3.5 mL ethyl acetate to remove the parent compound alachlor. A second elution with 3.5 mL methanol to remove alachlor ESA was collected in a separate test tube. The methanol extracts were evaporated to dryness under nitrogen at 45 °C (degrees Celsius) using a Turbovap evaporator (Zymark, Palo Alto, CA). The samples then were reconstituted with 10 mL distilled water and analyzed using an Alachlor RaPID assay kit (Strategic Diagnostics, Inc., Newark, DE). All samples were analyzed in duplicate. The reporting limit was 0.10 µg/L. In 1998, samples were analyzed for alachlor ESA by high-performance liquid chromatography with diode-array detection (HPLC/DAD). The reporting limit was 0.20 µg/L.

HERBICIDE USE

Since the 1989–90 regional-scale studies were conducted, two decreases have occurred in the maximum application rate of atrazine recommended on manufacturers' labels. In 1990, the manufacturers of atrazine voluntarily reduced the maximum recommended application rate for atrazine to 3 lb (pounds) active ingredient per acre per year for corn and sorghum. Prior to this, the recommended maximum application rate was 4 lb active ingredient per acre per year. The 1990 label change also restricted noncropland uses of atrazine to a maximum of 10 lb active ingredient per acre per year. This label change occurred because of concern about ground-water contamination and was applied to all products released for shipment after September 1, 1990.

In 1992, the manufacturers of atrazine again voluntarily reduced the maximum recommended application rate of atrazine on corn and sorghum to a range of 1.6 to 2.5 lb active ingredient per acre per year depending on soil organic residue and erosion potential. As much as 0.50 lb active ingredient per acre per year can be used in subsequent applications. The total of all applications cannot exceed 2.5 lb active ingredient per acre per year. A maximum of 1.6 lb active ingredient per acre per year is recommended on soil with less than 30-percent plant residue remaining on the surface. Most noncropland uses of atrazine are no longer recommended on manufacturers' labels. This label change applied to all products shipped for use after August 1, 1992.

As a result of the two voluntary label changes, the maximum recommended application rate for atrazine on corn and sorghum has been reduced by approximately 50 percent since the 1989–90 studies were conducted. However, atrazine typically has not been applied at the maximum recommended rate. The actual application rate for atrazine decreased about 10 percent from an average of 1.22 lb per acre in 1990 to 1.07 to 1.1 lb per acre in 1994–95 (U.S. Department of Agriculture, 1991, 1994, 1995).

Year-to-year variability in planted acreages and subsequent herbicide use can make comparisons of change between individual years misleading. A comparison of averaged 1989–90 herbicide use with averaged 1994–95 herbicide use is made here to account to some degree for the natural year-to-year variability in herbicide application. In the Midwestern United States, alachlor use was 56 percent less in 1994–95 (7,400 metric tons (MT)) than in 1989–90 (17,000 MT), atrazine use was 2 percent less in 1994–95 (17,600 MT) than in 1989–90 (18,000 MT), cyanazine use was 26 percent greater in 1994–95 (10,200 MT) than in 1989–90 (8,100 MT), and metolachlor use was 10 percent greater in 1994–95 (16,400 MT) than in 1989–90 (14,900 MT). Acetochlor was not used until 1994, but its use has increased rapidly (9,200 MT in 1995) (Gianessi and Puffer, 1991; U.S. Department of Agriculture, 1991–97). The estimated use of acetochlor, alachlor, atrazine, cyanazine, and metolachlor from 1963 through 1995 is shown in figure 2.

HERBICIDE CONCENTRATIONS

Herbicide concentrations in Midwestern streams were expected to change between 1989 and 1998 as a result of changes in herbicide use. One way to gain a measure of that change is to consider changes in the distribution of herbicide concentrations at a set of representative sites such as those used in this study over a period of years.

The percentage of samples with herbicides detected at or above the analytical reporting limit was greater in 1995 than in 1989 for five compounds (acetochlor, cyanazine, metolachlor, prometon, and propazine) and less in 1995 than in 1989 for three compounds (alachlor, metribuzin, and simazine). The detection frequency for atrazine was 100 percent for every year. Ametryn was detected twice, prometryn was detected only once, and terbutryn was not detected. The frequency of detection of deethylatrazine was the same in 1989 and 1995 (96 percent), but the frequency of detection of deisopropylatrazine was greater in

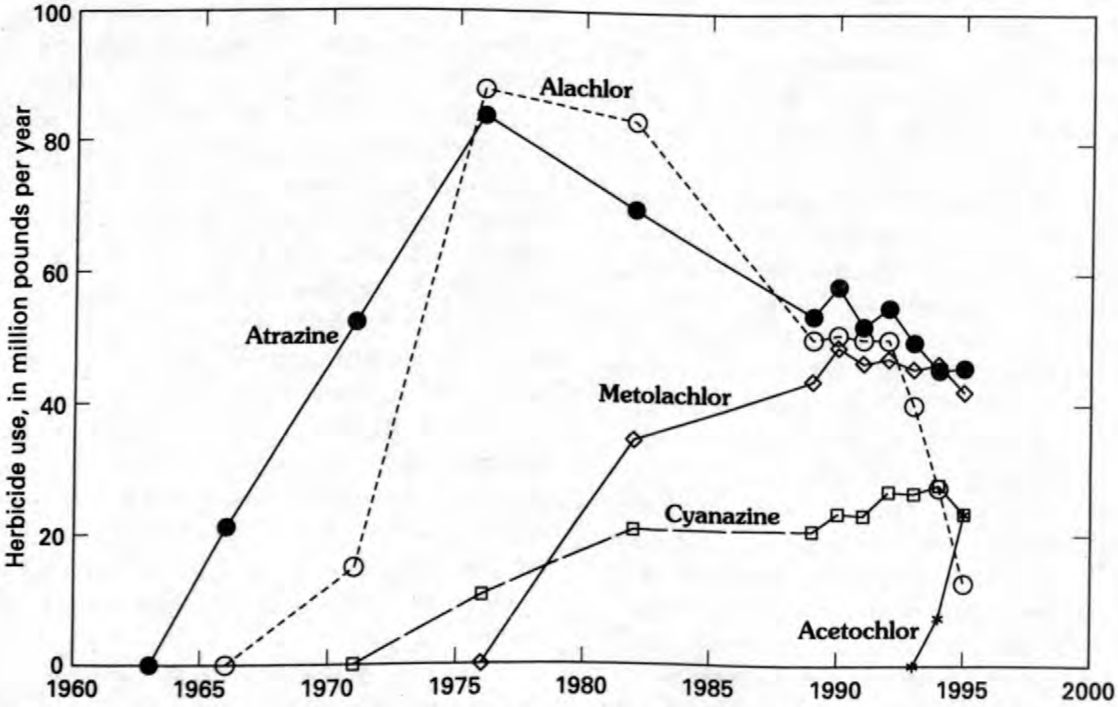


Figure 2. Herbicide use in Midwestern United States, 1963–95 (sources of data: 1964—Lin and others, 1995; 1966–89—Gianessi, 1992; 1990–95—U.S. Department of Agriculture, 1991, 1993, 1994, 1995).

1995 than in 1989. Alachlor ESA, cyanazine amide, deethylcyanazine, and deethylcyanazine amide were not analyzed in 1989 or 1990. The distribution of concentrations for eight herbicides and four herbicide metabolites is shown in figure 3 using boxplots. In figure 3, nondetections are plotted at the GC/MS reporting limit of 0.05 μg/L for the individual compounds.

The median concentrations of herbicides in selected Midwestern streams during post-application runoff were lower in 1995 than in 1989 for seven compounds (alachlor, atrazine, cyanazine, metribuzin, metolachlor, propazine, and simazine) and were the same (less than the detection limit) in 1989 and 1995 for four compounds (ametryn, prometon, prometryn, and terbutryn). Acetochlor was not used in 1989. The median of the sum of the concentration of 12 herbicides (total herbicides) was 22 μg/L in 1989, 11.4 μg/L in 1995, and 9.25 μg/L in 1998. The median alachlor concentration was 1.90 μg/L in 1989, 0.13 μg/L in 1995, and less than 0.05 μg/L in 1998. The median atrazine concentration was 10.9 μg/L in 1989, 5.54 μg/L in 1995, and 4.27 μg/L in 1998. The median cyanazine concentration was 2.65 μg/L in 1989, 1.35 μg/L in 1995, and 0.44 μg/L in 1998. The median metolachlor concentration was 2.50 μg/L in 1989, 1.66 μg/L in 1995, and 1.44 μg/L in 1998. In contrast, the median acetochlor concentration was less than 0.05 μg/L in 1994, 0.42 μg/L in 1995, and 0.72 μg/L in 1998. The median concentration of deethylatrazine and deisopropylatrazine both decreased between 1989 and 1995.

Another way to visualize the changes in herbicide concentrations over a period of years is to consider the distribution of their concentrations at a set of representative sites. The distribution of alachlor and atrazine concentrations in post-application runoff samples from Midwestern streams in 1989–90, 1994–95, and 1998 is shown in figures 4 and 5, respectively (nondetections are plotted at the reporting limit). Figure 4 shows the substantial decrease in alachlor concentration between 1989 and 1998. It also shows that the frequency of alachlor nondetections was much less in 1989–90 than in 1994–95 and 1998, and that the number of samples with concentrations of alachlor greater than 1.0 μg/L has decreased from 30 or more in 1989–90 to less than 10 in 1995 and 1998. Figure 5 shows that the decrease in atrazine concentrations was not as great as for alachlor. Still, atrazine concentrations were generally less in 1994–95 and 1998 than in 1989 and 1990.

DISCUSSION

A study was conducted to determine if changes in herbicide use between 1989 and 1998 would result in changes in herbicide concentrations during post-application runoff in Midwestern streams. In the Midwestern United States, alachlor use was much greater in 1989 than in 1995, whereas acetochlor was not used in 1989 but was commonly used in 1995. The use of atrazine, cyanazine, and metolachlor was about the same in 1989 and 1995. The median concentrations of atrazine, alachlor, cyanazine, and metolachlor were substantially higher in 1989–90 than in 1994–95 or 1998. The median acetochlor concentration was higher in 1998 than in 1995 or 1994. The results for alachlor and acetochlor suggest that changes in herbicide use do affect herbicide concentrations in streams.

However, the observed decreases in concentration for several other herbicides cannot be attributed solely to changes in the overall amount of their use. Other possible explanations for the decreases in herbicide concentrations during post-application runoff include increased use of split herbicide applications, decreased per-acre application rates, increased post-emergent application of traditionally pre-emergent compounds, and improved utilization of herbicide management practices. Application of the results of this study is limited by several factors. Most importantly, herbicide concentrations in Midwestern streams during post-application runoff are quite variable. Some changes in herbicide concentrations presented in this paper could be the result of unknown bias in the timing or method of sample collection and, hence, may be misleading.

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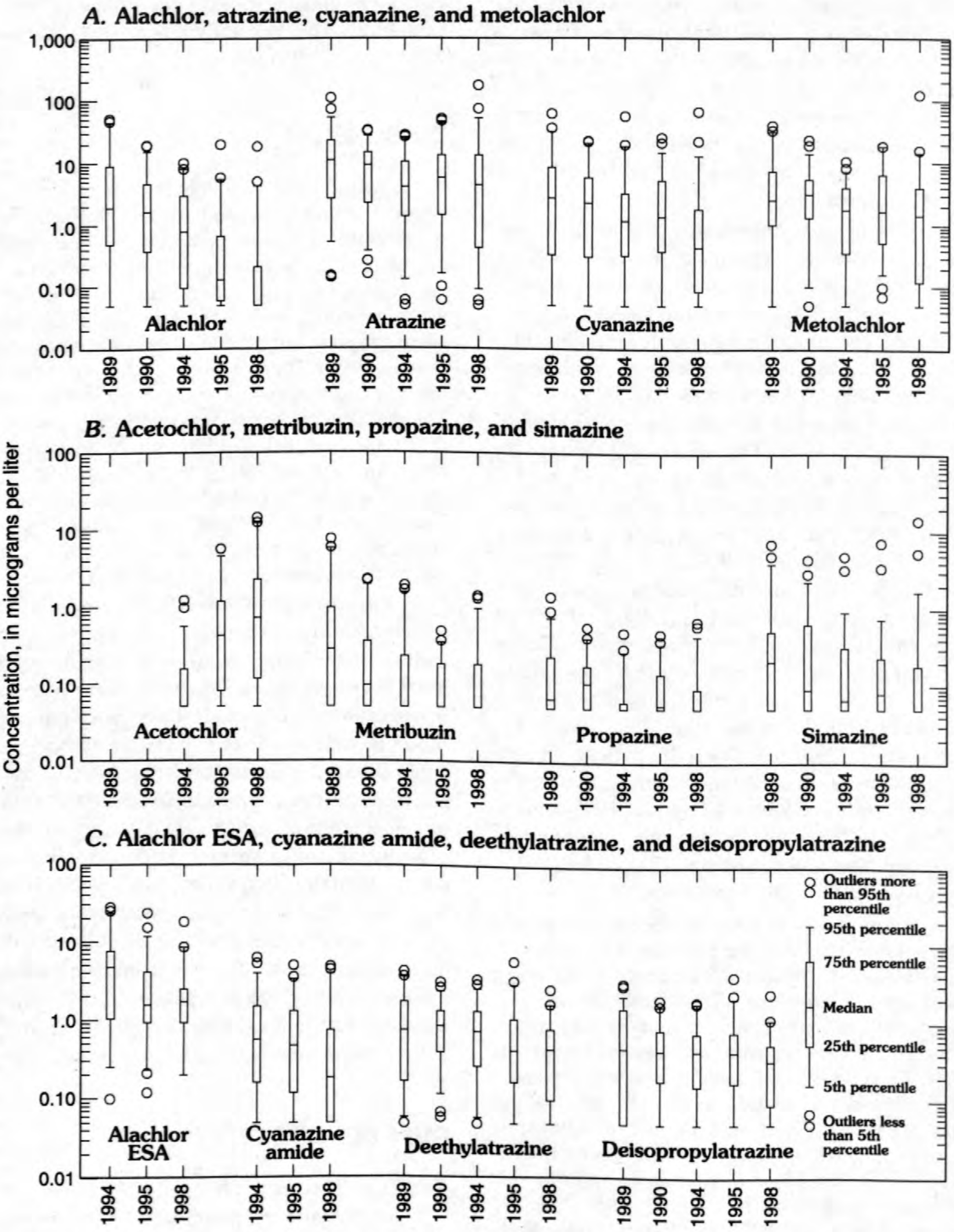

Figure 3. Concentrations in selected Midwestern streams during post-application runoff in 1989–90, 1994–95, and 1998 for *(A)* alachlor, atrazine, cyanazine, and metolachlor; *(B)* acetochlor, metribuzin, propazine, and simazine; and *(C)* alachlor ESA, cyanazine amide, deethylatrazine, and deisopropylatrazine.

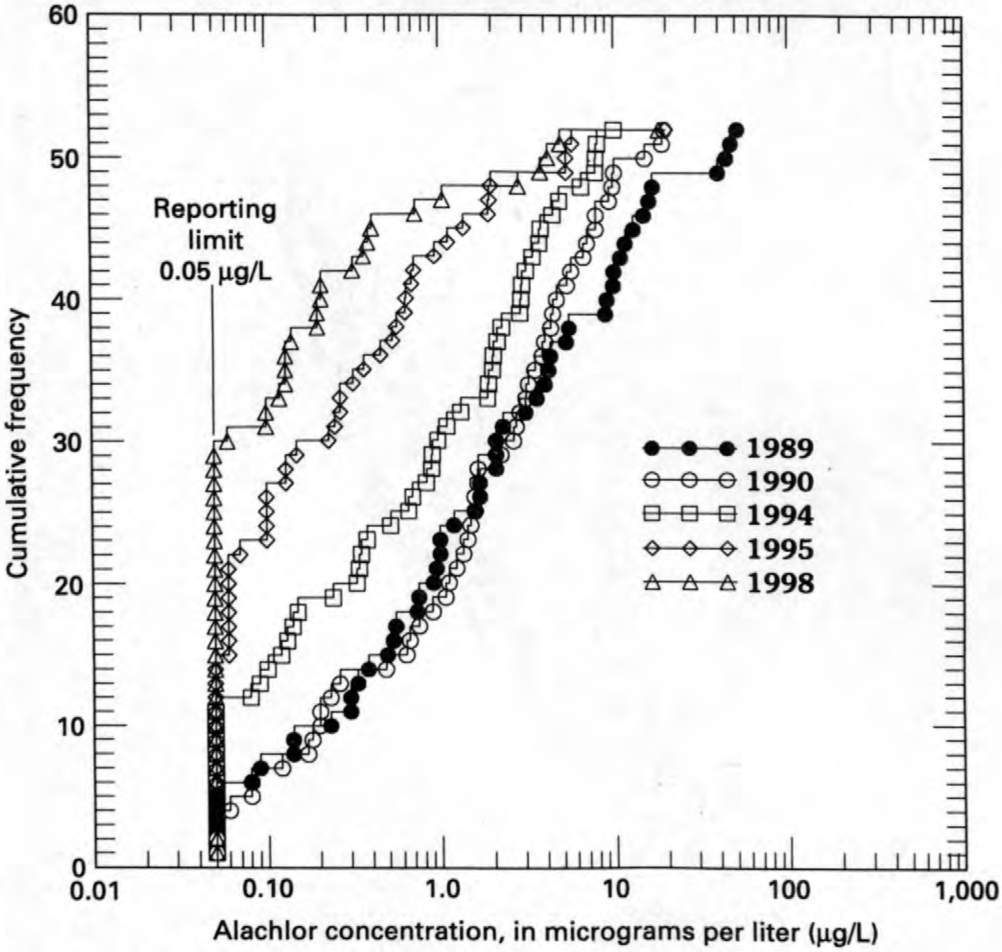

Figure 4. Distribution of alachlor concentrations in post-application runoff samples from Midwestern streams, 1989–90, 1994–95, and 1998.

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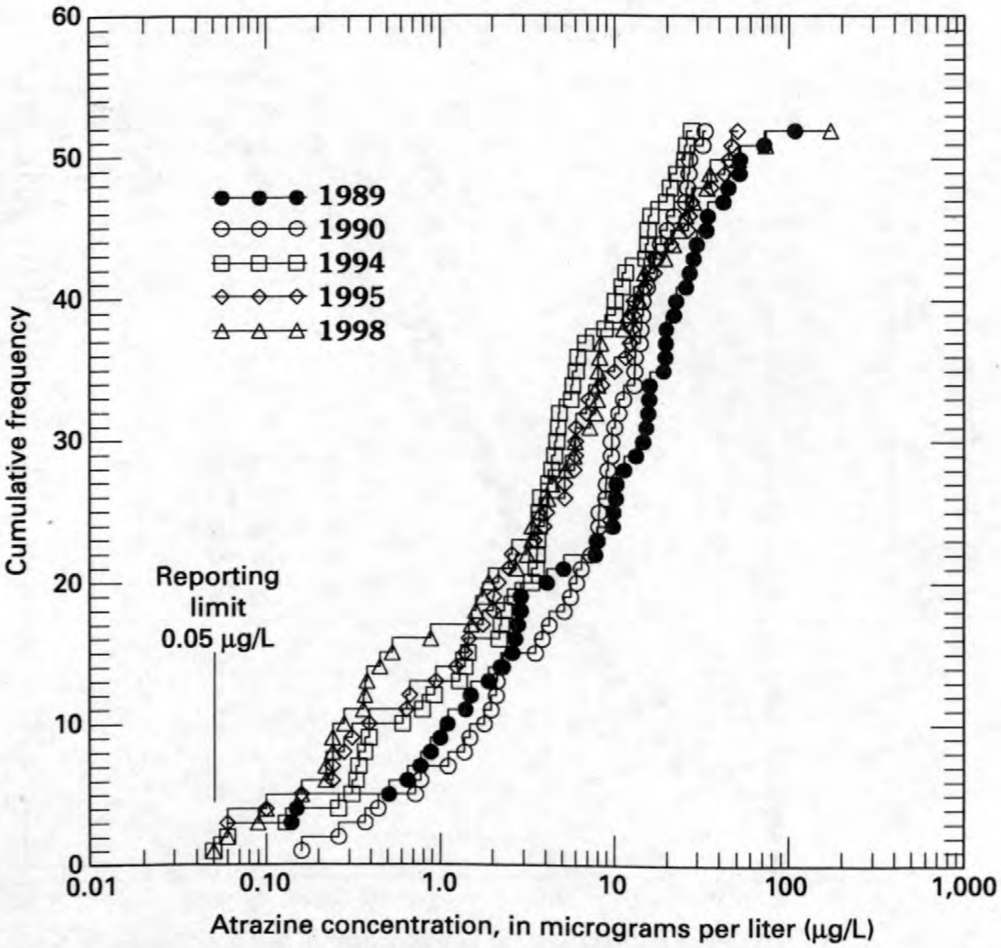

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An Ecological Risk Assessment of the Potential for Herbicide Impacts on Primary Productivity of the Lower Missouri River

by James F. Fairchild, Linda C. Sappington, and David S. Ruessler

ABSTRACT

The Lower Missouri River Basin has been drastically altered due to impoundment, channelization, and conversion of the floodplain to agriculture. Agricultural practices have led to both ecological and human health concerns related to herbicide use. We conducted a study to perform an ecological risk assessment of the potential impacts of herbicides on aquatic plant communities of the Lower Missouri River. Sixteen herbicides (atrazine, metribuzin, simazine, cyanazine, alachlor, metolachlor, chlorsulfuron, metsulfuron, triallate, EPTC, trifluralin, diquat, paraquat, dicamba, bromoxynil, and 2,4-D) were tested using one species of algae (*Selenastrum capricornutum*) and one floating macrophyte (*Lemna minor).* These herbicides represented nine chemical classes and several modes of action and were chosen to represent major current uses in the U.S. The triazinone herbicide metribuzin and the sulfonylurea herbicides chlorsulfuron and metsulfuron were highly toxic but do not necessarily represent a large aquatic risk due to the low rates of application. Bromoxynil, dicamba, 2,4-D, and EPTC exhibited low toxicity. Diquat, paraquat, triallate, and trifluralin were relatively toxic but exhibit low environmental mobility. Cyanazine, alachlor, atrazine, and metolachlor pose the greatest risk to aquatic plants. However, a comparison of these toxicity data to published information concerning application rates, chemical fate, and measured environmental concentrations indicates that adverse impacts of herbicides on non-target aquatic plant communities of the Lower Missouri River are unlikely. However, human health concerns will continue to regulate the use of these chemicals.

INTRODUCTION

Minimum tillage practices have led to increased use of herbicides in agriculture. In 1994 over 97% of corn and soybean acreages were treated with one or more herbicides (over 192 million pounds total application) in the Midwestern states of Illinois, Indiana, Iowa, Minnesota, Missouri, Nebraska, and Ohio (USDA, 1995). Herbicide runoff is a major component of non-point source agricultural pollution in aquatic systems. Water quality monitoring programs have determined that herbicide contamination of surface waters (Richards and Baker, 1993; Goolsby and Battaglin, 1995; Coupe et al., 1995), ground waters (Mills and Thurmon, 1994) and rainfall (Nations and Hallberg, 1992) are common and widespread. For example, a U.S. Geological Survey study of the Mississippi River Basin from 1989-1992 measured detectable levels of herbicides in 98% of post-plant samples analyzed (Goolsby and Battaglin, 1995). A survey of Lake Erie tributaries from 1983 to 1991 determined that herbicide contamination of rivers was widespread and especially common during the growing season of April to August (Richards and Baker, 1993).

The frequency and spatial extent of herbicide contamination of surface waters have led to numerous concerns about impacts on non-target aquatic organisms. The majority of herbicides exhibit low toxicity to fish and invertebrates (Mayer and Ellersieck, 1986). However, herbicides are toxic to a variety of

Table 1. Classification, mode of action, and use levels of herbicides tested with *Selenastrum* and *Lemna*.

Herbicide	Class	Mode of Action[1]	U.S. Annual Usage (#/yr X 10^3)[2]	Range of Use Rates (kg/ha)[1]	U.S. Annual Use Rank[2]
atrazine	triazine	inhibits photosynthesis	64,236	1.1 - 2.2	1
alachlor	acetanilide	inhibits protein synthesis	55,187	1.7 - 6.7	2
metolachlor	chloracetanilide	inhibits protein synthesis	49,713	1.4 - 4.5	3
EPTC	thiocarbamate	unknown	37,191	2.2 - 6.7	4
2,4,-d (salt)	phenoxy	auxin simulator	33,096	0.3 - 1.1	5
trifluralin	dinitroaniline	inhibits germination processes	27,119	0.6 - 2.2	6
cyanazine	triazine	inhibits photosynthesis	22,894	0.9 - 5.3	7
dicamba	benzoic acid	auxin simulator	11240	0.3 - 0.6	11
metribuzin	triazinone	inhibits photosynthesis	4,822	0.3 - 0.8	15
simazine	triazine	inhibits photosynthesis	3,964	2.2 - 4.5	20
triallate	thiocarbamate	inhibits cell elongation/division	3,509	1.1 - 1.7	22
paraquat (salt)	pyridine	free-radical formation	3,025	0.3 - 1.1	23
bromoxynil	benzonitrile	inhibits photosynthesis	2,627	0.2- 0.6	27
diquat	pyridine	free radical formation	166	0.4 - 0.6	77
chlorsulfuron	sulfonylurea	inhibits amino acid synthesis	77	.009 - .018	80
metsulfuron	sulfonylurea	inhibits amino acid synthesis	41	.004 - .012	87

[1] from Herbicide Handbook (1994).
[2] from Gianessi and Puffer (1991) and from USDA (1995).

aquatic plants including submerged macrophytes and algae (Fairchild et al., 1997; 1998) and have been suggested as potential causes for losses of aquatic plants in Midwestern streams (Menzel et al., 1984) and Chesapeake Bay (Forney and Davis, 1981; Kemp et al., 1985). Primary production of facultative and obligate aquatic plants is the primary energy basis for most aquatic ecosystems. Thus, herbicide impacts on primary producers are expected to have both direct and indirect impacts on the health of aquatic ecosystems.

The objective of this study was to conduct an ecological risk assessment for herbicides found in the Lower Missouri River Basin. Toxicity tests were conducted and interpreted in relation to application rates, chemical fate, and measured environmental concentrations to determine if herbicides represent an ecological risk to aquatic primary productivity of the river.

MATERIALS AND METHODS

Sixteen herbicides were tested using the standard test species of *Selenastrum capricornutum* and *Lemna minor*. Basic procedures and test conditions are presented in Fairchild et al. (1997). Herbicides were chosen to represent chemicals of current major agricultural use (Giannesi and Puffer, 1991; USDA, 1995) across a wide range of general classes and modes of action (Herbicide Handbook, 1994) (Table 1). Physical and chemical characteristics of the herbicides are

Table 2. Comparison of use rates, environmental behavior, runoff potential, and exposure potential based on runoff and aqueous half-life.

Herbicide	Use Rate (kg/ha)[1]	K_{oc}[2]	Soil $t_{1/2}$ (days)[2]	Runoff potential[2]	Aqueous $t_{1/2}$ (days)[3]	Exposure potential
Atrazine	1.1	160	60	high	47-193	high
Alachlor	2.2	190	14	medium	21	high
Metolachlor	2.1	200	20	high	75	high
EPTC	4.6	280	30	medium	no data	medium
2,4-D	0.4	20	10	high	1-7	low-medium
Trifluralin	0.9	1,400	60	medium	1-2	low
Cyanazine	2.3	168	20	high	103	high
Dicamba	0.4	2	14	high	30	high
Metribuzin	0.3	41	30	high	4	low
Simazine	0.7	138	75	high	30	high
Triallate	1.4	3,600	60	low	1	low
Paraquat	0.7	100,000	3,600	low	1-2	low
Bromoxynil	0.3	100	14	high	1-2	low
Diquat	0.5	100,000	3,600	low	1-2	low
Chlrosulfuron	0.01	1	30	high	no data	medium
Metsulfuron	0.003	61	61	high	29	medium

[1] from USDA (1995).

[2] From Wauchope, D.L.(1992), Interim Properties Database.

[3] Data sources: alachlor, 550 ha reservoir, Spalding et al. (1994)
atrazine, 0.1 ha ponds, Fairchild et al. (1994) and 550 ha reservoir, Spalding et al. (1994)
bromoxynil 0.01 ha pond, Muir et al. (1991) and 100 ml bacterial culture, Golovleva et al. (1988)
cyanazine, 550 ha reservoir, Spalding et al. (1994)
dicamba, 0.1 ha ponds, Scifres et al. (1973)
diquat, variety ecosystems, Reinert and Rodgers (1987)
metolachlor 550 ha reservoir, Spalding et al. (1994)
metribuzin, 0.1 ha pond, Fairchild et. al, 1996
metsulfuron, in-situ enclosures, Thompson et al. (1992)
paraquat, variety of habitats; Calderbank 1972 and 2 ha pond Way et al. (1970)
simazine, none given, Reinert and Rodgers (1987)
triallate, estimated based on K_{oc}
trifluralin, 30 L microcosm, Isensee et al. (1979) and 1 L microcosm, Huckins et al. (1986)
2,4-D, various reservoirs, Reinert and Rodgers (1987)

presented in Table 2. Herbicide application, transport, and exposure data was obtained from publications by the Water Resources Division, USGS (i.e. Goolsby et al., 1993; Goolsby and Battaglin, 1995; Coupe et al., 1995). Additional exposure estimates were calculated based on extensive literature that indicates that 1% is a proximate estimate of herbicide losses from agricultural fields (Baker et al., 1976; Wauchope, 1978). For screening purposes it is often assumed that 1% of applied chemical in a 10 ha watershed could enter a 1 ha basin with water 1-m deep (Jenkins et al., 1989). This is accuratefor herbicides that are relatively water soluble and

Table 3. Toxicity data and calculated risk for sixteen herbicides based on assumption of 1% herbicide runoff.

Herbicide	Use Rate (kg/ha)[1]	*Lemna* EC50 (ug/L)	*Selenastrum* EC50 (ug/L)	1% exposure estimate (ug/L)[2]	Desktop Risk Estimate[3]
atrazine	1.1	153	235	11	0.07
alachlor	2.2	198	10	22	2.20
metolachlor	2.1	343	77	21	0.27
EPTC	4.6	7,512	6,451	46	0.05
2,4-D salt	0.4	>100,000	41,772	4	0.01
trifluralin	0.9	170	673	9	0.05
cyanazine	2.3	705	27	23	0.85
dicamba	0.4	>100,000	36,375	4	0.00
metribuzin	0.3	37	43	3	0.08
simazine	0.7	166	1,240	7	0.04
triallate	1.4	>10,000	47	14	0.30
paraquat	0.7	51	559	7	0.14
bromoxynil	0.3	8,065	7,762	3	0.00
diquat	0.5	18	80	5	0.28
chlorsulfuron	0.01	0.7	135	0.1	0.14
metsulfuron	0.003	0.4	190	0.03	0.07

[1] from USDA (1995).

[2] 1% exposure estimate was based on 1% of chemical loss into a 1 ha basin (1m deep) receiving runoff from a 10 ha watershed.

[3] Risk rate calculated as 1% exposure estimate divided by most sensitive plant EC50 (*Lemna* or *Selenastrum*).

have a K_{oc} value less than 1,000. However, the 1% runoff factor overestimates dissolved losses of chemicals for many ionic (e.g. diquat and paraquat) or hydrophobic chemicals (e.g. trifluralin and triallate). These chemicals can be transported sorbed to eroded particles and eventually enter aquatic ecosystems. Thus, for desktop screening purposes in this study the 1% estimate was used. Risk was calculated by dividing the potential exposure concentration by the laboratory EC50 value. Risk values that exceeded 0.1 were assumed to indicate risk. For more accurate risk assessments mathematical models can be used to estimate exposure (e.g. SWRRB; PRZM, etc.) (Jenkins et al., 1989). However, models are more appropriate for use in edge-of field calculations and are not appropriate for use in predicting concentrations in large river systems. Thus, we relied on measured environmental data for the final herbicide risk assessment for the Lower Missouri River.

RESULTS AND DISCUSSION

The toxicities (96h EC50s) of the sixteen herbicides are presented in Table 3 along with representative application rates, exposure estimates based on the 1% runoff desktop calculation, and the calculated desktop risk

Table 4. Risk of herbicides to aquatic plants in Missouri River in relation to total use, percent transported, frequency of detections, and peak concentrations of herbicides measured in water at Hermann, MO. Measured risk values exceeding 0.1 indicate risk.

Herbicide	Herbicide Application Missouri Basin (metric tons)[1]	Total Herbicide Transported Missouri River (% applied)[1]	Frequency detection (n=44 samples)[1]	Measured concentration mean and range at Hermann, MO (ug/L)[2]	Most sensitive plant EC50 (ug/L)[3]	Measured risk[3]
Atrazine	6,280	1.1	100%	3.0 (0.4-5.7)	153	0.04
Alachlor	4,660	0.2	96%	0.4 (0.07-0.92)	10	0.09
EPTC	4,180	<0.1	13%	<0.05	6,451	0
Metolachlor	3,490	0.71	100%	1.0 (0.1-2.0)	77	0.03
Trifluralin	2,350	<0.1	47%	<0.05	170	0
Cyanazine	1,970	1.6	96%	1.6 (0.2-4.7)	27	0.17
Metribuzin	207	0.7	36%	0.07 (<.05-.19)	37	0
Simazine	52	2.1	86%	<0.05	166	0
Dicamba[4]	nm	nm	nm	nm	nm	0

[1] 1989 data from Goolsby and Battalgin (1993).
[2] Mean and range of 19 samples from April-June of 1991 from Coupe et al. (1995).
[3] Actual risk calculated as maximum measured concentration divided by most sensitive plant EC50.
[4] Dicamba not measured (nm) at Hermann, MO but categorized as no risk due to low toxicity (Table 3).

estimate. The triazine (atrazine, cyanazine, metribuzin, and simazine), acetanilide (alachlor and metolachlor), sulfonylurea (chlorsulfuron and metsulfuron), dinitroanaline (trifluralin), and pyridine (diquat and paraquat) classes of herbicides exhibited high toxicity to one or both plant species, with toxicities ranging from 0.4 to 198 ug/L (96h EC50) (Table 3).

One of the thiocarbamate herbicides (triallate) was toxic to *Selenastrum* at 47 ug/L (96h EC50) but was non-toxic to *Lemna.* In contrast four of the herbicides (bromoxynil, benzonitrile class; dicamba, benzoic acid class; 2,4-D, phenoxy class; and EPTC, thiocarbamate class) were relatively non-toxic and exhibited toxicity values greater than 6,000 ug/L (96h EC50) for both species.

Neither *Selenastrum* nor *Lemna* was uniformly more sensitive to the list of herbicides tested. *Selenastrum* was most sensitive to half (eight) of the herbicides, whereas *Lemna* was more sensitive to the other eight chemicals (Table 3). Major departures in sensitivity of both *Selenastrum* and *Lemna* occurred between chemicals within individual classes of the triazine, acetanilide, and thiocarbamate classes of herbicides (Tables 1 and 3).

Estimated exposure concentrations, based on the assumption of 1% runoff of chemical from a 10 ha watershed into a 1-m deep 1 ha basin, vary in direct proportion to the application rate due to the use of spatial constants in the calculation. The results of this risk assessment indicated that alachlor (risk=2.2), cyanazine (risk=0.85), triallate (risk=0.30), diquat (risk=0.28), metolachlor (risk=0.27), paraquat (risk=0.14), chlorsufuron (risk=0.14), metribuzin (risk=0.08), atrazine (risk=0.07), and metsulfuon (risk=0.07) pose the greatest risk to aquatic plants (Table 3). However, these risk estimates do not factor in the true degree of use, environmental mobility, and environmental half-life of the chemicals.

The risk assessment was refined by comparing plant sensitivity to measured herbicide concentrations from the Missouri River (Table 4). Major use chemicals such as atrazine, metolachlor, cyanazine, and alachlor are detected in over 95% of samples taken on an annual cycle (Table 4). However, neither average nor peak concentrations of herbicides in the Lower Missouri River exceed concentrations known to impact aquatic plants. Those chemicals that are most frequently detected are those that are widely applied, have low K_{oc} values, and high environmental persistence (Tables 2, 3, and 4). Cyanazine (actual risk 0.17) was the only herbicide found to exceed the 0.1 risk criterion (Table 4). However, this was based on the maximum measured concentration which is extremely conservative.

Alachlor, atrazine, and metolachlor represent lower, yet measurable risk. The remainder of the chemicals do not represent detectable risk due to low use, mobility, and persistence. Metribuzin, triallate, chlorsulfuron, metsulfuron, trifluralin, diquat, and paraquat are all highly toxic to aquatic plants. However, the relative ecological risk of these herbicides is reduced due to either lower application rates (e.g. metribuzin; chlorsufuron; metsulfuron), rapid dissipation rates (e.g. metribuzin and triallate), or sorption potential (e.g. triallate, trifluralin, diquat, and paraquat).

The data indicates that herbicides are unlikely to cause adverse impacts on primary productivity of the Lower Missouri River. Existing environmental data indicates that primary concerns for herbicides would occur in areas riparian areas receiving direct over-spray or excessive runoff (e.g. 1-10% of application) which may include ephemeral wetlands and farmed wetlands that exist in or near agricultural fields as temporary, seasonally-flooded habitats (Peterson et al., 1984). These data are not reflected in the current USGS data set at Hermann, MO which is intended to integrate exposures in the main river. Herbicide exposures and impacts in wetlands have not been widely studied and therefore represent a remaining data gap.

Although the ecological risk of herbicides in the Lower Missouri River is low, the maximum recommended level of atrazine in drinking water (3 ug/L) is frequently exceeded. Thus, human health concerns may drive future changes in the uses of these herbicides.

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Atmospheric Deposition of Nitrogen in the Mississippi River Basin

By Gregory B. Lawrence, Donald A. Goolsby and William A. Battaglin

ABSTRACT

Atmospheric deposition of nitrogen has been cited as a major factor in the overfertilization of forests (often termed nitrogen saturation) in the northeastern United States and as a contributor to the eutrophication of coastal waters, including the Gulf of Mexico near the mouth of the Mississippi River. Sources of nitrogen emissions and the spatial patterns of nitrogen deposition within the Mississippi River Basin, however, have not been fully assessed. The objectives of this study were to (1) quantify the spatial distribution of atmospheric nitrogen deposition throughout the Mississippi River Basin, and (2) relate the locations of emission sources to the spatial deposition patterns to infer patterns of atmospheric transport. Data collected through the NADP/NTN (National Atmospheric Deposition Program/National Trends Network) and CASTNet (Clean Air Status and Trends Network) were used for this analysis.

The highest rates of wet deposition of NO_3were in the northeastern part of the basin where electric utility plants are concentrated, whereas the highest rates of wet deposition of NH_4 were in Iowa, near the center of intensive agricultural activities in the midwest. The lowest rates of atmospheric nitrogen deposition are on the (windward) side of the basin, which suggests that most of the nitrogen deposited within the western basin is derived from internal sources. Atmospheric transport eastward across the basin boundary is greater for NO_3 than NH_4, but a significant amount of NH_4 is likely to be transported out of the basin through the formation of $(NH_4)_2SO_4$ and NH_4NO_3 particles, which greatly increases the atmospheric residence time of NH_4. This process may be a factor in the transport of nitrogen from the midwest to upland forest regions in the Northeast, such as the western Adirondack region of New York, where NH_4 constitutes 40 percent of total wet nitrogen deposition.

INTRODUCTION

Through human activities, the deposition of biologically available nitrogen from the atmosphere has increased to rates that are of similar magnitude to rates of natural fixation of N_2 (Vitousek and others, 1997). Atmospheric deposition of nitrogen has been recognized as a major factor in the overfertilization of forest ecosystems in the northeastern United States (often termed nitrogen saturation) and the acidification of freshwater lakes and streams (Stoddard, 1994; Aber and others, 1998; Fenn and others, 1998). Atmospheric deposition of nitrogen also has been identified as an important contributor to the eutrophication and hypoxia of Chesapeake Bay (Magnien and others, 1995) and the Gulf of Mexico (Dinnel, 1997). As part of an effort to evaluate possible causes of hypoxia in the Gulf of Mexico, a working group under the direction of the Committee on the Environment and Natural Resources (CENR) of the White House Office of Science and Technology was established in 1997 to quantify nitrogen cycling within the Mississippi River Basin; a task that included an assessment of nitrogen deposition from the atmosphere. The objectives of the atmospheric deposition component of this study were to (1) quantify the spatial distribution of atmospheric nitrogen deposition throughout the Mississippi River Basin and (2) relate locations of emission sources to the spatial deposition patterns to infer patterns of atmospheric transport.

APPROACH

The general approach used in this analysis entailed (1) the application of available data, (2) the development of empirical relations for regions for which data were not available, and (3) the use of information from peer-reviewed research publications.

Deposition of nitrogen from the atmosphere can be categorized as either wet deposition (rain or snow) or as dry deposition (particles or vapor deposited from the atmosphere during periods when precipitation is not falling). Wet deposition is monitored year round at about 200 sites across the United States through the NADP/NTN (National Acid Deposition Program/National Trends Network). Data from NADP/NTN sites within the Mississippi Basin were converted with a GIS (geographic information system) from point coverages to polygons that represent the 133 hydrologic accounting units (areas delineated by drainage divides) of the basin. This process entailed (1) creating a grid of 6.25-km^2 cells, (2) assigning nitrogen deposition values to each cell through inverse distance weighting of the point coverages, and (3) converting the grid coverages to polygons that each represent one of the 133 hydrologic accounting units.

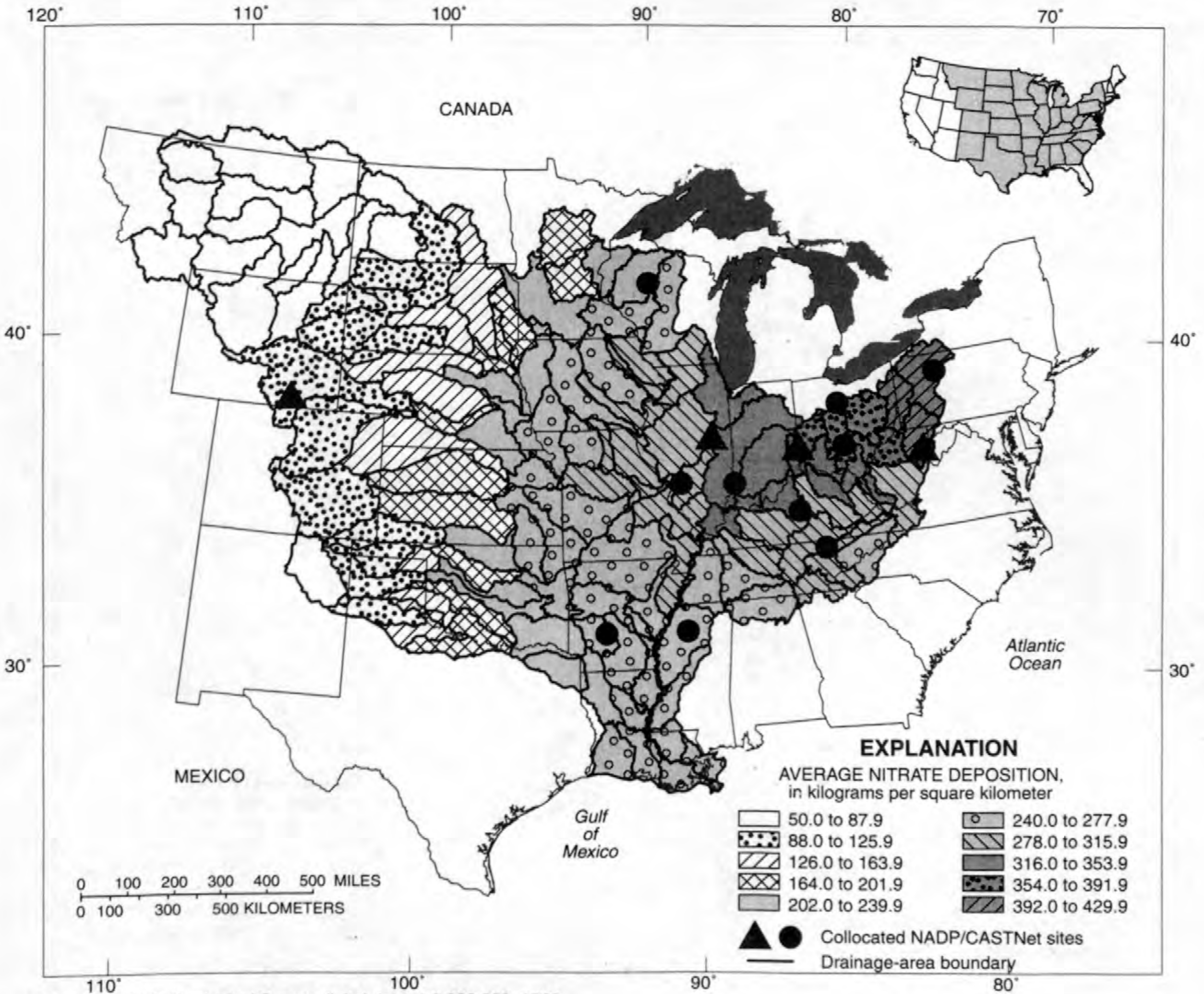
Dry deposition is monitored at about 60 sites through CASTNet (the U. S. Environmental Protection Agency Clean Air Status and Trends Network) through an inferential approach in which dry deposition-rates are obtained from air concentrations (measured with three-stage air samplers) and deposition velocites (the velocity with which gases and particles are deposited onto vegetation surfaces) determined by a mathematical model (Clarke and others, 1997). This approach enables deposition rates to be quantified for NO_3 and NH_4 particles and HNO_3 vapors. Because most of the CASTNet sites are east of the Mississippi River, the spatial distribution of dry deposition data was in adequate for the development of deposition polygons through interpolation. Thus, empirical relations between wet deposition and dry depostion were developed for each of the 14 sites within the Mississippi River Basin at which NADP and CASTNet stations were collocated.

SPATIAL PATTERNS OF DEPOSITION

No trend in the rates of wet deposition of NO_3 or NH_4 during 1984-96 was discerned from values representing the overall basin. The lowest deposition rates for both constituents occurred during the drought years of 1988-89. The highest rates of wet deposition of NO_3 within the basin were in an area that extends from central Ohio eastward to the basin boundary (fig. 1). These rates generally decreased southward and westward from Ohio. The highest rates of wet deposition of NH_4 were centered in Iowa and generally decreased in all directions; the lowest rates were in Montana (fig. 2).

Relations between wet deposition and dry deposition varied considerably among sites, but a positive correlation ($R = 0.42$, $p < 0.01$) was obtained when data from all sites were grouped. For all sites, the average value of total dry deposition (particulate NO_3 and NH_4 plus HNO_3 vapor) divided by the average value of total wet deposition (NO_3 plus NH_4) was 0.47. Because the variability among sites is high, application of this value throughout basin yields an imprecise estimate of dry deposition.

The values for components of wet and dry deposition of nitrogen for sites along a west-to-east transect in the basin for the period December 1, 1992 through November 30, 1993 are shown in figure 3. The deposition of all five components was lowest at the Wyoming site, and total nitrogen deposition at this site was less than half that at the Ohio site. The highest rate of wet deposition of NO_3 was measured at the West Virginia site, whereas the highest rate of wet NH_4 deposition was measured at the Illinois site, and the highest rate of HNO_3 deposition was observed at the Ohio site. The rate of total nitrogen deposition at the Illinois site was more similar to the rate at the West Virginia site than to the rate at the nearby Ohio site. Total dry deposition of nitrogen averaged for all sites, and all seasons, was composed of 81 percent HNO_3 vapor, 16 percent particulate NH_4, and 3 percent particulate NO_3.

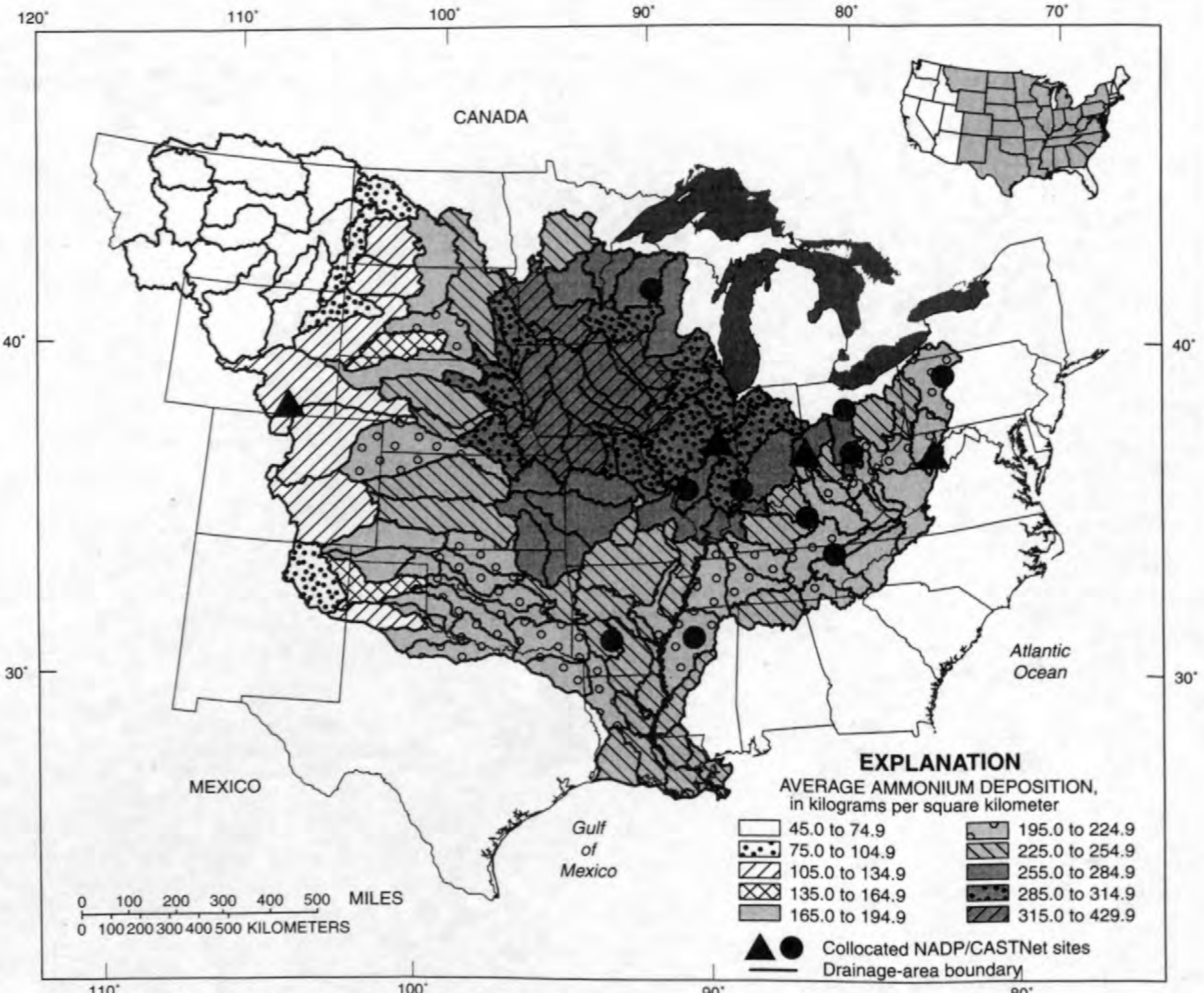


Base from U.S. Geological Survey digital data, 1:2,000,000, 1980
Albers Equal-Area Conic projection
Standard parallels 29°30' and 45°30', central meridian -96°00'

Figure 1. Wet deposition of NO_3, averaged for 1990-96, in the 133 accounting units that make up the Mississippi River Basin. Dry deposition rates for sites indicated by triangles are given in figure 3.

EMISSIONS SOURCES AND ATMOSPHERIC TRANSPORT

The regional patterns of wet deposition of NO_3 and NH_4 within the Mississippi River Basin reflect emissions sources and atmospheric-transport processes. The highest rates of NO_3 deposition were measured in Ohio and Pennsylvania (fig. 1), northeast of the concentration of electric utility plants in southern Indiana and western Kentucky (NAPAP, 1993). Fossil-fuel combustion is a known source of NO and NO_2, which are oxidized in the atmosphere to form HNO_3 vapor and particulate NO_3 (U. S. Environmental Protection Agency, 1997). Although HNO_3 vapor has a high deposition velocity and a relatively short residence time, it can react with other pollutants such as NH_3 to form particles with low deposition velocities that can be transported hundreds of miles. Particulate NO_3 also tends to have a low deposition velocity that facilitates long-range transport. Significant atmospheric transport of nitrogen from midwestern powerplants to the northeastern States has been well established (NAPAP, 1993).



Base from U.S. Geological Survey digital data, 1:2,000,000, 1980
Albers Equal-Area Conic projection
Standard parallels 29°30' and 45°30', central meridian -96°00'

Figure 2. Wet deposition of NH_4, averaged for 1990-96, in the 133 accounting units that make up the Mississippi River Basin. Dry deposition rates for sites indicated by triangles are given in figure 3.

Wet and dry deposition of NH_4 have generally been attributed to NH_3 emissions from high concentrations of livestock and nitrogen fertilization of croplands (Vitousek and others, 1997). Emissions from automobiles can also contribute atmospheric NH_3, but estimates of NH_3 emissions from automobiles did not exceed agricultural sources in the South Coast Air Basin of California, which includes Los Angeles and surrounding developed areas (Fraser and Cass, 1998). The highest rates of wet NH_4 deposition in the Mississippi Basin are centered in Iowa, a predominantly agricultural state.

Unlike the NO and NO_2 released from fossil-fuel combustion, NH_3 is emitted to the atmosphere in a highly water-soluble form that is readily removed from the atmosphere by precipitation As a result, NH_3 tends to be deposited closer to its sources than are other forms of nitrogen. An NH_3 transport model by Asman and van Jaarsvelt (1992) indicated that 46 percent of emitted NH_3 was deposited within 50 km of the source; 40 percent as dry deposition and 6 percent as wet deposition. Results from a separate modeling study (Ferm, 1998) indicated that 49 percent of NH_3 emitted in a 22,000-km^2 region in Sweden was deposited within this region; 21percent as dry deposition and 28 percent as wet deposition.

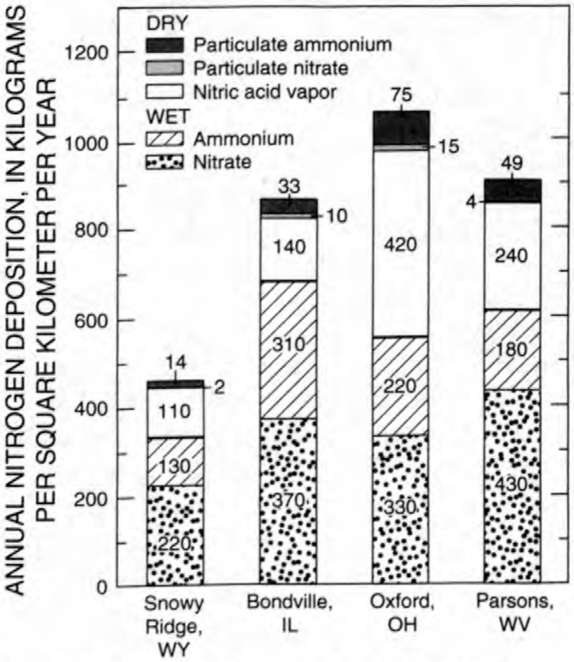


Figure 3. Chemical fractions of wet and dry nitrogen deposition at 4 sites selected along a west-to-east transect across the Mississippi River Basin, December 1992 through November 1993. Sites indicated as triangles on figures 1 and 2.

Transport of NH_3, depends on wind speed and reactions with other pollutants. Although about half of the emitted NH_3 tends to be deposited near its source, reactions with H_2SO_4 and HNO_3 form particulate NH_4, which is transported more readily than NH_3 (Ferm, 1998). High atmospheric concentrations of SO_2 and NO_x in areas where emissions of NH_3 are high can therefore lead to long-range transport of NH_3. Research in the Netherlands has shown that deposition of NH_x beyond 300 km of the source was halved approximately every 450 km; a pattern similar to that of SO_x compounds (Ferm, 1998). Thus, high emissions of SO_2 and NO_x in Illinois, Kentucky, Indiana, and Ohio are likely to enhance the transport of NH_4 from the agricultural regions in the central part of the Mississippi Basin to eastern sections of the basin and beyond. Long-range transport is the most likely explanation for the similarity between wet deposition rates of NH_4 measured in 1996 at NADP stations in Iowa and at Bennett Bridge, N.Y. (Table 1). The New York site is remote from sources of NH_3 emissions, but is located in an area with high rates of acid deposition.

On the basis of NADP and CASTNet data, NH_4 deposition represents about 35 percent of total nitrogen deposition in the Mississippi Basin, but the collection methods of both programs probably contribute to an underestimation of this fraction. Some of the NH_4 collected by NADP buckets may be converted to organic nitrogen through microbial assimilation between the time of deposition and the weekly collection (Vet and others, 1989). Also, the three-stage filter pack used in the CASTNet program is designed to collect NH_4 particles, but not NH_3, whose deposition velocity is about 5 times higher than that of the NH_4 particles (Ferm, 1998). The deposition of gaseous NH_3, therefore, is likely to represent a higher fraction of dry deposition close to sources, than indicated by CASNet measurements.

Table 1. Wet deposition of NH_4 and NO_3 for 1996 in kilograms per square kilometer per year, and the deposition ratio for these two ions.

Site	NH_4	NO_3	NH_4/NO_3
Bennett Bridge, New York	410	690	0.60
McNay Reseach Center, Iowa	280	210	1.3
Big Springs, Iowa	300	360	0.8

CONSIDERATIONS FOR THE NITROGEN BUDGET OF THE MISSISSIPPI RIVER BASIN

Despite the uncertainties of dry-deposition estimates, the data obtained by CASTNet are sufficiently reliable to indicate that, in general, dry-deposition rates are (1) positively correlated with wet-deposition rates, and (2) of similar magnitude to wet deposition rates. This information can be used in conjunction with NADP data to estimate total deposition of nitrogen (wet plus dry) in subregions of the

NADP data to estimate total deposition of nitrogen (wet plus dry) in subregions of the basin for the purpose of developing nitrogen budgets. The wet and dry deposition of NO_3 compounds should be considered a budget input because these compounds originate largely from the combustion of fossil fuels, and otherwise would be unavailable for biological utilization. Dry deposition of HNO_3 and NO_3 can be approximated throughout the basin by multiplying the wet deposition of NO_3 by 0.70 (dry deposition of NO_3 divided by wet deposition of NO_3, for the 14 sites at which wet and dry deposition stations were collocated). A value of 0.75 was obtained by Dinnel (1998) for this fraction of dry deposition of HNO_3 and NO_3 from CASTNet and NADP data from 1990-92.

Wet and particulate NH_4 represent a significant fraction of atmospheric nitrogen deposition throughout the basin. Most of the NH_4 deposition within the basin is likely to be from internal sources because (1) the region of highest NH_3 deposition is in the center of the basin, (2) half or more of emitted NH_3 is deposited within 300 km of the source, and (3) the lowest deposition is on the windward (western) side of the basin. The high variability of NH_4 deposition within the basin indicates, however, that some subbasins are net sources, whereas other subbasins are net sinks. Because most of the NH_4 emissions are either directly or indirectly the result of crop fertilization, budget estimates that include nitrogen fertilizer as an input would overestimate total inputs if atmospheric deposition of NH_4 was included. For purposes of the nitrogen budget, the atmospheric deposition of NH_4, therefore, should be considered an internal transformation rather than a basin input.

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Isotopic Tracing of Nitrogen Sources and Cycling in the Mississippi River Basin

By Carol Kendall, William A. Battaglin, Gilbert Cabana, Cecily C. Chang, Steven R. Silva, Stephen D. Porter, Donald A. Goolsby, Donald H. Campbell, Richard P. Hooper, and Christopher J. Schmitt

ABSTRACT

As part of five pilot studies, several different isotopic approaches for tracing nutrient sources, land uses, and nitrogen cycling processes that are contributing nitrogen to the Gulf of Mexico are being evaluated. Analysis of nitrate for both $\delta^{15}N$ and $\delta^{18}O$ enhances our ability to distinguish between variations in $\delta^{15}N$ due to mixing of sources and variations due to denitrification. Analysis of organic matter for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ provides information on the terrestrial versus aquatic origin of the material. The $\delta^{15}N$ of fish collected at large-river are evaluated as integrators of local variability in nitrogen sources. Preliminary data from these fish suggest that source signatures are being substantially overprinted by other processes at these sites.

INTRODUCTION

High nitrate levels in streamwater in the Mississippi River Basin pose potential problems for water supplies in the region, affect local ecosystem health, and are suspected of causing a large hypoxic zone in the Gulf of Mexico each summer. We are investigating several different isotopic approaches for tracing nutrient sources, land uses, and nitrogen (N) cycling processes that are contributing N to the Gulf of Mexico. In particular, we are evaluating the relative merits of measurements of the nitrogen and oxygen isotopic compositions of nitrate, and the carbon, nitrogen, and sulfur isotopic compositions of particulate organic matter (POM), dissolved organic matter (DOM), algae, fish, and invertebrates as environmental indicators.

STUDY DESIGN

Five pilot studies are in progress with stream samples collected as part of the U. S. Geological Survey's National Stream Quality Accounting Network (NASQAN) and National Water Quality Assessment (NAWQA) monitoring programs, and at Biomonitoring of Environmental Status and Trends (BEST) and National Contaminant Biomonitoring Program (NCBP) sites. To date, only about half of the collected samples have been analyzed for stable isotopes.

Nitrate samples were collected on exchange resins and analyzed for stable isotopic composition using the Silva and Chang methods described in Kendall (1998). The nitrogen and oxygen stable isotopic compositions (denoted $\delta^{15}N$ and $\delta^{18}O$, respectively) of nitrate are reported in units of permil (parts-per-thousand, denoted ‰) relative to the reference standards Air and V-SMOW, respectively. Organic samples were dried, homogenized, combusted, and analyzed by continuous-flow mass spectrometry. The carbon, nitrogen, and sulfur stable isotopic compositions (denoted $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$, respectively) of organic samples are reported in permil relative to the reference standards V-PDB, Air, and Cañon Diablo Troilite, respectively.

Below are descriptions of the types of samples collected for the 5 pilot studies:

1. Samples were collected during selected periods in 1996-98 from 7 sites on major tributaries, and at 17 sites on smaller rivers draining basins dominated by either row crop

agriculture, animal production, urban, or undeveloped land uses (Battaglin and others, 1997). Nitrate was analyzed for $\delta^{18}O$ and $\delta^{15}N$. Particulate organic matter (POM) and dissolved organic matter (DOM) were analyzed for $\delta^{13}C$ and $\delta^{15}N$, with a subset analyzed for $\delta^{34}S$.

2. Fish samples collected in 1986 and 1995 from some 50 locations within the Mississippi basin (and about 120 sites elsewhere in the USA) were analyzed for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$. We investigated the relations among total nitrogen concentration in the water, the $\delta^{15}N$ value of biota, and watershed land use, using the isotopic compositions of fish as integrators of the isotopic signal of riverine biota. Fish representing two trophic levels were collected and analyzed: invertebrate-eating fish (mainly carp and catostomids) and fish-eating fish (several species of bass).

3. POM samples were collected biweekly to monthly for over a year (1996-98) from some 15 sites in the basin (and at the rest of the NASQAN sites) and analyzed for $\delta^{13}C$ and $\delta^{15}N$; a subset were analyzed for $\delta^{34}S$. The isotopic compositions of POM provide information about the sources of the particulates (e.g., terrestrial detritus versus *in situ* production in the water column).

4. Intersite isotopic variability in POM and benthic algae (periphyton) samples was assessed by a NAWQA synoptic study conducted during August 1997 along 70 smaller tributaries in the basin. All samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$; a subset were analyzed for $\delta^{34}S$

5. Archived fish and invertebrate samples collected at selected NAWQA indicator sites (moderate-sized basins with homogeneous land use) from mid-1995 through 1998 are being analyzed now for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ to better understand the relations between land use and the isotopic compositions of prospective environmental isotopic indicators and integrators.

THEORETICAL BASIS

Many studies have shown that nitrate isotopes can be effective tools for distinguishing among potential sources of N in agricultural areas (Kendall, 1998) In particular, fertilizer and animal waste (including human waste) have very different $\delta^{15}N$ values (0 ± 2‰ versus +15 ± 5‰), so that the relative contributions from these sources can often be estimated by measuring the $\delta^{15}N$ of the nitrate in streams or groundwater. However, biogeochemical recycling of nitrate (especially denitrification) can cause large changes in $\delta^{15}N$, making such estimates problematic.

The first attempt to use $\delta^{15}N$ of nitrate to estimate sources of nitrate in major streams in the Mississippi basin (Kohl and others, 1971) elicited such a storm of protest from the soil science community (Hauck and others, 1972) that it has not been attempted again until now. Most of the criticism was aimed at the difficulty of determining source information from the $\delta^{15}N$ values of stream nitrate when the nitrate has been affected by denitrification. Analysis of nitrate for both $\delta^{15}N$ and $\delta^{18}O$ provides an enhanced ability to distinguish mixing from denitrification.

The theoretical basis of the " biomass isotope" studies is that the isotopic compositions of plankton and non-N fixing plants mainly reflect, to a large extent, the isotopic compositions of the dissolved N, C, and S in the environment that is being utilized by the biota and subsequently modified by various possible fractionating mechanisms in the plants. The isotopic compositions of the primary producers are then reflected by higher level organisms such as invertebrates and fish, as modified by mixed diets, trophic enrichments, and temporal/spatial variability in feeding behaviors. Hence, the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values of plants and organisms in the environment will reflect the interplay of " source" signatures (e.g., from agriculture, livestock, urban, atmospheric, soil), geochemical " process" signatures (e.g., resulting from denitrification, methane oxidation, sulfate reduction), and biochemical metabolic and catabolic fractionations (e.g., trophic enrichments, turnover rates, etc).

RESULTS AND DISCUSSION

Nitrate samples collected during the early spring through summer (the fall samples are currently being analyzed) show a 16‰ range in $\delta^{15}N$ and a 20‰ range in $\delta^{18}O$. Low-nitrate urban and undeveloped areas generally show the highest $\delta^{18}O$ and lowest $\delta^{15}N$ values, with values in the range expected for atmospheric nitrate and nitrate fertilizers. Otherwise, most other samples have $\delta^{15}N$ values that fall within the ranges expected for microbial nitrate derived from ammonium fertilizer, soil, and manure nitrogen sources.

The classic way to distinguish between mixing and recycling processes as major controls on the δ values and nitrate (NO_3) concentrations is to make plots of the δ values versus $1/NO_3$ and $\ln NO_3$. Linear trends on such diagrams indicate mixing and recycling, respectively, because mixing relations are hyperbolic and denitrification causes an exponential increase in $\delta^{18}O$ and $\delta^{15}N$ with decreasing nitrate concentrations (Kendall, 1998). Preliminary evaluation of data indicates substantial seasonal variations in δ values at most NAWQA indicator sites. The variability at some sites appears to be controlled mainly by mixing, but others show complicated mixtures of processes. Comparison of the nitrate data with other isotope data from POM or fish samples may help constrain the extent of possible riverine recycling of nutrients.

Preliminary $\delta^{18}O$ and $\delta^{15}N$ values for nitrate from large river (NASQAN) sites show that the values for each site in the upper Mississippi basin (Mississippi River at Clinton, Ohio River near Grand Chain, and Missouri River at Hermann) appear to cluster in distinct areas, and have a larger variation in $\delta^{18}O$ than in $\delta^{15}N$. Samples from Mississippi River at Thebes, which are a mixture of water from Clinton and Hermann, exhibit a large shift to lighter $\delta^{18}O$ and $\delta^{15}N$ values, possibly indicating the input of fertilizer from the basins in Eastern Iowa and Illinois that flow into the Mississippi between Clinton and Thebes. Samples from the Mississippi River at St. Francisville lie between those from Thebes and Grand Chain, as expected. More data will be required to confirm these observed patterns.

Samples collected from the Mississippi River at Thebes (MRTB) on 4/16/98, the Ohio River at Grand Chain (ORGC) on 4/23/98, and the Mississippi River at St. Francisville (MRSF) on 4/30/98 constitute a nearly Lagrangian sample set. The discharge, chloride load, $\delta^{15}N$, and $\delta^{18}O$ of the MRSF samples are consistent with simple mixing of the MRTB and ORGC waters. The combined discharge associated with the MRTB and ORGC samples is 103% of the discharge associated with the MRSF sample, and the chloride load calculated from these two samples is 92% of the chloride load of the MRSF sample. The $\delta^{15}N$ and $\delta^{18}O$ values predicted by mixing MRTB and ORGC water (+6.5‰ and +8.8‰, respectively) are very close to the values measured in the MRSF sample (+6.5‰ and +8.3‰, respectively). However, the nitrate and total N loads for the MRSF sample (6,070 and 7,495 tonnes/day) are only 83% of the sum of the nitrate loads and 70% of the sum of the total N loads in the MRTB and ORGC samples.

The mechanism for this loss of N is unclear. If the missing nitrate had been lost via progressive denitrification along the river reach between Grand Chain and St. Francisville, the $\delta^{15}N$ and $\delta^{18}O$ values of the remaining nitrate should have increased; however, there is no evidence for this in the isotopic compositions. Other possible processes that could account for the observed chemical and isotopic data include assimilation by aquatic plants (a process that generally causes minimal fractionation) or dilution by mixing with groundwater with a lower nitrate concentration but roughly similar isotopic composition. This analysis could be improved if samples were periodically collected from NASQAN stations using a Lagrangian strategy.

The C/N of the POM collected at NASQAN sites was generally in the range of 5 to 10, indicating that very little of the POM is terrestrial in origin; hence the isotopic compositions reflect processes in the water column. The $\delta^{13}C$ values ranged from -35 to -17‰, with seasonal variability ranging from 3 to 13‰. The $\delta^{15}N$ values ranged from +1 to +12‰, with seasonal variability ranging from a low of 3‰ (e.g., Missouri River at Hermann) to a high of 8‰ (e.g., Tennessee River at Paducah). Several sites showed a progressive increase in $\delta^{15}N$ over the year. The $\delta^{15}N$ values of POM showed the same general range of values as nitrate, indicating that assimilation of nitrate caused minimal fractionation.

The $\delta^{15}N$ of invertebrate-feeding fish (mostly carp and catostomids) which varied from +9 to +18‰ among sites, was significantly ($P<0.01$) related to total N. A similar relation was found for bass, but the $\delta^{15}N$ values were higher because of their higher trophic position. Preliminary statistical analysis showed little correlation of $\delta^{15}N$ with land use. However, there was a good positive relation of $\delta^{15}N$ of invertebrate-feeding fish and the percent of the watershed " exploited" by urbanization and agriculture.

The observation that fish from watersheds dominated by agriculture and fish from watersheds dominated by urbanization both had high $\delta^{15}N$ values is surprising. We had expected that the fish from agricultural areas would have lower $\delta^{15}N$ values because of the lower $\delta^{15}N$ values of the applied fertilizer. The absence of a fertilizer " signal" suggests a greater degree of recycling of the fertilizer N in large watersheds than anticipated. Hence, the $\delta^{15}N$ may not be an effective indicator of the source of nitrogen (fertilizer versus sewage) in large watersheds, but instead an indicator of the extent of N-fractionating processes such as denitrification and volatilization that accompany excess nitrogen loadings.

Since $\delta^{15}N$ has proved to be such an effective indicator of sources in smaller streams, we are now attempting to use archived fish samples from smaller watersheds of variable land use to determine at what scale the source signatures become " overprinted" by post-depositional biogeochemical recycling. NAWQA indicator sites, because they were chosen to have known, moderately homogeneous land uses (agricultural, urban, feedlot, and undeveloped), are an ideal " landscape-scale" set of sites to test whether the isotopic compositions of aquatic organisms collected in medium to large watersheds retain sufficient " source signal" to still provide land-use information.

SUMMARY

Preliminary data from our 5 pilot studies (only about half of the planned samples have been analyzed thusfar for stable isotopes) show encouraging signs that some of these isotope approaches will provide useful new information about sources and cycling of nutrients in the Mississippi Basin. Analysis of nitrate for both O and N isotopes will significantly improve our ability to distinguish between variations in $\delta^{15}N$ caused by mixing of sources versus denitrification. POM appears to be mainly autochthonous and the $\delta^{15}N$ values suggest minimal fractionation of nitrate during assimilation in the big-river sites. The $\delta^{15}N$ values of fish collected at large-river sites suggest that source signatures are being substantially overprinted by other processes at these sites.

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Determination of Chloroacetanilide Herbicide Metabolites in Water Using High-Performance Liquid Chromatography-Diode Array Detection and High-Performance Liquid Chromatography/Mass Spectrometry

By Kenneth A. Hostetler and E. M. Thurman

ABSTRACT

Analytical methods using high-performance liquid chromatography-diode array detection (HPLC-DAD) and high-performance liquid chromatography/mass spectrometry (HPLC/MS) were developed for the analysis of the following chloroacetanilide herbicide metabolites in water: alachlor ethanesulfonic acid (ESA), alachlor oxanilic acid, acetochlor ESA, acetochlor oxanilic acid, metolachlor ESA, and metolachlor oxanilic acid. Good precision and accuracy were demonstrated for both the HPLC-DAD and HPLC/MS methods in reagent water, surface water, and ground water. The average HPLC-DAD recoveries of the chloroacetanilide herbicide metabolites from water samples spiked at 0.25, 0.5, and 2.0 μg/L (micrograms per liter) ranged from 84 to 112 percent, with relative standard deviations of 18 percent or less. The average HPLC/MS recoveries of the metabolites from water samples spiked at 0.05, 0.2, and 2.0 μg/L ranged from 81 to 118 percent, with relative standard deviations of 20 percent or less. The limit of quantitation (LOQ) for all metabolites using the HPLC-DAD method was 0.20 μg/L, whereas the LOQ using the HPLC/MS method was at 0.05 μg/L. These metabolite-determination methods are valuable for aquiring information about water quality and the fate and transport of the parent chloroacetanilide herbicides in water.

INTRODUCTION

The chloroacetanilide herbicides—alachlor, acetochlor, and metolachlor—are an important class of herbicides in the United States. Together with the triazine compounds, chloroacetanilide herbicides compose the majority of pesticides applied in the Midwestern United States for control of weeds in corn, soybeans, and other row crops (Gianessi and Puffer, 1985). Alachlor and metolachlor have been used extensively for more than 20 years, whereas acetochlor application is relatively recent, applied extensively since March 1994 (Kolpin and others, 1996). chloroacetanilide herbicides have been shown to degrade more rapidly in soil than other herbicides, with half-lives from 15 to 30 days, whereas triazine half-lives are typically 30 to 60 days (Leonard, 1988).

Recent studies have reported the occurrence of chloroacetanilide metabolites in surface and ground water (Aga and others, 1996; Kolpin and others, 1996; Thurman and others, 1996). Kolpin and others (1996) found that metabolite concentrations in ground water may be at similar or even higher concentrations than the parent compounds, whereas in surface water, the parent compounds are more abundant in the spring after application and are replaced gradually by metabolites during the remaining growing season. In understanding the fate and transport of parent compounds, methodologies for the analysis of metabolites are crucial. These methods also are important for analytical verification of the metabolites in toxicological studies. Therefore, reliable methodologies for the analysis of chloroacetanilide metabolites are vital.

As with many other pesticide metabolites, high-performance liquid chromatography (HPLC) is needed for the analysis of chloroacetanilide herbicide metabolites because they are ionic compounds and are not sufficiently volatile for analysis by gas chromatography. HPLC-diode array detection (DAD) is very useful in determining metabolite concentrations, especially when the water sample is relatively free of humic materials and ionic surfactants that can cause chromatographic interference. Coupling HPLC with mass spectrometry (MS) yields more qualitative data and lower detection limits than HPLC-DAD analysis alone.

This paper addresses the development of reliable HPLC-DAD and HPLC/MS methods for the analysis of ethanesulfonic acid (ESA) and oxanilic acid metabolites of alachlor, acetochlor, and metolachlor in surface water and ground water. The HPLC-DAD method was derived from an analytical method for the analysis of alachlor ESA and alachlor oxanilic acid as reported by Macomber (1992). For application to the acetochlor and metolachlor metabolites, several modifications to that method were necessary to achieve chromatographic separation of metabolite peaks. The HPLC/MS method was derived from Ferrer and others (1997), with a minor modification to resolve co-eluting peaks on the chromatogram.

MATERIALS AND METHODS

HPLC-grade acetonitrile, methanol, and water, along with acetic acid, was obtained from Fisher Scientific (Pittsburg, PA). The analytical standard for acetochlor ESA was obtained from Zeneca Agrochemicals (Fernhurst, Haslemere Surrey, UK). Standards for alachlor oxanilic acid and acetochlor oxanilic acid were obtained from Monsanto Chemical Co. (St. Louis, MO), and the standard for alachlor ESA was obtained from the U.S. Environmental Protection Agency Repository (Cincinnati, OH). Metolachlor ESA was synthesized in the U.S. Geological Survey laboratory in Lawrence, KS, by Diana Aga (currently with University of Nebraska-Kearney, Kearney, NE). Metolachlor oxanilic acid was obtained from Robert Zablotowitcz of the Agricultural Research Service (Stoneville, MS). Standards solutions were prepared in methanol. The solid-phase extraction (SPE) cartridges (Sep-Pak) used to extract samples were obtained from Waters-Millipore (Milford, MA). These cartridges contained 360 mg (milligrams) of 40-µm (micrometer) C18- ($C_{18}H_{37}$) bonded silica.

Control surface-water samples were collected from Poison Creek in Valley County, Idaho. Control ground-water samples were collected from a well in Valley County, Idaho. Prior to extraction, all samples were filtered through 1-µm-pore glass-fiber filters into clean 4-oz (ounce) amber bottles. Before and between sampling, all sampling equipment was cleaned with detergent and thoroughly rinsed with tap water, then distilled water, then a 50:50 distilled water/methanol rinse.

The SPE procedure was performed using an automated Millipore Workstation (Waters, Milford, MA) as described by Thurman and others (1990). Each C18 cartridge was preconditioned as follows: 2 mL (milliliters) methanol, 2 mL ethyl acetate, 2 mL methanol, followed by 2 mL distilled water. A 100-mL sample was passed through the cartridge at a flow rate of 10 mL/min (milliliters per minute), and the cartridge was purged with air to remove excess water. The cartridge was eluted with 3 mL ethyl acetate, followed by a transfer step to remove the ethyl acetate (top layer, which contains the parent herbicides) from the residual water (bottom layer) in the ethyl acetate fraction. The cartridge then was eluted with 3 mL methanol, which removed the herbicide metabolites. The methanolic extract was spiked with 1 µg (microgram) 2,4-D (internal standard) then evaporated to dryness under a stream of nitrogen at 45 °C (degrees Celsius) using a Turbovap (Zymark, Palo Alto, CA). Because 2,4-D will not isolate using this SPE procedure, it is spiked into the methanol extract instead of the water sample and is used to normalize injection-volume variation and as a retention-time reference. For HPLC-DAD, the extract was reconstituted in 75 µL (microliter) of a solution containing 60 percent, pH 7.0, 25-mM (millimole) phoshate buffer and 40 percent methanol . For HPLC/MS, the extract was reconstituted in 75 µL of a solution containing 0.3 percent acetic acid, 24 percent ethanol, 35.7 percent water, and 40 percent acetonitrile.

For HPLC-DAD, 50 µL of extract was injected into a Hewlett-Packard 1090 HPLC (Hewlett-Packard, Palo Alto, CA) equipped with a DAD. The mobile phase consisted of 60 percent, pH 7.0, 25-mm phosphate buffer, 35 percent methanol, and 5 percent acetonitrile solution with a flow rate of 0.6 mL/min. The analytical columns consisted of a Phenomenex (Torrance, CA) 5-µm (micrometer), 250- x 3-mm C18 column coupled to a Keystone (Bellefonte, PA) 3-µm, 250- x 4.6-mm C18 column. Column temperatures were set at 60 °C to achieve better separation and peak shapes for the metabolites. A Hewlett-Packard HP Chemstation (Rev. A.03.03) was the application software used for instrument control and data processing.

For HPLC/MS, the 50-µL extract was injected into a Hewlett-Packard 1100 HPLC coupled to a Hewlett-Packard 1100 Mass Selective Detector (MSD). The mobile phase consisted of 0.3 percent acetic acid, 24 percent ethanol, 35.7 percent water, and 40 percent acetonitrile solution with a flow rate of 0.3 to 0.4 mL/min, depending on the backpressure (maximum of 400 bar). The analytical columns consisted of two Phenomenex 5-µm, 250- x 3-mm C18 columns coupled to one (or two, if within backpressure limitations) Phenomenex 3-µm, 150- x 2.0-mm C18 column. Using an extra column yields better resolution of the metabolite peaks. Column temperatures were set at 70 °C to achieve better separation and peak shapes. The MSD was operated using the electrospray chamber in negative ion mode. Prior to sample analysis, the MSD was tuned using a proprietary solution obtained from Hewlett-Packard. The drying gas flow was set at 6 L/min (liters per minute), the nebulizer pressure was 25 psi (pounds per square inch), the drying gas temperature was 300 °C, the capillary voltage was 3,100 V (volts), and the fragmenter voltage was 70 V. Table 1 summarizes the HPLC/MSD acquisition parameters. A Hewlett-Packard LCMS Chemstation (Rev. A.05.04[273]) was the application software used for instrument control and data processing.

DETERMINATION OF CHLOROACETANILIDE HERBICIDE METABOLITES

SPE and recovery for chloroacetanilide metabolites have been discussed in previous work (Thurman and others, 1990; Aga and others, 1994; Ferrer and others, 1997). In those studies, chromatographic separation was achieved only for a few of the herbicide metabolites specified in this paper. In the study described in this paper, each control surface- and ground-water sample was spiked with a standard containing all the ionic chloroacetanilide metabolites of interest. For purposes of accuracy and precision, chromatographic separation of the metabolites was essential.

HPLC-DAD Results

The phosphate buffer supplied sodium as a counter ion to the anionic metabolites, creating neutral species that interact with the column. Coupling two columns and maintaining the columns at 60 °C yielded enough metabolite-peak resolution for peak-height quantitation. In this case, it is not known exactly why using two columns with different particle diameters (5 µm and 3 µm) and column diameters (3 mm and 4.6 mm) gave better metabolite separation than using two identical columns. One hypothesis is that water capacity of the column is related to the particle diameter, giving rise to subtle differences in ionic interactions. The columns were configured so that the larger particle column is positioned before the smaller particle column for effective backpressure regulation

Table 1. Typical liquid chromatography/mass selective detector acquisition parameters

Parameter	Settings
Ions monitored	146, 160, 206, 264, 278, 314, 328 mass-to-charge ratio
Spray chamber mode	electrospray, negative mode
Drying gas temperature	300 degrees Celsius
Drying gas flow rate	6 liters per minute
Nebulizer pressure	25 pounds per square inch
Capillary voltage	3,100 volts
Fragmenter	70 volts
Analytical columns	two 5-micrometer, 250- x 3.0-millimeter C18 columns coupled to a 3-micrometer, 150- x 2.0-millimeter C18 column
Mobil phase	0.3 percent acetic acid in 24:36:40 methanol/water/acetonitrile
Flow rate	0.35 millimeter per minute

(smaller phase thickness gives higher backpressure). The analytical wavelength was set at 210 nm (nanometers), and DAD spectra were stored for every integrated peak with a peak height greater than 0.5 milliabsorbance units (mAU). Figure 1 shows a typical HPLC-DAD chromatogram of a 2.0-µg/L control reagent water sample.

Aliquots (123 mL) of reagent water (distilled), metabolite-free/interference-free surface water, and metabolite-free/interference-free ground water were spiked at 0.25, 0.5, and 2.0 µg/L with chloroacetanilide metabolites, making seven replicate samples at each concentration level. Each sample was extracted using the previously described automated SPE procedure. The average HPLC-DAD recoveries of the metabolites from the spiked water samples ranged from 84 to 112 percent, with relative standard deviations of 18 percent or less (tables 2–4). The limit of quantitation (LOQ) for this method was 0.2 µg/L.

HPLC/MS Results

A HPLC/MS method for the analysis of ethanesulfonic acids and oxanilic acids of alachlor, acetochlor, and metolachlor was reported by Ferrer and others (1997). The described HPLC system used an 5-µm, 250- x 3.0-mm C18 column, with a mobile phase consisting of 0.3 percent acetic acid in 24 percent methanol, 36 percent water, and 40 percent acetonitrilem solution. With this configuration, peak resolution was not achieved for alachlor ESA and acetochlor ESA, which have the same molecular ion (table 5). Thus, accurate quantitation of these metabolites was not possible. Chromatographic separation of alachlor ESA and acetochlor ESA was achieved with the same mobile phase by coupling two 5-µm, 250- x 3.0-mm C18 columns to one (or two, if backpressure permits) 3-µm, 150- x 2.0-mm C18 column. Figure 2 shows a total ion chromatogram (TIC) of a 0.05-µg/L control water

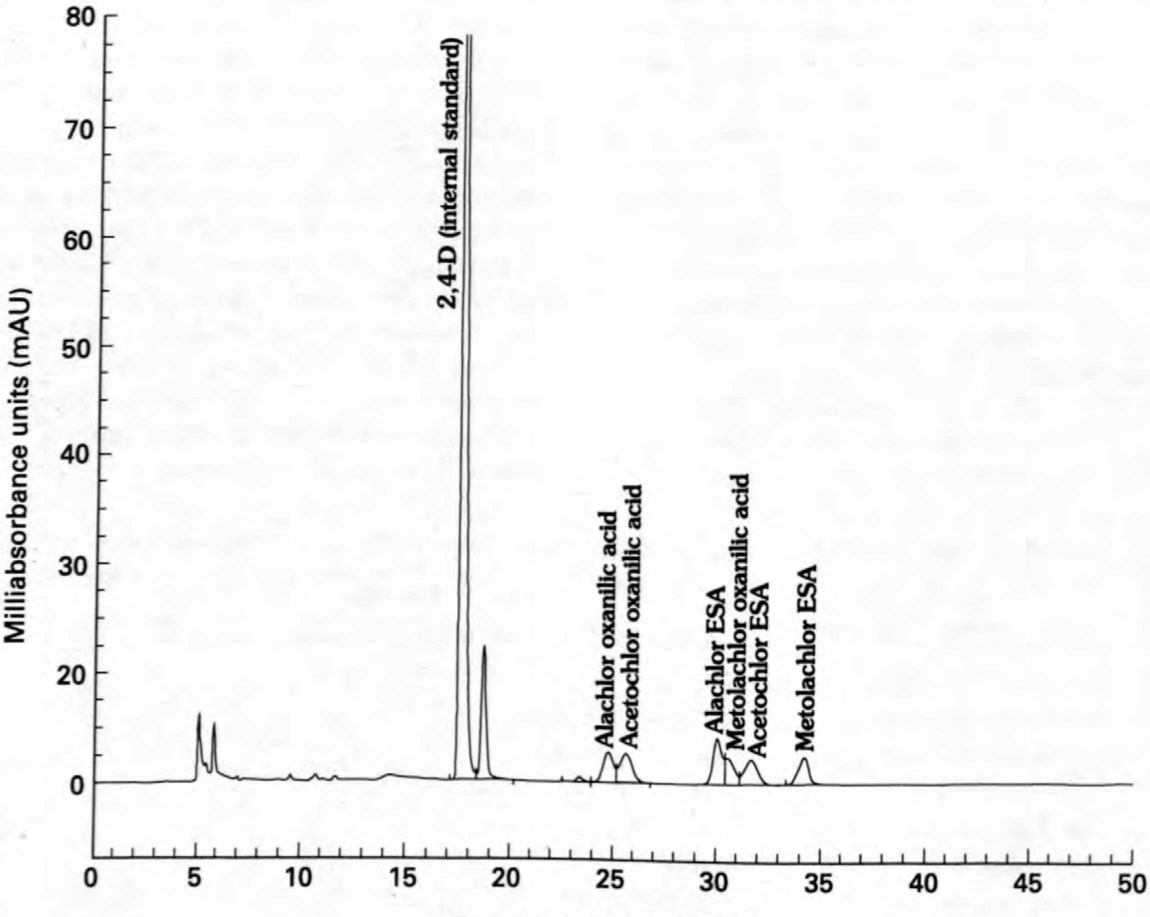

Figure 1. Typical chromatogram of a 2.0-microgram-per-liter control water sample using high-performance liquid chromatography-diode array detection.

Table 2. High-performance liquid chromatography-diode array detection method recoveries in reagent water

[µg/L, micrograms per liter; RSD, relative standard deviation]

	Reagent water					
	Seven replicate samples at 0.25 µg/L		Seven replicate samples at 0.5 µg/L		Seven replicate samples at 2.0 µg/L	
Metabolite	**Average recovery (percent)**	**RSD (percent)**	**Average recovery (percent)**	**RSD (percent)**	**Average recovery (percent)**	**RSD (percent)**
Alachlor ESA	100	10	102	5.5	100	2.4
Alachlor oxanilic acid	84	17	92	9.6	90	2.7
Acetochlor ESA	112	16	104	9.0	105	2.3
Acetochlor oxanilic acid	88	18	94	14	95	3.1
Metolachlor ESA	108	10	104	7.3	105	3.2
Metolachlor oxanilic acid	108	14	102	5.3	100	3.4

Table 3. High-performance liquid chromatography-diode array detection method recoveries in surface water

[µg/L, micrograms per liter; RSD, relative standard deviation]

	Surface water					
	Seven replicate samples at 0.25 µg/L		Seven replicate samples at 0.5 µg/L		Seven replicate samples at 2.0 µg/L	
Metabolite	**Average recovery (percent)**	**RSD (percent)**	**Average recovery (percent)**	**RSD (percent)**	**Average recovery (percent)**	**RSD (percent)**
Alachlor ESA	104	8.9	98	3.4	105	4.4
Alachlor oxanilic acid	92	14	96	7.4	100	6.3
Acetochlor ESA	100	16	104	8.0	100	5.4
Acetochlor oxanilic acid	84	14	94	7.0	100	4.9
Metolachlor ESA	108	12	102	4.9	105	4.6
Metolachlor oxanilic acid	108	16	100	3.5	100	5.7

Table 4. High-performance liquid chromatography-diode array detection method recoveries in ground water

[µg/L, micrograms per liter; RSD, relative standard deviation]

	Ground water					
	Seven replicate samples at 0.25 µg/L		Seven replicate samples at 0.5 µg/L		Seven replicate samples at 2.0 µg/L	
Metabolite	**Average recovery (percent)**	**RSD (percent)**	**Average recovery (percent)**	**RSD (percent)**	**Average recovery (percent)**	**RSD (percent)**
Alachlor ESA	103	9.3	98	4.6	103	3.2
Alachlor oxanilic acid	88	13	94	6.9	94	4.8
Acetochlor ESA	105	18	107	8.4	102	3.8
Acetochlor oxanilic acid	87	15	95	7.8	99	5.3
Metolachlor ESA	102	12	103	5.0	98	4.1
Metolachlor oxanilic acid	104	16	100	4.8	100	3.5

Table 5. Summary of typical molecular and fragmentation ions

[fragmenter voltage = 70 volts; m/z, mass-to-charge ratio]

Metabolite	Molecular ion (m/z)	Fragmentation ion(s) (m/z)
Alachlor ESA	314	314
Alachlor oxanilic acid	264	264, 160, 192
Acetochlor ESA	314	314
Acetechlor oxanilic acid	264	264, 146
Metolachlor ESA	328	328
Metolachlor oxanilic acid	278	278, 216
2,4-D (internal standard)	220	219, 161

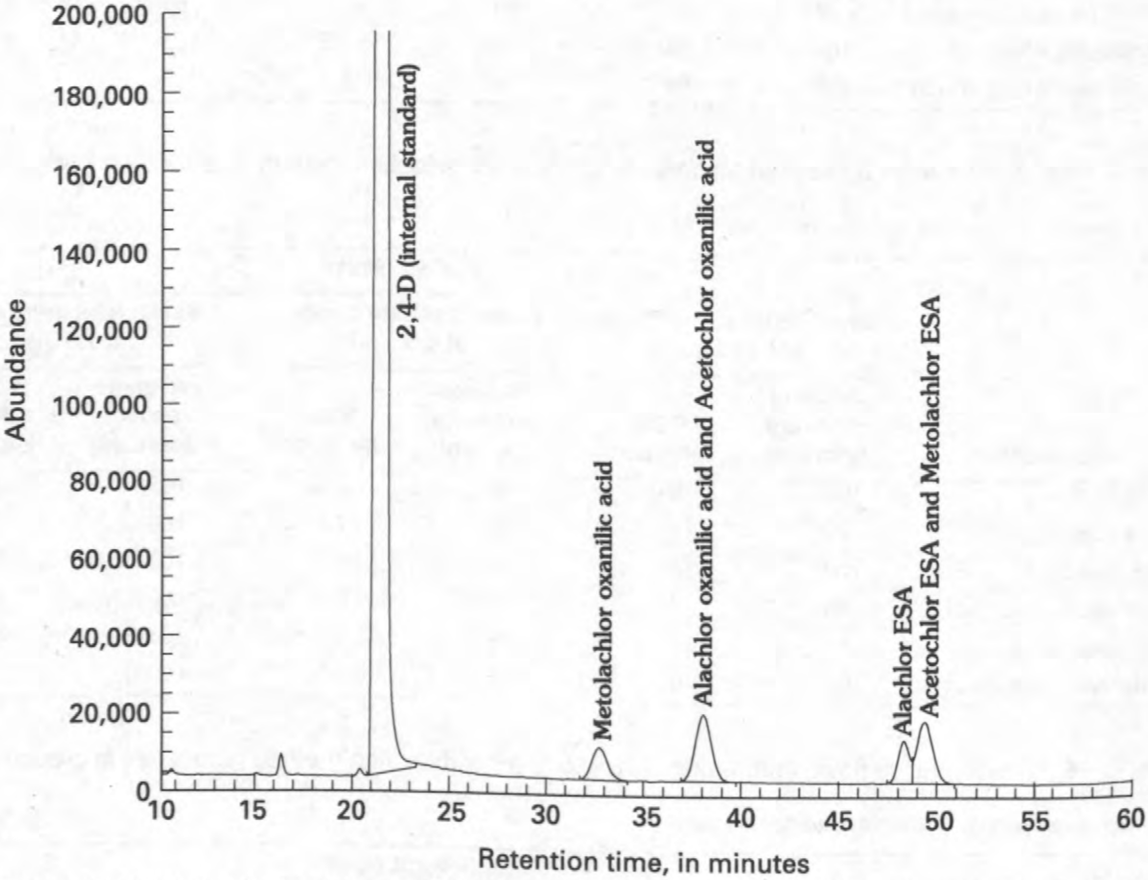

Figure 2. Total ion chromatogram (TIC) of a 0.05-microgram-per-liter control water sample using high-performance liquid chromatography/mass spectrometry.

sample. Figure 3 shows the extracted ion chromatogram for the molecular ion (314 mass-to-charge ratio) of alachlor ESA and acetochlor ESA with near baseline separation. The elution order of the metabolites using the HPLC/MS method differs from that of the HPLC-DAD method because the pH of the respective mobile phases are different.

Aliquots (123 mL) of reagent water, (distilled), metabolite-free surface water, and metabolite-free ground water were spiked at 0.05, 0.2, and 2.0 µg/L with the metabolites, making seven replicate samples at each concentration. Each sample was extracted using the previously described automated SPE procedure. The average HPLC/MS recoveries of the metabolites from the

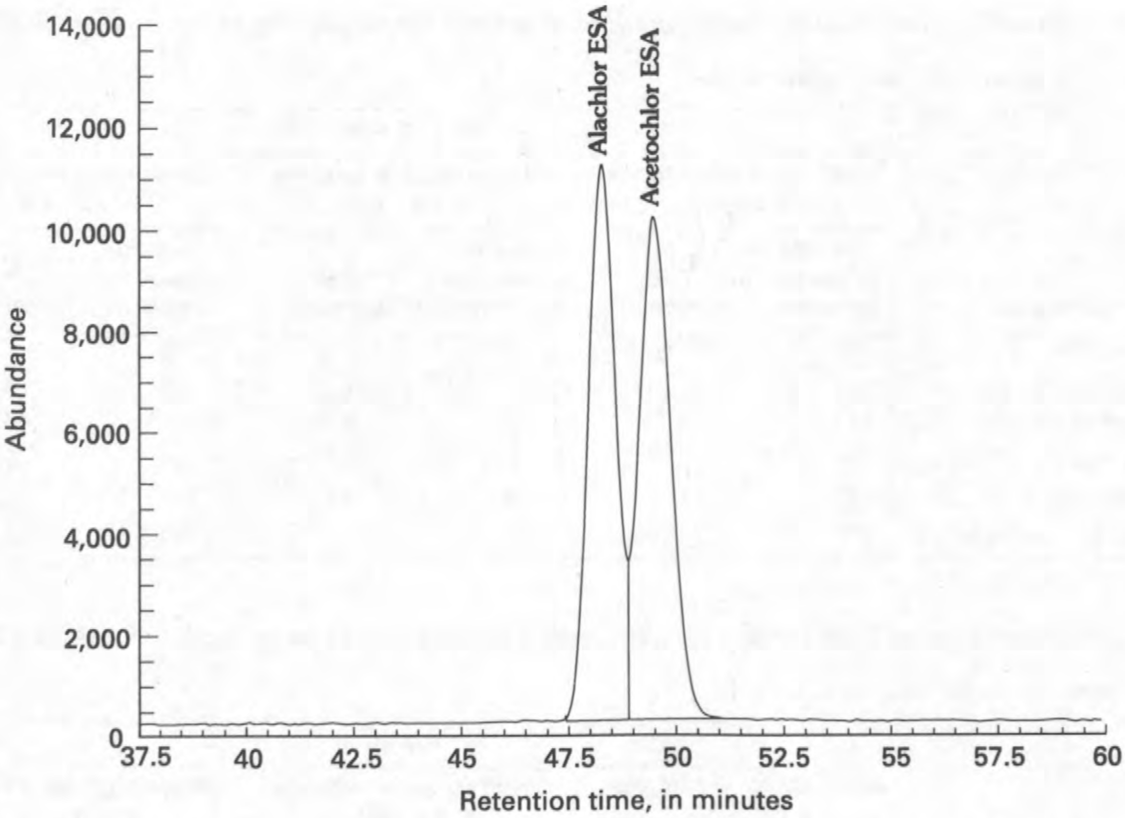

Figure 3. Selected ion chromatogram of a 0.05-microgram-per-liter control water sample for molecular ion 314 mass-to-charge ratio using high-performance liquid chromatography/mass spectrometry.

spiked water samples ranged from 81 to 118 percent, with relative standard deviations of 20 percent or less (tables 6–8). The limit of quantitation (LOQ) for this method was 0.05 µg/L.

DISCUSSION

Accurate and precise measurements of metabolite concentrations in surface water and ground water are obtained using the HPLC-DAD and HPLC/MS methods specified in this paper. Information about the fate and transport of the chloroacetanilide herbicides—alachlor, acetochlor, and metolachlor—in water can be acquired from the analysis of field-runoff water and ground water from nearby wells. These methods also can be useful for water-quality determinations and analytical verification in toxicological studies.

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Table 6. High-performance liquid chromatography/mass spectrometry method recoveries in reagent water

[µg/L, micrograms per liter; RSD, relative standard deviation]

	Reagent water					
	Seven replicate samples at 0.05 µg/L		Seven replicate samples at 0.2 µg/L		Seven replicate samples at 2.0 µg/L	
Metabolite	Average recovery (percent)	RSD (percent)	Average recovery (percent)	RSD (percent)	Average recovery (percent)	RSD (percent)
Alachlor ESA	95	19	100	5.4	100	4.9
Alachlor oxanilic acid	81	11	85	11	88	6.4
Acetolachlor ESA	117	20	125	6.3	110	7.4
Acetolachlor oxanilic acid	84	12	85	9.6	86	5.9
Metolachlor ESA	113	13	110	5.8	115	6.8
Metolachlor oxanilic acid	110	11	105	5	104	5.7

Table 7. High-performance liquid chromatography/mass spectrometry method recoveries in surface water

[µg/L, micrograms per liter; RSD, relative standard deviation]

	Surface water					
	Seven replicate samples at 0.05 µg/L		Seven replicate samples at 0.2 µg/L		Seven replicate samples at 2.0 µg/L	
Metabolite	Average recovery (percent)	RSD (percent)	Average recovery (percent)	RSD (percent)	Average recovery (percent)	RSD (percent)
Alachlor ESA	81	19	95	7.1	109	13
Alachlor oxanilic acid	90	10	99	6.2	109	10
Acetolachlor ESA	118	4.3	104	7.7	104	15
Acetolachlor oxanilic acid	90	8.1	101	6.1	111	11
Metolachlor ESA	92	11	101	6.3	114	10
Metolachlor oxanilic acid	90	9.5	100	5.9	110	12

Table 8. High-performance liquid chromatography/mass spectrometry method recoveries in ground water

[µg/L, micrograms per liter; RSD, relative standard deviation]

	Ground water					
	Seven replicate samples at 0.05 µg/L		Seven replicate samples at 0.2 µg/L		Seven replicate samples at 2.0 µg/L	
Metabolite	Average recovery (percent)	RSD (percent)	Average recovery (percent)	RSD (percent)	Average recovery (percent)	RSD (percent)
Alachlor ESA	104	16	100	4.8	100	3.5
Alachlor oxanilic acid	85	8.3	95	7.8	99	5.3
Acetolachlor ESA	102	12	103	5.0	98	4.1
Acetolachlor oxanilic acid	95	9.3	98	4.6	103	3.2
Metolachlor ESA	92	11	107	8.4	102	3.8
Metolachlor oxanilic acid	95	12	94	6.9	94	4.8

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Analysis of Selected Herbicide Metabolites in Surface and Ground Water of the United States

By Elisabeth A. Scribner, E.M. Thurman, and Lisa R. Zimmerman

ABSTRACT

One of the primary goals of the U.S. Geological Survey (USGS) laboratory in Lawrence, Kansas, is to develop analytical methods for the analysis of herbicide metabolites in surface and ground water that are vital to the study of herbicide fate and degradation pathways in the environment. Methods to measure metabolite concentrations from three major classes of herbicides—triazine, chloroacetanilide, and phenylurea—have been developed. Methods for triazine metabolite detection cover nine compounds; six compounds are detected by gas chromatography/mass spectrometry, one is detected by high-performance liquid chromatography with diode-array detection, and eight are detected by liquid chromatography/mass spectrometry. Two metabolites of the chloroacetanilde herbicides—ethane sulfonic acid and oxanilic acid—are detected by high-performance liquid chromatography with diode-array detection and liquid chromatography/mass spectrometry. Alachlor ethane sulfonic acid also has been detected by solid-phase extraction and enzyme-linked immunosorbent assay. Six phenylurea metabolites are all detected by liquid chromatography/mass spectrometry; four of the six metabolites also are detected by gas chromatography/mass spectrometry.

Additionally, surveys of herbicides and their metabolites in surface water, ground water, lakes, reservoirs, and rainfall have been conducted through the USGS laboratory in Lawrence. These surveys have been useful in determining herbicide and metabolite occurrence and temporal distribution and have shown that metabolites may be useful in evaluation of nonpoint-source contamination.

INTRODUCTION

Herbicides in surface and ground water are a major concern throughout the United States. Numerous studies have been completed by various government agencies, including the U.S. Geological Survey (USGS), and by chemical manufacturers to document occurrences of herbicides in ground water (Kolpin and others, 1996), rainfall (Goolsby and others, 1997; Pomes and others, 1998), and surface water (Thurman and others, 1992). However, there has been little study of the metabolites of these herbicides. Because metabolites are formed in the environment and transported to surface and ground water, it is important to understand herbicide use and the fate of herbicide metabolites.

Modern agricultural practices in the United States often require extensive use of herbicides for production of corn, soybeans, sorghum, and other row crops. Data compiled by Gianessi and Puffer (1991) indicate that about two-thirds of the 285 million kg (kilograms) of herbicides applied annually in the United States are used in crop production in the Midwest. In 1990 and again in 1992, because of concern about water contamination, manufacturers voluntarily reduced the maximum recommended application rate by 50 percent for atrazine, the most used herbicide on corn and sorghum. These application-rate changes have affected the frequency of detection of some of the herbicide metabolites (Scribner and others, 1998).

In 1987, a laboratory for organic geochemistry research was established at the USGS in Lawrence, Kansas. Since that time, analytical methods have been and continue to be developed to measure metabolite concentrations from three major classes of herbicides—triazine, chloroacetanilide, and phenylurea. From studies of degradation, fate, and transport of herbicides and their metabolites in soil and aquatic environments, numerous journal articles, book chapters, and USGS reports have been published.

The principal purpose of this paper is to list the analytical methods that have been developed to measure herbicide metabolites in surface and ground water. Listings of herbicide and metabolite surveys that have been conducted through the USGS laboratory in Lawrence also are summarized. Brief descriptions and illustrations depicting the degradation pathways for the parent herbicides also are presented.

ANALYTICAL METHODS DEVELOPED

Methods developed include use of gas chromatography/mass spectrometry (GC/MS), high-performance liquid chromatography with diode-array detection (HPLC/DAD), liquid chromatography/mass spectrometry (LC/MS), and solid-phase extraction (SPE) and enzyme-linked immunosorbent assay (ELISA) for analysis of triazine, chloroacetanilide, and phenylurea herbicide metabolites as shown in table 1. Triazine metabolites include deethylatrazine (DEA), deisopropylatrazine (DIA), hydroxyatrazine (HA), cyanazine acid (CAC), cyanazine amide (CAM), deethylcyanazine (DEC), deethylcyanazine acid (DCAC), deethylcyanazine amide (DCAM), and deisopropylprometryn. Six triazine metabolites are analyzed by GC/MS according to procedures described by Thurman and others (1990) and Meyer and

Table 1. Methods of analysis for triazine, chloroacetanilide, and phenylurea herbicide metabolites at the U.S. Geological Survey laboratory, Lawrence, Kansas

[GC/MS, gas chromatography/mass spectometry; HPLC/DAD, high-performance liquid chromatography with diode-array detection; LC/MS, liquid chromatography/mass spectrometry; SPE, solid-phase extraction; and ELISA, enzyme-linked immunosorbent assay]

Metabolite	Method			
	GC/MS	HPLC/ DAD	LC/MS	SPE/ ELISA
Triazine metabolites				
Deethylatrazine (DEA)	X		X	
Deisopropylatrazine (DIA)	X		X	
Hydroxyatrazine (HA)		X	X	
Cyanazine acid (CAC)			X	
Cyanazine amide (CAM)	X		X	
Deethylcyanazine (DEC)	X		X	
Deethylcyanazine acid (DCAC)			X	
Deethylcyanazine amide (DCAM)	X		X	
Deisopropylprometryn	X			
Chloroacetanilide metabolites				
Acetochlor ethane sulfonic acid (ESA)		X	X	
Acetochlor oxanilic acid (OXA)		X	X	
Alachlor ethane sulfonic acid (ESA)		X	X	X
Alachlor oxanilic acid (OXA)		X	X	
Metolachlor ethane sulfonic acid (ESA)		X	X	
Metolachlor oxanilic acid (OXA)		X	X	
Phenylurea metabolites				
3,4-dichloroaniline (DCA)	X		X	
3,4-dichlorophenylurea (DCPU)			X	
3,4-dichloromethylphenylurea (DCPMU)			X	
Trifluoromethylaniline (TFMA)	X		X	
Trifluoromethylphenylurea (TFMPU)	X		X	
Demethylfluometuron (DMFM)	X		X	

others (1993). Hydroxyatrazine is analyzed by both HPLC/DAD and LC/MS (Lerch and others, 1998). Since 1998, all triazine metabolites are analyzed by LC/MS except for deisopropylprometryn.

Two metabolites of the chloroacetanilde herbicides—ethane sulfonic acid (ESA) and oxanilic acid (OXA)—are detected by HPLC/DAD and LC/MS for acetochlor, alachlor, and metolachlor (Ferrer and others, 1997; Kalkhoff and others, 1998; Kolpin and others, 1998). Alachlor ESA is also analyzed by SPE and ELISA (Aga and others, 1994).

Phenylurea metabolites of diuron—3,4-dichloroaniline (DCA), 3,4-dichlorophenylurea (DCPU), and 3,4-dichloromethyphenylurea (DCPMU)—are analyzed by LC/MS. DCA is also analyzed by GC/MS. Phenylurea herbicide analysis also includes the metabolites of fluometuron—trifluoromethylaniline (TFMA), trifluoromethylphenylurea (TFMPU), and demethylfluometuron (DMFM)—which are detected by both GC/MS and LC/MS.

SURVEYS OF HERBICIDES AND METABOLITES

Surveys conducted in the Midwestern United States through the USGS laboratory in Lawrence are: 1989–90—surface-water runoff at 147 reconnaissance sites in a 10-State area (Scribner and others, 1993); 1990–91—rainfall samples from 81 collection sites (Goolsby and others, 1995); 1990–92—storm-runoff samples from nine stream basins (Scribner and others, 1994); 1991–98—samples from 303 well sites (Kolpin and others, 1993); 1992–93—samples collected from 76 reservoirs (Scribner and others, 1996); 1993—samples from the Mississippi River during flood stage (Goolsby and others, 1993); and 1994–95 and 1998—samples from 53 streams to help determine if changes in herbicide use resulted in a change in herbicide concentrations since the 1989–90 reconnaissance study (Scribner and others, 1998).

Further surveys of herbicides and their metabolites in the cotton-growing areas of the United States have been made to relate herbicide use to occurrence in streams during 1995–97 (Coupe and others, 1998). In 1996, surface-water samples were collected at 64 sites in the Mississippi Embayment and analyzed in conjunction with the USGS National Water-Quality Assessment (NAWQA) Program. Special emphasis in the 1996 survey was placed on streams in the Mississippi Delta as described in Thurman and others (1998).

DEGRADATION PATHWAYS

Concentrations of metabolites in water commonly may be equal to or even exceed concentrations of parent compounds. It has been found that metabolite concentrations in ground water often exceed parent compound concentrations for both triazine and chloroacetanilide herbicides, whereas in surface water the parent compound is most abundant after application of herbicide in the spring and is replaced gradually with metabolites throughout the growing season. In the fall, the metabolite concentrations may exceed concentrations of the parent compound (Kolpin and others, 1996, 1998; Kalkhoff and others, 1998).

Degradation of the phenylurea herbicides of diuron and fluometuron is similar. Both herbicides degrade by N-demethylation under aerobic conditions to metabolites (Ahrens, 1994; Field and others, 1997).

Triazine Metabolites

The triazine herbicides—atrazine, cyanazine, simazine, and propazine—are four compounds that have been used on corn and sorghum in the Midwestern United States. Application amounts of these herbicides in 1995 were 20 million kilograms of atrazine, 11 million kilograms of cyanazine, and 0.3 million kilograms of simazine. Propazine use has been discontinued (Gianessi and Puffer, 1991). Triazine herbicides degrade by various pathways to a series of metabolites. Atrazine degrades in soil through both biotic and abiotic reactions to the dealkylated metabolites, DEA and DIA, and the hydroxylated metabolite, HA (fig. 1). DEA may further degrade to the dealkylated hydroxymetabolites of didealkylatrazine (DDA), hydroxydeethylatrazine (HDEA), and hydroxydeisopropylatrazine (HDIA). DIA may further degrade to the hydroxymetabolites of DDA and HDIA. HA may degrade to dealkylated HDIA and HDEA (Lerch and others, 1998). The atrazine degradation pathway includes further dealkylation of DEA, DIA, and HA to the opening of the ammeline ring and eventual

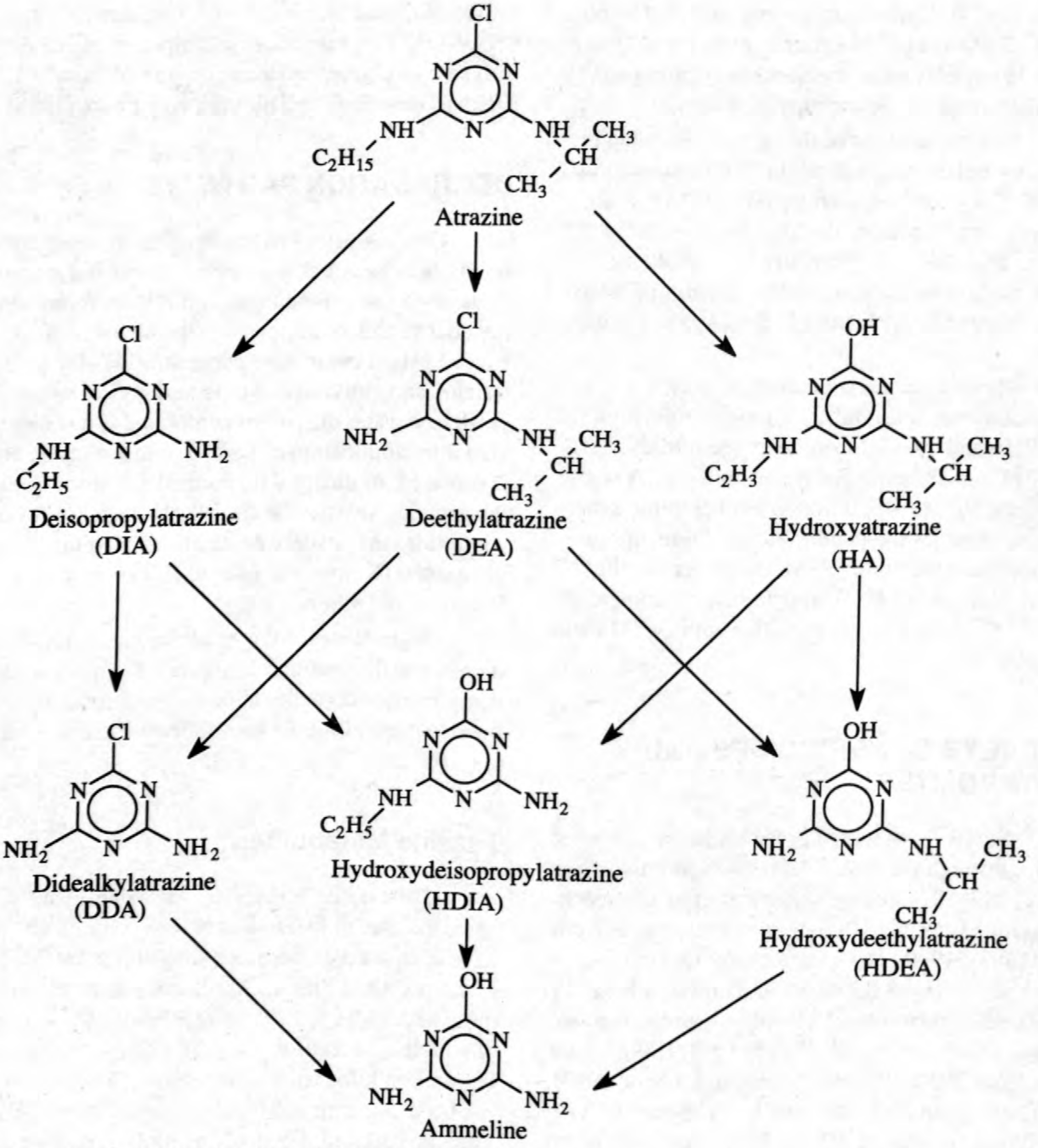

Figure 1. Pathways for degradation of atrazine.

mineralization to carbon dioxide and nitrogen gas (Gunther and Gunther, 1970). The degradation pathway of atrazine generally is well known and studied extensively.

Field-dissipation studies of the four chlorinated parent triazine herbicides atrazine, cyanazine, simazine, and propazine have found that they all degrade in soil in similar fashion and form at least one of two common dealkylated metabolites, DIA and (or) DEA. Figure 2 summarizes triazine degradation pathways described in Thurman and others (1994), which documents work on all four triazine compounds using field-dissipation studies.

Cyanazine is used primarily on corn in the upper Midwest. The work done by the USGS laboratory in Lawrence is the first major integrated research conducted on the geochemistry of cyanazine in surface water of the Midwest. Methods development, field-dissipation, and regional studies by laboratory personnel indicate that cyanazine is

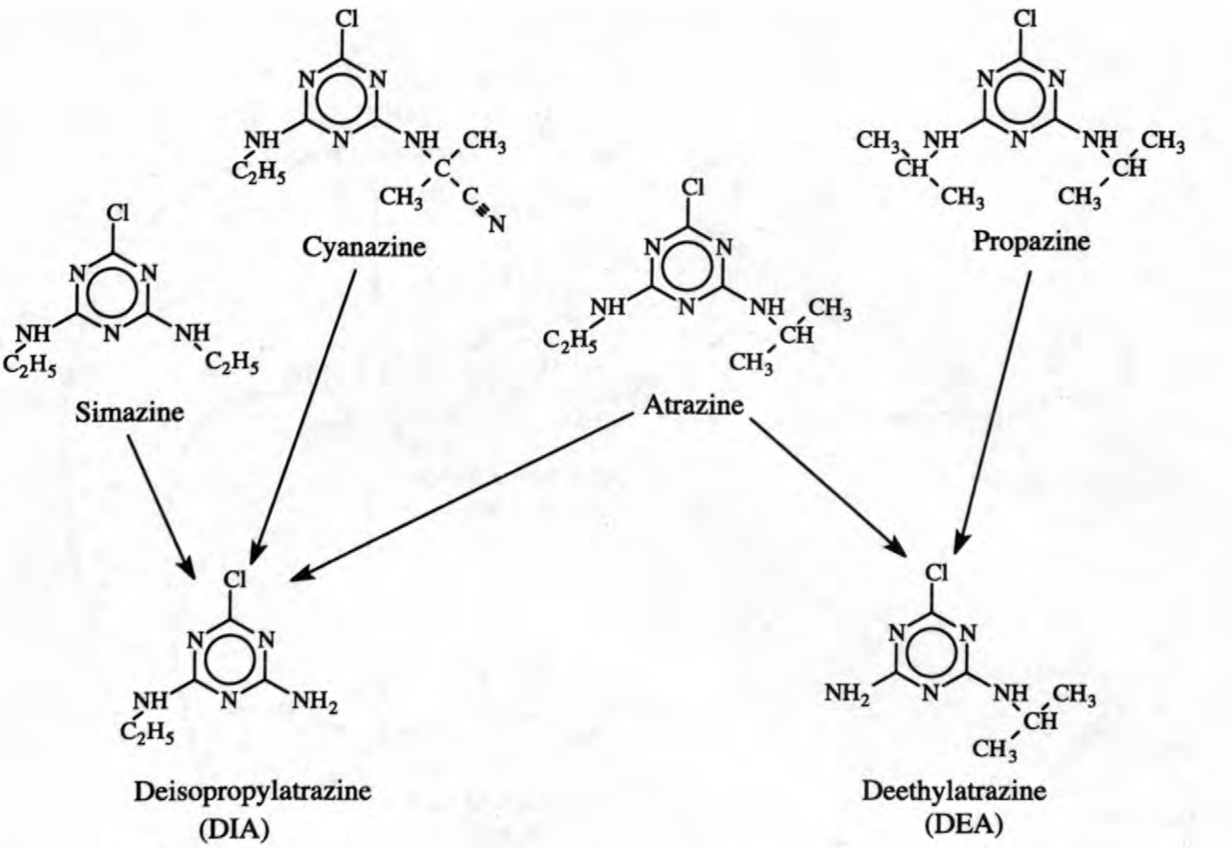

Figure 2. Pathways for degradation of atrazine, cyanazine, propazine, and simazine to DEA and DIA.

more labile than atrazine and that they both have a common metabolite, DIA (Meyer, 1994). Cyanazine degrades by deethylation to DEC, which degrades rapidly to DCAM. DCAM, in turn, degrades to DCAC, which further degrades to DDA. CAM is an important metabolite of cyanazine that is readily detected. CAM degrades to DCAM and CAC. CAC then degrades to DCAC and DIA, which further degrade to DDA. CAC and DCAC may be rapidly transported through the unsaturated zone (Meyer, 1994). Structures and degradation pathways for cyanazine and its metabolites are shown in figure 3.

Various studies in the Midwestern United States have demonstrated the importance of the two triazine metabolites, DEA and DIA, which were found to occur in water that has received parent triazine herbicides. These studies show that DEA has atrazine as its major source (98 percent) and only trace levels are derived from propazine (fig. 2). DIA has atrazine as its major source (75 percent) and cyanazine as a secondary source (25 percent). Trace amounts of DIA are contributed by simazine. Propazine and simazine do not contribute substantially to the DIA-to-DEA ratio in surface water (Thurman and others, 1994). When DEA is the major metabolite in the unsaturated zone, the deethlyatrazine-to-atrazine ratio (DAR) may be used to document the first major runoff of herbicides from nonpoint-source corn fields to surface water (Thurman and Fallon, 1996). The DAR in soil water quickly decreases from about 0.5 to less than 0.1 upon application of herbicide and the first major runoff occurrence in a basin. The DAR then gradually increases to values of approximately 0.4 to 0.6 during the harvest season. Atrazine and DEA have been reported frequently in ground water but less is known about the occurrence of HA in surface water. HA occurs at concentrations considerably less than atrazine or its metabolites, DEA and DIA (Lerch and others, 1998).

Other triazine herbicides analyzed by GC/MS include prometryn. Prometryn is a parent herbicide used in cotton-growing areas of the United States. Its dealkylated metabolite, deisopropylprometryn, also is analyzed by GC/MS. During a recent study in the Mississippi Embayment, prometryn was not detected in water samples at

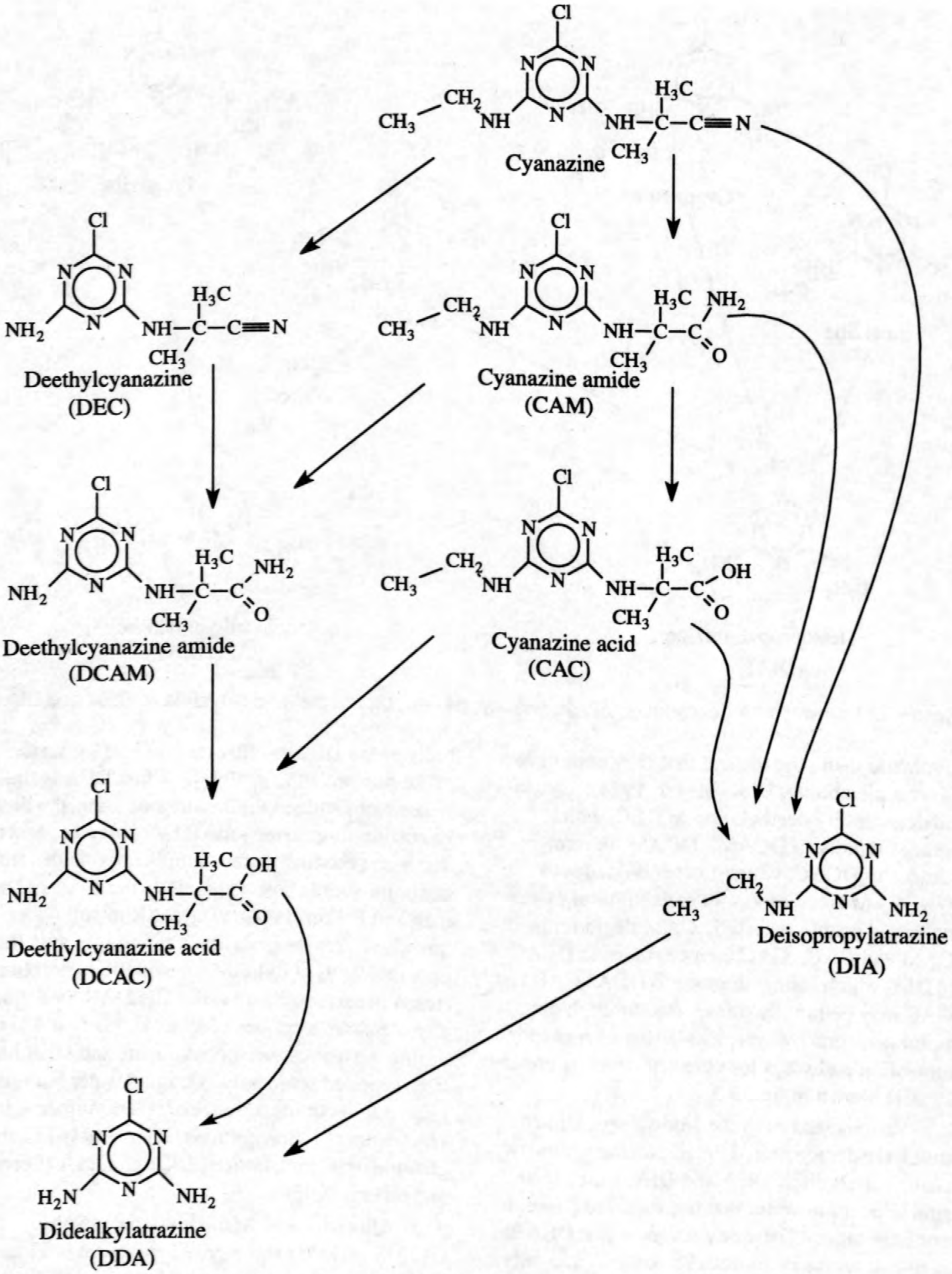

Figure 3. Pathways for degradation of cyanazine.

concentrations greater than 1.0 microgram per liter (µg/L), and deisopropylprometryn was not detected at concentrations greater than the reporting level of 0.05 µg/L (Thurman and others, 1998). The less extensive use of prometryn probably was responsible for the low concentrations in the samples analyzed (Coupe and others, 1998).

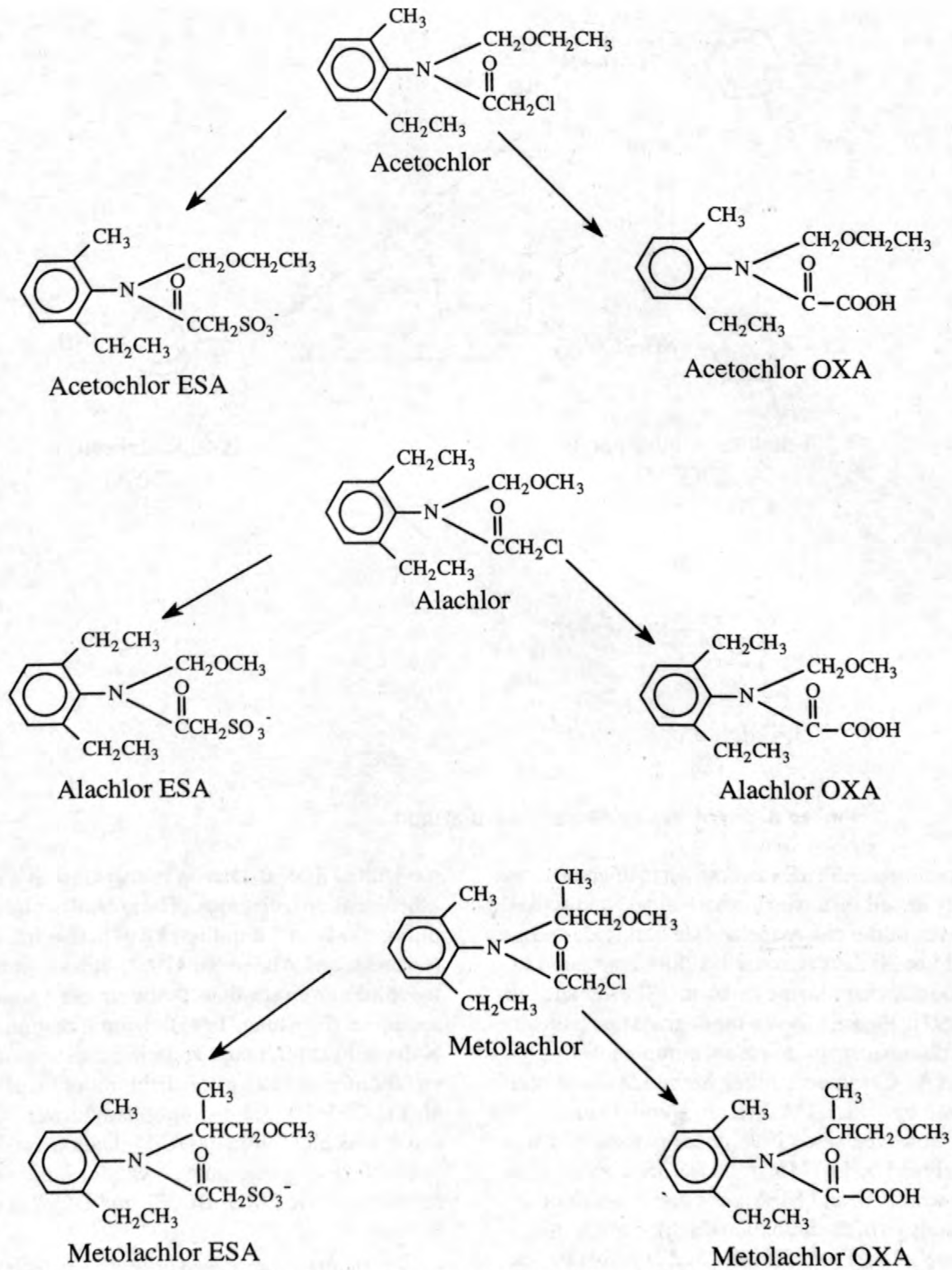

Figure 4. Pathways for degradation of acetochlor, alachlor, and metolachlor to ethane sulfonic acid and oxanilic acid.

Chloroacetanilide Metabolites

The chloroacetanilide herbicides—acetochlor, alachlor, and metolachlor—constitute the second major class of herbicides used in the United States. Together with triazine herbicides, chloroacetanilide herbicides account for the majority of herbicides applied to farmland in the Midwestern United States (Gianessi and Puffer, 1991). Alachlor and metolachlor have been used for more than 20 years (Thurman and others, 1996). Acetochlor was used for the first time in 1994. In general, chlo-

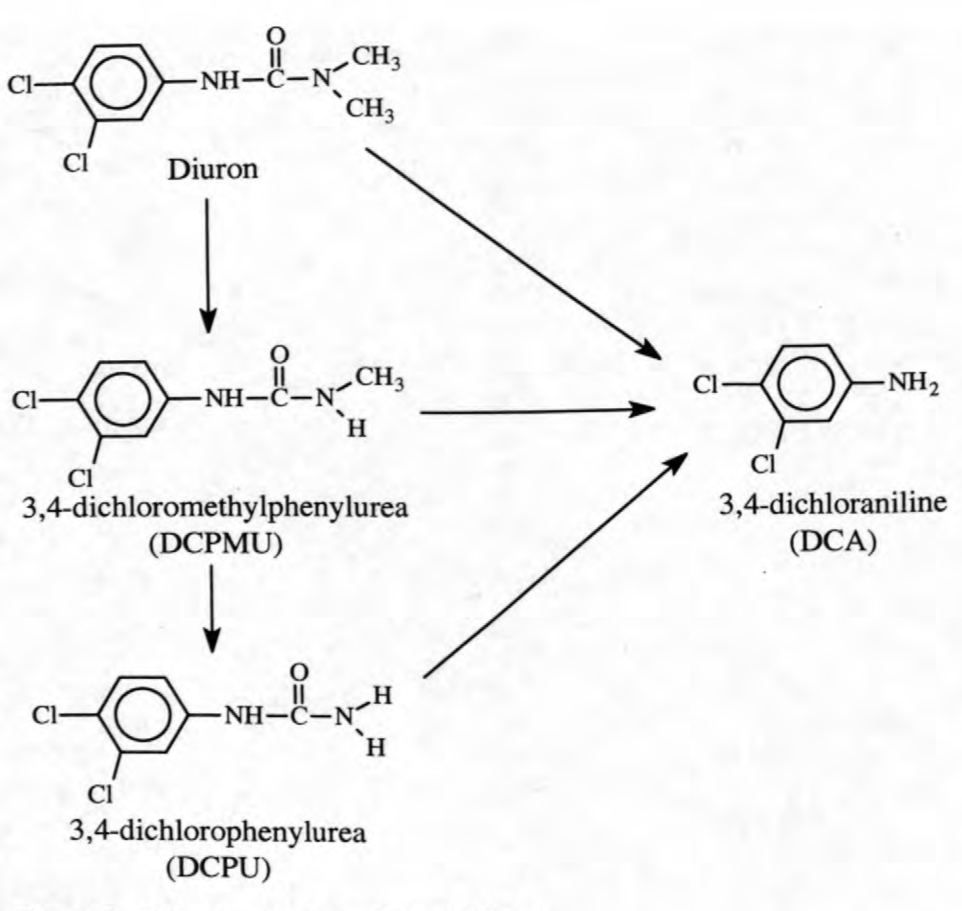

Figure 5. Pathways for degradation of diuron.

roacetanilide herbicides are known to degrade more quickly in soil than triazine herbicides, and typical half-lives of the chloroacetanilide herbicides range from 15 to 30 days (Leonard, 1988), compared to 30 to 60 days for triazine herbicides (Ferrer and others, 1997). Figure 4 shows the degradation pathway of the chloroacetanilide parent compounds to ESA and OXA. Chloroacetanilide herbicides have been analyzed by HPLC/DAD (Kolpin and others, 1998); however, since 1998, these metabolites also are analyzed by LC/MS at the USGS laboratory in Lawrence. SPE and ELISA are combined for the trace analysis of the herbicide alachlor and its major soil metabolite, ESA. The method is viable for the analysis of both surface- and ground-water samples and is comparable to GC/MS and HPLC analyses for alachlor and ESA (Aga and others, 1994).

Phenylurea Metabolites

The phenylurea herbicides, diuron and fluometuron, are used in the cotton-growing areas of the United States. Diuron is also used in many other areas on fruit crops. The annual application of diuron is about 1.8 million kg of active ingredient (Gianessi and Anderson, 1995). It was ranked as the third most hazardous pesticide to ground-water resources (Newman, 1995). Diuron degrades by N-demethylation under aerobic conditions to metabolites including 3,4-dichloromethylphenylurea (DCPMU), 3,4-dichlorophenylurea (DCPU), and 3,4-dichloroaniline (DCA) (Dalton and others, 1966). The degradation pathway for diuron and its metabolites, DCPMU, DCPU, and DCA, is shown in figure 5.

Fluometuron is used primarily in Mississippi and in the eastern coastal plain (Gianessi and Anderson, 1995) as a preemergent herbicide for broadleaf and grass control in cotton; therefore, the timing of the highest concentrations of fluometuron in surface water is much different than for corn because application times are different (4.7 average annual applications on cotton versus 1.2 average annual applications on corn). Application of fluometuron in the United States totaled about 1.5 million kg in 1996 at an average rate of approximately 0.81 kg per hectare of active ingredient (National Agricultural Statistics Service, 1997). Three fluometuron metabolites that have been analyzed are TFMA, TFMPU, and DMFM. In a study using GC/MS (Coupe and others, 1998), analyses showed that DMFM was the most common and was present in the highest concentration. TFMPU was not detected in any of the samples, and TFMA was detected infrequently and in low concentrations (Coupe and others, 1998). The degradation pathways of fluometuron and its metabolites are shown in figure 6.

SUMMARY

The U.S. Geological Survey laboratory in Lawrence, Kansas, was established in 1987 to enhance scientific knowledge in the field of organic geochemistry. Special emphasis has been on water-quality analysis as related to problem areas involving contamination of surface and ground water. Analytical methods continue to be developed to assess the nature, amount, and movement of herbicides and their metabolites in soil and water. This scientific work is important to define water quality and its relation to nonpoint-source contamination.

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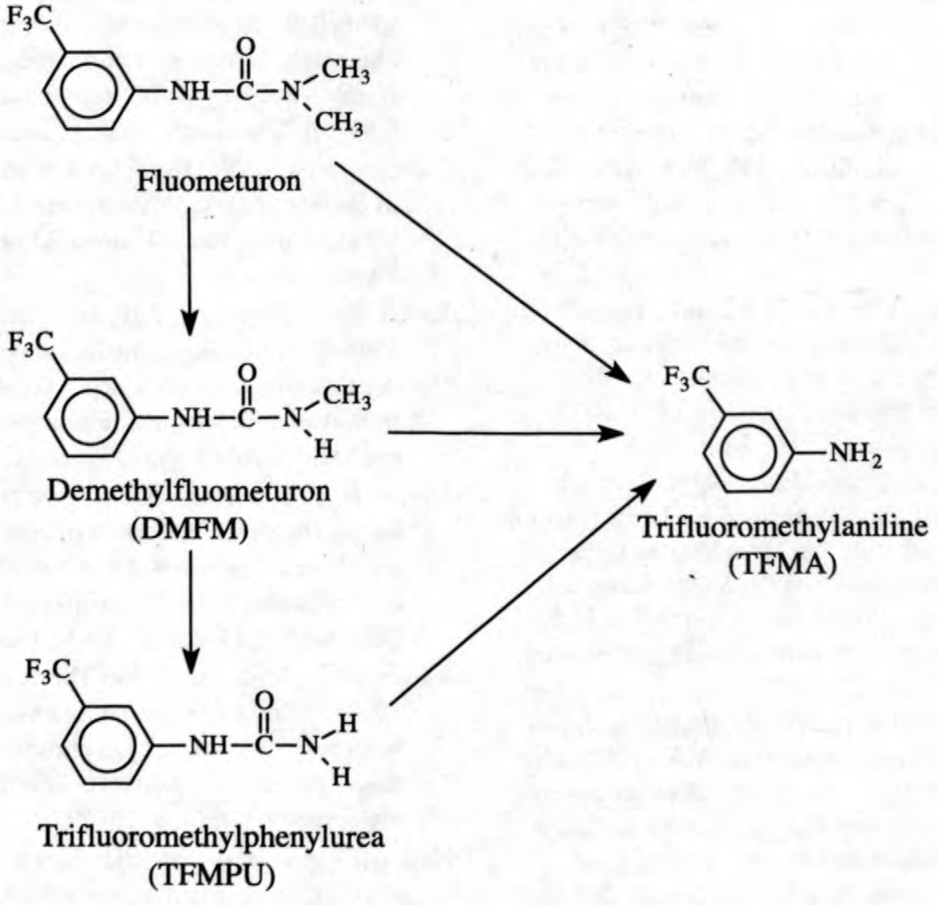

Figure 6. Pathways of degradation of fluometuron.

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Detection of Persistent Organic Pollutants in the Mississippi Delta Using Semipermeable Membrane Devices

By L.R. Zimmerman, E.M. Thurman, and K.C. Bastian

ABSTRACT

Persistent organic pollutants (POPs) are hazardous chemicals, with moderate to low volatility, that resist degradation and tend to accumulate in living tissues. Their persistence in various media facilitates their transport over long distances to remote regions where they have never been used. They have been found to present risks to human health and the environment in polar and other regions. In February 1997, a United Nations Environmental Program Governing Council Decision identified 12 specific POPs (aldrin, chlordane, DDT, dieldrin, dioxins, endrin, furans, heptachlor, hexachlorobenzene, mirex, polychlorinated biphenyls, and toxaphene) for which international action was deemed necessary to protect human health and the environment.

Semipermeable membrane devices (SPMDs) passively accumulate hydrophobic organic compounds when placed in an aquatic environment. They consist of a low-density polyethylene (LDPE) lay-flat membrane filled with 1 gram of a high molecular weight lipid, triolein, that cannot diffuse through the membrane. The LDPE membrane mimics a biological membrane in its ability to allow selective diffusion of organic compounds. After the deployment period, SPMDs are retrieved from the stream, dialyzed, cleaned up with gel permeation chromatography and silica gel, and analyzed using gas chromatography/ mass spectrometry (GC/MS) or gas chromatography/electron capture (GC/ECD) techniques.

From SPMDs placed in five Mississippi Delta streams in 1996 and 1997, the POPs aldrin, chlordane, DCPA, DDT, dieldrin, endrin, heptachlor, hexachlorobenzenes, mirex, nonachlor, and toxaphene were detected. In addition, two insecticides still in use, the organophosphate chlorpyriphos and the organochlorine endosulfan, were detected. Two low-solubility herbicides not detected commonly in surface water, pendimethalin and trifluralin, also were detected.

INTRODUCTION

Cotton is farmed intensively in the Mississippi River Delta, and pesticide use throughout the past several decades has been similarly intense. Many pesticides, especially those used prior to widespread environmental concerns, are long-lived and very hydrophobic. These tendencies cause the compounds to bioconcentrate in the fatty tissues of fish and other organisms. Many of these compounds can be classified as persistent organic pollutants (POPs). The presence of these pesticides in organisms has potential ramifications not only for the organisms directly affected but also for overall ecological health and for those segments of the human population that depend on subsistence fishing for a significant part of their diet.

Persistent Organic Pollutants (POPs)

POPs are hazardous chemicals, with moderate to low volatility, that resist degradation and tend to accumulate in living tissues. Their persistence in various media facilitates their transport over long distances to remote regions where they have never been used. They have been found to present risks to human health and the environment in polar and other regions (Wahlström, 1997). In February 1997, a United Nations Environmental Program Governing Council Decision identified 12 POPs (aldrin, chlordane, DDT, dieldrin, dioxins, endrin, furans, heptachlor, hexachlorobenzene, mirex, polychlorinated biphenyls, and toxaphene) for which international action was deemed necessary to protect human health and the environment.

Because POPs are present at such low concentrations in the aquatic environment, it is difficult to detect them with conventional chemical analysis, but they still may be important in the environment because they can accumulate in animal fat. Analyzing POPs in water samples is cumbersome because their low solubilities would require large sample volumes to detect concentrations at the levels common in the environment. Fish tissue has been the common method for analysis, but a biological sampling must consider additional complications such as analyte metabolism, disease, predation, migration, or the possible introduction of a foreign species.

Semipermeable Membrane Devices (SPMDs)

SPMDs are devices designed for passive, in-situ monitoring of aquatic contaminants. They consist of a low-density polyethylene (LDPE), lay-flat membrane filled with 1 g (gram) of a high molecular weight lipid, triolein, that cannot diffuse through the membrane. When placed in an aquatic environment, SPMDs passively accumulate hydrophobic organic compounds. The LDPE membrane mimics a biological membrane in its ability to allow selective diffusion of organic compounds. The development and applications of SPMDs have been reviewed recently by Huckins and others (1996).

The passive sampling of the hydrophobic organic chemicals is driven by lipid-water partitioning. Lipid-containing SPMDs have been used in several environmental settings (Huckins and others, 1990; Lebo and others, 1992; Prest and others, 1992), and their ability to concentrate trace levels of persistent and biodegradable organic contaminants has been demonstrated. Figure 1 shows a cross section of an SPMD deployed in an aqueous environment and illustrates the principals of SPMD sampling. The membrane transport corridor of the SPMD is winding and less than 1 nm (nanometer) in diameter. This prevents losses of the triolein to the environment yet allows permeation of smaller analyte molecules (Huckins and others, 1993). After the deployment period, the SPMDs can be retrieved from the stream, dialyzed, cleaned up with gel permeation chromatography and silica gel, and analyzed using chromatographic techniques.

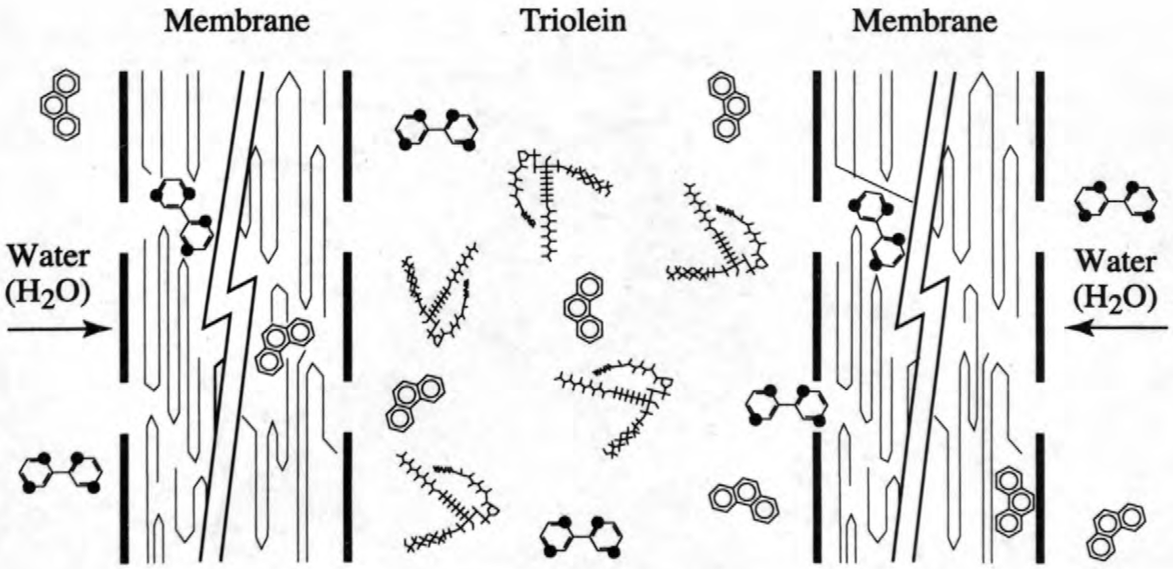
A close correlation exists between the organic compound's equilibrium triolein-water partition coefficient (K_{tw}) and its respective equilibrium octanol-water partition coefficients (K_{ow}). A compound's K_{tw} should closely approximate its K_{ow} (Chiou, 1985). Because log K_{ow} values are large for POPs (greater than 5), the capacity of triolein-containing SPMDs for POPs is high.

Soil half-life and other factors such as intensity of use, application methods, and climatic conditions are important in determining POP transport in surface water. Most of the older "first generation" chlorinated insecticides, such as chlordane, DDT, and dieldrin, are insoluble in water, have long soil half-lives, and large K_{oc} values. Consequently, they are transported primarily on sediment particles. Most of these first-generation chlorinated insecticides are banned and are no longer used in the United States, but they continue to persist in the Mississippi River Basin from previous use on cotton and because of their long soil half-lives. Also, because these chlorinated insecticides are relatively insoluble in water and have large K_{oc} values, they partition into the organic coatings of sediment or accumulate in the fatty tissues of fish and other stream biota (Moore and Ramamoorthy, 1984), which is not true for most other pesticides currently in use. Detections of selected POPs and other pesticides in part of the Mississippi Delta using semipermeable membrane devices (SPMDs) is described in this paper.

MATERIALS AND METHODS

Sampling Locations

SPMDs were deployed at five stream sampling sites in the Mississippi Delta for 31 days in June 1996 (fig. 2). The stream sampling sites were the Big Sunflower River at Anguilla, MS (site 1); Bogue Phalia at Leland, MS (site 2); Cassidy Bayou at Sumner, MS (site 3); Steele Bayou near Rolling Fork, MS (site 4); and the Yazoo River at Long Lake, MS (site 5). These streams are in the sparsely populated Yazoo River Basin, Mississippi's largest river basin, which consists of about 34,000 km^2 (square kilometers). In May and July 1997, SPMDs were deployed at only the Big Sunflower River near Anguilla, MS (site 1); Bogue Phalia at Leland, MS (site 2); and Steele Bayou near Rolling Fork, MS (site 4).



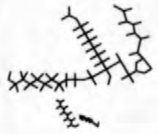
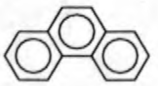
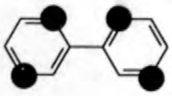
Compound	Symbol	Dimensions (nanometers) Length	Diameter
Triolein		2.7	2.8
Phenanthrene		1.2	0.8
2,2',5,5'-Tetrachlorobiphenyl		1.2	0.9

Figure 1. Schematic cross section of a semipermeable membrane device in an aqueous environment (modified from Huckins and others, 1993).

Sample Apparatus and Collection

SPMDs were acquired from Environmental Sampling Technologies (EST) of St. Joseph, MO. A total of 55 SPMDs were prepared for this study. Each SPMD consisted of a 81.4- x 2.54-cm (centimeter) strip of low-density, nonporous polyethylene tubing filled with 1 mL (milliliter) of purified (98 percent) triolein.

At each sampling site, the SPMDs were removed from their protective canisters and placed in stainless-steel deployment devices. A cylindrical deployment device of 0.32-cm stainless mesh with a center post for stacking the SPMD "spider" carriers was used. Each "spider" carrier consists of eight posts in which a single SPMD is woven to provide maximum available surface area for absorption of contaminants as shown in figure 3. Four SPMDs were placed in each deployment device. Each device was attached to weights to keep it submerged, while floats kept the SPMDs from resting on the bottom of the streams. The sampling sites were located on the downstream side of U.S. Geological Survey (USGS) gaging stations. The SPMDs were deployed in the streams for about 30 days in June 1996, May 1997, and July 1997.

A trip blank was designated for each site and consisted of a sealed canister containing a SPMD.

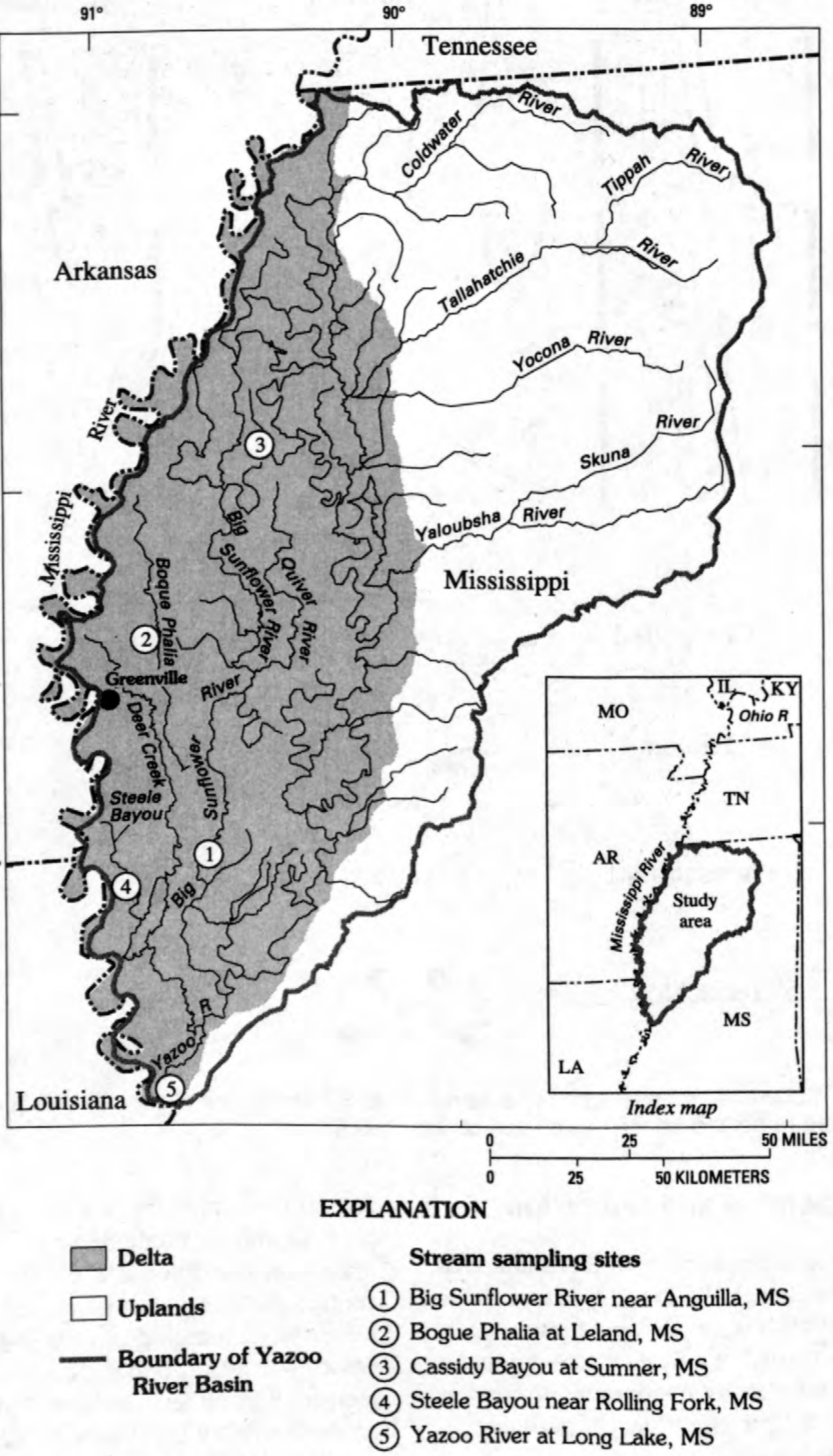


Figure 2. Location of sampling sites in part of the Mississippi Delta.

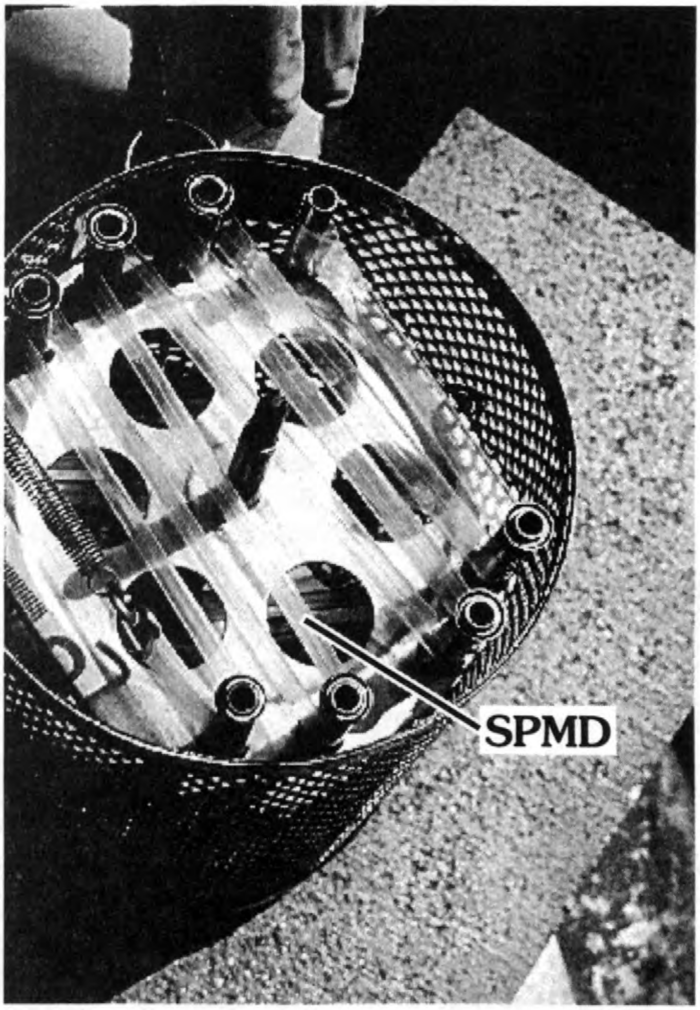


Figure 3. Spider carrier with semipermeable membrane device (SPMD).

The trip blanks were exposed during deployment to account for any atmospheric contamination of the SPMDs. The trip blank canisters were opened before the other canisters and remained open during the entire deployment period. After the deployment period, the SPMDs were removed from the stream, rinsed of sediment and debris, and shipped back to Environmental Sampling Technologies (EST) of St. Joseph, MO. During the recovery of the SPMDs, the trip blanks were re-exposed.

Extraction of Compounds

At Environmental Sampling Technologies the lipid fraction of each SPMD was removed and combined with other lipid fractions from the same sampling site. Trip blanks were extracted along with the other SPMDs. The extracts were recovered dialytically with a nonpolar solvent, hexane, from the lipid portion of the SPMD. This extract then was reduced, cleaned up, and enriched. The clean-up procedure used gel-permeation chromatography. This process removes any lipid that might have carried over during the dialysis extraction. Further cleanup by enrichment on an activated alumina and silica gel column was done. The enriched extracts were divided, and aliquots were analyzed, using chromatographic techniques, by the USGS National Water-Quality Laboratory (NWQL) in Arvada, CO; Environmental Sampling Technologies of St. Joseph, MO; and the USGS laboratory in Lawrence, KS.

Chromatographic Analysis

The NWQL and Environmental Sampling Technologies (EST) laboratories used gas chromatography/electron capture detection (GC/ECD) techniques. At the USGS laboratory in Lawrence, KS, gas chromatography/mass spectrometry detection (GC/MS) analysis of the extracts was done using a Hewlett-Packard Model 5890 Series II plus GC interfaced to a 5972 mass selective detector (MSD) (Palo Alto, CA). One microliter (1 µL) of each sample was injected in the splitless mode using an autoinjector. Separation of the compounds was accomplished with a fused-silica capillary column of 5 percent phenyl methyl silicone (Ultra 2) with a film thickness of 0.33 µm (micrometer), 30-m (meter) x 0.2-mm (millimeter) inside diameter (Hewlett Packard, Palo Alto, CA) with a 5-m guard column (Supelco, Bellefonte, PA). The column temperature was held at 60 °C (degrees Celsius) for 1 minute, ramped at 6 °C per minute to 200 °C, and then ramped at 30 °C per minute to 250 °C where it was held. Confirmation of the compounds was based on the presence of the molecular ion and two confirming ions, a retention-time match compared to external standards, and correct area ratios of the confirming ions. The compounds analyzed are listed in table 1.

Trip blanks were analyzed exactly as deployed samples and were used to define contamination of the SPMD concentrations during transportation and handling. Any concentrations detected in trip blanks were subtracted from SPMD concentrations from the stream sampling sites.

The SPMDs used in 1996 were mishandled, and, sample extract was lost. Because of the low mass of extract available, data from that sampling is qualitative only.

RESULTS AND DISCUSSION

POPs and Other Pesticides Detected

The POPs aldrin, chlordane, DCPA, DDT, dieldrin, endrin, hexachlorobenzenes (HCHs), heptachlor, mirex, nonachlor, and toxaphene were detected using SPMDs at the Mississippi Delta stream-water sampling sites. In addition, two insecticides still in use, the organophosphate chlorpyriphos and the organochlorine endosulfan, were detected. Figure 4 shows a chromatogram obtained from the Environmental Sampling Technologies (EST) laboratory using GC/ECD for the Big Sunflower River site (site 1, fig. 2) in May 1997. In figures 5, 6 and 7, calculated concentrations in the SPMDs for Big Sunflower (site 1, fig. 2), Bogue Phalia (site 2, fig. 2), and Steele Bayou (site 4, fig. 2) are shown, respectively.

Total POP concentrations varied little by site and sampling time with one exception. The May 1997 SPMDs sampled at Bogue Phalia (site 2) had a total concentration of detectable POPs much lower than the other sites and sampling times. This difference is most likely due to biofouling of the SPMDs on the exterior of the membranes. Biofouling can act as an impediment to flux across the membrane, thus slowing the effective sampling rate (R_s). This impedance factor is specific to each SPMD at any given point in time. There is potential for quantifying this impedance for a specific deployment by measuring the loss of a surrogate compound (contained within the SPMD) during deployment, but the SPMDs in this study did not contain any permeability reference compounds to act as surrogate standards.

Toxaphene, an insecticide POP consisting of a complex mixture of chlorinated camphenes, was a major source of chromatogram interference in 1996. Toxaphene was not analyzed in the 1997 SPMDs.

SPMDs have some advantages over traditional methods of water sampling. They can be standardized and may be representative of the thermodynamically dissolved organic phase in surface water. They can be deployed for long periods of time (days to months) and used to estimate the time-weighted mean concentrations of the hydrophobic organic compounds in the water body. For a given nonionic organic chemical, a SPMD will effectively sample 0.5 to 10 L/d (liters per day), depending on the chemical's hydrophobicity (as quantified by its water solubility or octanol-water partitioning coefficient (K_{ow}). A compound with log K_{ow} = 6 would need 200 days at a constant water concentration and at an effective sampling rate of 10 L/d to reach 90 percent equilibrium or more. However, during an initial period, the uptake rate into the SPMD is linear and first order (Huckins and others, 1993). The ambient "truly dissolved" water concentration (C_w) in nanograms per liter (ng/L) can be estimated on the basis of concentra-

Table 1. Pesticide class, compound name, and analysis type for compounds analyzed from semipermeable membrane devices

[IGR, insect growth regulator; OC, organochlorine, OP, organophosphate; Py, pyrethroid, SA, substituted analine]

Pesticide class	Compound name	Analysis type	Pesticide class	Compound name	Analysis type
IGR	methoprene	qualified	OC	nonachlor	qualified
None recognized	DCPA dacthal	quantified	Do.	o,p'-DDD	quantified
OC	aldrin	Do.	Do.	o,p'-DDD	Do.
Do.	alpha-HCH	Do.	Do.	o,p'-DDT	Do.
Do.	beta-HCH	Do.	Do.	p,p'-DDD	Do.
Do.	delta-HCH	Do.	Do.	p,p'-DDE	Do.
Do.	gamma-HCH (lindane)	Do.	Do.	p,p'-DDT	Do.
			Do.	toxaphene	qualified
Do.	cis-chlordane	Do.	OP	azinphos-methyl	Do.
Do.	trans-chlordane	Do.	Do.	chlorpyriphos	Do.
Do.	cis-nonachlor	qualified			
			Do.	dicrotophos	Do.
Do.	trans-nonachlor	quantified	Do.	fonofos	Do.
Do.	dieldrin	Do.	Do.	malathion	Do.
Do.	endosulfan	Do.	Do.	methyl parathion	Do.
Do.	endosulfan II	qualified	Do.	profenofos	Do.
Do.	endosulfan sulfate	Do.			
			Do.	sulprofos	Do.
Do.	endrin	Do.	Do.	terbufos	Do.
Do.	endrin aldehyde	Do.	Py	bifinthrin	Do.
Do.	heptachlor	quantified	Do.	cyfluthrin	Do.
Do.	heptachlor epoxide	Do.	Do.	cypermethrin	Do.
Do.	hexachlorobenzene	Do.	Do.	lambda-cyhalothrin	Do.
			Do.	permethrin	Do.
Do.	methoxychlor I	qualified	SA	pendimethalin	Do.
Do.	methoxychlor II	Do.	Do.	trifluralin	Do.
Do.	methoxychlor/ mirex	quantified			

tions in the SPMD's lipid (C_{spmd}) in nanograms per gram (ng/g), weight of the lipid in the SPDM (Wt_{spmd}, in grams) the fouling factor (f), the effective sampling rate (Rs), and the time of deployment (t) (Moring and Rose, 1997):

$$C_w = \frac{C_{spmd}*\mathrm{Wt}_{spdm}}{f*\mathrm{R}_s*\mathrm{t}}, \qquad (1)$$

The fouling factor (f) can be large due to sediment and other material accumulating on the SPMDs or the stainless-steel deployment device. Mississippi Delta streams carry substantial sediment, and biofouling was very obvious on the deployment devices at the time they were retrieved from the streams. Unfortunately, biofouling hinders the accuracy of SPMDs to indicate water concentrations unless an accurate fouling factor is determined. In this study, permeability reference compounds, which can be used to determine the extent of fouling, were not used. The effective sampling rate (Rs) of many hydrophobic compounds

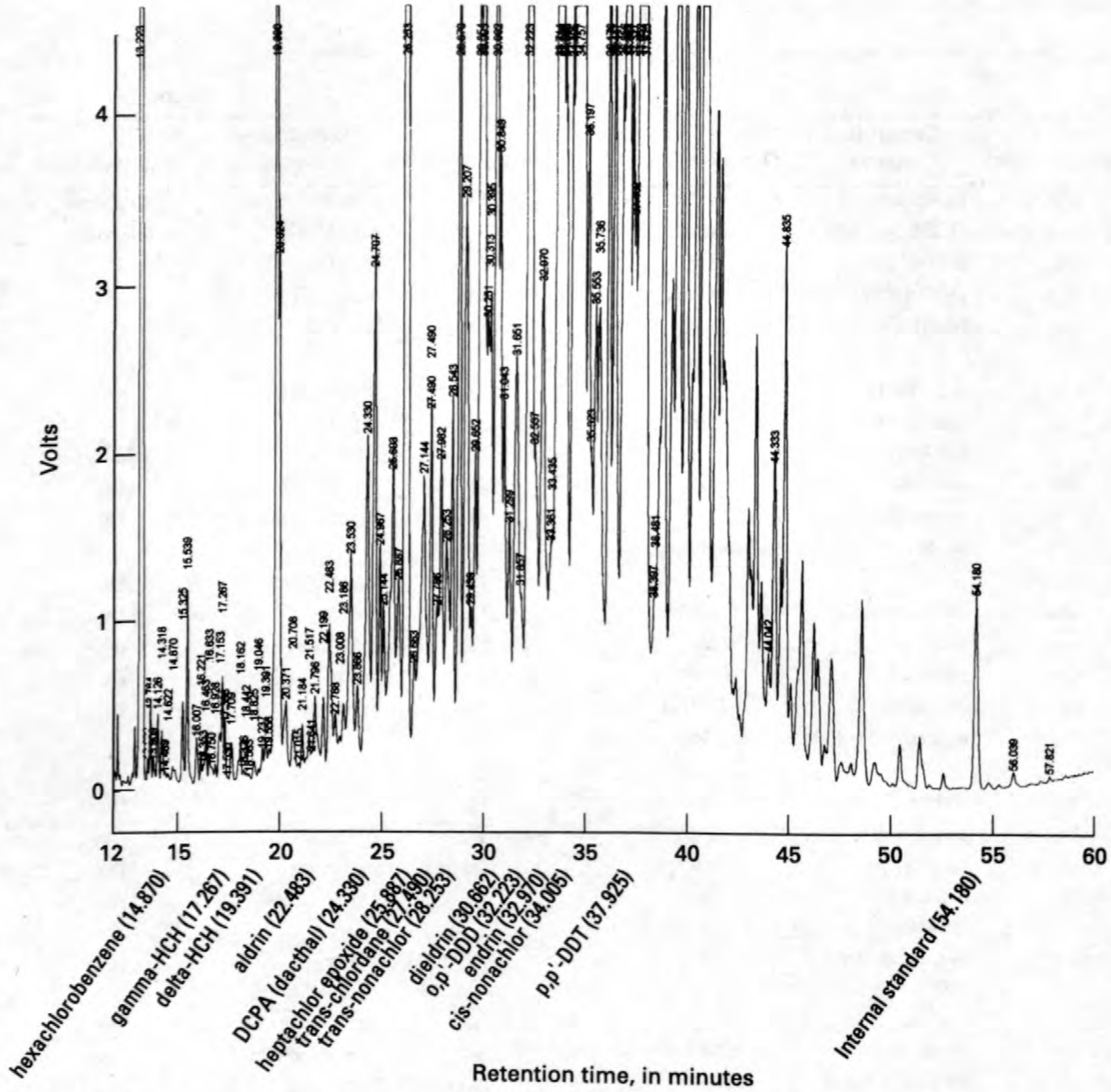


Figure 4. Chromatogram of semipermeable membrane device sample from the Big Sunflower River sampling site (site 1, fig. 2).

have already been determined, and more research is underway to expand the number of compounds.

Two herbicides not commonly detected in surface water, pendimethalin and trifluralin, were detected in some SPMD extracts. These two substituted-aniline herbicides are used extensively on cotton and other crops in the sampling basins (Gianessi and Anderson, 1995) but have been detected in surface-water samples only at very low concentrations (less than 0.1 µg/L) (Dayama and Coupe, 1997; Thurman and others, 1998). Considering their extensive use, the lack of pendimethalin and trifluralin detections in surface-water samples can be attributed to the low solubility (less than 1 mg/L) of these herbicides in water.

Comparison of SPMD Data to Fish-Tissue Data

Previously, fish tissue has been collected at sites within the Yazoo River Basin by State and Federal agencies (Mississippi Bureau of Pollution Control, 1984; Schmitt and others, 1990; Plunkett and others, 1997). These data have shown that total

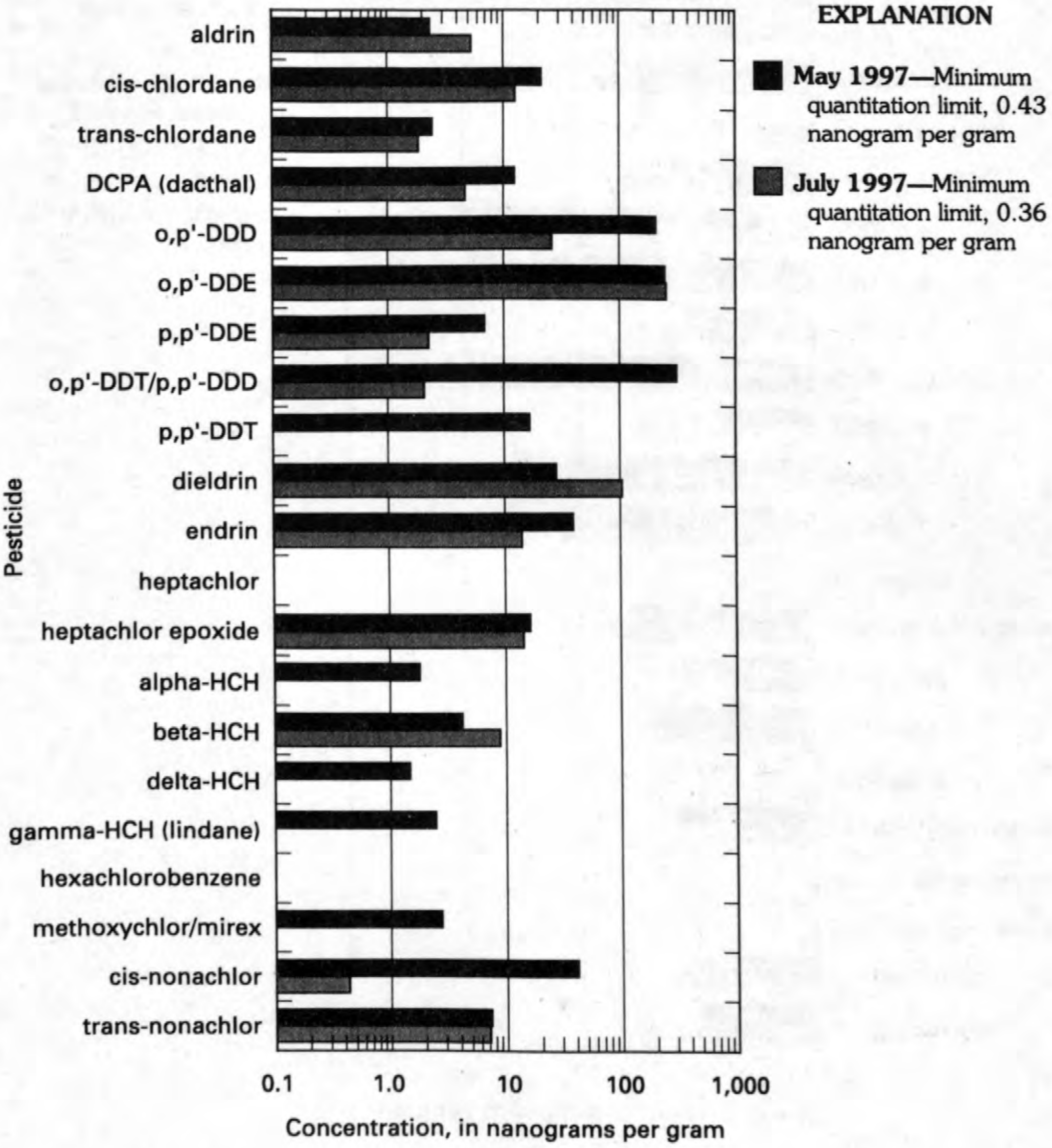


Figure 5. Pesticide concentrations in semipermeable membrane devices at Big Sunflower River sampling site (site 1, fig. 2).

DDT concentrations in the fish tissue collected from sites within the Yazoo River Basin are among the highest found in the United States. Kleiss and Justus (1997) provided fish-tissue analysis from tissue samples collected during the fall of 1995 and 1996 at the SPMD sampling sites (fig. 2). A tissue sample consisted of an eight-fish whole-fish composite of common carp (*Cyprinus carpio*) for organochlorine analysis. Figure 7 shows comparisons of the SPMD data to the fish-tissue data for DDT and its metabolites.

Concentrations of DDT and its metabolites detected in SPMD extracts were approximately 1,000 times smaller than those measured in fish tissues (fig. 8). If SPMD data can be corrected for fouling, SPMDs may provide first approximation of concentration of POPs in Mississippi Delta streams. Calculation of water concentrations from SPMD concentration may be possible, although potentially problematic.

CONCLUSIONS

The POPs aldrin, chlordane, DCPA, DDT, dieldrin, endrin, hexachlorobenzenes, heptachlor, mirex, nonachlor, and toxaphene were detected at

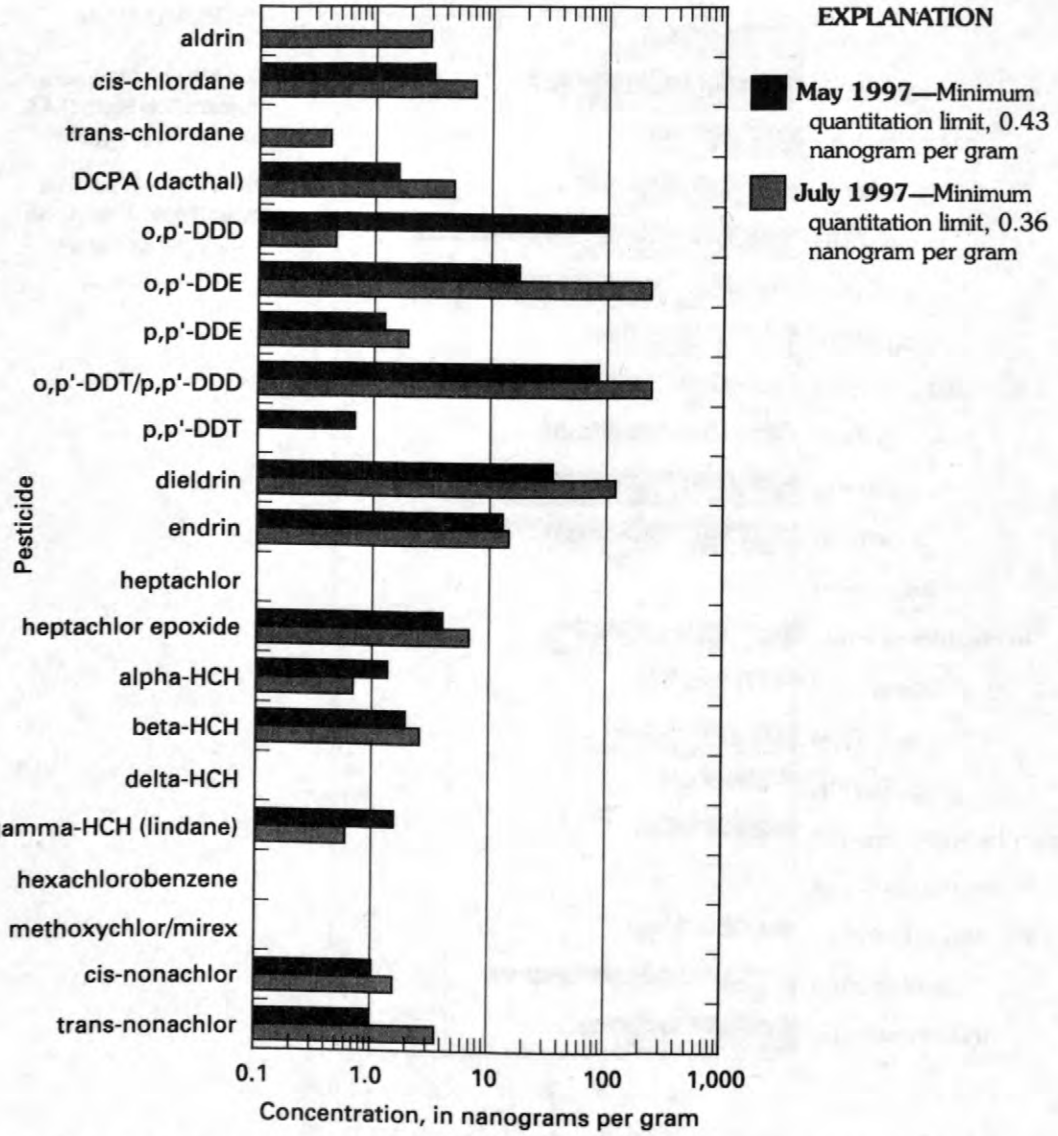


Figure 6. Pesticide concentrations in semipermeable membrane devices at Bogue Phalia sampling site (site. 2, fig. 2).

stream sampling sites within the Cotton Belt using SPMDs. In addition, two insecticides still in use, the organophosphate chlorpyriphos and the organochlorine endosulfan, were detected. These results, along with comparisons to fish-tissue data, suggest that SPMDs can provide an effective first approximation of biological accumulation of POPs in Mississippi Delta water when fouling factors are used. An unexpected finding was that two low-solubility herbicides not detected commonly in surface water, pendimethalin and trifluralin, were sequestered by SPMDs.

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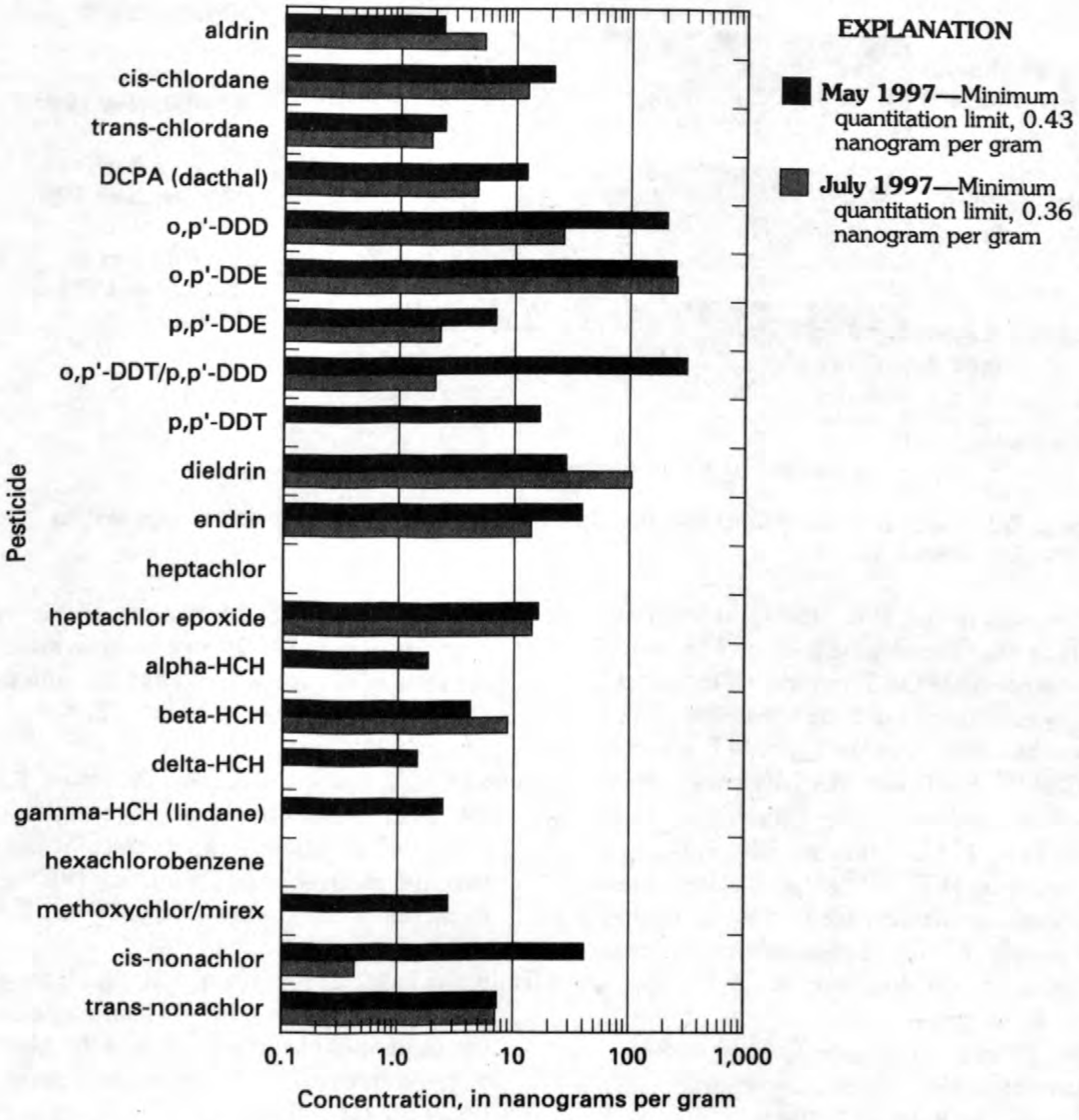

Figure 7. Pesticide concentrations in semipermeable membrane devices at Steele Bayou sampling site (site 4, fig. 2).

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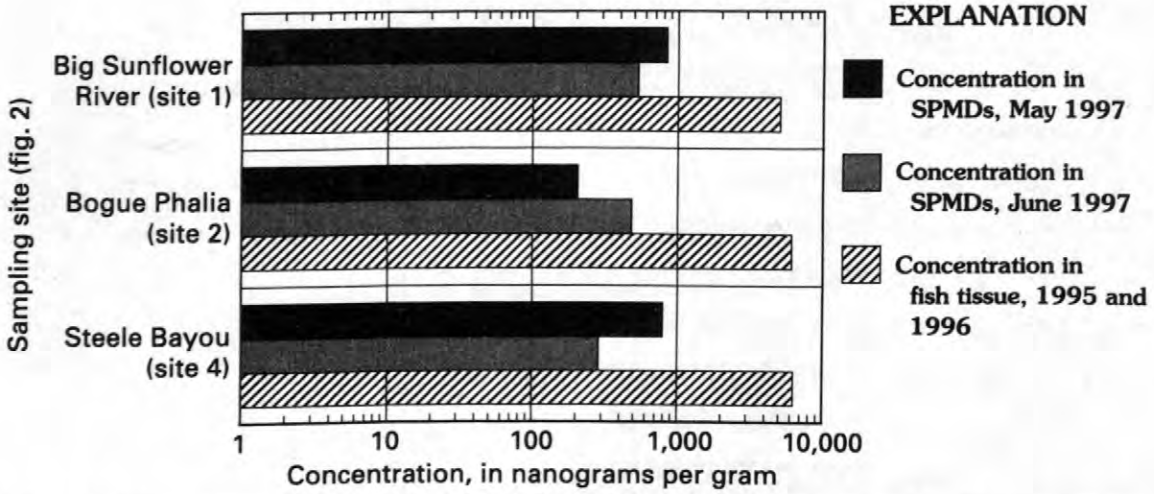

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SECTION D

Additional Research on the Effects of Contamination on Hydrologic Systems and Related Ecosystems

This section contains papers on a variety of topics that relate to the development of new methods to study contamination from nonpoint sources and new analytical methods to measure and detect contaminants at levels significant for the environment. In some cases, the Toxic Substances Hydrology Program directly funds the research. In other cases, the research uses methods that were originally developed by the Program. Some of the papers are reporting the results of research on the fate of contaminants in the environment conducted by other U.S. Geological Survey programs.

A variety of contaminants are discussed in the papers, including widely used pesticides and their metabolites, antibiotics that are used in livestock production, organometallic compounds, oxygenated gasoline additives such as methyl tert-butyl ether (MTBE), and common halogenated organic compounds. The link between contaminants and environmental health are also addressed by looking at potential effects of some contaminants on the health of fish.

The fate of these contaminants is being studied in rivers, streams, lakes, and ground water. Some of the compounds have existed in the environment for many years. Others are either newly created compounds or their environmental relevance and adverse effects on aquatic life are just being recognized. Developing methods to study the fate of these "emerging" contaminants is one of the many areas of research that is supported by the Program. The information presented in these papers is useful for developing better land- and water-management practices.

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Ratios of Metolachlor to its Metabolites in Ground Water, Tile-Drain Discharge, and Surface Water in Selected Areas of New York State

By Patrick J. Phillips, David A. Eckhardt, E. Michael Thurman, and
Stephen A. Terracciano

ABSTRACT

The transport and fate of the herbicide metolachlor and its principal metabolites - metolachlor ESA (ethanasulfonic acid) and metolachlor OA (oxanilic acid) - in ground water from Suffolk County, on Long Island, from tile drains in an agricultural area in central New York and in surface water from five streams in central and western New York, was evaluated in 1997-98. Comparison of (1) the ratio of metolachlor ESA to metolachlor (SAM ratio) and (2) the ratio of metolachlor ESA to metolachlor OA (SAO ratio) in well water from Suffolk County with these ratios in tile-drain runoff and in the five streams indicates that these ratios vary according to soil properties, hydrologic factors, and time since metolachlor application. Both ratios were lower in well-water samples from Suffolk County (which is dominated by coarse-grained soils), than in the tile-drain samples from central New York fields (which have finer grained soils). The lower SAM ratios for the Suffolk County well-water samples than for the tile-drain samples probably result from the more rapid transport and lesser degradation of metolachlor in the coarse soils than in the fine-grained soils. The lower SAO ratios in Suffolk County well water also result from rapid infiltration of metolachlor OA through the coarse grained soils. SAM ratios for the surface-water samples were lower than those for the well-water and tile-drain samples, probably because surface water has a shorter residence time in the soil than ground water; the shorter contact time with soil microbes and enzymes than in ground water allows less degradation of metolachlor to metolachlor ESA.

SAM and SAO ratios for samples from one of the tile drains decreased after rainstorms that followed metolachlor application. SAM ratios for this tile drain decreased from more than 500 to less than 1 during the storm, and SAO ratios decreased from about 3 to less than 2. These decreases are attributed to preferential flow that accelerated the movement of metolachlor and metolachlor OA into the subsurface during the storm. SAM ratios in the stream that receives discharge from the tile drains were lowest during stormflows in June 1998 that followed metolachlor application. These low postapplication SAM ratios are attributed to the water's short contact time with soil and, hence, minimal transformation of metolachlor to metolachlor ESA.

INTRODUCTION

The common presence of two major metabolites of metolachlor - metolachlor ethanasulfonic acid (metolachlor ESA) and metolachlor oxanilic acid (metolachlor OA) - in ground water and surface water in agricultural areas of Iowa (Kahlkhoff and others, 1998) indicates that these metabolites are readily formed in the soil and transported to ground water and to streams. In the Iowa study, more than 90 percent of the mass of acetanilide residues in ground water, and more than 80 percent of the acetanilide residues in surface water, consisted of the sulfonic and oxanilic acid degredates. Metolachlor is transformed to metolachlor ESA by the glutathione conjugation process (Field and Thurman, 1996) and to metolachlor OA by a process that is unknown, but probably biologically mediated within the soil.

Results of previous investigations of the occurrence of metolachlor and its metabolites in tile drain discharge from agricultural fields in central New York indicate that (1) metolachlor ESA and OA can persist in agricultural soils for 3 or more years after application (P. J. Phillips, U.S. Geological Survey, written commun., 1999), (2) metolachlor ESA concentrations in tile-drain discharge exceed those of metolachlor by a factor of 200 to 1,800, and (3) metolachlor ESA concentrations in tile-drain discharge generally exceed those of metolachlor OA by a factor of 2 to 5. Results also indicate that ratios of metolachlor ESA to metolachlor (SAM ratio) in the receiving stream are lower than those in tile-drain discharge, as a result of differing flow paths. Water in tile-drain runoff (and, by extension, ground water) has a longer period of contact with the soil, and thus, more time for chemical degradation through microbial and enzyme reactions than surface water and therefore has higher SAM ratios than surface water.

The ratio of deethylatrazine concentration to atrazine concentration (DAR) has been used in many studies to indicate hydrologic pathways along which atrazine is transformed into deethylatrazine. Low DAR values in surface water have been correlated with the "spring flush" of atrazine in the midwestern United States just after planting and pesticide application (Thurman and Fallon, 1996), and high DAR values in surface water typically occur in the months before application, or in the harvesting months, when surface water flow consists mostly of ground water (Thurman and others, 1991; Thurman and others, 1994). A corresponding seasonal change in the SAM ratio would probably indicate that, like the DAR, SAM ratios in surface water are probably controlled in part by timing of metolachlor application and the proportion of surface water consisting of ground water.

This paper describes how SAM ratios in surface water differ from those in ground water from wells on Long Island and tile drains beneath cultivated fields in central New York, and how these ratios vary according to soil properties and hydrologic factors; it also compares SAM and SAO ratios among the five upstate surface-water sites to indicate the seasonal variability of these ratios in relation to the timing of metolachlor application. Results of this paper will provide information on the fate and transport of metolachlor, which is one of the most heavily used herbicides in New York State (Gail Thelin, U.S. Geological Survey, written communication, 1998).

METHODS

Water samples were collected from three networks - 50 wells in Suffolk County, two tile drains in Montgomery County, and five surface-water sites in Montgomery, Onondaga, Tompkins, and Livingston County (fig. 1, table 1). Most of these samples were collected as part of a cooperative effort between the U.S. Geological Survey and the New York State Department of Environmental Conservation as part of the New York State Pesticide Monitoring Program. Some samples were collected as part of the U.S. Geological Survey National Water Quality Assessment Program.Sampling Sites

Sampling Sites

The 50 Suffolk County wells tap a shallow surficial sand aquifer of glacial sand and gravel. These wells were selected on the basis of known or suspected pesticide contamination. The samples were collected by the USGS and the Suffolk County Department of Health Services (SCDHS) from April through July 1998. Each well was sampled once during the study. Well-screen depths range from 9 to 202 feet below land surface, and thickness of the unsaturated zone at wells with a detection of metolachlor or metolachlor ESA ranges from less than 5 feet to 75 feet. Of the 50 wells sampled, 22 had a detection for metolachlor or its two metabolites. Most of the wells with a detection of any of these compounds are in agricultural areas. Metolachlor, which is widely used on potatoes in Suffolk County, has been detected previously in Suffolk County ground water by the SCDHS (Baier and Trent, 1998).

The two tile drains are in the Canajoharie Creek watershed (fig. 1) and underlie fields that are in a corn-soybean rotation. The tile drains were installed in the 1980's at a depth of 3 to 4 feet below

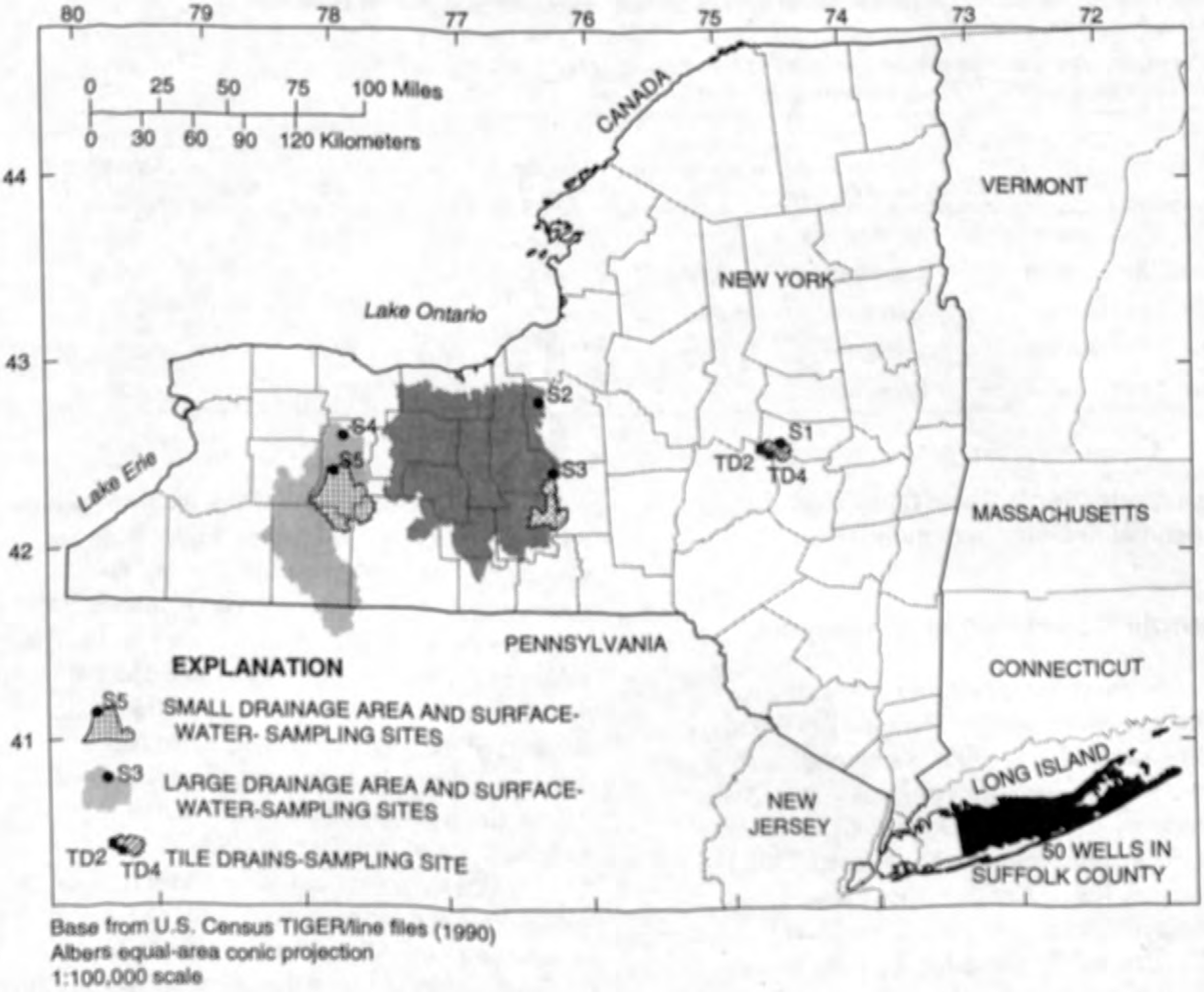


Figure 1. Location of Suffolk County, tile drain Network, and upstate surface water network.

land surface and are below the water table except in the late summer and early fall; thus, the tile-drain runoff is representative of shallow ground water beneath the fields. Parts of the fields overlying Tile Drain 4 (TD4) were treated with metolachlor in 1997 and 1998, but not during the 3 preceding years (1994-96), whereas the field overlying TD2 was treated in 1995, but not during 1996-98. The TD4 fields are underlain by an unconfined surficial aquifer that consists of as much as 20 feet of alluvial sand and gravel with some silt. The Tile Drain 2 (TD2) field is underlain by 9 to 40 feet of glaciolacustrine clay, which confines the underlying aquifer. Depth to water beneath both fields ranges from 3 to 8 feet below land surface (Hollister, 1997). Samples were collected from the tile drains once every 2 months from November 1997 through April 1998 and weekly from May through July of both years. The tile drains did not flow from mid-July through October of either year.

Samples were collected from the five upstate surface-water sites quarterly to monthly from April 1997 through May 1998, weekly during the postapplication period of June to July, and during selected storms in June and July. About 20 samples were collected at each site except Canajoharie Creek (which receives discharge from the two tile drains); 47 samples were collected at this site. Half the samples from each site were collected in June and July, the other half in the remaining months. Three of the sites (Canajoharie Creek, Fall Creek, and Canaseraga Creek) drain small (less than 350 square mile) agricultural watersheds - the other two (Seneca River and Genessee River) which drain watersheds of at least 1,500 square miles) include the Fall Creek and Canaseraga Creek watersheds,

Table 1. Land use within drainage areas of the five surface-water sites sampled for pesticides in upstate New York, 1990-98.

[mi^2, square miles. Land-use percentages based on satellite-imagery data collected in 1994 (U.S. Geological Survey, 1997). Locations are shown in fig. 1.]

Site no.	Site name and county		Drainage area (mi^2)	Percentage of drainage area				
				Pasture/ hay	Row crop	Forest	Urban	Open water
S1	Canajoharie Creek	- Montgomery	60	41	20	38	1	0.18
S2	Seneca River	- Onondaga	3160	21	31	40	3.3	6.6
S3	Fall Creek	- Tompkins	126	22	20	55	3.3	0.3
S4	Genesee River	- Livingston	1673	12	30	56	1.5	0.7
S5	Canaseraga Creek	- Livingston	334	12	34	52	2	0.28

respectively (fig. 1). These four watersheds contain substantial row-crop agriculture (table 1).

Sample Collection and Analysis.

Samples were collected according to procedures described by Shelton (1994) and Lapham and others (1995). Sample splits were sent to two laboratories - the National Water Quality Laboratory (NWQL) in Denver, Colo., and the USGS Organic Research Laboratory (ORL) in Lawrence, Kans. The parent compound (metolachlor) was analyzed at the NWQL (Zaugg and others, 1995); the detection limit for this method is 0.002 µg/L. The two metabolites were analyzed by high-performance liquid chromatography (HPLC) with diode array detection and quantification (Ferrer and others, 1997); the detection limit for the two metabolites is 0.2 µg/L. Blank samples collected and analyzed during this study showed no detections for metolachlor or its metabolites.

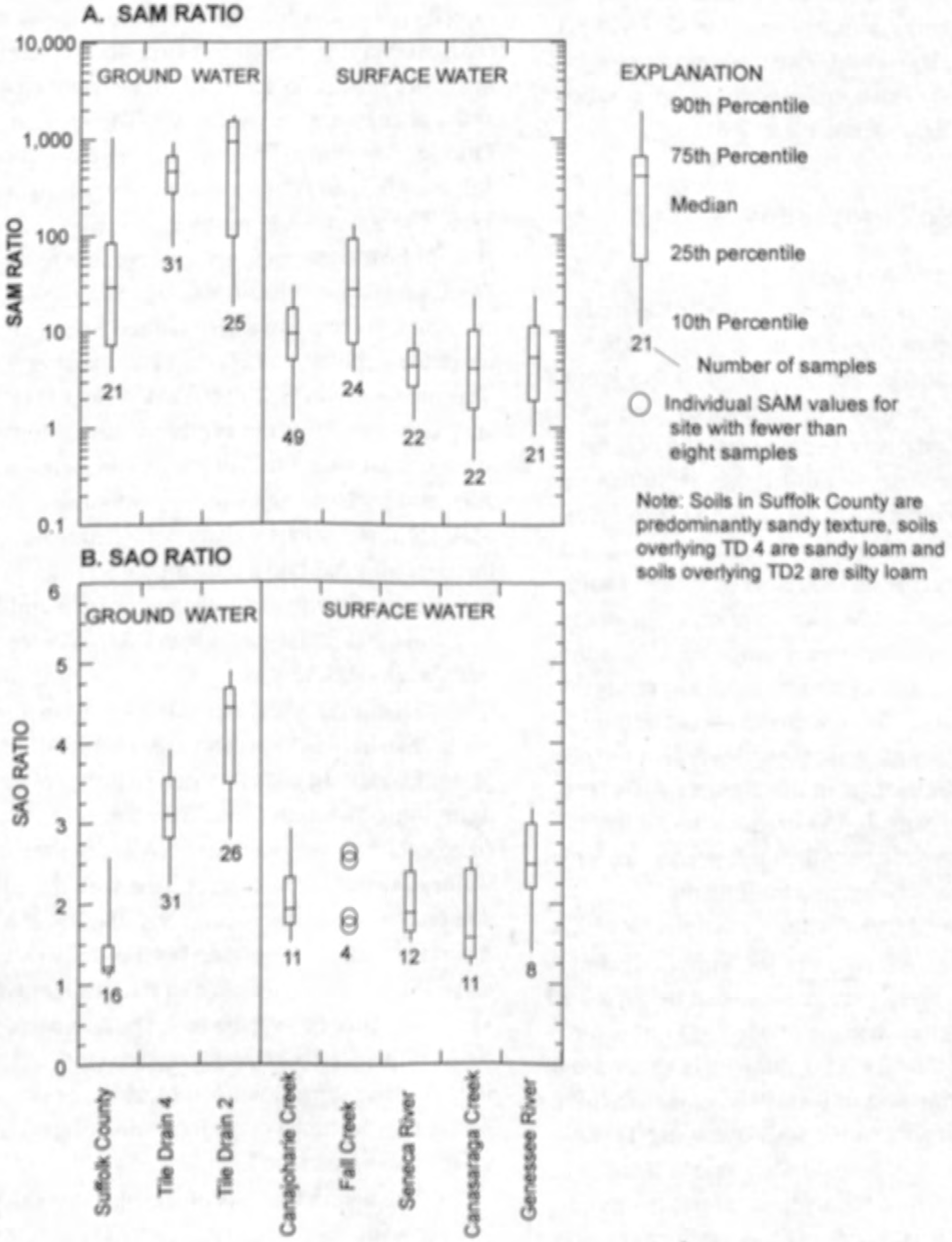
Calculation of SAM and SAO ratios

SAM ratios for samples with no detection of metolachlor were calculated as the metolachlor ESA concentrations divided by 0.001µg/L (one-half the detection limit of metolachlor). All but two samples with a detection of metolachlor ESA also had a detection for metolachlor; these two samples from wells in Suffolk County. The SAM ratios for these samples exceeded 1,000 and are the highest of any well-water samples. SAM ratios for samples with a detection of metolachlor but not metolachlor ESA were calculated as 0.1 (half the detection limit for metolachlor ESA) divided by the metolachlor concentration. Six well-water samples from Suffolk County had detectable metolachlor but no detectable metolachlor ESA; the calculated SAM ratios for these samples ranged from 7 to 38. These values are generally lower than the SAM ratios for the other well-water samples. A few (less than 10 percent) of the surface-water samples had a detection of metolachlor but not metolachlor ESA. All tile-drain samples had a detection of metolachlor and metolachlor ESA.

SAO ratios were calculated only for samples with a detection for both metolachlor ESA and metolachlor OA. All well samples and tile-drain discharge samples with a detection for metolachlor ESA also had a detection for metolachlor OA. Fewer than 40 percent of the surface-water samples with a detection for metolachlor ESA had a detection for metolachlor OA.

SAM AND SAO RATIOS IN GROUND WATER, TILE-DRAIN DISCHARGE AND SURFACE WATER

The highest median SAM ratios were in the tile-drain discharge samples, and the lowest were in the surface-water samples (fig. 2). This indicates that ratios of metolachlor ESA to metolachlor are higher in ground water (which includes tile drain and Suffolk County well water samples) than in surface water. The median SAM ratio for samples from TD2 was 950, and that for TD4 was 460; the SAM ratio for some samples from the tile drains exceeded 1,000. The median SAM ratio for the well-water samples from Suffolk County was 30 - markedly lower than the medians for the tile



Note: Soils in Suffolk County are predominantly sandy texture, soils overlying TD 4 are sandy loam and soils overlying TD2 are silty loam

Figure 2. Ratio of metolachlor metabolite concentrations in Suffolk County ground water, tile-drain discharge, and surface water samples: A. SAM ratio (ratio of metolachlor ESA to metolachlor concentration). B. SAO ratio (ratio of metolachlor ESA to metolachlor OA concentration).

drains. Median SAM ratios for all surface-water samples were less than 10 (fig. 2a) except those from Fall Creek.

The highest median SAO ratios were in the tile-drain discharge samples - 4.4 for TD2 and 3.1 for TD4 (fig. 2B) - and the well-water samples had the lowest (1.2). The surface-water samples ratios were intermediate - from 1.8 to 2.6.

Effects of Soil Properties

The lower SAM and SAO ratios in Suffolk County well-water samples than in the tile-drain discharge samples (fig. 2A) are attributable to differences in soil properties. The soils overlying TD2 and TD4 are silty loam and sandy loam, respectively, and contain more clay and organic matter than the coarse-grained soils of Suffolk County. The higher SAM ratios for TD2 samples than for TD4 samples reflect the finer texture and, hence, the longer residence time of water in soils overlying TD2. Metolachlor sorption is greater in fine-grained soils than metolachlor ESA; thus the TD2 samples generally had higher SAM ratios than the TD4 samples. The low SAM ratios for the Suffolk County well-water samples reflect the poor sorption of metolachlor in these coarse soils. The coarse soils of Suffolk County also have a lower organic carbon content, which minimizes microbial or soil enzyme activity, and inhibits the transformation of metolachlor to metolachlor ESA.

The low SAO ratios of the Suffolk County well-water samples (fig. 2B) also can be attributed the soil properties, and the lower SAO ratios for TD4 samples than for TD2 samples is attributed to preferential transport or formation of metolachlor OA in the coarser grained soils overlying TD4. Metolachlor OA is probably less stable than metolachlor ESA in the soil; therefore, the rapid infiltration of metolachlor OA through coarse soil into the aquifer would result in low SAO ratios in the underlying ground water. Once metolachlor OA has reached the aquifer, it is no longer subject to the activity of soil enzymes and microbes that cause degradation and alter the SAO ratio. The lower SAO ratios of Suffolk County well water than of tile-drain discharge are consistent with the observed difference in soil coarseness.

Effects of Hydrologic Factors

The lower SAM ratios in surface-water samples collected during the two postapplication months (June and July) than the other months (August-May) (fig. 3) result from the shorter residence time (contact time) of the water with soil, and soil enzymes and microbes. The median SAM ratio for Canajoharie Creek samples from June and July was 6.7, and that for the other months was 12.5. The lower SAM ratios in the two postapplication months are comparable to the low DAR values found in surface-water samples collected in the midwestern United States after pesticide application (Thurman and Fallon, 1996; Thurman and others, 1991). DAR values for the five surface-water sites (not presented here) show a similar seasonal pattern of lowest values in June and July, and highest values in other months. Metolachlor in surface water collected from August through May has had a longer contact time with the soil than surface water collected in June and July and, thus, has undergone greater degradation to metolachlor ESA.

The lower SAM ratios in upstate surface water than in tile-drain discharge or Suffolk County ground water are probably due to differences in hydrologic flowpaths. Transformation of metolachlor to metolachlor ESA is favored where water has significant contact time with the soil and soil enzymes and microbes; thus, the lower SAM ratios for surface water than for tile-drain discharge or well water is attributable to the surface water's short residence time in the soil. The anomalous high SAM ratios for Fall Creek samples (fig. 2, 3) indicate that some unknown local factor is promoting the transport or formation of metolachlor ESA in this watershed.

The few detections of metolachlor OA in surface-water samples make the SAO ratios difficult to correlate with hydrologic pathways. The median SAO ratios for surface-water samples were intermediate between those for the tile-drain discharge samples and those for Suffolk County ground water (fig. 2B). These data suggest that, in general, metolachlor OA is formed or transported more readily in ground water beneath coarse-grained soils than in surface water.

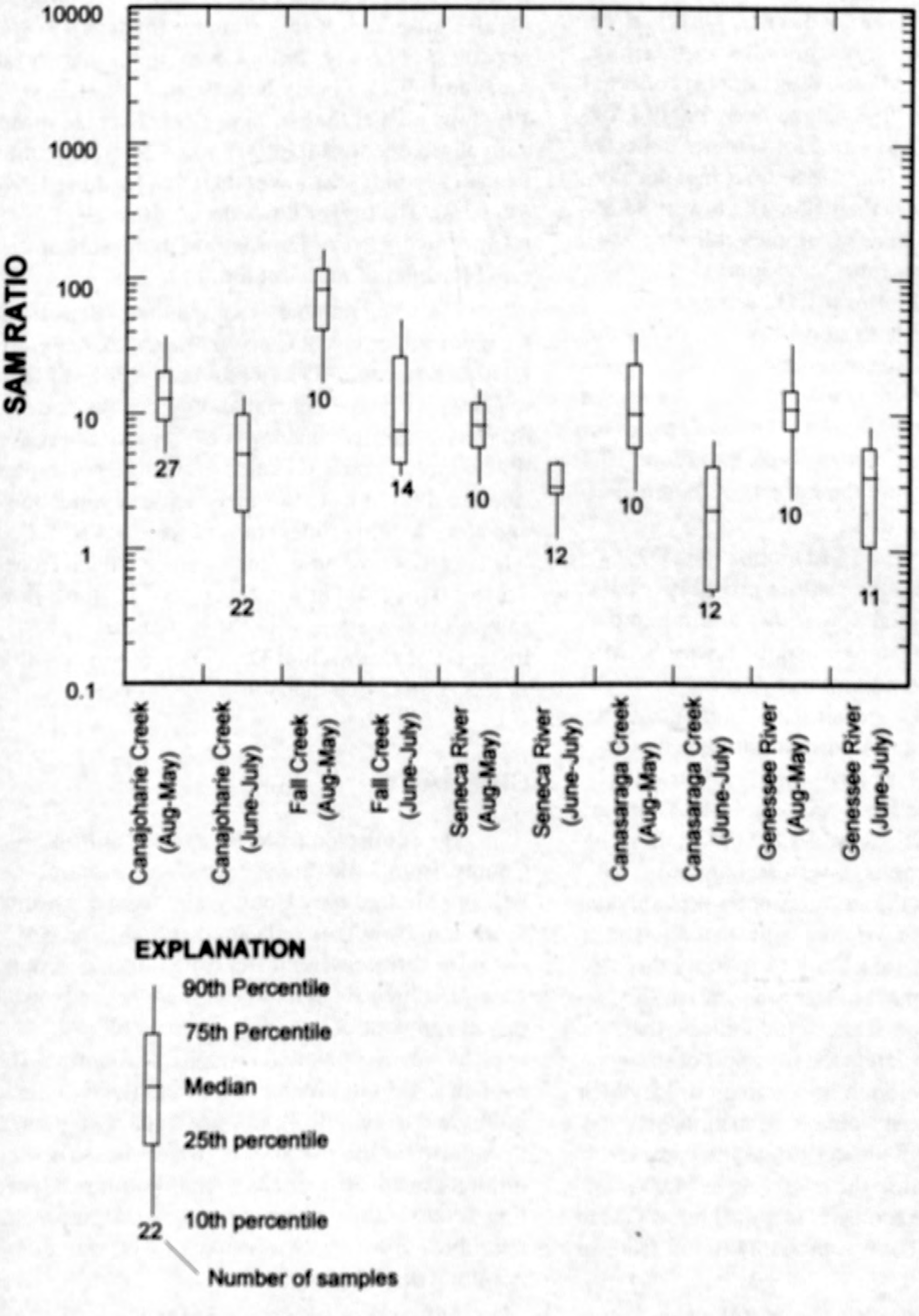


Figure 3. SAM ratios (ratio of metolachlor ESA to metolachlor concentrations) for five surface water sites, by post application months (June-July 1997-98) and other months (August-May 1997-98).

Effects of Storms

Samples collected from TD2 and Canajoharie Creek in June 1998 indicate that SAM and SAO ratios in tile-drain discharge and surface water change during storms just after metolachlor application. The SAM ratios for samples collected at TD2 before June 1998 ranged from 500 to 1,100 (fig. 4A), but the SAM ratios for samples collected after large storms in June 1998 (after metolachlor application) were less than 100. The lowest SAM ratios (less than 1) were in samples collected just after large storms on June 3 and June 15, 1998 (fig. 4A). The SAO ratios in TD2 samples also decreased in response to storms in June 1998 - the SAO ratios for non-storm samples collected from TD2 in June 1998 were similar to those for samples collected before June (fig. 4A). The SAO ratios for the two samples collected on June 3 and June 15, 1998 (less than 2) were the lowest SAO ratios obtained from this site.

The low SAM and SAO ratios for TD2 samples after the two June storms probably resulted from rapid preferential flow from the soil into the aquifer. These two samples had the lowest SAM and SAO ratios, the highest metolachlor concentrations (both greater than 7 µg/L), and the highest metolachlor OA concentrations (greater than 3 and 11µg/L), of any samples collected at TD2. Normally, the fine-grained soils that overlie TD2 result in high SAM and SAO ratios; thus, the elevated concentrations of metolachlor and metolachlor OA in the storm samples probably are the result of preferential flow paths that allowed metolachlor and metolachlor OA to move through the soil, with minimal contact time and, thus, minimal degradation. This would indicate that heavy rain can accelerate the transport of these compounds even through fine-grained soils, which generally retard the movement of metolachlor and metolachlor OA to a greater extent than coarse-grained soils. Because the overlying field was not treated in 1998, the source of metolachlor at TD2 in 1998 is uncertain, but was probably runoff from an adjacent field.

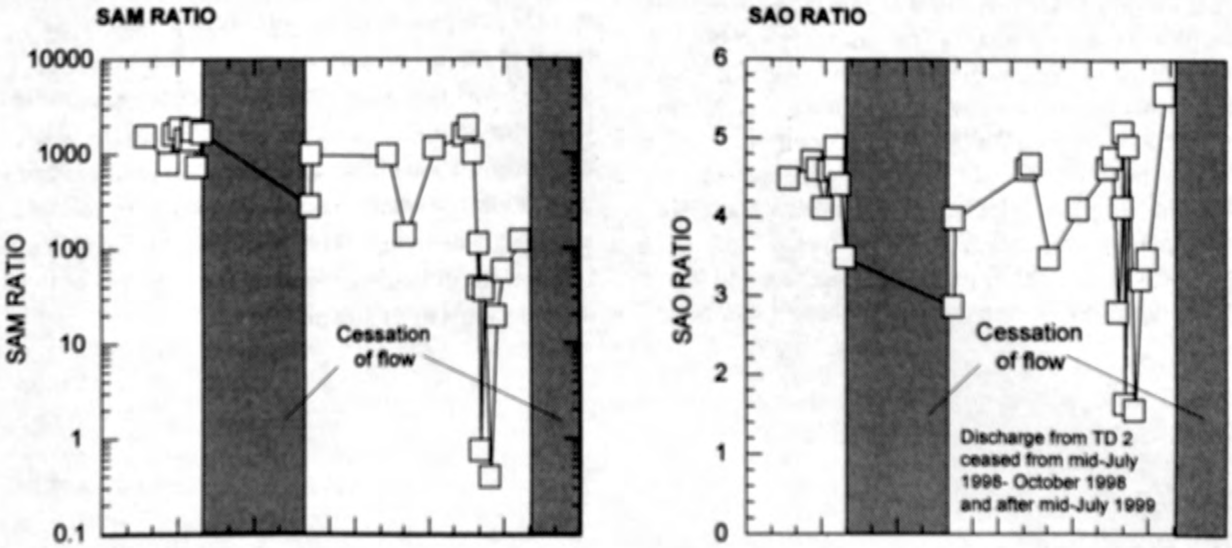
SAM ratios in surface-water samples from Canajoharie Creek also were lowest during stormflows just after metolachlor application in late May and early June (fig. 4B). The median SAM ratio for five stormflow samples collected in June 1998 was 0.4, and the median SAM ratio for six base-flow samples collected in June 1998 was 5.2. The SAM ratios for the June 1998 stormflow samples were the lowest obtained for Canajoharie Creek during the entire 1997-98 study. SAM ratios for all Canajoharie Creek samples for June 1998, regardless of flow conditions, were lower than those for June 1997, probably because rainfall in June 1998 was greater than in June 1997. Average mean daily discharge for June 1997 was 8.36 ft3/s ccubic feet per second) - far lower than that for June 1998 (45 ft3/s). The higher flows during June 1998 resulted in a short residence time in the soil, and rapid flushing of metolachlor.

The small number of samples with detections for metolachlor OA at Canajoharie Creek make the relation between SAO ratios and stormflow difficult to assess, yet some generalizations can be made. Stormflow samples had lower SAO ratios than base-flow samples, and SAO ratios of stormflow samples (those collected just after metolachlor application and some at other times) ranged from 1.9 to 1.4, whereas those for base-flow samples ranged from 2.2 to 3.0 (fig. 5). The low SAO ratios in storm-flow samples is consistent with the hypothesis that transport of metolachlor OA during storms results in less degradation than during dry conditions.

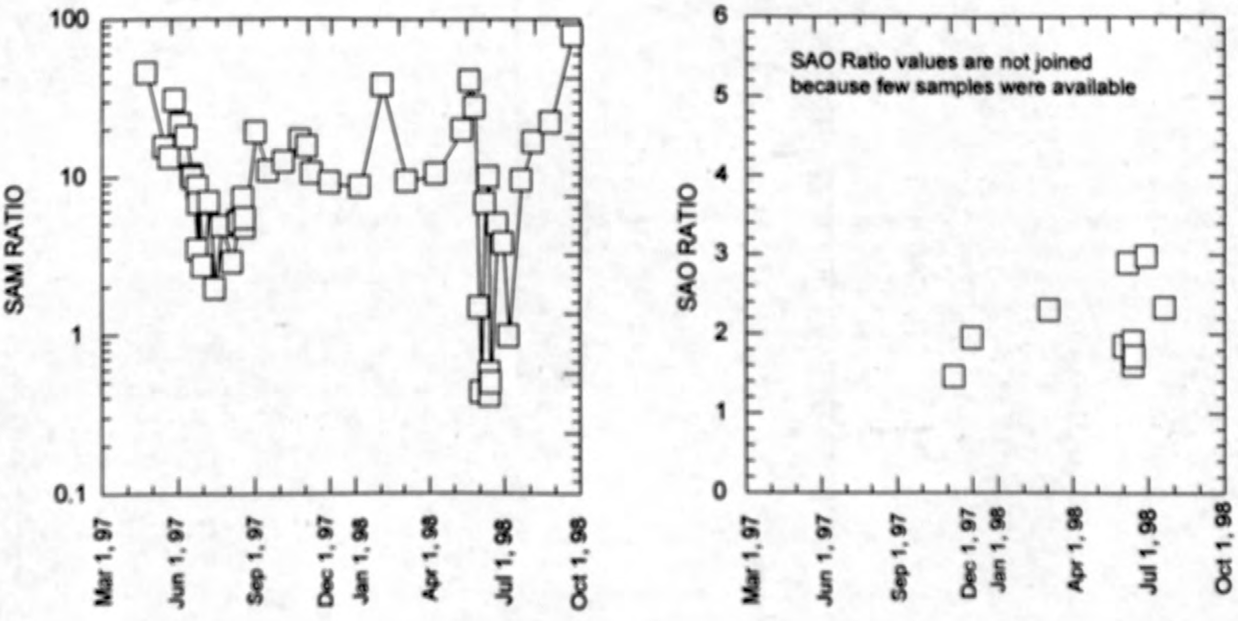
SUMMARY

Data collected from 50 wells in Suffolk County, from 2 tile drains beneath agricultural fields in Montgomery County, and from 5 streams in western New York indicate that SAM and SAO ratios for surface water differ from those for ground water. Differences in these ratios are related to soil texture, amount of time since metolachlor application, hydrologic flow paths, and storms. The median SAM ratio for samples from the two tile drains and from Suffolk County wells was greater than those for the five surface-water sites. These differences are attributable to the shorter residence time (contact time with the soil, soil enzymes, and microbes) of surface water than of well water or tile-drain discharge.

Differences between SAM ratios for Suffolk County well-water samples and those for tile-drain discharge also are related to differences in soil properties. The coarse-grained soils in Suffolk County correspond to low median SAM ratios, whereas the fine-grained soils overlying TD2



A. TILE DRAIN 2



B. CANAJOHARIE CREEK

Figure 4. SAM ratio (metolachlor ESA to metolachlor concentrations) and SAO ratio (metolachlor ESA to metolachlor OA concentrations) for samples collected from A. Tile Drain 2 between April 1997 and July 1998, and B. Canajoharie Creek between April 1997 and September 1998.

correspond to high median SAM ratios. The high SAM ratios for samples from the tile drain overlain by fine-grained soils probably reflect greater sorption of metolachlor in these soils. The coarse soils in Suffolk County allows rapid infiltration, and the low organic carbon content probably inhibits the transformation of metolachlor to metolachlor ESA. These characteristics also result in lower SAO ratios for well water than for tile-drain discharge.

The lowest SAM ratios in tile-drain discharge were in samples collected during storms just after metolachlor application. SAM ratios for TD2 generally were 500 or more, but decreased to less than 100 in response to storms of June 1998. SAO ratios for TD2 also decreased just after storms in June 1998 despite the fine-grained overlying soil that normally retards the movement of metolachlor and metolachlor OA. The low SAM and SAO ratios in TD2 samples in June 1998 were probably the result of rapid infiltration along preferential flow paths. SAM ratios for surface-water samples were lower for samples collected in the 2 months just after application (June and July) than in those from other months, when the contact time with soil and enzymes was longer. The lowest SAM and SAO ratios were in in Canajoharie Creek samples collected just after storms.

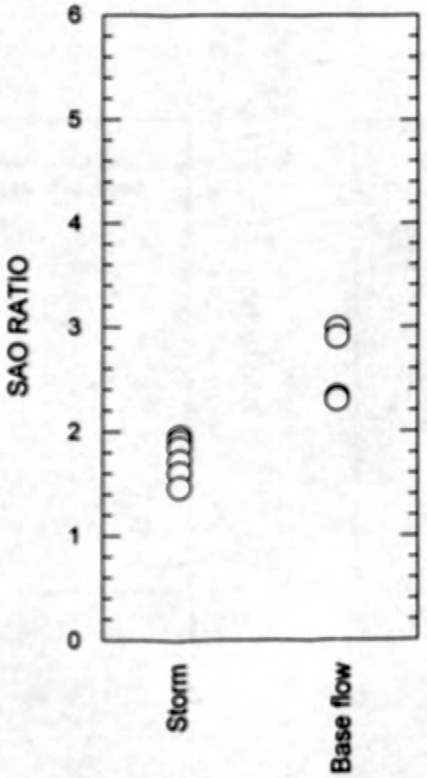


Figure 5. SAO ratio (metolachlor ESA to metolachlor OA concentration) for storm and baseflow samples collected from Canajoharie Creek from April 1997 through August 1998.

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Herbicides and their Metabolites in Cayuga Lake and its Tributaries, New York

by David A.V. Eckhardt, William M. Kappel, William F. Coon, and Patrick J. Phillips

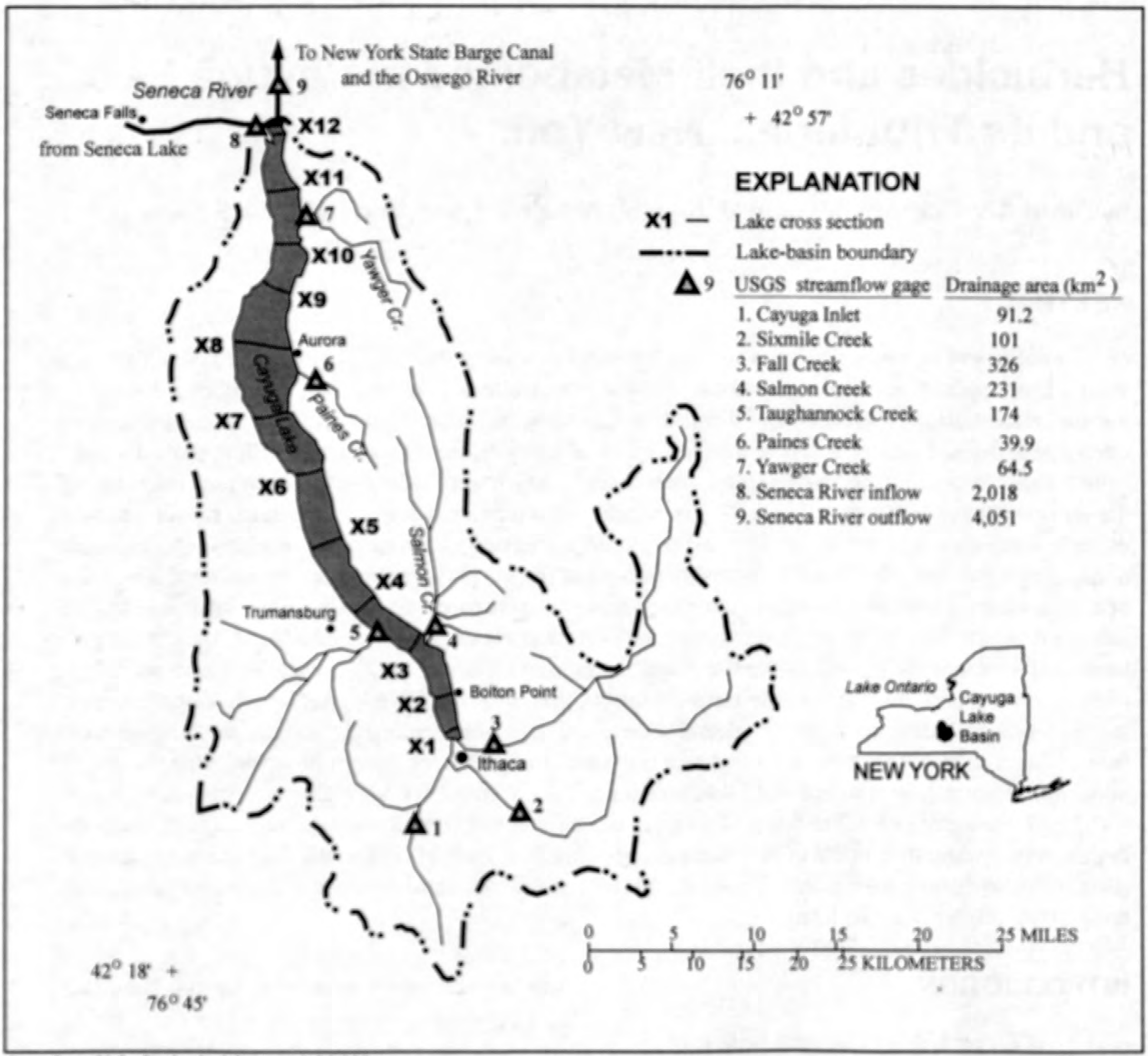
ABSTRACT

Analyses of stormflow samples collected from tributaries to Cayuga Lake in western New York shortly after application of atrazine and metolachlor to agricultural fields in June 1998 indicate that the highest concentrations, and the bulk of the loads of the two herbicides in the three tributaries was transported during peak flows. Concentrations of metolachlor metabolites remained high after the flow peaked as the soils drained. In contrast, deethylatrazine concentrations, which were generally low, increased only slightly during stormflow, apparently because the parent compound (atrazine) degrades at a much slower rate than metolachlor. Far more metolachlor-ESA (a degradation compound) was transported than any other pesticide or degradate; this indicates that it is relatively stable and mobile in the hydrologic environment. The ratios of a metabolite concentration to that of other metabolites and the parent compound in stream-water samples showed that base flow in the tributaries before the storm was enriched with metolachlor-ESA, but not with metolachlor or metolachlor-OA. After the storm, the ratio of metolachlor-OA to metolachlor increased markedly in the base flow; apparently metolachlor-OA that is formed in soils after pesticide application is readily leached but does not persist in ground water as much as metolachlor-ESA does. Water samples taken from Cayuga Lake in July, after the early-summer flush of pesticide residues in June, indicated fairly uniform concentrations of herbicides throughout the lake — from 0.2 to 0.6 µg/L for atrazine and from 0.05 to 0.3 µg/L for metolachlor. The ratios of the three metabolites to their parent compounds were significantly higher in lake water than in the three tributaries, possibly as a result of (1) the inflow of ground water that enters the lake directly from adjacent agricultural land, and (2) the transformation of the parent compounds during their residence in the lake.

INTRODUCTION

The Finger Lakes of western New York lie within 11 north-south trending basins that were deepened by Pleistocene glaciation. Cayuga Lake (fig. 1) occupies a deep glacial trough and is the longest of these lakes (61 km); it also has the largest drainage area (2,033 km^2). Its average width is 2.8 km, its maximum depth is 140 m (Birge and Juday, 1914), and its estimated water-retention time is 12 years (Oglesby, 1978). The predominant land use in the southern (upper) end of the basin is forest with some agriculture and urbanization; the extent of agricultural land increases northward, and the northern tributaries drain pastures and fields planted mostly in corn, soybean, and forage crops. Agricultural pesticides that are widely used in these areas are commonly detected in Cayuga Lake and its tributaries.

Cayuga Lake is used for public-water supply by several communities, and citizens are concerned about their exposure to pesticides in the drinking water. Water that is discharged from Cayuga Lake ultimately enters Lake Ontario through the Seneca and Oswego Rivers. Preliminary water-quality surveys of the Finger Lakes have shown that, of all the Finger Lakes, Cayuga Lake has been most severely affected by agricultural runoff that carries pesticide residues, and that its basin is a main source of pesticide loads from New York to Lake Ontario. For these reasons, Cayuga Lake was selected for detailed study.



Base from U.S. Geological Survey, 1:24,000

Figure 1. Locations of streamflow and lake-water sampling sites in Cayuga Lake basin, N.Y.

In 1998, the U.S. Geological Survey, in cooperation with New York State Department of Environmental Conservation, conducted an appraisal of pesticide occurrence in Cayuga Lake and its major tributaries as part of a statewide program for monitoring pesticides in surface water. The work included collection of stormflow samples from six major tributaries that together account for nearly half of the total area draining to the lake. The intent of the tributary sampling was to assess pesticide concentrations in storm runoff that directly followed the May and early June applications of herbicides to agricultural land. After this early-summer flush of pesticide residues to the tributaries, water samples were collected along 12 cross sections in Cayuga Lake for 2 consecutive weeks in late July to assess the spatial distribution and range of herbicide concentrations.

This paper presents results of (1) the sampling of June 17, 1998 storm runoff in three of the six tributaries to Cayuga Lake whose drainage basins are predominantly agricultural, and (2) the July 1998 sampling within the lake. Results are presented for two herbicides — atrazine and metolachlor — and their principal metabolites.

METHODS

Three tributaries — Yawger Creek, Paines Creek, and Salmon Creek (fig. 1, table 1) — were sampled after a storm on June17-18, 1998 that delivered about 50 mm of rain within 2 hours to the northeastern part of the Cayuga Lake basin. These tributaries drain land that is between 50 to 75 percent agricultural. Samples were collected and filtered by the methods of Shelton (1994). Pesticides were extracted from the sampled water by solid-phase methods (Meyer and others, 1993) and analyzed by gas chromatography with mass spectrometry (GCMS) (Thurman and others, 1990) and high-performance liquid chromatography (HPLC) (Ferrer and others, 1997). Target analytes were triazine and acetanilide herbicides, which are commonly used in the study area, and the main metabolites, or degradation products, of these herbicides.

Lake-water samples were taken 1 month after the storm runoff during 2 consecutive weeks in July at 12 cross sections in Cayuga Lake (fig. 1). Samples were taken at 3 to 5 equally-spaced locations along each section, and at each location water was sampled at the 2-m depth in the warm surface layer (epilimnion) and at the 30-m depth in the colder water (hypolimnion) below the thermocline. The lake-water samples were analyzed by two methods: (1) enzyme-linked immunosorbent assay (ELISA), which provided a field method for specific measurements of atrazine and metolachlor concentrations, and (2) solid-phase extraction and GCMS/HPLC, which provided concentrations of the suite of pesticides and their metabolites for which the stormflow samples had been analyzed.

HERBICIDE CONCENTRATIONS IN TRIBUTARIES TO CAYUGA LAKE

Water from Salmon Creek, which drains coarse-grained glacial deposits that contain aquifers that provide water for farm and rural homes, contains more ground water than Paines Creek, which drains an area of thin till overlying shale bedrock. Paines Creek basin produces sharp peak flows but little base flow to its stream, whereas the glacial deposits in Salmon Creek basin absorb more rainfall and thus reduce peak flows and prolong stormflow recessions. Yawger Creek drains an area of karstic limestone and also has prolonged runoff recessions and baseflows that are sustained by ground-water discharge after storms. Despite the differing hydrologic response of the three tributaries, pesticide concentrations in the stormflow samples showed similar patterns among the three basins.

Metolachlor

Concentrations of metolachlor and its metabolites in the tributaries increased as water discharge increased in response to rainfall. Concentrations are plotted in relation to discharges in figure 2, and loads are given in table 1. The maximum concentration of metolachlor in Paines Creek, whose basin is 78 percent agricultural, was 29 µg/L near the peak of the runoff. Concentrations in Salmon Creek (70 percent agricultural) were substantially lower than in Paines and Yawger Creeks; this was due in part to a storm (37 mm) earlier in June that produced rainfall in the lower part of Salmon Creek basin (but not in the other two basins) and caused the first flush in Salmon Creek to occur earlier than in Paines or Yawger Creeks. The lower concentrations in Salmon Creek also are consistent with the smaller amount of agricultural land in this basin than in the Paines or Yawger Creek basins. The highest metolachlor concentrations at all three sites occurred during peak flow, which indicates that the bulk of metolachlor is transported in storm runoff.

The metolachlor metabolites (metolachlor-ESA and metolachlor-OA) are formed by transformation of metolachlor as it moves through soil (Phillips and others, 1999). Concentrations of

Table 1. Pesticide loads in stormflow of three tributaries to Cayuga Lake, N.Y., June 1998

[km^2, square kilometers; mm, millimeters. Locations are shown in fig. 1.]

Site Name	Basin area (km^2)	Agricultural land use (percent)	Runoff duration (hours)	Runoff volume (mm)	Load (grams per square kilometer)				
					Atrazine	Deethyl-atrazine	Metolachlor	Metolachlor ESA	Metolachlor OA
Paines Creek	39.9	78	36	6.4	67	27	60	84	63
Yawger Creek	64.5	82	36	4.5	35	8	53	69	61
Salmon Creek	231	70	60	9.4	27	8	47	66	31

metolachlor-ESA in base-flow samples that were collected before the storm were several times higher than those of the parent metolachlor (fig. 2), which indicates that ground water discharging to the streams is enriched with the ESA metabolite. Concentrations of the metolachlor-OA metabolite in the initial baseflow were quite low, which suggests that this compound is less stable in ground water than the ESA metabolite and less preferentially formed or less mobile. Concentrations of the OA metabolite increased quickly during the rising flows, however, and its transport pattern became similar to that of the ESA metabolite, although at slightly lower concentrations. In general, the transformation of metolachlor into either metabolite appears fairly efficient in the glacially-derived soils of this region.

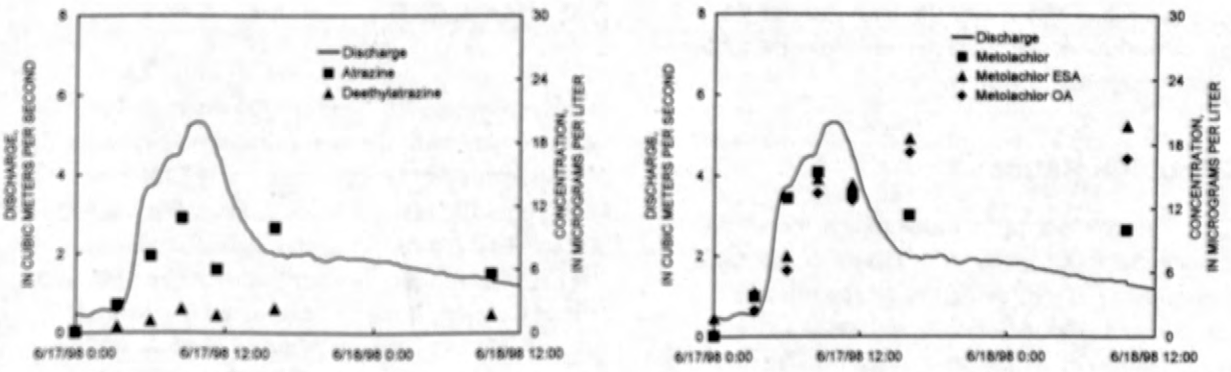
Concentrations of the metabolites during rising flows and peak flows were typically less than those of the parent (metolachlor), when the metolachlor that was transported from the fields to the stream in overland flow had only minimal soil-contact time (fig. 2). Metabolite concentrations increased as the discharge increased, however, which suggests that the metabolite mass that was formed and stored in the soil was quickly flushed into streams, probably through shallow subsurface flow paths on the adjacent hillslopes (interflow). Some of the metabolite mass in the runoff may have been residues that had remained in soils from the previous year. As the stormflow receded, the metabolite concentrations continued to increase and exceeded the parent concentrations; the metabolite concentrations remained high after the rain had ceased as soils drained (interflow) and ground-water discharge continued to contribute metabolite mass while the direct surface runoff gradually ceased.

Atrazine

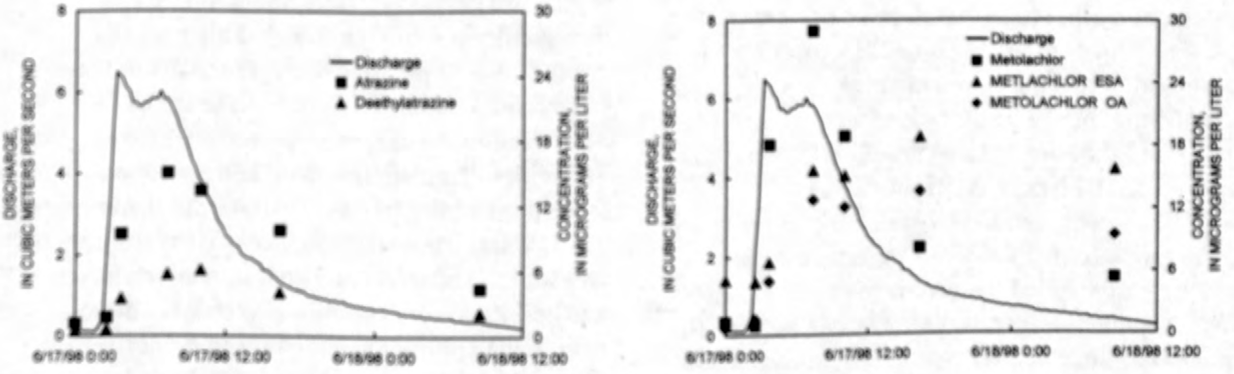
Atrazine concentrations in stormflow exceeded 10 µg/L in Yawger and Paines Creeks but were half of that value in Salmon Creek. Peak atrazine concentration in samples coincided exactly in time with peak metolachlor concentration. In contrast to the metolachlor metabolites, the atrazine metabolite (deethylatrazine) increased only slightly in stormflow and never exceeded values that were half those of its parent. Deethylatrazine concentrations remained relatively low compared to those of its parent and the metolachlor metabolites, probably because atrazine degrades at a much slower rate than metolachlor (Wauchope and others, 1992). The deethylatrazine that appeared in stormflow may have been resident in the soils and was flushed into streams, probably in interflow from the hill slopes.

Herbicide Loads

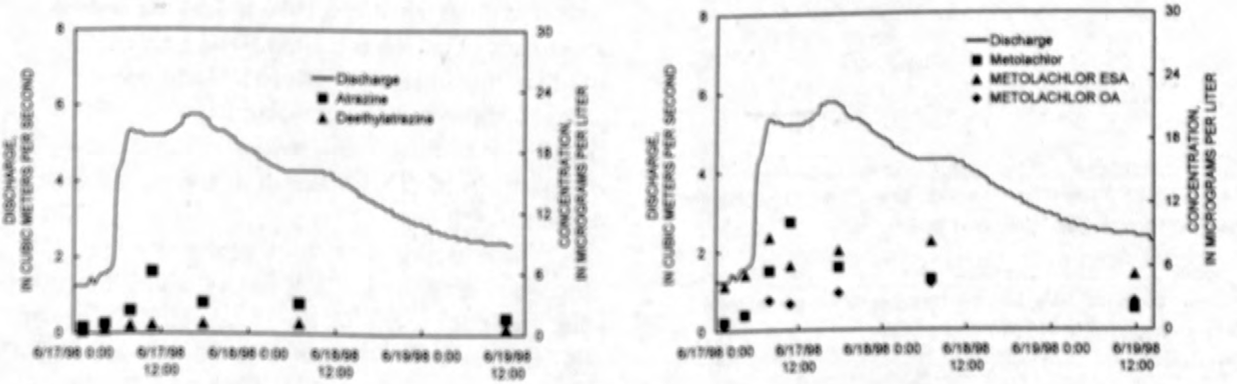
Mass loads of metolachlor, atrazine, and their metabolites and the total runoff volume per unit basin area are summarized in table 1. A comparison of loads among basins is complicated by (1) spatial differences in rainfall amount and intensity and pesticide use, and (2) the arbitrary selection of hydrograph duration (fig. 2), but certain conclusions can be made: (1) Far more metolachlor-ESA was transported than any other constituent; this indicates that it is relatively stable and mobile in the hydrologic environment. (2) Significantly less deethylatrazine was transported than any other constituent. (3) The smallest basin (Paines Creek) yielded the largest loads per unit area as a conse-quence of its high runoff volume and its high proportion of agricultural land use. (4)



A. YAWGER CREEK NEAR CAYUGA, NY



B. PAINES CREEK NEAR AURORA, NY



C. SALMON CREEK NEAR LUDLOWVILLE, NY

Figure 2. Concentrations of selected herbicides and their metabolites in three tributaries to Cayuga Lake, N.Y., during storm of June 17-18, 1998. (Locations are shown in fig. 1.)

The smallest herbicide loads per unit area were in Salmon Creek, which had the least amount of agricultural land, the most permeable soils, and the largest antecedent rainfall.

Metabolite Ratios

The concentration ratios of one metabolite to another metabolite, and to its parent compound, in a sample provide information on the relative abundance of the metabolite and assist in the interpretation of its fate and transport (Adams and Thurman, 1991; Phillips and others, 1999). The ratios in table 2 indicate that base flow in the three tributaries before the June storm contained more metolachlor-ESA than metolachlor or metolachlor-OA. The ratios of metolachlor-ESA to its parent decreased to less than 1 during peak flow, when the parent compound concentrations were highest from their rapid flush in surface runoff. After the peak flow, the ratios increased again through the stormflow recession; the most notable increase was in the ratios of metolachlor-OA to metolachlor, which nearly tripled the prestorm values in base flow. Apparently, metolachlor-OA can be formed and remains in soil after pesticide application, but it does not appear to persist as a ground-water contaminant as does metolachlor-ESA.

Table 2. Median ratios of metabolite concentrations to concentrations of parent compounds and other metabolites in surface waters of Cayuga Lake basin, N.Y., June-July 1998

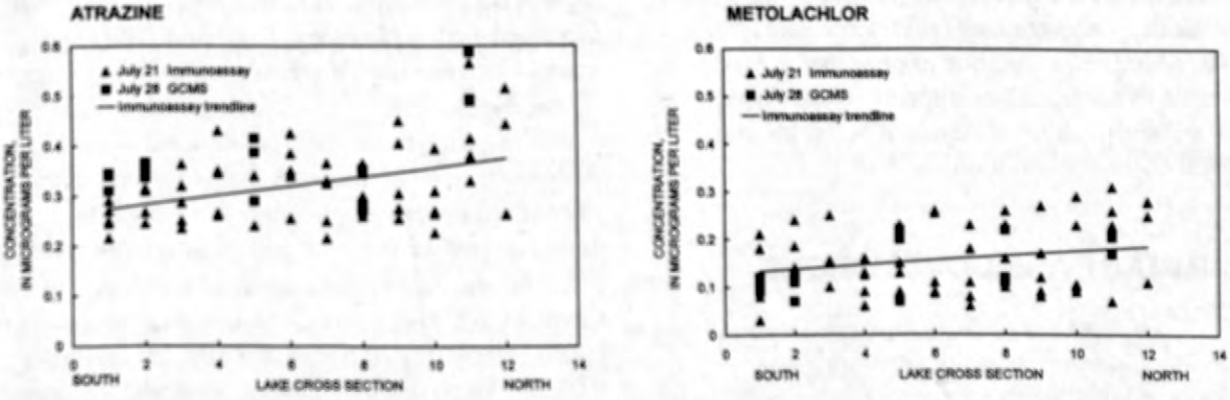
Water sample	Deethyl-atrazine/ Atrazine	Metolachlor ESA / Metolachlor	Metolachlor OA / Metolachlor	Metolachlor ESA / Metochlor OA
Tributaries, June 1998				
Baseflow	0.40	4.1	0.57	6.8
Peakflow	.21	.60	.42	1.2
Recession	.38	2.6	1.7	1.7
Cayuga Lake, July 1998				
Shallow	.79	8.0	3.2	2.4
Deep	.87	18	7.0	2.6

HERBICIDE CONCENTRATIONS IN CAYUGA LAKE

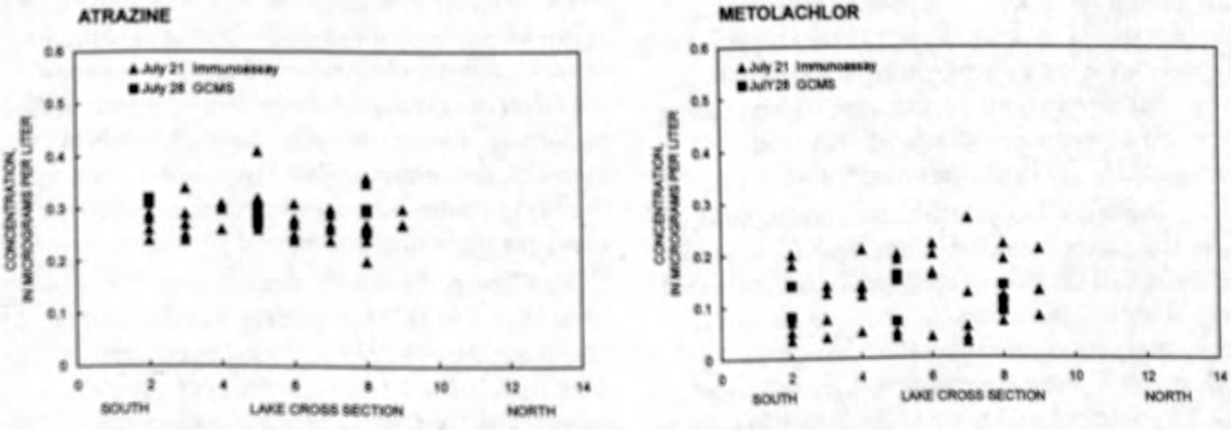
The early-summer tributary runoff was relatively warm (20° C) and soon dispersed into the lake's upper, warm layer (epilimnion). In general, herbicide concentrations were fairly uniform throughout the lake — from 0.2 to 0.6 µg/L for atrazine and from 0.05 to 0.3 µg/L for metolachlor (fig. 3). The two northern tributaries (Yawger and Paines Creeks), however, produced a seasonal loading in June 1998 that caused slightly higher herbicide concentrations above the thermocline (in the epilimnion) in the northern part of the lake than in the southern part, as shown by the statistically significant trend lines (atrazine $p = 0.009$; metolachlor $p = 0.09$) in fig. 3. This result is consistent with the hypothesis that parts of Cayuga Lake basin that have a high percentage of agricultural land can produce seasonal loadings of herbicides that cause elevated concentrations in those parts of the lake that receive the loadings.

Water below the thermocline (hypolimnion) was well mixed from the previous winter's turnover, and herbicide concentrations were less variable than in the epilimnion and showed no north-to-south trend. The range in concentrations below the thermocline remained fairly constant throughout the year, as indicated by analyses of water samples collected quarterly since 1996 at Ithaca's public-water supply intake at Bolton Point near the southern end of the lake (fig. 1). Herbicide concentrations below the thermocline were also slightly lower than above it, as shown by t-test comparisons of group means (atrazine $p = 0.001$; metolachlor $p = 0.03$).

Degradation rates for metolachlor and atrazine apparently become nearly negligible after entering streams and lakes (Buser, 1990; Mueller and others, 1997). The range and distribution of metabolite concentrations in Cayuga Lake could not be defined because the data were insufficient. The ratios given in table 2, however, indicate that (1) metolachlor-ESA concentrations were from 8 to 18 times greater than those of metolachlor, (2) metolachlor-OA concentrations were from 3.2 to 7 times greater than those of metolachlor, and (3) deethylatrazine concentrations were about 17 percent lower than those of atrazine. The ratios for all three metabolites were significantly higher in lake water than in the tributaries; this indicates that



A. ABOVE THERMOCLINE (DEPTH 2 METERS)



B. BELOW THERMOCLINE (DEPTH 30 METERS)

Figure 3. Concentrations of atrazine and metolachlor above and below thermocline at selected cross sections of Cayuga Lake, N.Y., July 1998. (Locations are shown in fig. 1.)

the herbicide and metabolite concentrations in the tributaries might not be representative of all water flowing into the lake. For example, metabolite-enriched ground water that enters the lake directly from adjacent agricultural land might have contributed to the high ratios observed in the lake; this could be especially true in areas such as the Yawger Creek basin, where the karstic limestone provides direct flowpaths to the lake. Also, the long water-retention period for Cayuga Lake (about 12 years; Oglesby, 1978) could provide sufficient time for transformation of the parent compounds in the lake.

A lake volume of 9.4 x 10^9 m^3 (Birge and Juday, 1914) and an average atrazine concentration of 0.30 µg/L (from the data in fig. 3) indicate that the amount of atrazine present in the lake in 1998 was 2.8 metric tons. The amount of metolachlor, based on an average metolachlor concentration of 0.15 µg/L, was 1.4 metric tons. The ratios given in table 2 indicate that the metabolite mass in the lake were about 18 metric tons of metolachlor-ESA, 7.0 metric tons of metolachlor-OA, and 2.3 metric tons of deethylatrazine. As mentioned previously, the GCMS results for water samples collected at Ithaca's public-water supply at Bolton Point, which takes water from below the thermocline near the southern end of the lake, has shown no time trend in pesticide concentrations (1996 to present). The lake's long retention time implies that at least a decade of continued monitoring would be required for a determination of trends in herbicide and metabolite concentrations.

SUMMARY AND CONCLUSIONS

An appraisal of pesticide occurrence in Cayuga Lake and its major tributaries was conducted in 1998 as part of a program with the State of New York for monitoring pesticides in water. The work included (1) collection of stormflow samples in three major tributaries during June 1998, shortly after herbicide applications to agricultural land, and (2) collection of water samples from Cayuga Lake at 12 cross sections on 2 consecutive weeks 1 month later. Results are presented for two pesticides that are widely used in corn and soybean agriculture (atrazine and metolachlor) and their main metabolites.

The highest metolachlor concentrations in all three tributaries occurred during peak flow, which indicates that the bulk of metolachlor is transported in stormflow. The metabolites persisted at high concentrations during streamflow recession as the soils drained, and as interflow and ground-water discharge contributed more to the streamflow than direct surface runoff after the rain had ceased. Concentrations of the atrazine metabolite (deethylatrazine), by contrast, increased only slightly during stormflow and were never more than half the values of the parent compound. Deethylatrazine concentrations remained relatively low compared to those of the parent compound and to the metolachlor metabolites, probably because atrazine degrades at a much slower rate than metolachlor. Significantly more metolachlor-ESA was transported than any other herbicide residue; this indicates that it is relatively stable and mobile in the aquatic environment.

The ratios of one metabolite to another metabolite, and to the parent compound, in streamflow samples indicated that base flow in the three tributaries before the June 1998 storm was more highly enriched with metolachlor-ESA than with metolachlor or metolachlor-OA. These ratios decreased during peak flow, when the concentrations of the parent compound were highest from the rapid flush in overland runoff, but increased during the recession period. The most marked increase during recession was in the ratio of metolachlor-OA to metolachlor, which were more than three times the base-flow values. Apparently, metolachlor-OA that is formed in the soils after pesticide application and is mobile during stormflow does not persist in ground water.

In general, concentrations of herbicides in Cayuga Lake were fairly uniform throughout the lake — from 0.2 to 0.6 µg/L for atrazine and from 0.05 to 0.3 µg/L for metolachlor. Seasonal loadings cause slightly higher concentrations in the epilimnion in parts of the lake near outlets of tributaries that drain areas of extensive agricultural activity in the northern part of the lake's basin. Water in the hypolimnion was well mixed from the previous winter turnover of the lake, and herbicide concentrations were less varied and did not show the effect of seasonal loadings that were seen in the epilimnion. Identification of temporal trends in herbicide concentrations will require at least a decade of continued monitoring because the lake's water-retention time can exceed 12 years.

Metolachlor-ESA concentrations in the lake were from 8 to 18 times greater than those of the parent metolachlor; metolachlor-OA concentrations were from 3.2 to 7 times greater than those of metolachlor, and deethylatrazine concentrations were about 17 percent lower than those of atrazine. These ratios were significantly higher in lake water than in tributary stormflow. The difference may be due in part to (1) the inflow of metabolite-enriched ground water that enters the lake directly from adjacent agricultural land, and (2) the transformation of the parent compounds during their residence in the lake.

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Methyl Tert-Butyl Ether (MTBE) in Lakes in Byram Township, Sussex County, New Jersey, 1998 and Vulnerability of Ground Water in Lakeside Communities

By Otto S. Zapecza and Arthur L. Baehr

ABSTRACT

Water samples were collected from four lakes in Byram Township, Sussex County, N.J., in the summer of 1998 as part of an investigation of the occurrence of volatile organic compounds (VOCs) in domestic wells in lakeside communities. Cranberry Lake and Lake Lackawanna are surrounded by densely populated communities where the use of gasoline-powered watercraft is prevalent and water is supplied by lakeside wells. Forest Lake is surrounded by a densely populated community where the use of gasoline-powered watercraft is prohibited. Stag Pond is privately owned, is situated in a sparsely populated area, and is not navigated by gasoline-powered watercraft.

Samples were collected from Cranberry Lake in early summer and again in late summer 1998. Concentrations of the gasoline oxygenate methyl tert-butyl ether (MTBE) ranged from 1.6 to 15.0 μg/L (micrograms per liter) on June 24 and decreased with depth. The depth-related concentration gradient is attributed to density stratification caused by the temperature gradient that is present in the lake during the early summer. MTBE concentrations ranged from 7.4 to 29.0 μg/L on September 8 and were uniform with depth, as was water temperature, indicating that the lake was vertically mixed. On the basis of these concentration profiles, the mass of MTBE in Cranberry Lake was estimated to be 15 kilograms on June 24 and 27 kilograms on September 8. These mass estimates are equal to the amount of MTBE in 52 and 95 gallons, respectively, of gasoline that consists of 10 percent MTBE by volume. The increase in MTBE mass from June to September is a result of the net accumulation of this ether oxygenate in the lake during the summer season, when use of gasoline-powered watercraft is at its peak. Samples were collected from Lake Lackawanna on September 9. Concentrations of MTBE ranged from 3.7 to 14.0 μg/L. Like those in Cranberry Lake the previous day, VOC concentrations and water temperatures were nearly uniform with depth. The mass of MTBE in Lake Lackawanna on September 9 was estimated to be 6 kilograms, which is equal to the amount of MTBE in 21 gallons of gasoline that consists of 10 percent MTBE by volume. All VOC concentrations were less than 0.2 μg/L in samples collected from Forest Lake on September 8, 1998, and from Stag Pond on the following day.

Maximum MTBE concentrations measured in samples from several depths at one site on Cranberry Lake on November 5 and December 16, 1998, were 1.1 and 0.38 μg/L, respectively. The difference in MTBE concentrations in the lake between September 8 and November 5 is attributed to the decrease in MTBE mass input to the lake resulting from the significant decline in the use of recreational watercraft after the Labor Day weekend and to natural volatilization processes.

Preliminary assessment of ground-water quality in 14 areally distributed wells around Cranberry Lake during November and December 1998 shows a high frequency of detection of MTBE -- approximately 93 percent. The extent to which Cranberry Lake functions as a nonpoint source of MTBE to wells and the effect of lakeside land use on ground-water quality is currently being studied. A regional assessment of the occurrence of MTBE and other VOCs in lakes and ground water is needed to determine the effect of the use of oxygenated gasoline on water quality in lakeside environments throughout northern New Jersey.

INTRODUCTION

MTBE is an oxygenate that has been added to gasoline for more than a decade to enhance fuel octane. Current Federal law (Clean Air Act of 1990) requires the use of fuel oxygenates to increase burning efficiency and reduce atmospheric concentrations of carbon monoxide and ozone in some metropolitan areas that have severe air-pollution problems (Squillace and others, 1996). MTBE is the most widely used gasoline additive and one of the most commonly produced organic compounds in the United States (Reisch, 1994). The widespread detection of MTBE in ground water and surface-water bodies used for drinking-water supplies has raised concern among environmental regulators, health officials, water purveyors, and the public. The State of New Jersey has set a maximum contaminant level of 70 µg/L for MTBE (N.J. Department of Environmental Protection, 1997). The U.S. Environmental Protection Agency has classified MTBE as a possible human carcinogen and has issued a drinking-water advisory for aesthetic concerns of 20 to 40 µg/L (micrograms per liter) (U.S. Environmental Protection Agency, 1997). In addition, several states, including California (California Department of Health Services, 1998) and Maine (Smith and Kemp, 1998), have established or are in the process of establishing more stringent drinking-water criteria for MTBE.

MTBE is more soluble, less volatile, and less reactive than other constituents of gasoline such as benzene, toluene, ethylbenzene, and xylenes (BTEX) (Zogorski and others, 1997); therefore, when oxygenated gasoline is spilled, MTBE is typically the dominant gasoline constituent present in the contaminated water. The use of MTBE in gasoline across the country is a topic of considerable debate (Andrews, 1998) as scientists and regulators evaluate the benefits of improved air quality in relation to the widespread occurrence of MTBE in ground and surface water.

During the summer of 1998, the U.S. Geological Survey (USGS) became involved in an investigation, coordinated by the Sussex County Department of Health, of MTBE in wells in a neighborhood of Cranberry Lake in Byram Township, Sussex County, N.J. Cranberry Lake is a densely populated community characterized by picturesque surroundings, bathing beaches, and facilities for power boating and water sports typical of many lakeside communities in northern New Jersey. Many of these lakes were formed or enlarged with dams, and water from wells surrounding these lakes is the major source of water supply for the residents. The combination of clustered well withdrawals and maintained lake levels makes lake/well interaction likely. Therefore, a study was initiated to determine whether VOCs are present in Cranberry Lake and, ultimately, whether lake-water quality is related to ground-water contamination. The study was expanded to include sampling for VOCs in three additional lakes in Byram Township: Lake Lackawanna, which is surrounded by a densely populated lakeside community similar to Cranberry Lake, where clustered well withdrawals and the use of gasoline-powered water craft is prevalent; Forest Lake, which is adjacent to a densely populated community where the use of gasoline-powered watercraft is prohibited; and Stag Pond, a lake situated in a sparsely populated area where the use of gasoline-powered watercraft is prohibited.

Byram Township is in the southeastern corner of Sussex County in the New Jersey Highlands (New England physiographic province) in northwestern New Jersey, about 50 miles west of New York City. The Highlands province is a northeast-southwest-trending system of folded and faulted Precambrian and Paleozoic rocks that form a terrain characterized by forested, hilly uplands and narrow valleys. The bedrock underlying Byram Township consists of a complex suite of metamorphic and igneous crystalline rocks composed predominantly of hornblende granite, syenite, and gneiss (Volkert and others, 1989). The Township lies just north of the Pleistocene terminal moraine of the Wisconsin glacier. Erosion and deposition associated with the glaciation explain, in part, the presence of many lakes throughout the Highlands and Valley and Ridge provinces of northwestern New Jersey (fig. 1).

This paper summarizes results of the lake sampling study performed during the summer of

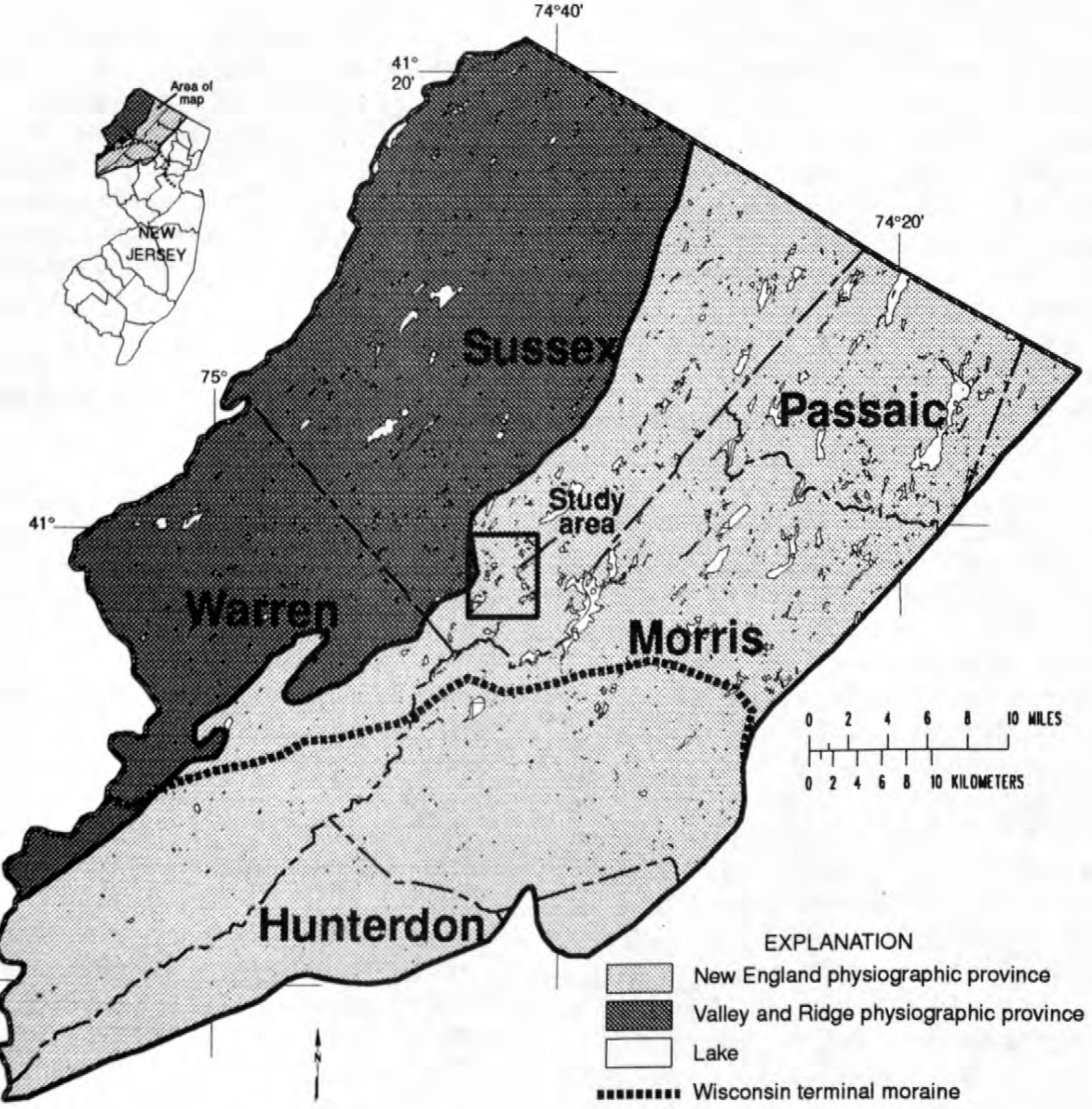

Figure 1. Location of study area, lakes and physiographic provinces, northwestern New Jersey. (From Baehr and Zapecza, 1998)

1998 (Baehr and Zapecza, 1998) and provides new information on the seasonal variability of MTBE in Cranberry Lake and the vulnerability of ground water in lakeside environments to contamination.

LAKE SAMPLING

Cranberry Lake is a 187-acre, man-made lake that was created with a dam in the 1830's. Ground water from fractured crystalline rock is the major source of water supply for approximately 500 residences surrounding Cranberry Lake. About 160 of these residences are served by four small water companies; most of the other residences have individual domestic wells. A bathymetric map produced in 1992 shows that the deepest parts of the lake are about 16 ft (feet) deep and that the average depth is about 7 ft (Coastal Environmental Services, 1992). On June 24, 1998, samples were collected at a depth of 3 ft below the lake surface at 12 locations (fig. 2). Depth measurements at these locations agreed closely with the 1992 bathymetric map. At three locations, where the lake is relatively deep, a second sample was collected at a depth of 10 ft. On September 9, 1998, samples were collected near 9 of the 12 locations at which samples had been collected on June 24 to assess conditions at the end of the summer. At the three sites where two samples had been collected previously, samples were collected at depths of 3, 6, and 10 ft. Depth measurements at the sampling sites indicate that the lake surface was about 1.2 ft lower than on June 24, as a result of the dry summer.

Lake Lackawanna is a 109-acre, man-made lake that was created with a dam in 1910. Ground water from fractured crystalline rock on its western shore and from stratified glacial drift on its eastern shore is the major source of water for approximately 300 residences surrounding Lake Lackawanna. No water companies serve the Lake Lackawanna community and, therefore, all ground water withdrawn is from domestic wells. A bathymetric map (Perrone, 1989) shows that the maximum depth of the lake is about 10 ft and its average depth is about 4 ft. On September 9, 1998, samples were collected at a depth of 3 ft below the lake surface at five locations (fig. 2). At two of these locations, a second sample was collected 5 ft below the lake surface. Lubbers Run, the stream that discharges to Lake Lackawanna, was sampled once near the lake inlet.

Forest Lake was created in the 1950's. Ground water serves as the source of water supply for approximately 400 residences surrounding Forest Lake, which are served by a water company that operates two wells. One well is open to stratified glacial drift and the other is open to fractured crystalline rock. Forest Lake is about 44 acres in size. On September 8, 1998, its maximum and average depths were estimated to be 15 and 11 ft, respectively. On September 8, 1998, samples were collected from Forest Lake at a depth of 3 ft below the lake surface at four locations (fig. 2). At two of these locations, a second sample was collected 10 ft below the lake surface.

Stag Pond is a privately owned natural lake with an area of about 33 acres in a sparsely populated area. On September 9, 1998, its maximum and average depths were estimated to be 25 and 16 ft, respectively. On September 9, 1998, samples were collected from Stag Pond at a depth of 3 ft below the lake surface at two locations (fig. 2). A discussion of sampling and analytical procedures and a complete list of VOC constituents analyzed for and their concentrations are given in Baehr and Zapecza (1998).

MTBE CONCENTRATIONS AND MASS ESTIMATES

Concentrations of MTBE measured in synoptic samples collected from Cranberry Lake on June 24 and September 8 are listed in table 1. Data obtained from Lake Lackawanna on September 9 are summarized in table 2. The only other VOCs detected in any of the samples were tert-amylmethyl ether (TAME) and BTEX, but these compounds were present at significantly lower concentrations than MTBE. MTBE and TAME were detected in every sample from Cranberry Lake and Lake Lackawanna. No VOC concentration in any sample from Forest Lake or Stag Pond exceeded 0.2 μg/L.

The concentrations of MTBE in samples collected 3 ft below the surface of Cranberry Lake on June 24 ranged from 10.1 to 15.0 μg/L; therefore, MTBE was evenly distributed across the upper part of the lake (table 1). MTBE concentrations were lowest (1.6, 3.8, and 4.3 μg/L) in samples from 10 ft below the surface. The difference in concentration with depth is attributed to density stratification caused by the vertical temperature gradient that is present in Cranberry Lake in the early summer. Temperature profiles at the three sites where samples were collected at multiple depths are shown in figure 3.

MTBE mass estimates, although approximate, can be used to scale the source strength and for comparison with estimates of MTBE mass at the end of the summer. The estimate of MTBE mass in Cranberry Lake on June 24 is 15 kg (kilograms). This estimate was derived by numerically integrating concentrations over the volume of the lake. The

Base from U.S. Geological Survey 1:24,000 digital raster graphic

Figure 2. Lake sampling sites, Sussex County, New Jersey. (From Baehr and Zapecza, 1998)

Table 1. Concentrations of MTBE in samples from Cranberry Lake, Sussex County, N.J., 1998

[Concentrations in micrograms per liter; sample depth in feet below lake surface; --, no data]

Site number	Sample depth	Concentration	
		June 24	Sept. 8
1	3	13.6	27.3
2	3	13.2	--
3	3	13.9	22.0
3	6	--	21.9
3	10	4.32	28.1
4	3	12.5	29.0
5	3	12.2	--
6	3	10.1	7.98
6	6	--	8.00
6	10	1.56	7.40
7	3	11.0	19.8
8	3	14.0	20.1
8	6	--	20.6
8	10	3.75	20.5
9	3	13.2	20.8
10	3	14.2	18.3
11	3	14.2	--
12	3	15.0	23.8

estimated MTBE mass of 15 kg in Cranberry Lake on June 24 is equivalent to 52 gal (gallons) of a gasoline that is 10 percent MTBE by volume, an MTBE content representative of gasoline used in the region. If the density of gasoline is assumed to be 0.75 grams per cubic centimeter, 10 percent MTBE by volume corresponds to 281.6 grams of MTBE per gallon of gasoline.

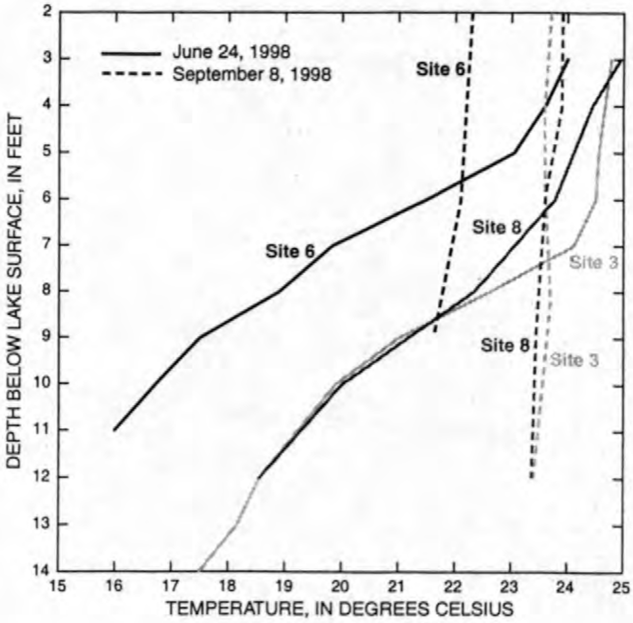

Figure 3. Temperature as a function of depth at sites at which samples were collected at multiple depths, Cranberry Lake, Sussex County, New Jersey, summer 1998 (From Baehr and Zapecza, 1998)

MTBE concentrations in samples collected from Cranberry Lake on September 8 ranged from 7.4 to 29.0 μg/L (table 1). At the three sites where three samples were collected at different depths, MTBE concentrations and water temperatures were nearly uniform (fig. 3), indicating that the lake was vertically mixed. Concentrations were lowest in samples from site 6, which is at the shallow end of Cranberry Lake, nearest the inlet (fig. 2). The mass estimate for MTBE in Cranberry Lake on September 8 is 27 kg. This estimate was obtained by numerically integrating concentration over the volume of the lake with the assumption that concentrations are constant with depth. The estimated mass of MTBE in Cranberry Lake on September 8 is equivalent to 95 gal of a gasoline that is 10 percent MTBE by volume, or about 1.8 times the estimated MTBE mass present in the lake on June 24, as a result of the net accumulation of this ether oxygenate in the lake during the summer, when the use of gasoline-powered watercraft is at its peak.

MTBE concentrations in samples collected from Lake Lackawanna on September 9 ranged from 3.7 to 14.0 μg/L (table 2). The concentration was lowest at site 5, which is near the Lubbers Run

Table 2. Concentrations of MTBE in samples from Lake Lackawanna, Sussex County, N.J., 1998

[Concentrations in micrograms per liter; sample depth in feet below lake surface; <, less than]

Site number	Sample depth	Concentration Sept. 9
1 Lubbers Run	1.5	<0.20
2	3	12
3	3	14
3	5	14
4	3	11
5	3	3.7
6	3	12
6	5	12

inlet, in the shallowest part of the lake. Concentrations of all VOCs, including MTBE, in a sample from Lubbers Run just upstream from Lake Lackawanna were less than 0.2 µg/L. At the two sites where two samples were collected at different depths, MTBE concentrations were unrelated to depth and water temperature was nearly constant with depth, indicating that the lake was vertically mixed. The mass estimate calculated for MTBE in Lake Lackawanna on September 9 is 6.0 kg, which is equivalent to 21 gal of a gasoline that is 10 percent MTBE by volume.

MTBE concentrations recorded in Cranberry Lake and Lake Lackawanna during the summer of 1998 are among the highest observed by the USGS in ambient ground and surface water nationwide. The major benefit derived from oxygenated gasoline is in its use in automobiles; however, because gasoline formulation is not specific to use, oxygenated gasoline is also used to power watercraft. Recreational boating has been associated with MTBE occurrence in lakes in Nevada and California (Boughton and Lico, 1998; Reuter and others, 1998). Direct exhaust of unburned fuel into the water from marine engines is the likely source of MTBE in the lakes. There are no marinas on Cranberry Lake or Lake Lackawanna. Repeated direct spillage of gasoline into the lake, because of improper handling, in the amounts calculated on the basis of the estimated MTBE mass is unlikely.

SEASONAL VARIATIONS IN MTBE CONCENTRATIONS IN CRANBERRY LAKE

In order to determine seasonal concentrations of MTBE in Cranberry Lake and any potential carry-over amount from year to year, samples were collected for VOC analysis on November 5 and again on December 16, 1998. Samples were collected from a foot bridge that crosses the lake directly over site 1 (fig. 2). On each of these days two samples were collected, one at a depth of 3 ft below lake surface and another at a depth of 10 ft below lake surface. Total depth of the lake at this location during this sampling was about 11.5 ft. Temperature measurements indicated that no thermocline existed at the time of sampling. MTBE concentrations at the 3- and 10-ft sampling depths were 1.1 and 1.0 µg/L, respectively, on November 5 and 0.38 and 0.37 µg/L, respectively, on December 16. In the absence of a thermocline, it is reasonable to assume that these concentrations generally reflect lakewide conditions, as previous samplings indicated.

The MTBE loss from the lake between September 8 and November 5 was likely caused by a major decrease in MTBE input to the lake because of the significant decline in use of recreational watercraft after the Labor Day weekend and natural volatilization processes. Increased surface-water inflow or outflow is not considered to be a primary factor because conditions during this period included drought, low lake levels, and an absence of overflow at the dam. Seasonal sampling of Cranberry Lake, Lake Lackawanna, and other, larger lakes surrounded by lakeside communities is planned to assess in greater detail the seasonal variation in and spatial distribution of MTBE and other compounds in lakes in northern New Jersey.

VULNERABILITY OF GROUND WATER IN LAKESIDE COMMUNITIES

The occurrence of MTBE and other VOCs in wells surrounding Cranberry Lake is also being investigated as part of this study. During November and December 1998, 14 wells were sampled as part of a preliminary areal assessment of ground-water quality. Raw water from these wells was analyzed for VOCs, major ions, nutrients, detergents, radon, pH, alkalinity, and dissolved oxygen. Wells were selected on the basis of availability of well-construction data, areal distribution around the lake, and owner permission. Sampled wells typically were cased to a depth of 50 ft below land surface; below this depth, they were open to the fractured rock, with total depths ranging from 75 to more than 500 ft. MTBE was detected in 13 of the 14 water samples, for a detection frequency of about 93 percent. Detectable concentrations ranged from 0.12 to 19.8 μg/L with a median concentration of 0.435 μg/L. Although the sample size is small, the frequency of detection of MTBE appears to be significantly greater from that found in random domestic ground-water sampling in other parts of New Jersey. Data collected as part of the USGS National Water Quality Assessment Program indicate a 48-percent detection frequency of MTBE above a detection limit of 0.10 μg/L in 23 samples collected from domestic wells in crystalline-rock aquifers of the New Jersey Highlands. A 7-percent detection frequency of MTBE above the 0.10-μg/L detection limit was recorded in 30 samples collected from domestic wells screened in the Kirkwood-Cohansey aquifer system in the southern Coastal Plain of New Jersey (J.A. Hopple and P.E. Stackelberg, U.S. Geological Survey, written commun., 1999).

The degree to which Cranberry Lake functions as a nonpoint source of MTBE to the community's ground-water supply is being investigated. Additional lake and ground-water sampling is planned to determine the relation between MTBE concentrations in wells and those in the lake during the year. Preliminary plans are to resample the Cranberry Lake wells during late summer 1999. Sampling is also planned for fractured-rock and glacial wells surrounding Lake Lackawanna. The list of analytes will be expanded to include potential tracers indicative of lake-water quality, including copper sulfate, which is used in the lake as an herbicide, and radon, which would be expected to be present in lower concentrations in the lake than in the aquifer.

Lakeside residential land use is unique in that gasoline handling is more common than in other types of residential areas because of the high rate of ownership of recreational watercraft. At Cranberry Lake, the near-vertical alignment of fractures in the crystalline rocks and the thin or nonexistent overburden increase the ground-water vulnerability to contamination that is associated with this type of land use. In early 1998, in response to residential concerns about poor drinking-water quality, the Sussex County Department of Health coordinated the sampling of approximately a dozen wells in a local neighborhood within Cranberry Lake for VOCs. Several wells within a 200-ft radius contained MTBE in concentrations of hundreds of micrograms per liter. Subsequent sampling of a subset of these wells by the USGS in July 1998 confirmed these concentrations. The source of the MTBE in these wells has not been determined. There are no gasoline storage tanks in the area, and results of analysis for detergents and biological constituents indicates that septic-tank effluent is not a source. The most likely source is small-volume spillage of gasoline in a localized area. Moreover, point-source contamination may be chronic rather than an isolated incident, as other occurrences of MTBE and chlorinated VOCs in Cranberry Lake community wells have been reported (Steven Stiansen, Sussex County Health Department, written commun., 1998).

CONCLUSIONS

The occurrence of MTBE and other VOCs in lakes and lakeside wells in Byram Township, Sussex County, N.J., has prompted concern about the vulnerability of drinking-water supplies in Cranberry Lake, Lake Lackawanna, and similar lakeside communities. The use of oxygenated fuel in watercraft and the persistence of MTBE in water can cause a lake to become a nonpoint source of MTBE to the water supply as a result of lake/well interaction and direct withdrawals of lake water for domestic use. Lakeside residential development is a unique land use that involves the frequent handling and use of gasoline. The ground-water resource is vulnerable to contamination from the land surface as a result of the permeable fractured-rock geology. A regional assessment of the occurrence of MTBE and other VOCs in lakes and ground water is needed to determine the effect of the use of oxygenated gasoline on water quality in lakeside environments throughout northern New Jersey.

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Halogenated Organic Compounds in Endocrine-Disrupted Male Carp from Las Vegas Wash and Lake Mead, Nevada

By Thomas J. Leiker, Hugh E. Bevans, and Steven L. Goodbred

ABSTRACT

Adult common male carp from Las Vegas Wash, Las Vegas Bay, and Callville Bay were collected and tested for the concentration of vitellogenin in blood. The presence of vitellogenin was confirmed in the male carp from Las Vegas Wash and Bay. Extracts of fish tissue of adult male carp were analyzed by capillary gas chromatography/mass spectrometry. The extracts contained low microgram-per-kilogram concentrations of common chlorinated pesticides typically found in the environment. The extracts also contained a series of chlorinated and brominated organic compounds that have not been previously reported in this water system. These compounds range in concentration from low to high micrograms-per-kilogram. Nonyl phenols and nonyl phenol ethoxylates were also detected at hundreds of microgram-per-kilogram concentrations.

INTRODUCTION

Las Vegas Wasteway is a tributary to Las Vegas Wash and Las Vegas Bay of the Lake Mead National Recreation Area, Nevada. Lake Mead is used for fishing and water sports, public water supply, and habitat for the endangered Razorback Sucker. Vitellogenin, a lipophosphoglcoprotein, --not normally present in male fish--was confirmed in male carp. Its presence suggests endocrine disprution. Vitellogenin may be produced in male fish after exposure to compounds that may exhibit estrogenic activity. The purpose of this article is to identify and describe the halogenated organic compounds present in whole-body, adult male carp that have been tested for vitellogenin.

EXPERIMENTAL

Male carp were collected from Las Vegas Wash by seining and from Las Vegas Bay and Callville Bay (reference site) by electroshocking from a boat. Blood samples were collected and analyzed for vitellogenin (Bevans and others, 1996).

Fish tissue samples then were submitted for chemical analysis. Individual fish samples were prepared for analysis in accordance with the U.S. Geological Survey's approved methods (Leiker and others, 1995). The procedure is as follows: A 10-g (gram) aliquot of homogenized tissue is mixed with Na_2SO_4. The sample and Na_2SO_4 mixture is Soxhlet* extracted with methylene chloride for 24 hours. After extraction, the extract is concentrated by means of Kuderna-Danish to a volume of 5 mL (milliliters). A 1-mL aliquot is removed for lipid determination. A 2-mL aliquot of the remaining extract is passed through a gel permeation chromatographic column to isolate low molecular weight compounds of

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*The use of brand, firm, and trade names in this article is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey.

interest from endogenous lipid material. The extract is again concentrated by means of Kuderna-Danish to a volume of 1 mL. The 1-mL extract is passed through an adsorption chromatographic column containing 5 g of 8.5 percent water deactivated alumina; 3 g of 2 percent water deactivated silica, and 0.5 cm (centimeter) of sodium sulfate. This column is used to separate the extracts into two fractions. The first fraction is eluted with 30 mL of hexane and contains nonpolar organic compounds, such as, but not limited to, *p,p'*-DDE and polychlorinated biphenyls. The second fraction is eluted with 25 mL of 50 percent (volume/volume) acetone in hexane and contains chlordane components, DDT, DDD, HCH isomers, dieldrin, endrin, toxaphene, and other more polar organic components. After concentrating the extract to about 0.5 mL, each fraction is analyzed twice using capillary gas chromatography/mass spectrometry (GC/MS). All gas chromatographic separations are performed with a 30-m (meter) by 0.25-mm (millimeter) inside diameter capillary column coated with 5 percent diphenyl and 95 percent dimethyl polysiloxane. The first GC/MS analysis is conducted using electron ionization. The second GC/MS analysis is conducted using electron-capture negative ionization, a technique that selects organic compounds that are highly electronegative. These compounds, in many cases, are not detected under normal GC/MS analysis using electron ionization. Quantitation is based on an internal standard.

RESULTS AND DISCUSSION

The data suggest that Las Vegas Wash and Las Vegas Bay are more highly contaminated than Callville Bay. This result is most likely related to discharge from Las Vegas Wash and entering Las Vegas Bay. Callville Bay seems to be isolated from any significant discharge. Selected compounds that have been identified in whole-body male carp samples from Las Vegas Wash, Las Vegas Bay, and Callville Bay are listed in table 1. Figures 1 and 2 show the mean concentration of selected organic compounds that have been identified and quantified in individual fish tissue extracts from the three sampling sites. Figure 3 shows the distribution of vitellogenin in fish plasma from the three sampling sites. Nonyl phenols and nonyl phenol ethoxylates are suspected endocrine disrupters. Their detection in these samples was unexpected. The concentration of these compounds is significantly higher than other compounds identified in the extracts. The data suggest that the appearance of vitellogenin might be a result of compounds entering Las Vegas Wash and Las Vegas Bay, because vitellogenin has been detected in male fish from those two sites but has not been detected in male fish from Callville Bay. The data also suggest that discharge from Las Vegas Wash is impacting Las Vegas Bay and potentially might impact Lake Mead.

SUMMARY

In general, tissue samples from Las Vegas Wash and Las Vegas Bay have higher concentrations of vitellogenin and organic contaminants than samples collected from Callville Bay. The production of vitellogenin in male carp might be related to an individual contaminant or a combination of several contaminants that are present in the water column. Some of the compounds that have been detected in the tissue extracts are suspected endocrine disrupters. Their presence, however, does not imply a direct correlation with vitellogenin. Their presence does suggest that these compounds might be tested to determine if they are related to vitellogenin production in male carp.

Table 1. Selected organic compounds detected in male carp tissue samples from Lake Mead National Recreational Area

[D-Detected at greater than 1 ug/kg, ND-Not detected at 1 ug/kg]

Compound	Las Vegas Wash	Las Vegas Bay	Callville Bay
cis-Chlordane	D	D	D
trans-Chlordane	D	D	D
DCPA	D	D	D
p,p'-DDE	D	D	D
Dieldrin	D	D	D
Endosulfan I	D	D	D
Endosulfan II	D	D	ND
Endosulfan sulfate	D	D	D
alpha -HCH	D	D	ND
beta -HCH	D	D	ND
gamma -HCH	D	D	ND
Heptachlor epoxide	D	D	ND
Hexachlorobenzene	D	D	D
cis -Nonachlor	D	D	D
trans -Nonachlor	D	D	D
Oxychlordane	D	D	ND
Pentachloroanisole	D	D	D
Polychlorinated biphenyls	D	D	D
Tetrabromodiphenyl ether	D	D	ND
Pentabromodiphenyl ether	D	D	ND
Heptachlorostyrene	D	D	ND
Octachlorostryene	D	D	ND
Nonyl phenols	D	D	ND
Nonlyl phenols ethoxylates	D	D	ND

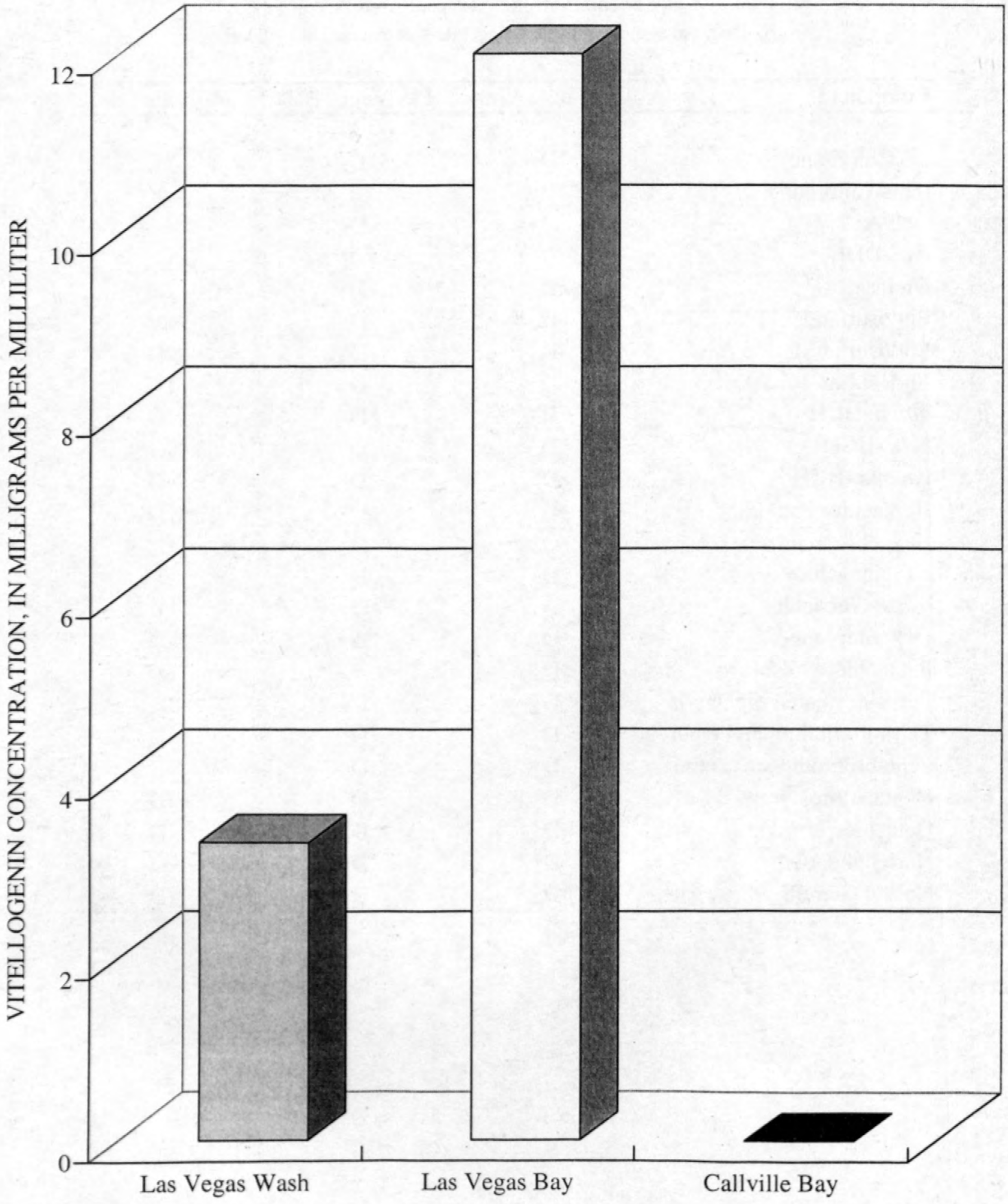

Figure 1. Mean concentration of viltellogenin in plasma from individual male carp samples collected from Las Vegas Wash, Las Vegas Bay, and Callville Bay.

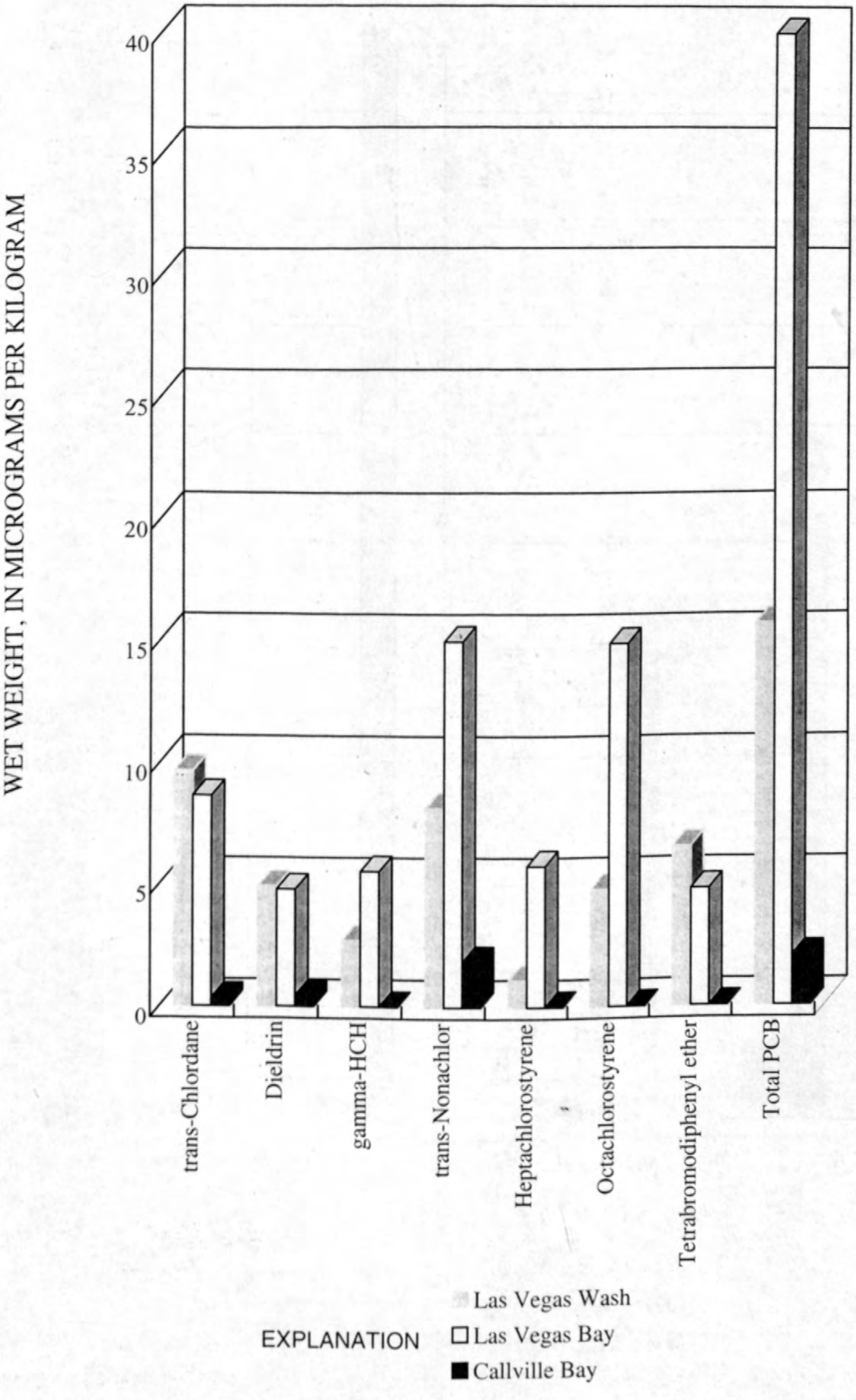

Figure 2. Mean concentration of selected compounds found in individual male carp samples collected from Las Vegas Wash, Las Vegas Bay, and Callville Bay.

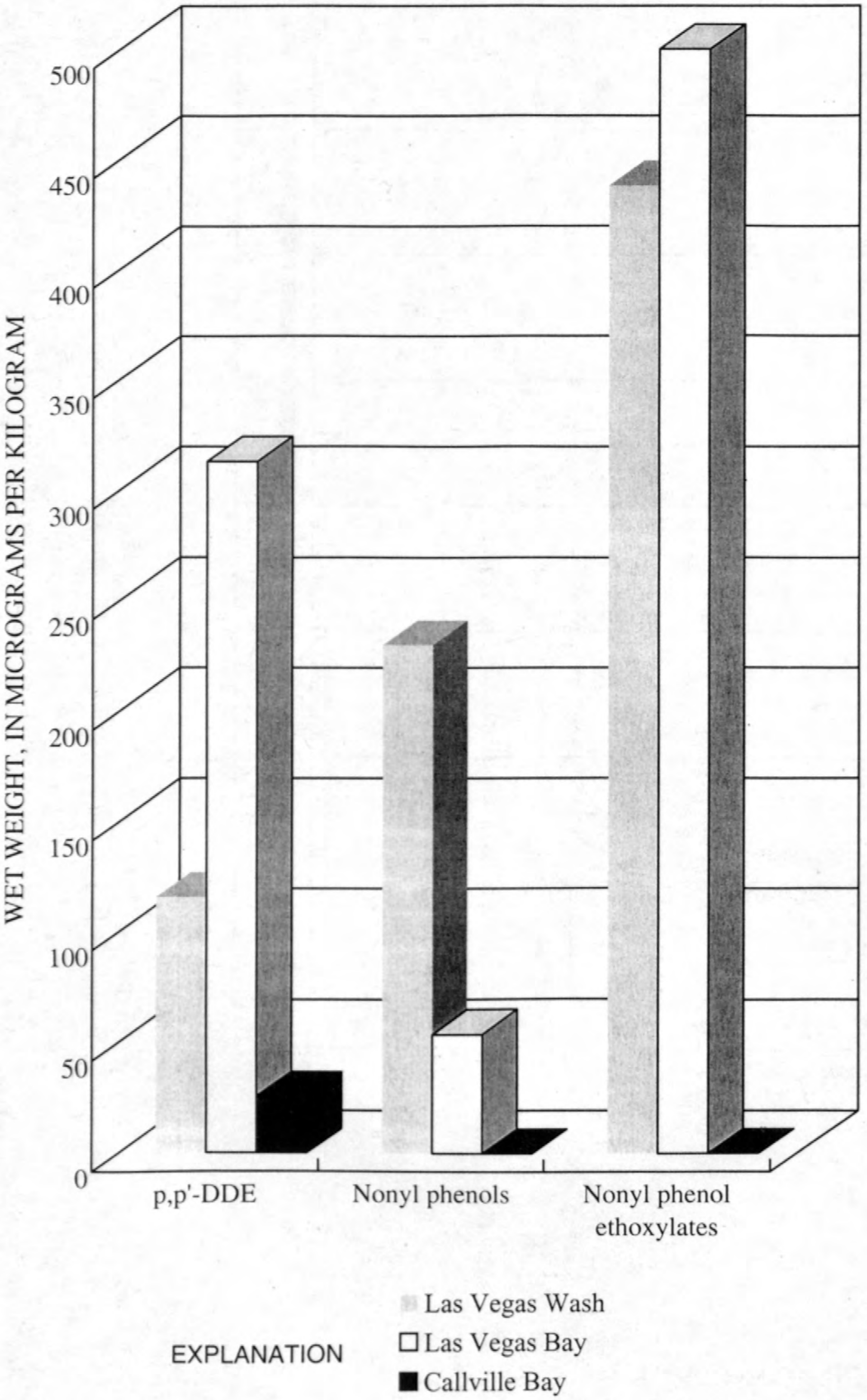

Figure 3. Mean concentration of selected compounds found in individual male carp samples collected from Las Vegas Wash, Las Vegas Bay, and Callville Bay.

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How DOC Composition May Explain the Poor Correlation Between Specific Trihalomethane Formation Potential and Specific UV Absorbance

By Miranda S. Fram, Roger Fujii, James L. Weishaar, Brian A. Bergamaschi, and George R. Aiken

ABSTRACT

Trihalomethane formation potential, ultra-violet light absorbance, and aromatic carbon content of dissolved organic carbon in natural water from grab sampling in watersheds across the USA and from detailed sampling in one watershed, the Sacramento-San Joaquin Delta, California, were examined in order to better understand the compositional nature of trihalomethane precursor material. Specific trihalomethane formation potential and specific ultra-violet light absorbance were not tightly correlated in either dataset, indicating that ultra-violet light absorbance cannot be used for accurate prediction of specific trihalomethane formation potential in drinking water derived from a broad spectrum of watershed types. Specific trihalomethane formation potential was not closely related to the aromatic carbon content of dissolved organic carbon isolates or to the partitioning of the dissolved organic carbon by nonionic macroporous resins, suggesting that neither of these measures are related to the compositional features of the dissolved organic carbon responsible for trihalomethane formation.

INTRODUCTION

Dissolved organic carbon (DOC) in natural water reacts to form trihalomethanes (THM) and other disinfection by-products when this water is chlorinated for use as drinking water. Aromatic-rich humic substances within the DOC have long been considered the precursors for THM, and ultra-violet (UV) absorbance measurements have been used to infer the aromatic content of natural water for the purpose of predicting THM formation (Rook, 1977; Reckhow and others, 1990). Absorbance of UV light is a standard proxy for aromatic carbon content (Traina and others, 1990; Chin and others, 1994), and along with DOC, is being considered as a surrogate measurement for trihalomethane formation potential (THMFP) that will indicate when additional water-treatment strategies are required.

In this study, we examine the relation among THMFP, UV absorbance, and aromatic carbon content of DOC in natural water from grab samples in watersheds across the USA that were collected as part of the National Water Quality Assessment (NAWQA) Program, and from detailed sampling in one watershed, the Sacramento-San Joaquin Delta, California (Delta), to better understand the compositional nature of THM precursor material. New U.S. Environmental Protection Agency (EPA) regulations that will take effect during the next several years will mandate the use of water-treatment strategies designed to lower the concentration of reactive DOC in water prior to chlorination in order to reduce THM concentrations in finished drinking water. Greater understanding of the relation between THM formation potential and compositional nature of the DOC is essential for the design of effective water-treatment strategies.

This report presents an analysis of the relations among THMFP, UV absorbance, DOC content, and percent aromaticity of DOC in samples from the Delta and NAWQA datasets. Complete data and full interpretations are reported in Fujii and others (1998), Bergamaschi and others (1999), and several forthcoming reports.

The majority of the Sacramento-San Joaquin Delta data (Twitchell Island) are compiled from a study done by the U.S. Geological Survey (USGS) in cooperation with the California Department of Water Resources Municipal Water Quality Investigation Program and as part of the USGS National Drinking Water Initiative (Fujii and others, 1998). The remaining Delta samples were collected as part of the USGS Toxic Substances Hydrology Program (Delta channel water) or the NAWQA Program (Sacramento and San Joaquin Rivers). DOC isolation and nuclear magnetic resonance characterization of the Delta channel water samples was done as part of the the USGS Toxic Substances Hydrology Program (Bergamaschi and others, 1999), and all other analyses of Delta samples were done as part of the USGS National Drinking Water Initiative. We gratefully acknowledge NAWQA Program units in Alabama, Alaska, California, Colorado, Illinois, Indiana, Louisiana, Massachusetts, Minnesota, Montana, New Jersey, New York, Ohio, Pennsylvania, South Carolina, Tennessee Texas, and Wisconson, that collected water samples for us. Analyses of the NAWQA samples were made as part of the USGS National Drinking Water Initiative, and USGS research sponsored by the EPA.

EXPERIMENTAL METHODS

The DOC present in Delta water is thought to be a mixture of river-borne DOC and DOC derived from Delta peat islands. Whole water samples were collected from the Sacramento and San Joaquin Rivers upstream of the Delta, from drainage ditches and ponds on Twitchell Island in the Delta, and from piezometers installed in two types of soil on Twitchell Island. More than 600 whole-water samples collected during a 3-year period were analyzed for DOC concentration, UV absorbance at 254 nanometers (nm), and THMFP. Measurements of DOC were made with a Shimadzu TOC-5000A total organic carbon analyzer and UV measurements were made with a Perkin-Elmer Lambda 3B ultraviolet/visible spectrophotometer using a 1-centimeter (cm) cell. (The use of trade-names in this report is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey.) Aliquots of sample were reacted with chlorine to form THM using a modified version of EPA Method 510.1, following the reactivity-based dosing method (Krasner and Sclimenti, 1993; California Department of Water Resources, 1994). THM concentrations were measured using a Tekmar ALS2016 and LSC2000 purge and trap apparatus, and a Hewlett-Packard 5890 II gas chromatograph outfitted with an electron capture dectector, following a modified version of EPA Method 502.2.

To provide material for further chemical analysis, DOC was isolated from selected water samples from Twitchell Island drainage ditches and piezometers, and from main Delta flow channels. DOC was fractionated and isolated from water sample of about 140 liters using sequential XAD-8 and XAD-4 resin extractions. The XAD-8 resin retains the larger, more hydrophobic materials, including humic and fulvic acids, while the XAD-4 resin retains the smaller, more hydrophilic materials (Aiken and others, 1992). Materials are sorbed to the resins at pH 2 and then eluted at pH 13. Isolates were freeze-dried until use, and reconstituted isolates were analyzed for DOC concentration and THMFP.

Carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra for the freeze-dried DOC isolates were obtained by solid-state, cross polarization, magic angle spinning spectroscopy using a 200 megahertz Chemagnetics CMX spectrometer. We assign resonance in the region 0-60 parts per million (ppm) as aliphatic, 60-90 ppm as heteroaliphatic, 90-110 ppm as anomeric, 110-160 ppm as aromatic, and 160-190 as carboxylic. Integrated areas in these regions were normalized to the aggregate area and the proportion of each functional assignment expressed as a percentage of the whole.

Water samples were collected at 92 sites in 18 states in conjunction with sampling by NAWQA Program. The sampling sites encompassed a wide spectrum of types of watersheds that provide water for drinking water purposes. DOC in the whole water samples was fractionated using XAD-8 resin. The XAD-8 resin retains the more hydrophobic compounds within the DOC, including the humic and and fulvic acids. Whole water, effluent from the

XAD-8 resin (the unretained fraction of the DOC), and the eluate from the resin all were analyzed for DOC content, UV absorbance at 254 nm and THMFP.

RESULTS AND DISCUSSION

Whole Water

The NAWQA and Delta whole water samples show well-defined linear relations between DOC concentration and UV (fig. 1). In order to compare the two datasets, we focus on data with UV absorbance of less than 0.4 (in units of per cm), which includes all of the NAWQA samples, and all of the Sacramento and San Joaquin River samples as well as many Twitchell Island surface water samples in the Delta sample set. Linear regressions indicate both the NAWQA and Delta whole water samples form well-defined lines, as indicated by high coefficients of determination (r^2) values of 0.71 and 0.91 respectively (table 1). However, the two linear regressions yielded statistically distinct lines (t-test of difference between two slopes, $\alpha = 0.05$, P

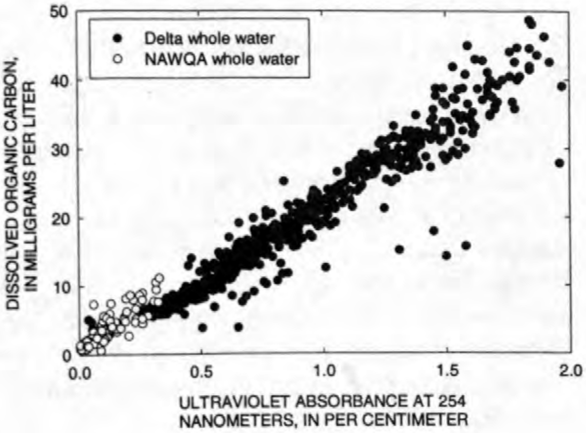

Figure 1. Relation between ultraviolet light absorbance at 254 nanometers and dissolved organic carbon content for whole water samples from the Sacramento-San Joaquin Delta, California and from a national sampling of watersheds the National Water Quality Assessment Program. UV absorbance measured in a 1-cm path length cell.

Table 1. Results of linear regressions of data for ultraviolet absorbance, dissolved organic carbon concentration, trihalomethane formation potential, specific ultraviolet absorbance, and specific trihalomethane formation potential for samples from the Sacramento-San Joaquin Delta, and the Sacramento and San Joaquin Rivers, California and from a national sampling of watersheds by the National Water Quality Assessment Program

[Equations for slope, intercept, coefficient of determination (r^2), and confidence interval for prediction of Y values at $\alpha = 0.05$ are from Zar (1984). Equation for percentage difference between measured and predicted Y values is from Rathbun (1996). Fig., figure in which data appears; NAWQA, samples national sampling of watersheds by the National Water Quality Assessment Program; Delta UV<0.4, samples from the Sacramento-San Joaquin Delta and Rivers with ultraviolet absorbance less than 0.4 cm^{-1}; Rivers, Sacramento and San Joaquin Rivers, UV; ultraviolet absorbance; DOC, dissolved organic carbon; THMFP, trihalomethane formation potential; SUVA, specific ultraviolet absorbance; STHMFP, specific trihalomethane formation potential; mg/L, milligrams per liter; µmol/L; micromoles per liter; mmol/mol, millimoles per mole]

fig.	Data set	X	Y	Slope	Intercept	r^2	Percentage difference	Prediction interval
1	NAWQA	UV	DOC	24.1	1.04	0.71	31.5	2.66 mg/L
1	Delta, UV<0.4	UV	DOC	15.1	1.59	.91	13.5	1.46 mg/L
2	NAWQA	UV	THMFP	20.0	- .01	.83	21.8	1.71 µmol/L
2	Delta, UV<0.4	UV	THMFP	12.4	1.04	.73	31.5	1.83 µmol/L
3	Delta	SUVA	STHMFP	27.4	8.65	.07	11.0	4.27 mmol/mol
3	Rivers only	SUVA	STHMFP	24.9	.33	.80	8.2	1.93 mmol/mol

< 0.001; Zar, 1984) for the two sets of data. The Delta samples lie on a line with shallower slope than the NAWQA samples, indicating presence of DOC with higher UV absorbance. This result is consistent with the presence of a greater proportion of peat-derived DOC in the Delta samples than in the NAWQA samples. DOC derived from reducing environments such as peat soils, generally contains a high proportion of phenolic structures (Fujii and others, 1998), and aromatic structures, including phenols, absorb UV light (Rao, 1975).

The utility of a linear regression between two water-quality variables, such as DOC and UV absorbance, to the development of water-treatment strategies, depends on how accurate a prediction of the independent variable the regression equation gives. This accuracy can be expressed as the prediction interval for the independent variable at the given confidence level ($\alpha = 0.05$ in this report). The prediction interval for the NAWQA regression is ±2.67 milligrams per liter (mg/L) DOC, whereas the prediction interval for the Delta regression is only ±1.46 mg/L DOC (table 1). All of the Delta samples lie within the prediction interval for the NAWQA samples, but the inverse is not true. Rathbun (1996) used the average percentage difference between measured and predicted values of the independent variable to assess the accuracy of prediction. Percentage difference for the Delta regression is much smaller than that for the NAWQA regression (table 1), which is in agreement with the lower prediction interval determined for the Delta regression. The larger scatter in the NAWQA data may be because that dataset includes a "national average" of watersheds. Each watershed type may have a different relation between DOC and UV absorbance, so that a national sampling represents the sum of many individual relations.

Both datasets also show linear relations between molar THMFP and UV absorbance, although the data are considerably more scattered for samples with UV less than 0.4 cm^{-1} (fig. 2). Again comparing data with UV absorbance less than 0.4 cm^{-1}, the slopes of the two regressions are significantly different (t-test of difference between two slopes, $\alpha = 0.05$, $P < 0.001$; Zar, 1984). The Delta samples lie on a line with shallower slope than the NAWQA samples, indicating that the excess aromatic carbon in the Delta samples is relatively unreactive with respect to THM formation during chlorination. The prediction interval is ±1.71 micromoles per liter (µmol/L) for the NAWQA samples and ±1.83 µmol/L the Delta samples, and the percentage differences are large for both (table 1), suggesting that UV absorbance alone is not a highly accurate predictor of THMFP.

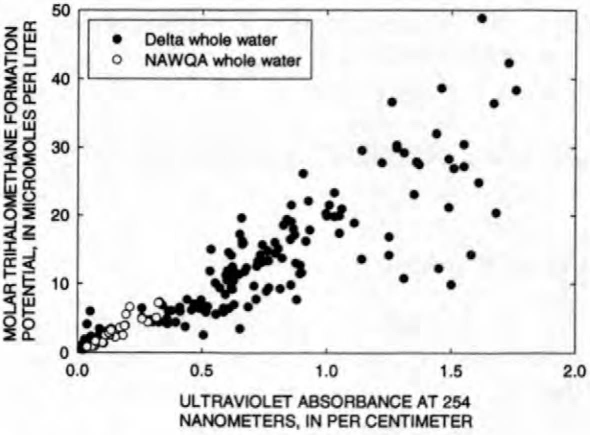

Figure 2. Relation between ultraviolet light absorbance at 254 nanometers and molar trihalomethane formation potential for whole water samples from the Sacramento-San Joaquin Delta, California and from a national sampling of watersheds the National Water Quality Assessment Program.

Specific properties

Delta samples

In order to examine the effect of carbon composition, we use the following carbon-normalized parameters: specific trihalomethane formation potential (STHMFP), defined as millimoles (mmol) of THM formed per mole (mol) of DOC, and specific UV absorbance (SUVA), defined as the UV absorbance divided by the DOC concentration (the units of SUVA are liters per milligram per centimeter, L/mg/cm). In the Delta samples, STHMFP ranged from 2 to 16 mmol/mol, and SUVA ranged from 0.01 to 0.09 L/mg/cm (fig. 3), indicating large variability in the reactivity and composition of the DOC. Although SUVA and STHMFP for the full range of whole water from the Delta region generally were positively correlated (fig. 3), the relation between the two parameters was not well defined, as evidenced by the very large prediction interval (STHMFP predicted to only ± 4.3 mmol/mol for a given SUVA) and small coefficient of determination ($r^2 = 0.07$) for the linear regression (table 1). The Delta results indicate that the connection between STHMFP and DOC composition is not directly related to properties measured by SUVA.

STHMFP and SUVA for water from the Sacramento and San Joaquin Rivers did show a meaningful linear relation (fig. 3) (prediction interval of ± 1.93 mmol/mol and coefficient of determination, $r^2 = 0.80$). The linear regression of STHMFP and SUVA data for the Sacramento and San Joaquin River samples yielded a percentage difference of 8.2 percent, the lowest percentage difference in the dataset, and quite comparable with percentage difference observed for samples from the Mississippi River system by Rathbun (1996). A large river system may average DOC from its entire watershed, thus obscuring the signal from DOC derived from specific sources, such as wetlands. Thus, a linear relation between STHMFP and SUVA may be an accurate enough method for predicting STHMFP for some water systems – but only for systems in which the temporal and spatial variation in DOC composition is very small because of extensive mixing of large amounts of water. However, there is no reason to expect that all such systems will show the same relation betweeen STHMFP and SUVA. Several types of organic structures that are likely to be present in the DOC pool are capable of absorbing UV light, including aromatic rings and conjugated dienes and carbonyls (Rao, 1975). Experiments on model compounds indicate high yields of THM can be produced by chlorination of aromatic structures, such as phenolic compounds, as well as nonaromatic structures, such as enolizable β-di-ketones (Larson and Weber, 1994). Not all of these nonaromatic structures are capable of absorbing UV light (Rao, 1975). No two water systems have exactly the same sources of DOC, therefore, one would expect DOC from every water system to contain a unique combination of molecular structures and thus, to have a different relation between STHMFP and SUVA.

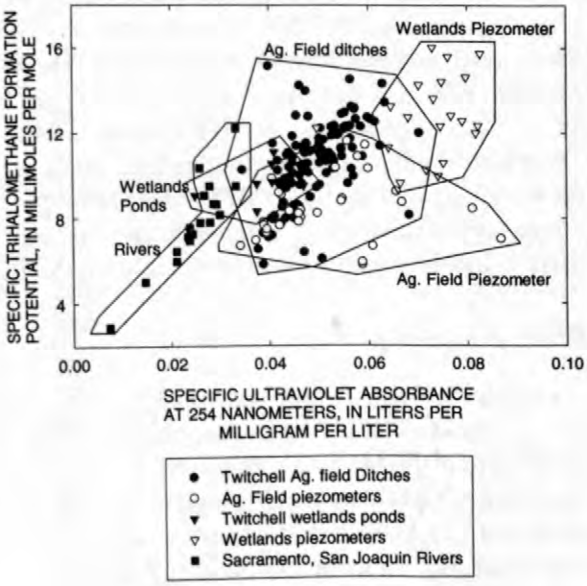

Figure 3. Relation between specific trihalomethane formation potential and specific ultraviolet light absorbance at 254 nanometers for whole water samples from the Sacramento-San Joaquin Delta, California the National Water Quality Assessment Program.

Comparison of water samples taken from different soils and locations suggests that the lack of correlation between DOC aromaticity (as estimated by SUVA) and STHMFP may be due to the presence of an unreactive aromatic fraction in the DOC derived from peat soils. DOC derived from all of the Twitchell Island drainage and soil water samples contained more aromatic carbon than DOC derived from Sacramento and San Joaquin main stem river water samples, as indicated by the markedly higher SUVA values (t-test of differences between two means, $\alpha = 0.05$, $P < 0.001$; Zar, 1984) (fig. 3). This result was expected, given the abundance of peat soils on Twitchell Island. Although the highest STHMFP values were measured in some Twitchell Island samples, many of the Twitchell samples have STHMFP values between 6 - 12 mmol/mol, which is indistinguishable from most of the main stem river samples (fig. 3). The mean STHMFP value for the Twitchell Island samples is statistically the same as the mean STHMFP value for the Sacramento and San Joaquin River samples (t-test of differences between two means, $\alpha = 0.05$; Zar, 1984). The observation that SUVA values for the Twitchell Island samples are higher than those in the River samples, but STHMFP values are the same, suggests that some of the aromatic carbon derived from the peat soils must be unreactive.

NAWQA samples

The NAWQA whole water and fractionated samples have STHMFP values ranging from 3.9 to 16.5 mmol THM/mol DOC and SUVA values ranging from 0.007 to 0.052 L/mg/cm (fig. 4). Although STHMFP and SUVA were positively correlated (fig. 4), there was no well-defined relation as evidenced by the small coefficient of determination ($r^2 = 0.39$) and large prediction interval (± 6.4 mmol THM/mol DOC) for the linear regression (table 1). Separation of the whole water, effluent, and eluate samples did not yield linear regressions with statistically different slopes or intercepts (t-test of difference between two slopes and two intercepts, $\alpha = 0.05$; Zar, 1984). These results indicate that the connection between STHMFP and DOC composition is not directly related to properties measured by SUVA – just as concluded from the Delta results. DOC in the whole water, effluent, and eluate subsamples of the NAWQA samples did vary in composition. Mean SUVA and STHMFP for the effluent samples (mean = 0.025 units/mg/L, standard deviation, s = 0.010; and 6.87 mmol/mol, s = 2.19, respectively) are lower than values for the eluate samples (0.035 units/mg/L, s = 0.006; and 9.22 mmol/mol, s = 2.33, respectively) (t-test of difference between two means, a = 0.05, $P < 0.001$; Zar, 1984). This difference indicates that the fraction of the DOC retained by the XAD-8 resin contained slightly more aromatic carbon and was slightly more likely to form THM than the unretained fraction. However, mean SUVA and STHMFP values for the whole water and eluates, and mean STHMFP for whole water and effluents cannot be distinguished statistically (t-test of difference between two means, a = 0.05; Zar, 1984). The XAD-8 resin retained 30-75 percent of the DOC in the NAWQA whole water samples, and the eluates accounted for 20-80 percent (mean 56 percent, s = 13 percent) of the total THMFP of the whole water sample. These results suggest that the compositional properties determining retention on XAD-8 resin are only very weakly related to the compositional properties determining reactivity to form THM. The connection between the efficiency of DOC removal from water and the reactivity of the DOC removed has implications for the design of water-treatment strategies. Tests of various coagulation, flocculation, sedimentation, filtration, and adsorption methods in operating water-treatment plants and in bench-scale studies have shown that the amount of DOC removed and the amout of THM precursor carbon removed are not necessarily correlated (Lykins and Clark, 1989).

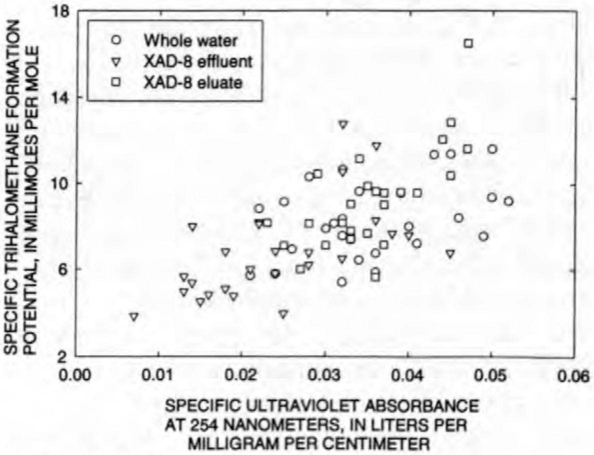

Figure 4. Relation between specific trihalomethane formation potential and specific ultraviolet light absorbance at 254 nanometers for whole water samples, XAD-8 effluents. and XAD-8 eluates from a national sampling of watersheds the National Water Quality Assessment Program.

DOC isolates

DOC from a subset of the Delta samples was isolated and analyzed by ^{13}C-NMR to determine its chemical composition and aromatic carbon content. Although the isolation technique extracted only 40-80 percent of the total DOC in these samples, isolation permits analysis and direct comparison of DOC chemical composition and STHMFP. For the isolates, STHMFP ranged from 5 to 9 mmol/mol, and the percentage of aromatic carbon from 11 to 25 percent (fig. 5). The percentage of aromatic carbon and STHMFP do not show a significant relation (fig. 5) (linear regression $r^2 = 0.09$), indicating that aromatic carbon content alone does not determine the extent of THM formation.

Further evidence that aromatic carbon content does not determine THMFP is apparent when comparing XAD-8 and XAD-4 isolates. XAD-4 isolates contain only 52-82 percent of the aromatic carbon in the corresponding XAD-8 isolates (fig. 5), and should have distinctively lower STHMFP if aromatic carbon content alone determines STHMFP. However, the mean STHMFP of the XAD-4 isolates (6.48 mmol/mol; s = 1.6 mmol/mol) was 87 percent of the mean STHMFP of the XAD-8 isolates (7.43 mmol/mol; s = 1.9 mmol/mol), and the two populations were significantly different only at less than the 85 percent confidence level (t-test of difference between two means; Zar, 1984) (fig. 5). Thus, either nonaromatic carbon in the XAD-4 isolates was reactive, or some of the aromatic carbon in the XAD-8 isolates was unreactive. Since SUVA is used as a surrogate measurement of DOC aromaticity (Traina and others, 1990; Chin and others, 1994), the presence of reactive nonaromatic carbon or unreactive aromatic carbon would result in poor correlation between SUVA and STHMFP as observed in the NAWQA and Delta whole water samples.

CONCLUSIONS

1. SUVA and STHMFP in water from the NAWQA sampling and the Delta sampling are not tightly correlated, indicating that SUVA cannot be used for accurate prediction of THMFP in drinking water derived from a broad spectrum of watershed types, and that DOC derived from different environments, such as wetlands, within the watershed has distinct composition and reactivity. However, the results from the Sacramento and San Joaquin River samples suggest that it may be possible to establish the relation between SUVA and STHMFP for specific systems.
2. STHMFP is not related to the aromatic carbon content of DOC isolates from Delta water, providing a compositional basis for the lack of correlation between SUVA and STHMFP.
3. Carbon released from Delta peat islands may contain a significant fraction of aromatic material that does not form THM upon chlorination.
4. Retention of DOC by XAD-8 resin is weakly associated with reactivity of DOC to form THM, suggesting that water treatment techniques for DOC reduction may not necessarily achieve commensurate THMFP reduction in drinking water.

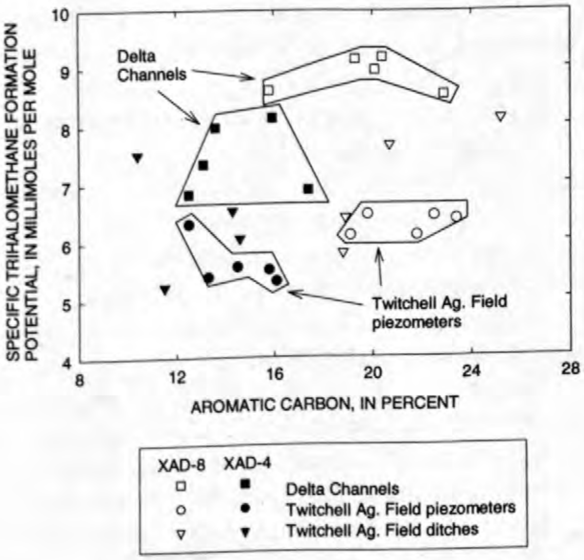

Figure 5. Relation between specific trihalomethane formation potential and percentage of aromatic carbon for XAD-8 and XAD-4 dissolved organic carbon isolates from water samples from the Sacramento-San Joaquin Delta, California. Percentage of aromatic carbon measure by carbon-13 nuclear magnetic resonance spectroscopy.

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Wastewater Analysis by Gas Chromatography/Mass Spectrometry

By Gregory K. Brown, Steven D. Zaugg, and Larry B. Barber

ABSTRACT

The U.S. Geological Survey National Water Quality Laboratory and National Research Program have developed a custom analytical method for the determination of compounds typically found in domestic and industrial wastewater. The method was developed in response to increasing concern over the impact of endocrine disrupting chemicals in wastewater on aquatic organisms. Compounds were isolated by continuous liquid-liquid extraction with methylene chloride and determined by capillary-column gas chromatography/mass spectrometry using selected-ion monitoring. The method focuses on the analysis of the alkylphenolethoxylate nonionic surfactant compounds that are persistent indicators of wastewater. Other compounds are representative of food additives, antioxidants, fire retardants, plasticizers, industrial solvents, disinfectants, fecal sterols, polycylic aromatic hydrocarbons, and high-use domestic pesticides. The method detection limits range from 0.05 to 0.2 microgram per liter for single compounds and about 0.2 microgram per liter for the sum of the isomeric compounds, such as nonylphenol and nonylphenolethoxylates.

INTRODUCTION

Industrial and domestic waste must be managed effectively to meet the challenges of increasing population, stringent regulatory requirements, and aging water treatment facilities. To meet these challenges, specific analytical methods are available to monitor chemical compounds in wastewater. However, because of the complexity of the sample matrix, several analytical methods are required to determine polar and non-polar organic compounds in the dissolved and suspended phases that may impact water quality. Several compounds are regulated by the U.S. Environmental Protection Agency (USEPA), and appropriate analytical methods are generally available (USEPA, 1995) to monitor for them in industrial wastes or in discharge from wastewater-treatment facilities. Other compounds known to be toxic to aquatic life are currently unregulated even though many, such as nonylphenolethoxylates (NPEO's) are on the USEPA Toxic Substance Control Act Priority Testing List (USEPA, 1996). To improve the measurement of the impact of wastewater discharge practices on water quality, the U. S. Geological Survey (USGS) National Water Quality Laboratory (NWQL) and National Research Program (NRP) have developed a custom analytical method. This method uses representative compounds from several compound classes, to monitor unregulated and regulated contaminants.

METHODS

The custom method is suitable for analysis of complex water samples such as wastewater effluents. One-liter samples, collected in amber glass bottles and stored at 4 °C, are necessary to achieve the low level of detection. Because many of the target compounds are common food additives (caffeine, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT)), or found in detergents and plasticizers (nonylphenol-NP, NPEO), care must be taken to avoid sample contamination. Field processing blanks and method blanks are used to monitor low-level contamination of samples.

To meet the needs of USGS projects, the NWQL has included several sewage indicators such as pesticides, caffeine, and alkylphenols to select USEPA targeted compounds and developed a custom wastewater method. Other semi-volatile organic compounds that have been detected frequently in samples at the NWQL since 1995 were also added to the method even though many are currently unregulated.

Samples are extracted by continuous liquid-liquid extraction (CLLE) using methylene chloride as the extraction solvent. The unique design of the extractor (fig. 1) uses a porous glass frit that disperses methylene chloride solvent in micro-droplets, improving extraction efficiency compared to conventional designs. Extraction of whole-waters samples by this technique has been in use at the NWQL since 1994, and CLLE has become the extraction method of choice for a wide range of polar and non-polar organic compounds.

Once extracted, the CLLE sample volumes are reduced to 500 microliters prior to analysis by gas chromatography/mass spectrometry (GC/MS). Operation of the mass spectrometer in the selected-ion-monitoring (SIM) mode is necessary to achieve detection levels ranging from 0.05 to 0.20 micrograms per liter, which are required for most environmental samples.

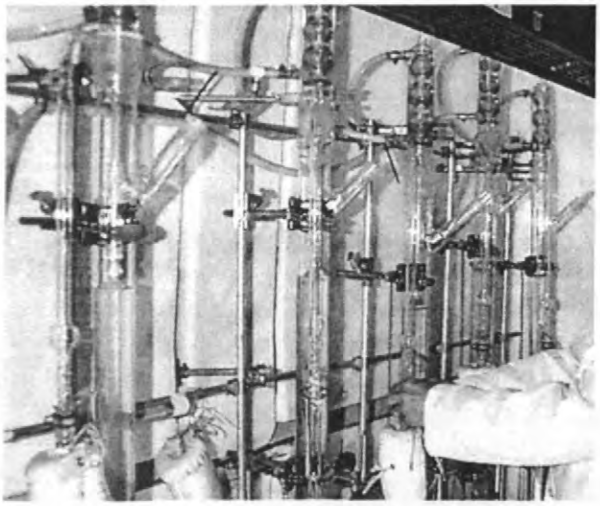

Figure 1. Continuous liquid-liquid extractors with solvent-dispersing frits.

RESULTS

In 1998, 10 USGS projects submitted 126 samples for analysis by this method. Table 1 lists the detection frequency for the industrial and domestic compounds used to identify potential sources of contamination in wastewater samples.

Table 1. Wastewater, method compounds, their use, and percent frequency of detection in 126 samples from diverse sources.

Compound	Use/Source	%
tetrachloroethylene	solvent	11
2-(2-butoxyethoxy) ethylacetate	solvent	8
phenol	disinfectant	15
trichlosan	antibacterial	17
4-cresol	preservative	9
benzaldehyde	flavor	12
acetophenone	fragrance	19
caffeine	stimulant	22
butylated hydroxyanisole	antioxidant	11
butylated hydroxytoluene	antioxidant	8
2,6-di-*t*-butylbenzoquinone	antioxidant	14
2,6-di-*t*-butylphenol	antioxidant	3
4-nonylphenol	detergent	25
4-nonylphenol monoethoxylate	detergent	21
4-nonylphenol diethoxylate	detergent	13
4-octylphenol monoethoxylate	detergent	17
4-octylphenol diethoxylate	detergent	6
1,4-dichlorobenzene	fumigant	12
1,3-dichlorobenzene	fumigant	4
naphthalene	fumigant	18
2,6-dimethylnaphthalene	gasoline	5
phenanthrene	hydrocarbon	19
anthracene	hydrocarbon	11
fluoranthene	hydrocarbon	18
pyrene	hydrocarbon	17
benzo(a)pyrene	hydrocarbon	4
tri-(dichloroisopropyl) phosphate	fire retardant	12
tri-(2-chloroethyl) phosphate	fire retardant	10
lindane	pesticide	1
cis-chlordane	pesticide	4
dieldrin	pesticide	5
methyl parathion	pesticide	1
diazinon	pesticide	11
chlorpyrifos	pesticide	3
carbaryl	pesticide	3
17-*beta*-estradiol	estrogen	2
cholesterol	fecal sterol	21
3-*beta*-coprostanol	fecal sterol	20
bisphenol A	polymers	10
phthalic anhydride	plastics	12
tributyl phosphate	plasticizer	14
triphenyl phosphate	plasticizer	13
2-butoxy-ethanol phosphate	plasticizer	15
bis-(2-ethylhexyl) phthalate	plasticizer	16
bis-(2-ethylhexyl) adipate	plasticizer	11
codeine	analgesic	6

The wide range of compounds detected (table 1), demonstrates the usefulness of this method for identifying anthroprogenic contaminants over a wide range of sample types. The method also was used on wastewater and river waters samples collected from the Great Lakes region in March, 1998. The river water samples were collected at the surface, near the bank, whereas the wastewater effluents were collected at the outfall. All samples appeared relatively clear of sediment, and none of the samples were filtered prior to extraction.

The analytical results for these samples are sumarized in table 2 and figure 2. All 8 sewage treatment plant (STP) samples on the right side of the table show elevated octyl- and nonylphenol-ethoxylate levels over the river water samples.

Table 2. Results for analysis of river and wastewater samples collected March, 1998, as part of NRP-USEPA study. [concentrations in micrograms per liter unless otherwise indicated; nd, not detected at about 0.01 microgram per liter]

	Sampling Locations										
COMPOUND	**IR**	**DR**	**MR**	**ST**	**NS**	**CA**	**SE**	**ME**	**BL**	**JI**	**DE**
total organic carbon (mg/L)	7.0	6.9	10	7.6	6.3	7.6	8.5	12	9.6	17	12
4-methylphenol	nd	nd	nd	nd	nd	0.2	0.0	nd	nd	nd	0.8
4-*t*-butylphenol	nd	nd	nd	0.1	0.2	nd	0.3	0.3	0.1	0.3	1.0
4-*t*-pentylphenol	nd	nd	nd	0.3	nd	nd	nd	0.3	nd	0.3	0.9
2,6-di-*t*-butylphenol	nd	nd	nd	nd	nd	nd	nd	0.2	0.1	nd	0.1
2,6-di-*t*-butylbenzoquinone	nd	0.2	nd	0.5	0.6	0.6	0.9	0.8	0.8	0.7	1.4
butylated hydroxyanisole	nd	0.3	nd	0.6	0.6	0.6	1.2	1.3	0.9	0.2	0.2
butylated hydroxytoluene	nd	nd	nd	0.0	0.0	0.1	0.1	nd	0.3	0.0	0.1
4-*n*-octyphenol	nd	nd	nd	nd	nd	nd	nd	0.2	nd	nd	nd
4-octylphenol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.4
4-nonylphenol (total)	nd	1.1	nd	1.7	1.6	1.4	1.5	2.1	0.9	2.5	19
4-nonylphenol monoethoxylate (total)	nd	1.5	nd	4.0	13	5.8	6.0	11	1.5	9.0	55
4-nonylphenol diethoxylate (total)	nd	0.5	nd	5.0	7.0	3.7	2.9	19	0.8	28	110
4-nonylphenol triethoxylate (total)	nd	nd	nd	nd	nd	nd	nd	2.0	nd	3.0	17
4-nonylphenol tetraethoxylate (total)	nd	nd	nd	nd	nd	nd	nd	1.2	nd	nd	5.0
4-octylphenol monoethoxylate	nd	nd	nd	0.5	1.0	0.5	0.6	6.0	0.3	0.8	3.2
4-octylphenol diethoxylate	nd	nd	nd	1.5	1.5	0.8	0.7	4.0	nd	6.0	25
4-octylphenol triethoxylate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	22
caffeine	0.2	0.2	0.1	0.1	0.1	0.3	0.1	2.4	0.2	0.7	4.0
bisphenol A	nd	0.2	nd	0.4	nd	0.6	nd	nd	nd	0.9	2.7
coprostanol	nd	nd	nd	0.5	0.6	0.3	0.2	1.3	0.1	0.8	14
cholesterol	0.6	0.6	0.6	1.2	1.1	1.0	0.7	2.2	0.6	1.7	8.0
Surrogate Standards*	%	%	%	%	%	%	%	%	%	%	%
d_{21}-butylated hydroxytoluene	55	47	57	38	70	91	36	43	70	21	65
d_6 bisphenol A	70	94	111	108	110	114	104	122	114	94	120
4-*n*-nonylphenol	71	91	104	82	120	125	95	134	131	96	123
17-α-estradiol	84	82	92	99	75	105	80	109	111	83	126
$^{13}C_6$ 4-*n*-nonylphenol	91	80	149	90	122	124	108	130	137	107	131
$^{13}C_6$ 4-*n*-nonylphenol monoethoxylate	80	129	126	137	152	137	155	206	142	210	257
$^{13}C_6$ 4-*n*-nonylphenol diethoxylate	35	43	51	51	48	41	74	113	46	109	17
$^{13}C_6$ 4-*n*-nonylphenol trithoxylate	35	43	51	51	48	41	74	113	46	109	17

STP - Sewage treatment plant
IR, Illinois River, Ottawa, Ill.
DR, Des Plains River, Joliet, Ill.
MR, Minnesota River, Minneapolis, Minn.
ST, Stickney STP, Cicero, Ill.
NS, North Side STP, Skokie, Ill.
CA, Calumet STP, Chicago, Ill.
SE, Seneca STP, Eagan, Minn.
ME, Metropolitan STP, Ramsey, Minn.
BL, Blue Lake STP, Shakopee, Minn.
JI, Jones Island STP, Milwaukee, Wisc.
DE, Detroit STP, Detroit, Mich

*High percent recovery values for ^{13}C labeled surrogate compounds are due to compounds being spiked at low concentration levels (1 microgram per liter).

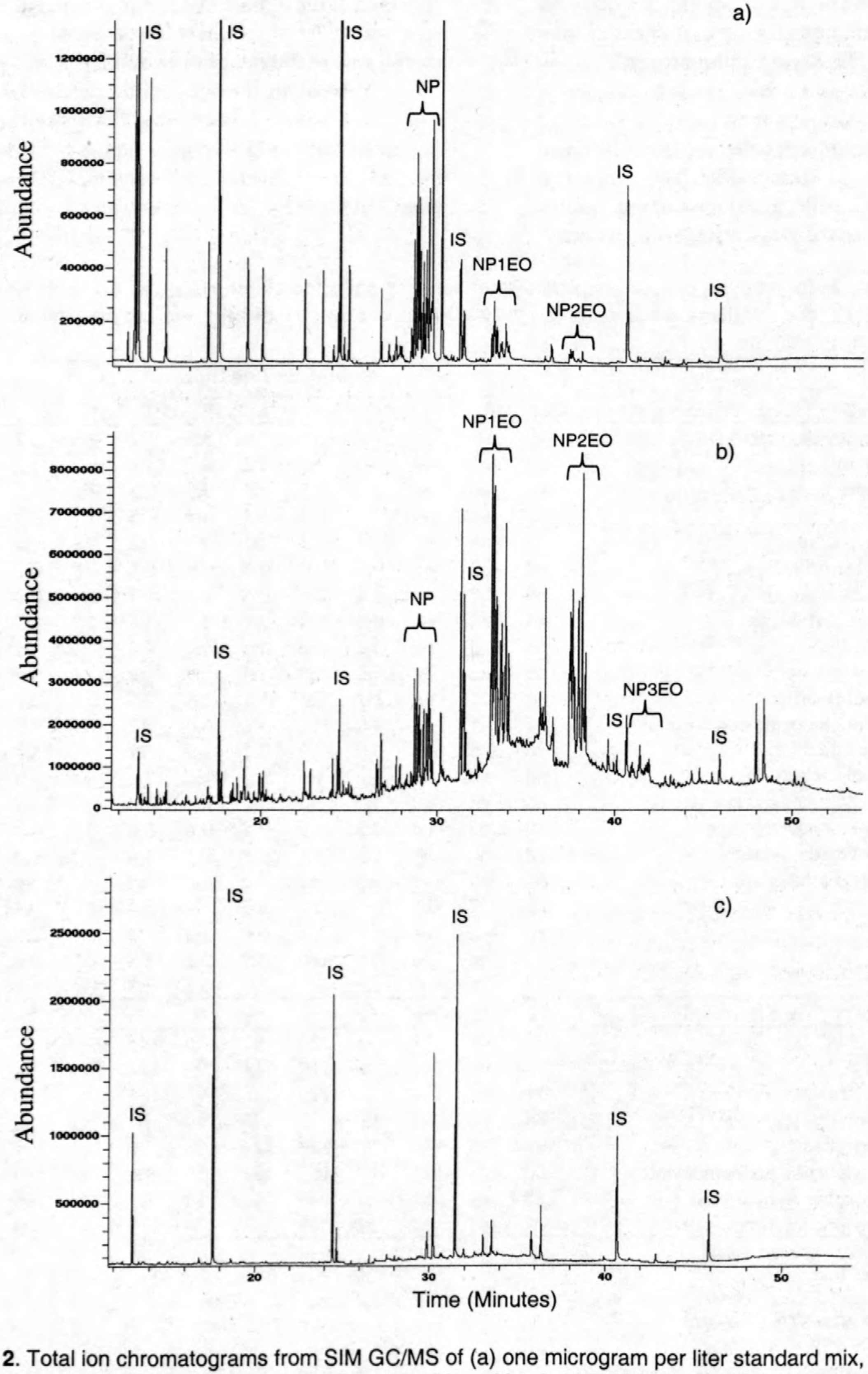

Figure 2. Total ion chromatograms from SIM GC/MS of (a) one microgram per liter standard mix, (b) Detroit wastewater, March 1998, and (c) Minnesota River, March 1998. [IS, internal standard; NP, 4-nonylphenol; NP1-3EO, 4-nonylphenol-mono, di, or triethoxylate]

The STP samples also have higher concentrations of antioxidants (BHA, BHT), as well as the oxidized form, 2,6-di-*t*-butylbenzoquinone. Several surrogate compounds were used in this study to monitor method efficiency and reproducibility. The surrogate standards consist of representative deuterated, carbon 13 labeled, or straight chained (*n*) compounds. Total organic carbon (TOC) values are also given for reference.

The use of SIM mass spectrometry decreases the number of compound peaks seen, and increase the instrument sensitivity by monitoring only ions specific to the compounds of interest. The CLLE GC/MS chromatograms (fig. 2) collected in the SIM mode show the isomeric nature of the NP and NPEO homologs. Under the GC/MS conditions used in this method, approximately 13 separate isomeric peaks are resolved for NP and each NPEO homolog. Resolution and response of NPEO homologs decrease with an increase of EO units with NP4EO being about the maximum quantifiable homolog. The chromatogram from the Minnesota River (fig. 2c) shows a sample with relatively few wastewater compounds present.

DISCUSSION

The method provides an efficient means of detecting several anthropogenic compounds that otherwise might not be possible by USGS or USEPA approved methods. More than one half of the target compounds have been detected in recent samples submitted to the NWQL by projects specifically designed to monitor municipal wastewater. Many of the compounds are endocrine-system disrupters (NP and NPEO's), whereas others, such as caffeine and fecal sterols, are excellent indicators of wastewater. Even though the method is not particularly sensitive for the fecal sterols, it has proven to be an effective way to identify their presence at somewhat higher concentration levels than might be obtained by a more specific method.

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Biomonitoring of Environmental Status and Trends (BEST) Program: Contaminants and Related Effects in Fish from the Mississppi, Columbia, and Rio Grande Basins

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ABSTRACT

The Biomonitoring of Environmental Status and Trends (BEST) Program was initiated, in part, as a revision and expansion of the National Contaminant Biomonitoring Program (NCBP). One aspect of the BEST program focuses on monitoring contaminants and effects across broad geographic areas. This approach is currently being tested in the Mississippi, Columbia, and Rio Grande basins. The overall objectives of this project are to describe the occurrence and distribution of contaminants and their effects on fish in the three basins; to quantitatively evaluate the performance of aquatic methods used by the BEST program; and to evaluate potential collaborations with the National Stream Quality Accounting Network (NASQAN-II) and the National Water Quality Assessment (NAWQA) programs of the USGS-Water Resources Division. Fish were collected from 46 sites in the Mississippi River basin (1995); 16 sites in the Columbia River basin (1997); 10 sites in the Rio Grande basin (1997); and from a reference site in West Virginia. Sites were located at the historic NCBP fish monitoring stations in all three basins; at NASQAN-II water quality sampling sites in the Columbia and Rio Grande basins; and at NAWQA sites in the Mississippi Embayment and Eastern Iowa Basins study units within the Mississippi River basin. The primary species targeted at each site were common carp (*Cyprinus carpio*) and largemouth bass (*Micropterus salmoides*); other species, mostly other black basses (*Micropterus* spp.), percids (*Stizostedion* spp.), salmonids, suckers (Catostomidae), and catfish (Ictaluridae) were collected as alternates, depending on habitat and location. Individual fish (about 40 per station) were analyzed for reproductive biomarkers (vitellogenin and sex steroid hormones), histopathological alterations, macrophage aggregates, EROD activity, lysozyme activity, and general fish health measures (organosomatic and ponderal indices, observations of grossly visible lesions, deformities, and parasites). Organochlorine (pesticides and total PCB's) and elemental (heavy metals and metalloids) contaminant analyses and the H4IIE bioassay for dioxin-like activity were performed on fish samples composited by species and sex. In the Mississippi basin, DDT (mostly as *p,p'*-DDE) residues in fish remained sufficiently high in the southern parts of the watershed to represent a hazard to sensitive species of fish-eating birds. Toxaphene residues also remained evident. The combined results of organochlorine chemical, H4IIE bioassay, and biomarker analyses also indicated the presence of other organic contaminants in the lower Mississippi valley. Cyclodiene pesticides (dieldrin, endrin, and chlordane) were present in many agricultural areas, especially in the Corn Belt. Concentrations of these pesticides were also elevated at Memphis, Tennessee, where there is a point-source. Selenium concentrations were sufficiently high to constitute a hazard to piscivorous fishes and wildlife in the upper Arkansas River, where levels have been increasing for approximately 10 years. As expected, mercury concentrations were higher in the predator species than in bottom fish. The occurrence of vitellogenin in plasma and of ovarian cells in the testes of male fish from several sites, along with abnormal ratios of sex steroids, suggest that fish from some sites are exposed to endocrine-modulating substances.

BACKGROUND

Development of the Biomonitoring of Environmental Status and Trends (BEST) program was initiated in 1991 by the U.S. Fish and Wildlife Service (FWS) in part as a revision and expansion of the National Contaminant Biomonitoring Program (NCBP). The BEST program also sought to address contaminant issues on National Wildlife Refuges. The NCBP originated as part of the National Pesticide Monitoring Program (NPMP) --a multi-agency effort to document temporal and geographic trends in persistent contaminant concentrations through the collection and chemical analysis of environmental media (Johnson et al. 1967). As part of its NPMP activities, the FWS periodically collected and analyzed freshwater fish from a national network of stations that was last sampled completely in 1986 (Schmitt and Bunck 1995; Schmitt et al. in press). By the mid-1980's, the FWS had re-named its monitoring program NCBP in anticipation of adding biological monitoring and assessment components. The BEST program incorporates FWS planning for this expanded monitoring. In 1993, the BEST program, as well as curatorial responsibility for the historic NCBP data bases and sample archives, were transferred to the National Biological Service (NBS). The NBS became the Biological Resources Division (BRD) of the U. S. Geological Survey (USGS) in 1996.

The present goal of the BEST program is to monitor, identify, and understand the effects of contaminants on the Nation's biological resources, especially those managed by the Department of the Interior (DOI), and to provide scientific information for guiding management actions to DOI agencies. To achieve this goal, the BEST program employs a variety of approaches including assessment of existing information, development of biomonitoring methods, technical assistance, and monitoring a suite of contaminants and contaminant effects across broad geographic areas.

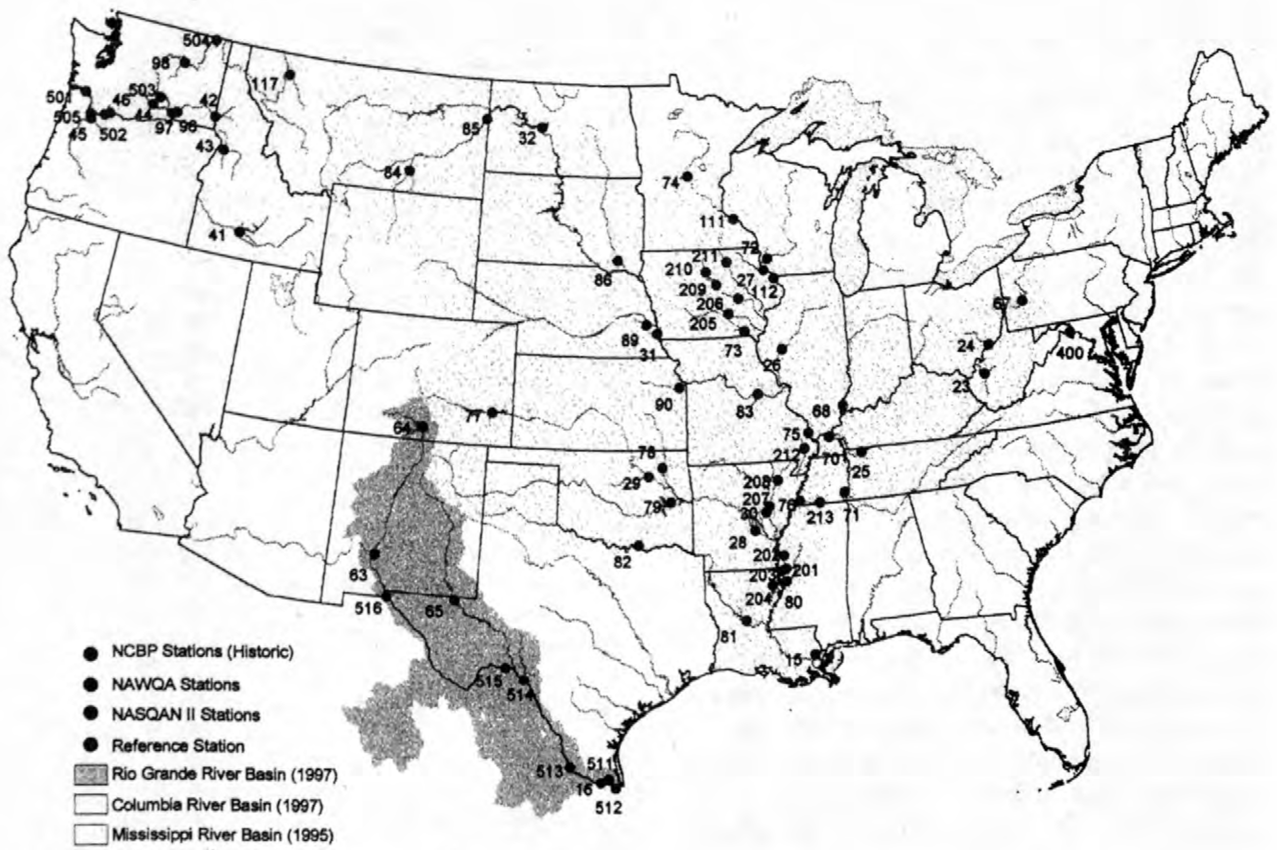
Large Rivers Pilot Project

The pilot project described here evolved from two primary needs: (1) to field test the suite of monitoring methods selected for use in the BEST program (NBS 1996); and (2) to update information on the distribution and concentrations of persistent toxic contaminants in large rivers. The latter need arose because the NCBP fish network was last sampled in its entirety in 1986, prior to the extensive flooding of 1993 and 1995 in the Midwest and South. To meet these combined needs, BEST program methods that could be performed on fish collected to meet the NCBP collection goals and requirements were incorporated into the project.

The NCBP fish network was designed to monitor spatial and temporal trends in the concentrations of bioaccumulable contaminants over large expanses of the U.S. The network was based on periodic sampling at "fixed" stations chosen to reflect the general concentrations of accumulative toxins in the environment. Fish were collected periodically from a national network of stations located at or near key points, such as at the confluences of major tributaries and below dams, in the larger rivers of the 50 states and in the open waters of the Great Lakes. The overriding assumption was that periodic collections and analyses of whole fish from key locales would integrate large expanses of time and space with respect to the concentrations of the measured contaminants. Results could then be generalized to taxa other than those collected and used to assess the risk to piscivorous fishes and wildlife represented by the contaminants measured in the fish.

During the period spanned by the NPMP-NCBP, the use of bioaccumulable pesticides and other contaminants in the U.S. was regulated, and concentrations in biota and other media declined (e.g., Schmitt et al. in press). Concomitantly, concerns about new pesticides and the cumulative effects of exposure to multiple contaminants and to other environmental stresses increased. Traditional contaminant monitoring programs, including the NCBP, relied almost exclusively on chemical analyses of selected environmental media. Although such programs provide useful information on the distribution and magnitude of the chemicals, they provide little insight regarding the effects of the measured contaminants on biota. In addition, chemical analyses alone provide no information about

Figure 1. Locations sampled in 1995 and 1997.



contaminants or their metabolites and degradation products that either do not persist or accumulate sufficiently to measure; for which there are no methods or analytical standards; or about the cumulative effects of multiple contaminants or contaminants and other environmental factors.

For the above-stated reasons, the BEST program incorporates a weight-of-evidence approach that includes bioassays and biomarkers (biological responses to contaminants at the sub-organism level) in addition to chemical analyses to monitor and assess the effects of contaminants on biota. The suite of methods, including the subset selected for evaluation in this project, was based on maximum sensitivity, cost-effectiveness, and the ability of the methods to detect a wide variety of contaminants and their effects in freshwater fish (NBS 1996). In this paper we present an overview of the large rivers pilot project and some preliminary findings.

Project Objectives and General Approach

The specific objectives of the project are to: (1) document the occurrence and distribution of contaminants and their effects on fish in the Mississippi, Columbia, and Rio Grande basins; (2) quantitatively evaluate the performance of aquatic methods used by the BEST program. (3) evaluate the logistic and administrative feasibility of implementing a large-scale monitoring program through partnerships with other bureaus, USGS divisions and programs, universities, and BRD research centers; and (4) evaluate opportunities for collaboration with the National Stream Quantity Accounting Network (NASQAN-II--Hooper et al. 1996) and National Water Quality Assessment (NAWQA--Hirsch et al. 1988) programs of the USGS-Water Resources Division.

METHODS OF STUDY

Sampling Locations

A total of 71 sites in the three basins were sampled, as was a reference site located at the BRD research center in Leetown, WV. (Figure 1, Table 1). The study sites included NCBP stations in each basin (34 of the 38 NCBP stations in the Mississippi River basin; 10 stations in the Columbia River basin; and four stations in the Rio Grande basin). In the Rio Grande and Columbia River basins, fish were also collected at 12 NASQAN-II sites (Bartish et al. 1997). The NASQAN-II program, which presently operates in four major U.S. river systems, collects water on a regular basis for analysis of hydrophilic pesticides, nutrients, and elemental contaminants (including metals). Sediments and additional water quality data are also collected at NASQAN II sites (Hooper et al. 1996). The NCBP and NASQAN II sites are typically located in large rivers, often at or near confluences or other key features such as dams. In the Mississippi River basin (Schmitt et al. 1995), fish were also collected in two NAWQA study units (13 sites)--four in the Eastern Iowa Basins Study Unit and nine in the Mississippi Embayment Study Unit (Figure 1, Table 1). The NAWQA sites are

Table 1. Locations sampled by the BEST program in 1995 and 1997. Station 400 was a reference site.

Sta. No.	River	Nearest city or feature	State
15	Mississippi	Luling	LA
23	Kanawha	Winfield	WV
24	Ohio	Marietta	OH
25	Cumberland	Clarksville	TN
26	Illinois	Beardstown	IL
27	Mississippi	Guttenburg	IA
28	Arkansas	Pine Bluff	AR
29	Arkansas	Keystone Reservoir	OK
30	White	Devall's Bluff	AR
31	Missouri	Nebraska City	NE
32	Missouri	Garrison Dam	ND
33	Missouri	Great Falls	MT
67	Allegheny	Natrona	PA
68	Wabash	New Harmony	IN
69	Ohio	Cincinnati	OH
70	Ohio	Metropolis	IL
71	Tennessee	Savannah	TN
72	Wisconsin	Woodman	WI
73	DesMoines	Keosauqua	IA
74	Mississippi	Little Falls	MN
75	Mississippi	Cape Girardeau	MO
76	Mississippi	Memphis	TN
77	Arkansas	John Martin Res.	CO
78	Verdigris	Oologah	OK
79	Canadian	Eufaula	OK
80	Yazoo	Redwood	MS
81	Red	Alexandria	LA
82	Red	Lake Texoma	TX
83	Missouri	Hermann	MO
84	Big Horn	Hardin	MT
85	Yellowstone	Sidney	MT
86	James	Olivet	SD
89	Platte	Louisville	NE
90	Kansas	Bonner Springs	KS
111	Mississippi	Lake City	MN
112	Mississippi	Dubuque	IA
201	Big Sunflower	Anguilla	MS
202	Bogue Phalia	Leland	MS
203	Steele Bayou	Rolling Fork	MS
204	Tensas	Tendal	LA
205	S. Skunk	Oskaloosa	IA
206	Iowa	Morengo	IA
207	Cache	Cotton Plant	AR
208	Cache	Egypt	AR
209	S. Fork Iowa	New Providence	IA
210	Iowa	Rowan	IA
211	Cedar	St. Charles City	IA
212	Lt. River Ditch	Moorehouse	MO
213	Wolf	LaGrange	TN
16	Rio Grande	Mission	TX
63	Rio Grande	Elephant Butte Res.	NM
64	Rio Grande	Alamosa	CO
65	Pecos	Red Bluff Lake	TX
511	Rio Grande	Harlingen	TX
512	Rio Grande	Brownsville	TX
513	Rio Grande	Falcon Dam	TX
514	Rio Grande	Amistad Res.	TX
515	Rio Grande	Langtry	TX
516	Rio Grande	El Paso	TX
41	Snake	Hagerman	ID
42	Snake	Lewiston	ID
43	Salmon	Riggins	ID
44	Yakima	Granger	WA
45	Willamette	Oregon City	OR
46	Columbia	Cascade Locks	OR
96	Snake	Ice Harbor Dam	WA
97	Columbia	Pasco	WA
98	Columbia	Grand Coulee	WA
117	Flathead	Creston	MT
501	Columbia	B. A. Terminal	OR
502	Columbia	Warrendale	OR
503	Columbia	Vernita Bridge	WA
504	Columbia	Northport	WA
505	Willamette	Portland	OR
506	Columbia	Vancouver	WA
400	Leetown Res.	Leetown	WV

typically located in lower-order streams; as such, their inclusion allows for an examination of the issue of scale relative to biomonitoring results. Extensive additional information on land use, land cover, and the condition of the aquatic community is also collected at NAWQA sites.

Specific Methods Included in the Pilot Study

The methods selected for the pilot were those BEST program methods for aquatic habitats (NBS 1996) that could be applied to fish (Table 2). The fish carcasses were analyzed for elemental and organic chemical contaimnants following NCBP protocol (Schmitt et al. in press). Extracts of the carcass samples were tested with the H4IIE rat hepatoma cell bioassay, a sensitive *in vitro* method for detecting and quantifying planar halogenated hydrocabons such as the polychlorinated dioxins and biphenyls (Tillitt et al. 1991). Liver tissue was assayed for ethoxyresorufin-O-deethylase (EROD) activity, an indicator of exposure to polycyclic and polyhalogenated hydrocarbons (Pohl and Fouts 1990). Fish were examined and scored using a quantitative health assessment based on the methods of Goede (1989), Adams (1990), and Adams et al. (1993). Selected tissues and organs were also examined histopathologically for evidence of parasites, tumors, and other lesions potentially indicative of chemical exposure (Hinton et al. 1992).

Several markers of endocrine modulation or disruption in fish that had been identified as promising by the BEST program (NBS 1996) and which were being field tested by the NAWQA program (Goodbred et al. 1994; 1996) were also incorporated into the suite of fish health indicators. These included plasma concentrations of reproductive hormones (Guillette et al. 1994) and the protein vitellogenin, an egg yolk precursor (Folmar et al 1996). In addition, the composite fish carcass samples were analyzed for stable isotopes of nitrogen ($\delta^{15}N$), a potential indicator of trophic position and nitrogen source (Cabana and Rasmussen 1996), as a corollary variable and tracer of sewage inputs.

Many contaminants are also known or suspected to suppress immune system function in animals (e.g., Matthews et al. 1990; Hutchinson and Simmonds 1994), and immune system indicators were consequently among the methods included (NBS 1996) The immune system indicators are splenic macrophage aggregates and plaxma lysozyme activity (Blazer et al. 1994).

Field Procedures

Sampling was conducted in the fall of 1995 in the Mississippi River basin and in the fall of 1997 in the Columbia and Rio Grande basins. The preferred collection method was D.C. electrofishing from a boat. Seining or hook-and-line capture was also permitted. In keeping with NCBP protocol, one piscivorous and one bottom-dwelling species was sought at each site. The primary species targeted at each site were common carp (*Cyprinus carpio*) and largemouth bass (*Micropterus salmoides*) as the bottom-dweller and piscivore, respectively. Other fishes, mostly other black basses (*Micropterus* spp.), percids (*Stizostedion* spp.), salmonids, suckers (Catostomidae), and catfishes (Ictaluridae), were collected as alternates depending on habitat and location. These fishes were selected because the responses of the biological markers have been documented in them and because they were expected to be the most widespread in their distributions. In addition, these taxa had been collected historically at many NCBP sites (Schmitt et al. in press) and are on the list of NAWQA target taxa (Crawford et al. xx). At NCBP, NASQAN-II, and reference sites, the collection target was 40 fish (10 of each sex of each of two species). At the NAWQA sites, only one species was normally collected (common carp at 11 sites, largemouth bass at one site, and both species at one site).

Following capture, fish were held alive until processed for sample collection (generally < 4 h). Fish were weighed and measured, and samples of blood and liver (the latter cryogenically frozen for the EROD assay, preserved for histopathology); and of gill, kidney, spleen, and gonads (preserved for histopathology) were collected. Weights of the liver (most species), spleen, and gondads were also determined as part of the necropsy-based health assessment. Plasma was obtained from the blood samples by centrifugation and cryogenically frozen for analysis of lysozyme activity, sex steroid hormone concentrations (i.e., testosterone, 11-ketotestosterone, and 17β-estradiol), and vitellogenin. Individual fish were then composited by species and gender for chemical extraction and analysis, including the H4IIE bioassay. Details of the laboratory procedures are given elsewhere (see references listed in Table 2).

Table 2. Methods incorporated into the large rivers pilot project.

Method	Description	Tissue(s) examined	Sensitivity	Primary reference(s)
Histopathology	Microscopic examination for the presence of lesions; can provide early indication of chemical exposure	Liver, gill, gonads, spleen, and kidney	Overall organism health and contaminants	Hinton et al. (1992)
Ethoxyresorufin - O-deethylase (EROD) activity	Enzyme induction by planar hydrocarbons	Liver	PCBs, PAHs, dioxins, and furans	Pohl and Fouts (1980); Kennedy and Jones (1994)
Lysozyme activity	A disease resistance factor that can be suppressed in the presence of contaminants	Blood plasma	Overall organism health	Blazer et al. (1994)
Macrophage aggregate analysis	Macrophages are important in the immune system, serving as a first line of defense for the organism and as an antigen processing cell	Spleen, hemopoetic kidney, and liver	Multiple contaminants including PAHs and metals	Blazer et al. (1994)
H4IIE bioassay	A screening tool to determine the presence of certain classes of planar halogenated compounds	Whole fish (composites)	PCBs, dioxins and furans; and PAHs	Tillitt et al. (1991)
Vitellogenin	A precursor of egg yolk, normally synthesized in the liver of female fish	Blood plasma	Endocrine modulating compounds	Folmar et al. (1996)
Chemical analyses	Organochlorine chemical residues and elemental contaminants	Whole fish (composites)	Specific analytes	Schmitt et al. (in press)
Somatic indices	The relative mass of some organs is often indicative of chemical exposure	Gonads, spleen, liver	Overall organism health	Grady et al. (1993)
Stable N isotopes (^{14}N and ^{15}N)	The ratio of ^{15}N to ^{14}N (δ^{15}N) increases with trophic position and sewage pollution	Whole fish (composites)	Trophic position, nitrogen sources	Cabana and Rassmussen (1996)
Necropsy-based fish health assessment	Visual assessment of external/internal anomalies (e.g., lesions, parasites, tumors), which may indicate contaminant-related stress	All	Overall organism health	Goede (1996); Adams et al. (1993)

PRELIMINARY RESULTS

A total of 2,323 fish were collected in the three basins during 1995 and 1997. Of these, 72% were common carp and largemouth bass. Common carp were collected at 63 sites and largemouth bass at 35 sites. The predominant alternative species in the Mississippi River basin were smallmouth bass (*Micropterus* *dolomieu*), spotted bass (*M. punctulatus*), goldeye (*Amphiodon alosoides*) and sauger (*Stizostedion canadense*). In the Columbia River basin the predominant alternative species were largescale sucker (*Catostomus macrocheilus*), smallmouth bass and northern squawfish (*Ptychocheilus oregonensis*), and in the Rio Grande basin they were channel catfish (*Ictalurus punctatus*), and white bass (*Morone chrysops*).

Concentrations of organochlorine chemicals at NCBP sites in the Mississippi River basin were generally lower in 1995 than when last sampled in 1986 (Schmitt et al. in press); almost no unmetabolized *p,p*'-DDT (the parent insecticide) was present, and concentrations of many pesticides were below detection levels. DDT-derived residues (mostly *p,p'*-DDE) in fish from historic use on cotton remained evident in the southern parts of the watershed; concentrations were highest at the NAWQA sites in the Mississippi Embayment Study Unit. Concentrations of toxaphene, which was also used extensively and almost exclusively on cotton, followed a similar pattern. Total DDT concentrations in fish from these sites, although much lower than historically reported values, nevertheless remained sufficiently high to represent a hazard to sensitive species of fish-eating birds (Anderson 1975; USEPA 1980). Because toxaphene is a complex mixture of chemicals that weather at different rates, it is not possible to assess the ecological risk of weathered mixtures based on total concentrations.

Point sources of contaminants (i.e. pesticide manufacturing or formulating facilities) remained evident at some large river NCBP sites in the Mississippi basin; conversely, concentrations of some agricultural chemicals were higher at NAWQA sites, which are typically located on lower order streams. As noted above, concentrations of toxaphene and DDT (as *p,p*'-DDT) remained evident at many sites in the south, especially in the Mississippi Embayment Study Unit. Cyclodiene pesticides (dieldrin, endrin, and chlordanes, including heptachlor epoxide) were present in many agricultural areas, especially in the Corn Belt; concentrations were elevated relative to other areas of the Mississippi River system in the Eastern Iowa Basins Study Unit. These pesticides were also present in high concentrations in fish from the Mississippi River at Memphis, Tennessee, where there is a history of cyclodiene pesticide contamination from a site of synthesis (Schmitt et al. 1990; in press). Concentrations of cyclodiene pesticides approached levels of concern for fish-eating wildlife at Memphis (Eisler 1990).

In contrast to concentrations of organochlorine chemicals, which generally decreased since the NCBP stations were last sampled in 1986, concentrations of mercury increased at some sites in the Mississippi River basin. As expected, mercury concentrations were higher in the predator species (e.g., largemouth and smallmouth bass, walleye) than in bottom fish (common carp, suckers). Greatest concentrations were detected in largemouth bass from the Mississippi River at Memphis. Opinions on the toxicity of mercury to fish-eating wildlife vary; according to some (Yeardley et al. 1998), concentrations at some sites exceeded toxicity thresholds.

Selenium concentrations in the Mississippi basin were generally low except for one site on the upper Arkansas River (John Martin Reservoir). At that site, concentrations in common carp were sufficiently high to constitute a risk to the fish themselves as well as to fish-eating wildlife (Lemly 1996). Selenium concentrations in common carp from John Martin Reservoir have been increasing for approximately 10 y (Schmitt et al. in press).

Greatest dioxin-like activity, as measured by the H4IIE bioassay, occurred in fish from NAWQA sites in the Mississippi Embayment Study Unit. Combined results of organochlorine pesticide analyses and those from the H4IIE bioassay and EROD measurement suggest the presence of other organic contaminants in the lower Mississippi River valley.

Histopathological analysis revealed the presence of ovarian cells (oocytes) in male bass from five sites. At one site (Mississippi River at Lake Pepin, MN), oocytes were present in 8 of the 11 male smallmouth bass examined. Vitellogenin was present in the serum of male largemouth bass from at least 9 sites. Seemingly abnormal ratios of sex steroid hormones were also found in common carp and bass from several sites. Collectively, these findings suggest that fish from some sites are exposed to endocrine-modulating substances.

RELATED ACTIVITIES OF THE BEST PROGRAM

In addition to the large rivers pilot study described here, the BEST program is conducting and supporting a number of related activities. These include in-depth reviews and syntheses of extant information on the biomarkers being evaluated in this project alsong with the development of supporting data bases and bibliographies. Additional projects include: (1) An assessment of contaminant information from Atlantic Coast estuaries to determine suitable avian wildlife species for environmental monitoring. (2) Examination of fish and wildife mortality incidence reports as a means for identifying new contaminant problem areas. (3) Providing technical assistance to the FWS in the conduct of standardized, comprehensive assessments of contaminant threats to National Wildlife Refuges. And (4) Conducting pilot projects with the National Park Service to further evaluate and refine the contaminant assessment process developed for FWS refuges.

Acknowledgments

Most of the field portions of this study were conducted by FWS Environmental Contaminants Specialists duty-stationed at Ecological Services Field Offices throughout the Mississippi River, Columbia River, and Rio Grande basins, and by NAWQA biologist at the WRD District offices in Mississippi, Illinois, and Texas. T.J. Kubiak of the FWS was a co-investigator in the 1995 portion of the study and coordinated much of the early FWS support. M. Wilson and S. Zylstra of the FWS were co-investigators and coordinated 1997 field activities. N.D. Denslow, of the University of Florida-Gainesville, supervised the vitellogenin analyses.

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A Model Fish System to Test Chemical Effects on Sexual Differentiation and Development

By Diana M. Papoulias, Douglas B. Noltie, and Donald E. Tillitt

ABSTRACT

A model system was characterized which may be used as an in vivo screen for effects of chemicals or environmental mixtures on sexual differentiation and development of reproductive organs and gametes. We evaluated the effects of a model environmental estrogen, ethinyl estradiol (EE_2), on the d-rR strain of medaka, *Oryzias latipes,* using a nano-injection exposure. Gonad histopathology indicated that a single injection of 0.5-2.5 ng EE_2/egg can cause phenotypic sex-reversal of genetic males to females. Sex-reversals could be detected as early as seven days post-hatch. Sex-reversed males had female-typical duct development and the secondary sex characteristics we measured were generally consistent with phenotype, with the exception of a few EE_2-exposed XX and XY females which possessed ambiguous anal fins. Using discriminant analysis, we determined that the presence or absence of the secondary sex characteristic, a dorsal fin notch, was a very reliable indicator of gonadal sex. No instances of gonadal intersexes were observed. Ethinyl estradiol also appeared to reduce growth but not condition (weight at a given length) and exposed XX females appeared to have a higher incidence of atretic follicles relative to controls. Our results suggest that estrogenic chemicals may influence sexual differentiation and development and that the medaka model is well suited to assessing these effects. (The complete manuscript of this work was submitted to the journal Aquatic Toxicology, December, 1998).

INTRODUCTION

This study was part of an on-going characterization of the d-rR medaka in which we are evaluating the effects of known estrogenic, androgenic, anti-estrogenic, and anti-androgenic chemicals on sexual differentiation and development.

The recent focus on endocrine disrupting chemicals (EDC) has led to extensive efforts to develop screening assays that detect exposure or demonstrate effects of this group of chemicals on wildlife species (Ankley and others, 1998). However, to date, no test has been accepted that screens chemicals for effects on the primary germ cells (PGC) with subsequent alteration of sexual differentiation of reproductive organs and gametes. Such information is particularly important for those species (fishes, amphibians, birds) for which chemically- or environmentally-induced sex-reversal has been documented.

The d-rR strain of medaka, *Oryzias latipes*, is an ideal organism with which to observe the effects of chemicals on sexual development and differentiation. The males of this strain possess a sex-linked color gene for xanthathin deposition whereas the females are deficient in this gene (Hyodo-Taguchi and Egami, 1989). The orange-red males and white females produced can be distinguished as early as two days post-hatch.

Based on extensive study of the effects of natural and synthetic steroids on fish sexual development, Yamamoto (1962) concluded that sexual differentiation requires that the PGCs be directed towards spermatogenesis or oogenesis by exposure to an endogenous inductor substance, possibly an androgenic or estrogenic hormone, during a particular period in development. Therefore, EDCs which are transferred to the oocyte during gametogenesis, and are available to the developing embryo prior to hatch or sexual differentiation, can pose a risk to the embryo.

In this model, embryos of the d-rR strain of medaka were exposed to a model EDC by nano-injection. Directly exposing the embryos closely simulates maternal transfer of contaminants and is applicable for testing those EDCs which bioaccumulate and are lipophilic.

The objectives of this report are to characterize the response of the d-rR medaka to a single exposure of ethinyl estradiol (EE_2), a model environmental estrogen which is found in sewage effluents. Effects on sexual differentiation are assessed by comparing genetic sex to phenotypic sex of newly-hatched and adult fish. Effects on sexual development are assessed through evaluation of the gonadosomatic index, gonad and gonadal duct histopathology, and secondary sex characteristics.

METHODS

Test Organism

The d-rR strain of medaka was a generous gift of Dr. Akihiro Shima, University of Tokyo, Japan. In this strain, either (r) or (R) genes are carried by the X or Y chromosome, respectively. Crossovers or genetic imbalances occur at a rate of about 0.005-0.5%, making color a reliable marker of genetic sex (Hishida, 1965). In addition to the genetic marker, there is distinct sexual dimorphism in the medaka. Males are typified by a dorsal fin notch and a convex anal fin with a serrated margin. Females lack the dorsal notch and have a more concave anal fin with a smooth margin.

Preparation

Eggs were collected in the morning soon after fertilization the day before exposure. Embryos were embedded in a solid 1% agarose matrix which had been set-up in square 36-grid petri dishes. The agarose matrix provided the necessary stability for injecting the embryos. The dish was then filled with well water and stored in a cool place (~20°C) to slow embryo development until injection (prior to completion of the gastrula stage).

Embryo Injection

Embryos were exposed following a slightly modified nano-injection procedure described by Walker and others (1996). Immediately prior to injection, dead embryos or embryos that had completed epiboly were replaced and the well water covering the embryos was exchanged for a sterile saline solution. Aluminosilicate capillary tubes were pulled on a pipette puller to make needles with a 5-10 μ OD tip when beveled at a 15°. Needles were filled either using a vacuum or a pipettor. Triolein was used as the carrier solvent and was filter-sterilized before use. Carrier solvent, steroid working solutions, and filled needles were stored at 4°C under nitrogen. Eggs were injected with five concentrations (0, 0.005, 0.05, 0.5, 2.5, or 5.0 ng/egg) of EE_2 dissolved in the carrier triolein or triolein alone. Injections of material were made directly into the oil globule using a picoinjector and micro-manipulator. The injected embryos were left in the agarose at room temperature until the following day.

Embryo Incubation and Larval Growout

Approximately 24 h after injection, the embryos were inspected for mortalities; survivors were maintained in 27°C well water in 150-ml glass side-arm test tubes. Embryos were rolled in the water during incubation by a gentle stream of air. Upon hatch, the medaka larvae were held in flow-through containers suspended in stainless steel raceways supplied with a constant flow of well-water until they reached maturity.

Fish Characterization and Procedures

Fish were identified as genetic males (XY, orange-red) or genetic females (XX, white), and then euthanized at 5 mm total length (TL) or as adults. Morphological observations and measurements were made on a total of 145 adult fish averaging 30±2 mm TL and 0.26± 0.06 g body weight (WT). Larvae, whole adults, and mature gonads were preserved in Bouin's solution. Fish of 5 mm TL were used to assess the volume of the developing gonad and the number of PGCs. In medaka, as for many teleosts, gametogenesis (germ cell division and differentiation) in females begins weeks before that in males, making PGC number and gonad volume useful indicators of male or female phenotypic sex. Adults were measured (TL) and weighed (blotted dry), and were used to assess growth, gonadosomatic index (GSI), sex-reversal, gonad and gonadal duct histopathology, and development of secondary sex characteristics.

Volume of the early gonad was calculated using image analysis (Optimas®) by digitizing the area of serial cross-sections of the gonad of the 5 mm larvae, and computing a volume. The number of PGCs in each cross-section were also recorded by enumerating observable PGC nuclei. Histological analysis followed standard procedures for processing, dewaxing, hydration, and dehydration. All sections were cut at 7µ and stained using a modified Masson trichrome stain.

RESULTS

Growth and Survival

Survival from embryo to adult was greater than 60% for all but the 5.0 ng EE_2/egg dose. Only a 2% survival was observed at 5.0 ng EE_2/egg.

A general linear models analysis of the TL and body WT data indicated that neither weight nor length differed significantly within treatment group among adult males, females, and sex-reversed males ($p>0.09$). However, males tended to be heavier than females at all doses, whereas sex-reversed males were intermediate between these; both TL and body WT correlated negatively and significantly with exposure to EE_2 for males, females, and sex-reversed males.

Gonad Size

Nearly all the female fish examined were in the pre- to early vitellogenic stage (Iwamatsu and others, 1988). The GSI for XX females was approximately ten times that of XY males but was the same as that for XY females ($p<0.0001$; Figure 1). The uninjected and triolein controls did not differ. While there was no significant effect of EE_2 on GSI of those fish that were not sex-reversed ($p>0.05$), ovarian weights correlated negatively and significantly with increasing dose of EE_2.

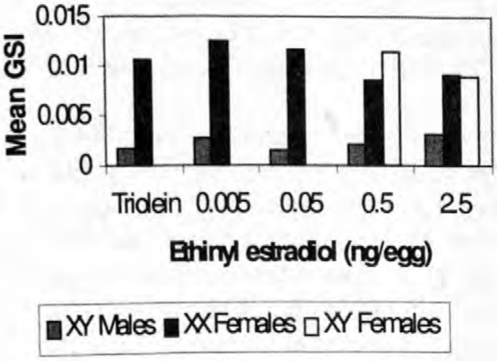

Figure 1. Gonadosomatic index (GSI; gonad weight/(body weight less gonad weight)) for XY males, XX females, and XY females (mean ± SD). The GSI for XX females was approximately ten times that of XY males, but was the same as that for XY females ($p<0.0001$). There was no significant effect of EE_2 on GSI of those fish that were not sex-reversed ($p>0.05$).

Gonad and Duct Histopathology

Histological examination of the gonads of the adults indicated that an injection of 0.5 or 2.5 ng EE_2/egg could cause complete sex-reversal in genetic males. The most effective concentration for sex reversal was 2.5 ng/egg. One anomalous sex-reversed female was observed at the 0.005 ng/egg concentration. No intersexes were observed. Degenerating or atretic oocytes were observed in almost all the ovaries of the 0.5 and 2.5 ng/egg dosed fish; few were present in the two lowest doses, and nearly none were observed in the triolein or uninjected controls. Individuals at the highest dosages of EE_2 typically exhibited very small ovaries bearing a few late vitellogenic or mature oocytes, the remainder being previtellogenic. Both XX and XY ovaries displayed the described characteristics.

In contrast, the morphology of the testes appeared normal in nearly all specimens, with most having sperm in the efferent ducts. However, of six XY males treated with 0.5 ng EE_2/egg only two individuals had testes resembling the controls, with all stages of germ cells present and sperm in the ducts. The remaining individuals had very few or no sperm in their efferent ducts. One individual receiving this dosage appeared sterile, its testis was devoid of germ cells. The testes of individuals exposed to 0.05 ng EE_2/egg generally appeared less mature (most germ cells at early stages of spermatogenesis) and with less sperm in their ducts than did those of the lowest dose or control groups.

Sex-reversed males had typical female ducts, with separate openings for the genital and urinary tracts. Ethinyl estradiol-exposed males which were not sex-reversed possessed normal male morphologies, with the fusion of the urinary and gonadal ducts within the body cavity occurring just proximal to forming a single urogenital opening. Ducts in all but the sex-reversed fish seemed unaffected by the EE_2 exposure.

Secondary Sex Characteristics

Our objective was to discriminate between genetic males and females in order to identify sex-reversals by their misclassification according to several phenotypic attributes. We conducted a stepwise discriminant analysis based on an overall sample of 145 fish described by 9 attributes (anal fin convexity, anal fin serration, dorsal fin notch, abdominal distention, all scored presence/absence; urogenital papilla [UGP] size, scored incrementally from 1 to 4; anal fin length, anal fin width, TL, all measured in mm; body WT measured in grams). This analysis identified the 11 sex-reversals that occurred. The phenotypic attributes which together made significant contributions to the classification included dorsal fin notch, abdominal distention, and TL.

Our attempt to identify which of the external attributes were of most use in predicting the correct genetic sex also proved successful. A repeat of the analysis above, using phenotypic sex as the correct group designator, correctly assigned our fish to functional gender using only a single variable, dorsal fin notch The remaining attributes, while informative, were insufficiently diagnostic. A serrated and convex anal fin was generally consistent with male phenotype although some feminized males and sex-stable females displayed them. Sex-stable males bore longer and wider anal fins than did fish which were either genotypically or phenotypically females. Anal fin growth in length and width appeared to be retarded in feminized males such that overall shape was more similar to XX females. The UGP of sex-reversed males were more female than male-like with respect to size. Whether the abdomen was distended or not was dependent on the stage of vitellogenesis in females. Except for the vitellogenic females, abdomen for both male and female phenotypes was not distended.

Primary Germ Cells

Only data for 2.5 ng EE_2/egg dose were assessed since the greatest percent of sex-reversals occurred at this dose. As expected, gonadal volumes and number of PGCs were greater in uninjected or triolein control females than control males, indicating that sexual differentiation had occurred in the 5 mm larvae (Table 1).

Table 1. Mean volume of gonads and number of PGCs in medaka larvae injected as embryos with ethinyl estradiol (n=5). Treatments sharing a letter are not significantly different (*p*>0.05).

	Genetic ♀ (XX)		Genetic ♂ (XY)	
Dose ng/egg)	Vol. (x10^{-5}) mm^3±SD	Num. PGCs± SD	Vol. (x10^{-5}) mm^3±SD	Num. PGCs± SD
Con.	17.2 ± 3^{a}	212±49d	$5.3 \pm .3^{c}$	40 ± 4^{f}
Triolein	12.6 ± 3.6^{ab}	129±29e	4.5 ± 1.6^{c}	55 ± 28^{f}
2.5	10.7 ± 2.4^{b}	97±19e	12.6 ± 4.2^{ab}	132 ± 27^{e}

Gonad volumes and PGC numbers for EE_2-exposed females were also larger than those for control males and not significantly different from values for triolein-exposed females. Males exposed to 2.5 ng EE_2/egg had gonad volumes and PGC numbers similar to both triolein-control and EE_2-exposed females, indicating that sex-reversal had occurred in these exposed males.

DISCUSSION

Our studies demonstrate that a single injection of EE$_2$ into medaka embryos prior to the period of sexual differentiation can result in feminization of males. To our knowledge, the only estrogens which have been injected into fish embryos are estrone (Gresik and Hamilton, 1977) and EE$_2$ (this study).

We demonstrated that EE$_2$-induced sex-reversal coincides with sexual differentiation and can be identified as early as a few days post-hatch. In medaka, as in many teleosts, female and male phenotypes are first differentiated based on the activity of their PGCs within 24 h after hatch. Those PGCs which enter mitosis first are identified as oogonia, while they are refered to as spermatogonia in those larvae in which no division is taking place. Up until this point of differentiation, the male and female PGCs are morphologically the same and are considered to be bipotent with respect to their fate as future sperm or ova (Reinboth, 1982). Our observations of gonad size and PGC number indicate that sex-reversal has already occurred by the time the medaka larvae reached 5 mm in length (about six days post-hatch). The injected EE$_2$ was present for at least eight days before and four days after hatch, at which time the yolk and oil droplet were completely absorbed.

The relative potencies of EE$_2$ compared with other estrogens in effecting sex-reversal, either by nano-injection or via the diet, corresponds with their respective binding affinities for the estrogen receptor. This suggests that sex-reversal is induced through the estrogen receptor.

Given that only a single white (XX) male was found in the lowest treatment, we believe that this individual was more likely to have been one of the normal exceptions to the white female (X^rX^r) and orange-red male (X^rY^R) color pattern which is expected to occur at low frequencies in the d-rR strain population.

It should be noted that in our study, the gonads of treated fish displayed varying degrees of pathology. Atretic oocytes were much more common in the ovaries of XX and XY EE$_2$-treated fish. Atresia is believed to be uncommon in physiologically healthy females (Wallace and Selman, 1981). Factors such as environmental stress, poor nutrition, or stressors that could decrease gonadotropin levels (e.g. anti-gonadotropic substances), are believed to be the major causes leading to atresia (Saidapur, 1978). The high incidence of atresia we observed in EE$_2$-exposed XX and XY females suggests that embryonic exposure to EE$_2$ may have disrupted the development of gonadotropin-producing cells, or in some other way impaired the release or uptake of gonadotropins.

Development of male or female secondary sex characteristics is influenced by the type of gonad present. Generally, these characteristics are male-positive, in that testosterone production by the gonad results in dimorphisms between males and females (Okada and Yamashita, 1944). Estrogen does not induce development of secondary sexual characteristics, except for the UGP. Thus, a sex-reversed fish would be expected to display secondary sexual characteristics which would match its gonadal phenotype and not its genotype. Although the secondary sex characteristics we measured (size and shape of anal fin, dorsal fin notch, UGP, and size of abdomen) generally followed the phenotype of the gonad independent of the genetic sex, we also found the anal fin was slightly more ambiguous among a few individual XY females and EE$_2$-exposed XX females.

There were lethal and growth-related effects from exposure to EE$_2$ in addition to effects on sexual differentiation of the gonad and consequent development of secondary sex characteristics. Embryo mortality was 91% at the 5.0 ng EE$_2$/egg dose with only two females surviving. We found no reports of EE$_2$-induced fish mortality in the literature. However, E$_2$, estrone, and estriol are all reportedly toxic when administered via diet at high concentrations (Yamamoto, 1961, 1965; Yamamoto and Matsuda, 1963). Control XX females tended to be less heavy (although not significantly so) than XY males and the males, which were sex-reversed were smaller than the XY males but larger than XX females. It also appears that EE$_2$ had a suppressive effect on growth, but not condition (the TL-body WT relationship). The length and weight of medaka exposed, from hatch, to estrone in their food showed a similar negative influence, and unexposed control males were larger than XX females (Fineman and others, 1974a 1974b).

However, since the duration, timing of exposure, and route of administration of steroid were quite different in the latter experiments relative to our studies, direct comparisons must be made cautiously.

CONCLUSIONS

Our results suggest that estrogenic chemicals may influence sexual development. However, the normal process of sexual differentiation in fishes is not yet completely understood. Thus, the specific mechanism through which exposure to exogenous steroids during the period of sexual differentiation may result in sex-reversal or impaired sexual development is unknown. The medaka model presented here is well suited to testing chemicals for their effects on sexual differentiation and development. The same endpoints could be evaluated with water bath exposures of embryos, alone or in combination with egg injection, to further simulate exposure scenarios for both bioaccumulative and water soluble chemicals. Our studies have demonstrated the benefit of using the d-rR strain for testing for effects of chemicals on sexual differentiation. Utilizing the sex-linked color gene marker and gender-specific characteristics of the PGCs allows detection of an effect as early as two weeks after exposure. Furthermore, growing the fish to maturity (approximately three months) allows the evaluation of additional endpoints for assessment of effects on sexual development.

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The Potential for Contaminated Ground Water to Adversely Affect Chinook Salmon (*Oncorhynchus tshawystcha*) under Exposure Conditions Simulating the Hanford Reach of the Columbia River, Washington, USA

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ABSTRACT

The Hanford Reach of the Columbia River is a free flowing stretch that exists within the Hanford Nuclear Reservation in south central Washington, USA. Operations at the reservation have resulted in contamination of ground water with chromium and other chemical and radiological constituents. Ground water discharges into the Columbia River occur in areas where chinook salmon (*Oncorhynchus tshawystcha*) frequently spawn and may influence fertilization, egg survival, and health of alevins and parr. This paper describes the problem and outlines the framework for research designed to determine the potential for chromium to adversely affect chinook salmon. This research is to be performed by the Biological Resources Division from October 1998 to December 1999.

INTRODUCTION

The Hanford Nuclear Reservation in south central Washington is a 900 square kilometer area claimed by the federal government in 1943 as a site for the production of plutonium (Figure 1)(Geist, 1995). The location was ideal because it was remote, sparsely populated, and most importantly, had a readily available supply of cold water from the Columbia River. Because of national security concerns, public access and river development projects were restricted until 1971 (Dauble and Watson, 1997). Extensive dam building and development occurred throughout the Columbia River Basin from 1943 to 1971 and led to severely reduced populations of chinook salmon (*Ocorhynchus tshawystcha*). The 90 km section within the Hanford Reservation was not developed, and today, the Hanford Reach remains a free flowing stretch of the Columbia River and is the only remaining area where significant mainstem spawning occurs in the Columbia River (Dauble and Watson, 1990). The Hanford Reach of the Columbia River is regulated by upstream dams, but is the last unimpounded stretch of the mainstem Columbia River.

Large quantities of Columbia River water were used to cool nuclear reactors and cooling water was treated with sodium dichromate to prevent corrosion and mineral collection within the pipes (Peterson and others,1996). During operations, cooling water with associated radionuclides and chromium were discharged directly to the river and also entered ground water through leakage of pipes and seepage from retention areas. Today, groundwater at the Handford site continues to be contaminated with chemical and radiological constituents (Geist and others, 1994). The hydraulic head of the ground water aquifers in the 100 Area (National Priority

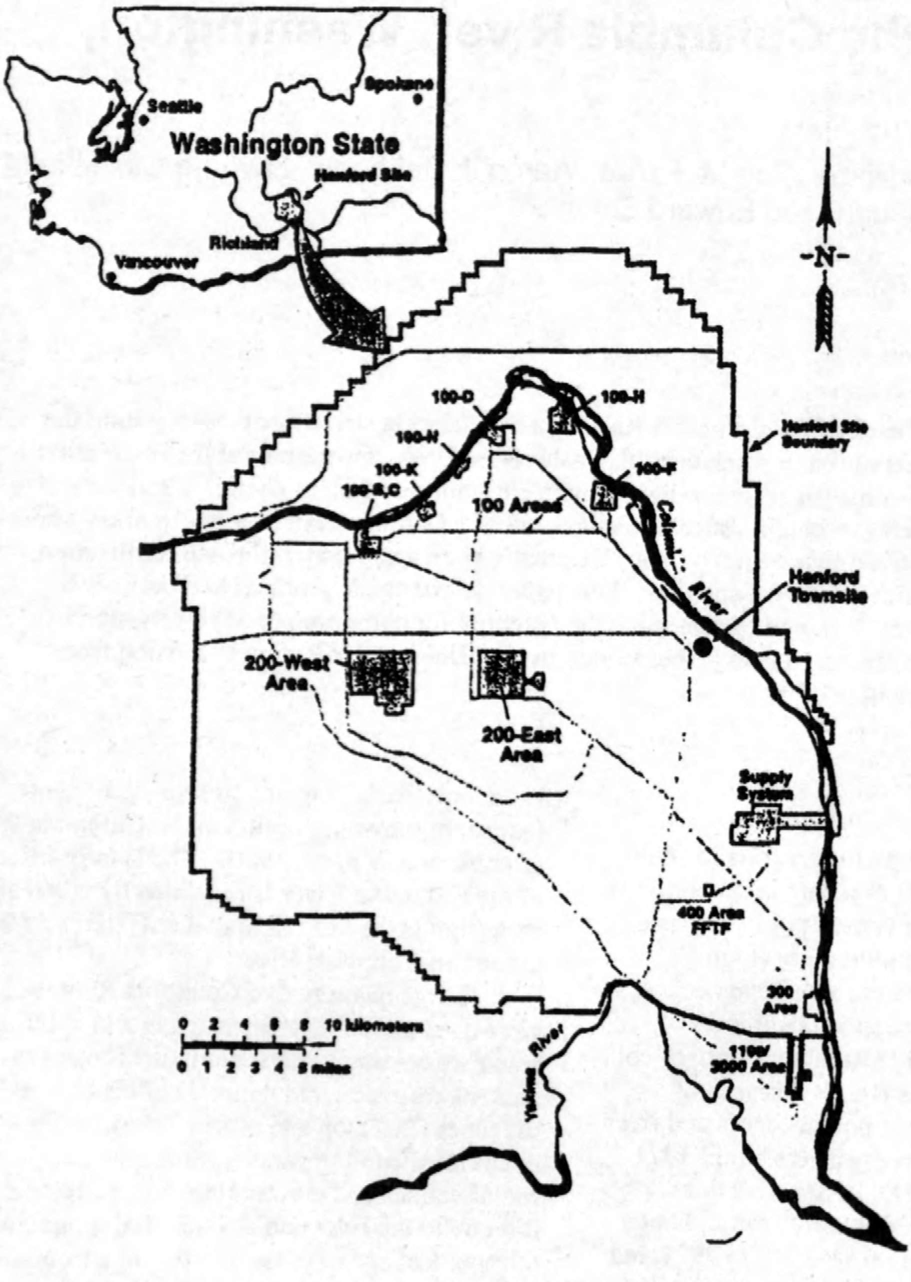

Figure 1. Map of the Hanford Reach of the Columbia River.

List Site) are higher in elevation than that of the Columbia River and results in discharge from the aquifer into the Columbia River through shoreline springs and seeps (Figure 1). The ground water is hydraulically connected to the river with peak aquifer discharges occurring during low river flows (fall and winter) and minimum aquifer discharges occurring during high river flows (spring and summer) (Geist and others, 1994).

The use of the Hanford Reach for fall chinook spawning and rearing has dramatically increased since 1960 (Becker, 1985; Dauble and Watson, 1990). The 10 year average adult escapement increased from 27,660 (1964-1973) to 54,661 (1983-1992). This increase is pronounced when compared with the rest of the mid and upper Columbia River where chinook runs have declined during the same time period. Spawning occurs in close proximity to the 100 Area where contaminated ground water is entering the River. Adult chinook spawn in variable water depths, water velocities, and substrate types (Swan and others, 1988). Spawning in the Hanford Reach begins in mid-October, peaks in mid-November, and ends in late November (Dauble and Watson, 1997). Egg and fry development within the redds take place from mid-October to May during low river flows that result in peak aquifer discharges. Based on the mid-November peak redd abundance and ambient temperatures, eggs would become eyed in early December, hatch in late December, and alevins would emerge from the redds in late February. Upon emergence, fry move out of the main river channel into shallow, slow moving, near shore and backwater habitat (Dauble and Watson, 1990; Dauble and others 1989). Juveniles remain in the Hanford Reach from February to mid-July feeding on macroinvertebrates (Becker, 1973). Outmigrating begins in May and is usually completed by July at 5-7 months of age, 60-70mm in length, and 3-4gm in weight (Olson and Foster, 1956).

One contaminant of major concern, and associated with the 100 Area groundwater and seeps, is chromium. The concentrations of chromium in the groundwater upwellings (Hope and Peterson, 1996) exceed the chronic ambient water quality criteria (AWQC, 11 μg/L) for the protection of aquatic life, established by the U.S. Environmental Protection Agency (USEPA, 1986) and the State of Washington. The Department of Energy currently has activities underway to pump and treat chromium at the Hanford facility, and reduce the amounts of hexavalent chromium released into the Hanford Reach. However, the critical nature of the Hanford Reach as spawning habitat for the chinook salmon, makes it essential to determine if current water quality standards protect chinook salmon (Geist, 1997). In particular, additional information is needed to determine if the current standards protect early life stage survival and development. While some data do exist on the effects of hexavelent chromium on salmon (Olson and Foster, 1956, Buhl and Hamilton 1991), previous studies did not investigate the direct effects on fertilization, effects on alevin exposure only, recovery of exposed alevins, or physiological impairment.

The early life stages of chinook salmon are most likely to come in constant contact with elevated chromium and these stages have been shown to be the most sensitive to contaminants (McKim, 1977). Chromium may hamper fertilization success by directly acting on the fertilized egg to cause death of the embryo (Billard and Roubaud, 1985). Or chromium may react with the sperm and egg individually to impede fertilization. If fertilization is successful, chromium may affect the survival of early lifestages (Olson and Foster, 1956; Benoit, 1976). While it has been documented that elevated concentrations of chromium reduce survival (Buhl and Hamilton, 1991), and to a lesser extent, growth (Olson and Foster, 1956; Benoit, 1976), information has not been gathered on the relevance of recovery periods on these toxicological effects. In the Hanford Reach, chromium that moves from the ground water upwellings becomes diluted extensively. Thus, as young fry begin to emerge from the redds, they may no longer be exposed to elevated concentrations of chromium. The effects of chromium exposure to alevins, as monitored by post-exposure recovery of fry during later development, will mimic the exposure situation present in the Hanford Reach.

Chinook salmon will be present in the Hanford Reach for 5-7 months, and it is important to understand health effects as related to chromium exposure. It is unclear what the exposure concentration might be through contaminated surface water or diet, but long-term health effects from continuous exposure is not well understood in either early life or parr stages

(Geist and others, 1994). An understanding of the physiological responses (pathology) associated with chromium exposure can be used to supplement fish population or water and sediment monitoring. Evaluations based on the residue concentrations and physiological condition (e.g. increased lipid peroxidation) of fish integrate the actual exposure to pollutants (dose) and effects of these exposures on fish survival and growth (Farag and others, 1994; Farag and others, 1995). Further associations of tissue chromium accumulation, oxidative stress, and growth reduction would add more weight to a determination of fish health impairment. This weight of evidence approach uses all of the information gathered to determine the health status of a fish population.

We will meet two objectives by investigating the health status of salmon exposed to chromium during the early and parr stages. First, the effects of chromium on survival and growth of chinook salmon will be explained in terms of the health status of individual fish. Additionally, tools can be developed in the laboratory under controlled conditions, that can later be used to assess the health of fish in the Hanford Reach.

The objectives of this proposed study are to assess the effects of chromium on chinook salmon under exposure conditions similar to those of the Hanford Reach of the Columbia River. This objective will be accomplished in three tasks: Task 1, (Fertilization), the potential for chromium to adversely affect gametes and their fertilization in chinook salmon; Task 2, (Early Life Stage), determine the effects of chromium on the early development of chinook salmon; Task 3, (Fish Health), determine degree of fish health impairment of chinook salmon exposed to chromium.

EXPERIMENTAL PROCEDURES

General

Experimental water will simulate that of the Columbia River surface and pore water in the Hanford Reach and known to be associated with the location of spawning redds (Hope and Peterson, 1996; Geist, 1997). Experimental water will be adjusted to a hardness of 80 mg/L as $CaCO_3$; pH, alkalinity, and conductivity will be in a range consistent with Columbia River conditions. Experimental water temperature will match seasonal conditions: December through March, 5°C; March through July, 10°C Wiggins and others, 1997). Geist (1997) documented that the hyporheic zone (where river water and ground water mix) is generally warmer than the river water. However, data from samples collected between November and March indicate that the temperature of the hyporheic zone minus the river water is only 1°C. Experimental water will be prepared by blending laboratory well water with deionized water produced by reverse osmosis. Experimental water produced in this way will eliminate the use of surface water and the potential for fish pathogens to be introduced to the experiment and influence test results. Experimental water will be produced in 5,600L batches and analyzed to insure quality is within 5% of the experimental design in terms of hardness, alkalinity, conductivity, and pH. Unless otherwise indicated, experimental water was used. Photoperiod will be adjusted to simulate time of year of the exposure.

The range of chromium concentrations tested in the experimental water will be from 0 to 120 µg/L. This range of concentrations are above and below the chronic AWQC for chromium, 11 µg/L (USEPA, 1986) and the State of Washington. This concentration range is also representative of concentrations in pore water sampled from the intergravel substrates in locations where salmon spawn (Giest, 1997; Hope and Peterson, 1996). Specific concentration are stated with each task.

Gametes and eyed embryos of chinook salmon will be obtained from the McNenny State Fish Hatchery, Spearfish, South Dakota. These eggs will be certified disease free prior to any testing. The disease free status is essential in assuring that toxicity testing is performed on healthy test organisms, increases reliability of results, and is a recommended standard procedure (ASTM, 1993). This source of chinook salmon eggs has been used in past Natural Resource Damage Assessments (Blackbird Mine Site, Idaho; Marr and others, 1995).

Task 1: Fertilization

Gametes will be taken from chinook salmon brood stock between October and November of 1998. This is the normal time for gametogenesis in fall adult chinook salmon and the stock will be checked weekly for ovum and sperm formation. We will use a pooled source of eggs and sperm from a minimum of three females and three males to perform the following three tests: 1) toxicity of chromium to the ovum, the ovum survival test; 2) toxicity of chromium to sperm, the sperm survival test; and 3) toxicity of chromium to fertilization, the fertilization test.

A physiological saline (PS) solution will be used in the ovum and fertilization test; and a physiological saline solution with sperm extender (PS/SE) will be used in the sperm test (Billard and Roubaud, 1985). The physiological saline solution will consist of a standard 1% NaCl solution buffered to pH 9.0; the PS/SE will be the same solution with KCl added (30 mM) to prevent the spermatozoa from becoming motile. Ova and sperm can survive for several hours in these solutions. In all three tests, there will be six treatment concentrations of chromium: 0, 5, 11, 24, 54, and 120 μg/L. The six chromium treatments will be incorporated into the PS or the PS/SE so that when diluted with the appropriate amount of sperm or ovum the desired concentration of chromium is achieved. Each treatment will be replicated four times for a total of 24 treatments.

Ovum Survival Test

Ova will be divided into 24 treatment lots of 150-200 eggs, each and mixed with 10 ml of PS containing the appropriate chromium concentration. After 15 min, the liquid will be removed from each treatment and replaced with 10ml fresh uncontaminated PS solution followed by insemination with 1ml of intact sperm. This will be a 15 min ovum exposure.

Sperm Survival Test

Sperm will be diluted with PS/SE containing the appropriate chromium concentration (1ml sperm:10ml PS/SE) to obtain 24 treatment lots. The sperm, PS/SE, and chromium will be mixed, and left standing for 15 min. Sperm will be separated from the PS/SE exposure treatments by centrifuging for 10 min at 1800g, followed by replacement of 10ml fresh uncontaminated PS/SE. The exposed sperm will be used to inseminate 24 lots of about 150-200 ova, each previously diluted in 10ml of PS. This will be a 15 min sperm exposure.

Fertilization test

Ova (150-200), sperm (1ml), and 10ml of PS containing the appropriate chromium concentrations will be mixed together to achieve the 24 treatment lots. This will be a 65 min exposure of egg and sperm during fertilization and water hardening.

In all three tests, ova and sperm will be mixed for 5 min followed by rinsing and water hardening in Hanford experimental water according to standard procedures (Piper and others, 1982). Water hardening will last for one hour and is the process by which water is absorbed into the eggs and fills the perivitelline space between the shell and yoke. The eggs become turgid during this process and additional water exchange is minimal during further development. In the fertilization test, exposure to chromium will continue through water hardening. After water hardening, eggs will be rinsed and transferred into incubators.

Eggs from all three tests will be incubated in McNenny hatchery water (temperature, 11°C; hardness, 360 mg/L as $CaCO_3$; alkalinity, 210 mg/L; and pH, 7.6) for 10 days. The eggs will be cleared in 10% acetic acid solution for 2 min and percent fertilization will be determined. The embryo of fertilized eggs will turn an opaque white and become visible through the translucent chorion. At 10 days the embryo will have a definite optic lobe developed with an elongated somite and will be easily distinguished from an unfertilized germinal disk.

Task 2: Early Life Stage

Eyed eggs of chinook salmon will be exposed to 5, 11, 24, 54, and 120 μg/L of chromium and a control treatment with no chromium added. The test will be conducted in a modified Mount and Brungs (1967) flow-through diluter system. Temperature will be maintained at 5 ± 2 °C by chilling the exposure water before it enters the diluter and submerging the exposure aquaria in a temperature-controlled water bath.

To initiate the test, two groups of 50 eggs each will be placed into 177-mL glass hatching containers and suspended into each of four exposure aquaria. The aquaria will be covered with black plastic to shield the eggs from light during incubation, and gentle aeration will be used to provide continuous circulation of the exposure water. On the median hatch date, the alevins will be released into the exposure aquaria. On the median swim-up date, the chromium exposure will be discontinued and the alevins will be maintained in the aquaria in chromium-free water until 30 days after the medium swim-up date.

During the exposure, egg mortality and hatching will be monitored and recorded daily. Dead eggs will be removed from the hatching containers and discarded. Alevin mortality and deformities will be monitored daily and dead alevins will be removed from the aquaria and discarded. The development of alevins will be monitored daily to document the sequence and timing of critical developmental stages including; hatch, onset of movement, side plough, upright plough, free swimming, and exogenous feeding following Dill (1977). The tanks will be video taped weekly to develop an accurate count of these developmental patterns among the test populations. Quantitative measures of the form and frequency of movements will be made during the alevin/free swimming transition. Samples of alevins containing 15 fish each will be taken from each of the four replicate exposures at the following times: after hatching, mid-way through yolk absorption, at swim-up, and at 30-days post swim-up. This fish will be analyzed for tissue residues of chromium, DNA strand breakage, and lipid peroxidation (see Task 3 for further details of physiology measurements). Two fish from each replicate will be collected at swim-up and at 30-days post swim-up for histological analyses. At the end of the exposure and at 30 days post swim-up all surviving alevins in each treatment will be measured for total length and weighed to determine growth.

Task 3: Parr Health

The goals of this experiment are two-fold. First, data gathered from this experiment will further explain toxicological responses on growth and survival documented during the early lifestage experiment. Because fry at the end of the early lifestage experiment are small, it will be difficult to interpret the results in terms of individual tissue responses. And, therefore, explain the mechanistic processes involved during chromium action on fish. Second, this experiment will provide useful information to interpret effects of chromium on fish in the Hanford Reach. Health parameters used in the laboratory can also be performed on fish collected in the field.

Measurements will be performed to assess physiological impairment caused by chromium. For example, researchers have documented that chromium causes lipid peroxidation (Susa and others, 1996). Lipid peroxidation results in damage to polyunsaturated fatty acids located in the cell membrane. This damage can decrease fluidity, increase leakiness, and inactivate membrane-bound enzymes. An ultimate result may be cell death and tissue damage (Halliwell and Gutterirdge, 1985; Wills, 1985). Chromium can form intermediates that react with DNA (Outridge and Scheuhammer, 1993). These reactions have been associated with DNA damage measured in the form of DNA strand breakage (Aiyar and others, 1990). Therefore, lipid peroxidation and DNA strand breakage will be measured, in addition to histology and tissue metal accumulation, to document physiological impairment during this study.

Eyed eggs will be maintained in a Heath^R incubator at a temperature of 10 ± 2 °C and hardness of approximately 150 mg $CaCO_3$/L. Mortalities will be documented and removed daily. At hatch, the fish will be moved to flow-through culture tanks with a flow of 4 L/min. The fish will be fed at least a 5% wet weight ration of a commercial biodiet daily. The daily food ration will be split between two feedings.

The experimental phase will begin during the parr stage of fish by randomly distributing 35 fish in each of 12 test chambers receiving experimental water with a flow-through proportional diluter system. The circular chambers will have a 20-L capacity with dimensions of 43.2 cm X 35.6 cm and a volume of 20,510 cubic cm. The fish will be allowed to acclimate in the experimental chambers for at least five days before the start of the experiment. Thus, the experiment will be conducted for a period of 100 days beginning with parr fish. Eyed embryos, larvae, and parr will be handled so as to minimize stress in accordance with the ECRC-Columbia Animal Welfare Plan and the Region 6 U.S. Fish and Wildlife Service, Fish Health Policy.

Chromium in stock solutions will be delivered to eight test chambers via automatic pipettes (Micromedic Systems AP, Model #25000FW). Two test concentrations of 11 and 24 µg/L chromium (hereafter, referred to as 11 and 24) will be maintained in each of four replicate chambers. Four chambers without chromium added will be used as controls. Thus, a total of 12 experimental (four control, four with 11 µg/L Chromium, and four with 24 µg/L) units will be maintained. Each chamber will receive 8 L/hr for 10 volume additions per day. Experimental units will be checked daily for mortality and observations on behavior.

At Day 60 and at the termination of the experiment, samples will be collected for fish health measurements. An external necropsy assessment will be made on all sacrificed fish and lengths and weights will be recorded. One whole fish will be collected from each replicate chamber for measurements of tissue metal accumulation. DNA strand breakage, lipid peroxidation, histological anomalies, and tissue metal accumulation will be evaluated in 2 to 4 fish from each replicate. Gill lamellae, liver (free of the gall bladder), kidney, and intestine will be removed immediately from the 10 individual fish. Samples for histology will be collected from 2 fish from each replicated chamber and fixed in 10% neutral buffered formalin. It should be noted that spleen and skin samples will also be collected for histological examinations. Samples for DNA strand breakage, lipid peroxidation, and tissue metals will be collected from four fish from each replicate, frozen with liquid nitrogen, and stored at -90°C. At a later date, these samples will be ground with liquid nitrogen and composited by tissue to result in one sample from each replicate chamber. Aliquots of these composites will be measured for DNA strand breakage, lipid peroxidation, and tissue metals.

Samples will be collected from the remaining two fish for additional measurements of DNA strand breakage. For example, both the anterior and posterior portions of the kidney will be sampled to distinguish between effects related to immune functions of the kidney (i.e. anterior section) to effects on the excretory processes (i.e. posterior portion). This information can be used to explain the mechanisms of observed toxicity. These data are also necessary to make comparisons between data gathered from samples of whole, ground tissue and those from specific locations within a tissue. Whole tissue samples would be less labor intensive to collect in field situations. However, it must be documented that this method is sufficiently sensitive to correspond with other toxicological effects. Fish will not be fed for 24 h prior to sampling.

Analysis of Water and Tissue (All Tasks)

Exposure water will be monitored once per week for dissolved oxygen, pH, alkalinity, hardness, and conductivity. More frequent monitoring will be performed if conditions dictate. Samples of exposure water will be taken weekly to monitor total chromium exposure concentrations. One hundred mL samples of exposure water from each treatment will be filtered using a Nalgene7 300 filter holder. Each filtered sample will be transferred to a pre-cleaned, 125 ml I-Chem7 polyethylene bottle, acidified to 1% HNO_3, and analyzed with ICP-MS. At each time of total chromium sampling, one additional sample will be extracted from the low, middle, and high chromium exposures for analysis of Cr(VI) (hexavalent chromium). The Cr(VI) water samples will be filtered as above, then immediately put on ice and shipped by overnight carrier (or hand delivered) to the analytical laboratory. Upon receipt, the analytical laboratory will immediately conduct extraction or ion-exchange separation of the Cr(VI) species. The treated sample containing only the Cr(VI) will then be acidified to 1% HNO3 for analysis by ICP-MS or graphite furnace atomic absorption spectrophotmetry. For analysis of chromium in tissue, samples will be lyophilized, acid digested with microwave heating, and analyzed by either ICP-MS or graphite furnace atomic absorption spectrophotometry.

RESULTS AND INTERPRETATION

The data from these experiments will be

used to determine the toxicity of chromium to fertilization, alevin survival, and to health of alevins and parr of chinook salmon. The results will be compared with environmental concentrations of chromium in the Columbia River to evaluate the potential for chromium to adversely affect salmon. This research is to be performed by the Biological Resources Division from October 1998 to December 1999.

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A Radioimmunoassay Method to Screen for An~~it~~tibiotics in Liquid Waste at Confined Livestock Operations, with Confirmation by Liquid Chromatography/Mass Spectrometry

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ABSTRACT

Approximately one-half of the 50 million pounds of antibiotics produced in the United States are used in agriculture. Because of the intensive use of antibiotics in the management of confined livestock operations, the potential exists for the transport of these compounds and their metabolites into the Nations's water resources. A commercially available radioimmunoassay method, developed as a screen for tetracycline antibiotics in serum, urine, milk, and tissue, was adapted to analyze water samples at a detection level of approximately 1.0 part per billion. This method has a semiquantitative analytical range of 1 to 20 parts per billion. Six liquid waste samples from hog lagoons, 14 surface water samples, and 3 ground-water samples were used to test the radioimmunoassay method as a screen for tetracycline antibiotics. The radioimmunoassay tests yielded positive results for tetracycline antibiotics in samples from all six hog lagoon samples and one surface-water sample. Dilutions of 10 to 100 fold of the hog lagoon samples indicated that tetracycline antibiotic concentrations ranged from approximately 1 to several hundred parts per billion in liquid hog waste. A new liquid chromatography/mass spectrometry method was used to confirm the radioimmunoassay and also to identify the tetracycline antibiotics to which the radioimmunoassay test was responding. The new liquid chromatography/mass spectrometry method with online solid-phase extraction and a detection level of 0.5 microgram per liter confirmed the presence of chlortetracycline in one hog lagoon sample and in one surface-water sample.

INTRODUCTION

A wide variety of pharmaceutical compounds has been detected at low concentrations in some of the lakes, rivers, and ground water in Europe (Holm and others, 1995; Buser and others, 1998; Raloff, 1998). These findings have raised concern, not only that pharmaceutical compounds are present in our water systems but that they may have detrimental effects on ecological and human health. In a recent literature review of studies on pharmaceutical compounds in the environment, virtually no studies were found on the potential effects of the presence of these compounds on the environment (Halling-Sorenson and others, 1998).

Natural and synthetic antimicrobials are one class of prescribed pharmaceutical compounds that are of human health concern because of increased bacterial resistance. More than 50 million pounds of antibiotics are produced in the United States per year, and about one-half of these are used for agriculture (Levy, 1998). Approximately 40 percent of the antibiotics that are produced are used for livestock (for example, swine, poultry, and cattle) and the majority of these antibiotics are given in subtheraputic doses as feed additives to enhance growth (Levy, 1998). Antibiotics are also used in aquaculture and sprayed on fruit trees to inhibit fungal growth. The interest in pharmaceutical compounds in the environment is new, and for the most part, methods have not been developed to concentrate and analyze these compounds at levels at which they probably occur in the environment (Raloff, 1998).

Immunoassay is a screening technique developed for use in the clinical health sciences that has been readily adopted for use by environmental scientists over the last decade primarily for pesticides in water and sediment. However, immunoassays for pharmaceutical compounds currently are used in biological media where the concentrations are relatively high. To assess the use of these tests on environmental samples where the concentrations are usually low, the techniques must be modified to lower their limits of detection. In the same way, quantitative extraction and identification techniques, such as solid-phase extraction (SPE), liquid chromatography (HPLC), and liquid chromatography/mass spectrometry (LC/MS), for pharmaceutical compounds were developed for biological samples. Because many of these compounds are at much lower concentrations in the environment and are often polar, the challenge for environmental chemists is to modify or develop new extraction and analysis methods for the identification of pharmaceutical compounds in water and sediment.

The tetracyclines are a widely used class of antibiotics in the United States. Currently chlortetracycline, and oxytetracycline are 2 of only 10 antibiotic compounds licensed for use as growth promoters for livestock in the United States (Swick, 1996). It has been shown that tetracycline-resistant bacteria in swine outflow can pass this resistance on to bacteria commonly found in soil (Haack and others, in press). Because little is known about the occurrence, fate, and transport of antibiotics in the environment, it is important to begin assessing where and at what levels they may occur in our water resources. This paper presents the results for a modified radioimmunoassay (RIA) procedure for screening tetracycline antibiotics in water, evaluates the method as a screening tool for source areas and surface and ground water, and compares the results of the RIA to a newly developed LC/MS method with online SPE.

SAMPLING AND ANALYTICAL METHODS

Sampling and analytical methods were tested and documented during this investigation. The following sections describe the sampling and analytical procedures developed for the RIA and LC/MS methods.

Sampling

Water samples were collected as part of investigations being conducted for the U. S. Environmental Protection Agency (USEPA) Neuse River Research project and the U.S. Geological Survey (USGS) Toxic Substances Hydrology, Emerging Contaminants Program. Two to four liters (L) of water or liquid hog waste were collected at each site packed in ice, and shipped in coolers to the USGS North Carolina District Research Laboratory (NCDRL) in Raleigh. The samples were filtered either through a 0.2-micron glass fiber filter in the field or in the NCDRL. All of the samples are stored at 3 degrees Celsius (^{0}C) in refrigerators at the NCDRL and the USEPA National Exposure Research Laboratory (NERL) Research Triangle Park, N.C. The hog-waste samples were centrifuged at 3,000 revolutions per minute (RPM) for 40 minutes in a swing bucket centrifuge and then prefiltered through a paper filter under vacuum before being filtered through a glass-fiber filter.

Radioimmunoassay

Radioimmunoassay analyses were conducted by USGS and USEPA researchers at NERL. Charm II[1] RIA tests (Charm Sciences Inc.; Malden, Mass.) for tetracycline antibiotics were used to analyze the samples. This tetracycline RIA test recently was approved by the Food and Drug Administration for use in determining safe levels of tetracycline antibiotics in milk (Smucker, 1998). The procedure used for the RIA analyses was modified from the company-supplied instructions for urine analysis in the following manner: (1) the powdered MSU extraction buffer supplied by the manufacturer with the tests was diluted into 100 milliliters (mL) of ultrapure water (10X buffer) instead of 1 L of water (normal-strength buffer), (2) instead of adding 13.3 microliters (µL) of sample to 2 mL of MSU normal-strength buffer, 0.5 mL of the 10X buffer was added to 4.5 mL of sample, and (3) the chlortetracycline standard pellet supplied by the manufacturer was diluted in 200 mL of ultrapure water instead of 10 mL of water to make a 200-parts per billion (ppb) standard solution. Serial dilutions then were made to produce 100-, 50-, 20-, 10-, 5-, 1-, and 0.5-ppb solution standards.

Chlortetracycline has less affinity for the RIA test antibodies than the other commonly used tetracycline antibiotics, oxytetracycline and tetracycline (Smucker, 1998). Thus, the level of detection established with the chlortetracycline standard ensures that the other tetracyclines also will respond to the RIA at that level or less.

The procedure that was used is as follows: 4.5 mL of sample, standard, or ultrapure water and 0.5 mL of 10X buffer was added to a borosilicate test tube. A binder tablet, containing the antibody bound to a microbial cell was then added to the sample and vortexed for 10 seconds (s). Next, the tablet containing the

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[1] The use of firm, trade, or brand names is for identification only and does not imply endorsement by the U.S. Geological Survey.

tritium (^{3}H)-labeled chlortetracycline was added to the sample and vortexed for 15 s. The sample was incubated at 35 ^{0}C for 5 minutes (min) and centrifuged at 3,300 RPM for 5 min. Following this procedure, the liquid was decanted and 0.3 mL of water was added to the test tube and gently vortexed for 2 s to break up the pellet at the bottom of the test tube. Next, 3 mL of scintillation fluid was added to the test tube and vortexed. The sample then was allowed to sit undisturbed for 1 min before being analyzed in the Charm 6600[1] scintillation counter. The ^{3}H activity was analyzed for 1 min and the average count per minute (CPM) was recorded.

Samples were run in sets of six, which included one negative control, one standard, and four samples. The average negative control was calculated after all the samples were run. The negative control point (NCP), the cutoff point for separating a positive and negative response, was set at three standard deviations below the average CPM. A standard linear regression curve was developed for the 1 through 20-ppb standards by using the natural log (ln) of the CPM and the ln of the concentration of the standards. Samples that tested at concentrations of 20 ppb or more were diluted with ultrapure water at factors of 10, 20, and 100 and reanalyzed until their CPM was in the working range of the standard curve.

Liquid Chromatography/Mass Spectrometry

The LC/MS analyses were performed in the USGS Organic Geochemistry Research Laboratory (Lawrence, KS) by using a Hewlett Packard[1] 1100 series LC and a diode array detector (DAD) interfaced with an electrospray MS and on-line SPE. A 250 x 3 millimeter (mm) LC column packed with 5-micrometer (µm) diameter C_{18} was used to separate the tetracycline compounds. The extraction conditions were as follows: 50 mL of sample was automatically pumped through a C_{18} SPE disk and eluted with the mobile phase solvents described next. The LC mobile phase was 80 percent channel A containing 0.1 percent trifluoroacetic acid (TFA) in a 2:7:91 mixture of methanol/acetonitrile/water (MeOH/ACN/H_2O) and 20-percent channel B containing 100 percent MeOH. This mixture was held 1 min and then ramped to 25 percent channel A and 75-percent channel B over 15 min at a flow rate of 0.4 mL/min. The DAD monitoring wave length was 355 nanometers (nm). The electrospray MS parameters that were used were positive ion mode, a fragmentor ion voltage of 70 volts, and selected ion monitoring (SIM) for the following ions: 444, 445, 446, 461, 462, 479, 481, and 501. The purity of the quantitative standards of chlortetracycline, oxytetracycline, and tetracycline was 79, 97, and 98 percent respectively. The standard solutions of chlortetracycline concentrations were corrected for purity.

RESULTS AND DISCUSSION

Results of the modified RIA procedure for tetracycline antibiotics are presented in the following sections. Also presented is a comparative analysis of a subset of samples analyzed by RIA and a new LC/MS method.

Radioimmunoassay Methodology

In order to analyze water samples with a lower level of detection than the procedures offered by the manufacturer, three parameters were considered -- the size of the test tube required for the analyzer, the need to increase the amount of sample to be analyzed, and the amount and strength of the buffer to add to the sample to maximize the CPM of the negative control samples. It was found that some sample spilled during vortexing if more than 5.5 mL of sample and buffer combined was added to the 10-mL test tube. Based on the detection levels for the urine procedure, it was determined that 4 to 4.5 mL of sample would be required to achieve a detection level of 1 to 10 ppb for water samples. From this information it was deduced that 0.5 mL of buffer and 4.5 mL of sample was the maximum amount that could be added to the sample test tubes without losing any sample while vortexing.

Based on these requirements, four sets of negative control samples with different buffer strengths were analyzed in duplicate. For this experiment 4.5 mL of ultrapure water was added to eight test tubes. Next, each set of two test tubes received 0.5 mL of one of the following: water, normal-strength, 10x, or 100x buffer. The negative controls with the 10x buffer had the highest CPM.

The modified RIA procedure for the tetracycline antibiotics resulted in an average negative control CPM of 1,934 ± 106.5 (one standard deviation (sd); n = 26) and an established NCP of 1,614 CPM; three standard deviations below the average CPM of the negative controls. The average CPM for the 1 ppb standards was 1,337 ± 90.6 CPM (1 sd; n = 3) and was always more than 180 CPM less than the NCP. The CPM for the 0.5-ppb standards were less than the NCP in 50 percent of the samples. These data indicate that the RIA procedure can be reliably used to detect samples with 1 ppb of chlortetracycline, but the procedure will give a false negative 50 percent of the time in samples containing 0.5-ppb chlortetracycline.

The analysis of standards showed that the CPM of the 1-, 5-, and 10-ppb standards varied but did not overlap and that the CPM values of the 10- and 20-ppb standards were separated in approximately 75 percent of the analyses. The CPM range from approximately 1,400 to 410 for the 1 through 20-ppb standards, respectively. A log-log linear regression of the CPM and concentration of the 1- through 20-ppb standards had a correlation coefficient (r) of 0.96 ($n = 16$; $p < 0.1$). The CPM data from the analysis of the 50-, 100-, and 200-ppb standards were less than that of the 20 ppb standards; however, the CPM of these standards overlapped with each other. The CPM for these standards ranged from 390 to 290. These data indicate that the samples contained more standard than the ^{3}H-labeled antibodies could effectively compete with to give scintillation readings reliable enough to estimate concentrations greater than 20 ppb.

Radioimmunoassay Results

The concentrations determined from the tetracycline RIA for samples with CPM less than the NCP are shown in figure 1. The data from the hog waste samples indicate that the concentrations ranged from approximately 1 to nearly 800 ppb (fig. 1). These data also indicate that the tetracycline antibiotics are commonly used in confined livestock operations for swine and that the tetracycline compounds are able to withstand very active microbial environments.

Of the 17 surface- and ground-water samples analyzed, only one sample had a CPM less than the NCP. The tetracycline concentration of this sample was less than 1 ppb. These data indicate that although tetracycline antibiotics commonly are used in both swine and poultry, they generally are not transported into surface and ground water at concentrations greater than 1 ppb. In this sense the modified RIA method is not sensitive enough to detect antibiotics at the levels in

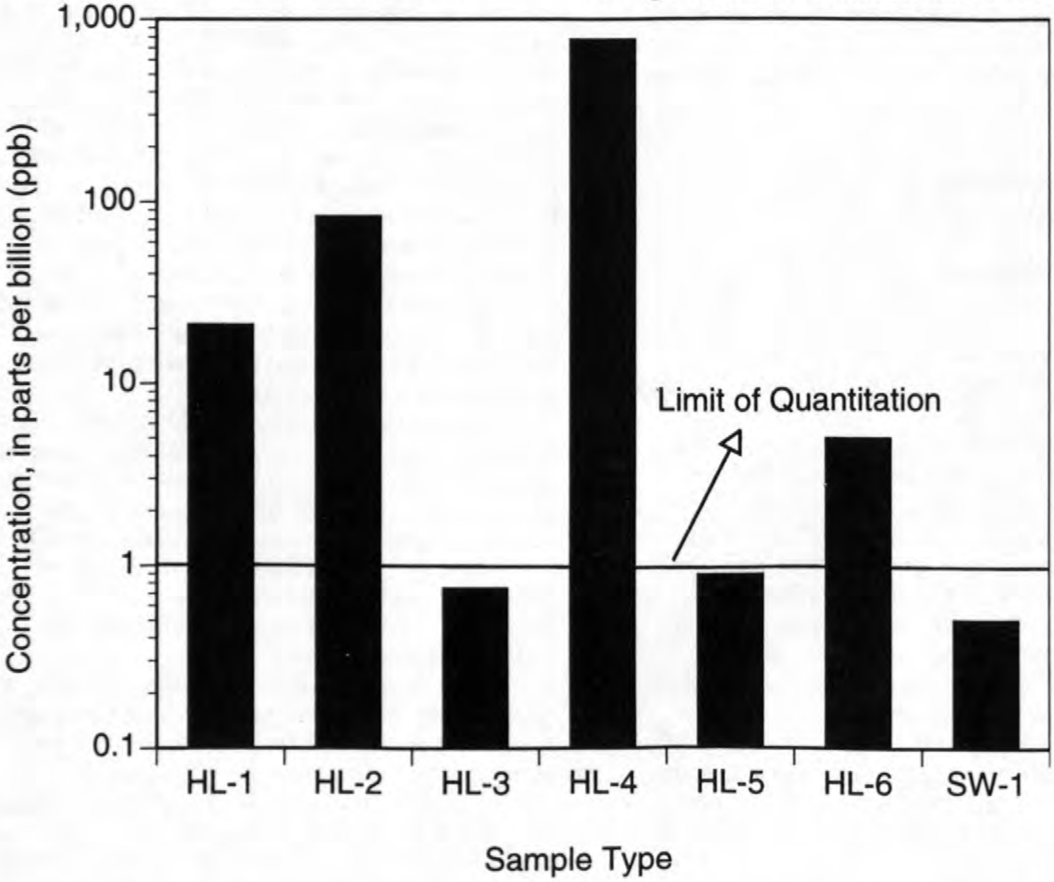

Figure 1: Graph showing tetracycline radioimmunoassay concentrations for selected samples from hog lagoons (HL) and surface water (SW) with positive responses.

which they may occur in streams or in ground water. However, these data also suggest that this class of antibiotics does occur in high concentrations at sources where transport can occur.

The presence of antibiotics in hog lagoons is a potentially important finding because of increased antibiotic resistance in bacteria. Currently it is not known whether bacteria that are present in the hog lagoons are developing resistance to the antibiotics introduced into these microbially active environments. Also it is uncertain to what extent resistant bacteria or their plasmids may be transported from these areas into surface and ground water where resistance may be transferred to other bacteria. To confirm the RIA results a new LC/MS was used to analyze a subset of samples.

Comparison of Radioimmunoassay and Liquid Chromatography/Mass Spectrometry

Table 1 shows 4 samples analyzed by LC/MS for conformational analysis, identification of the tetracycline compounds detected by the RIA tests, and for comparison of the RIA and LC/MS. The LC/MS analyses show that chlortetracycline was present in the hog lagoon and one of the surface-water samples in which a positive response was obtained from the RIA and that tetracycline compounds were not detected by RIA in the samples where it was not detected by LC/MS (table 1). These data indicate that the modified RIA procedure was an effective screen for the presence of tetracycline antibiotics in water and liquid waste samples.

Table 1. Concentrations of selected samples analyzed by radioimmunoassay (RIA) and liquid chromatography/mass spectrometry (LC/MS) as chlortetracycline in (ppb, parts per billion; µg/L, micrograms per liter; HL, hog lagoon; SW, surface water; and GW, ground water sample; nd, not detected).

Sample Name	RIA (ppb)	LC/MS µg/L
HL_5	5	5.0
SW_1	(+)[1]	0.5
SW_2	nd	nd
GW_1	nd	nd

[1] CPM greater than the NCP and less than 1.0 part per billion (ppb)

These initial RIA and LC/MS comparison data indicate that there is good agreement between the two methods; however, a larger data set needs to be established before this is confirmed. For example, research has shown that tetracycline compounds form epimers as they pass through an animal and that this results in isomers being excreted that are different from the predominant isomer of the tetracycline administered to the animal (Blanchflower and others, 1997; Kennedy and others, 1998).

In the LC/MS analyses, the mass spectra obtained from the hog lagoon sample was the same as for the chlortetracycline standard, but the retention time was different, due to the difference in the isomeric composition of the chlortetracycline. Currently the affinity of these excreted chlortetracycline isomers to the RIA test antibodies is not known. It may be that these excreted isomers do not bind with the same affinity to the antibodies in the RIA test as the predominant isomer of which the chlortetracycline standard is mostly composed. This is an area that warrants further investigation.

CONCLUSIONS

A modified procedure was developed to analyze water samples at a detection level of approximately 1 ppb for a commercially available RIA used for testing the tetracycline class of antibiotics in biological media. The modified RIA test detected tetracycline compounds at concentrations ranging from 1 to several hundred ppb in all hog lagoon samples that were tested. A positive response of less than 1 ppb was elicited from the modified RIA tests in only 1 of 17 surface- and ground-water samples. The level of nondetection among the water samples indicates that antibiotics, if present, in surface and ground water are generally at concentrations less than 1 ppb.

Conformational analysis of a subset of the samples by LC/MS supported the RIA analyses and proved that the RIA was responding to chlortetracycline. To more fully determine the extent to which the RIA and LC/MS methods agree a larger set of samples needs to be analyzed.

The LC/MS retention time data indicate that the chlortetracycline isomer in the samples is different from the predominant isomer of the standard. This study indicates that the RIA method presented is a reliable screen for tetracycline compounds in liquid waste from hog lagoons and in water samples at concentrations as low as 1 ppb. The data from the hog lagoons indicate that antibiotics may be present in high levels in areas where they may be transported into surface and ground water

The data also suggest that a more sensitive method should be developed for analyzing antibiotic compounds in water that is located away from the contributing contaminant sources.

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Trends in Sediment Quality in Response to Urbanization

By Peter C. Van Metre and Edward Callender

ABSTRACT

Trends in hydrophobic contaminants identified in reservoir and lake sediment cores in three watersheds dominated by post-1960 urbanization follow several consistent patterns. Since the 1970s, trends include decreases in lead, increases in arsenic and zinc, decreases in PCBs (polychlorinated biphenyls) and organochlorine pesticides (with the occasional exception of chlordane), and large increases in PAHs (polycyclic aromatic hydrocarbons). Trends in PAHs in a core from a fourth lake, where development mostly occurred before 1940, indicate highest concentrations in about 1950, which contrasts with trends in the newer urban watersheds. In this paper, we attempt to answer the question, "Is the sediment quality (and water quality as influenced by hydrophobic contaminants) getting better or worse?" The approach used is to normalize sediment-core concentrations of these contaminants to toxicity-based sediment quality guidelines, then sum the normalized concentrations. The results of this approach indicate that overall sediment quality improved from about 1950 to 1970 in the lake in an older watershed, where improvements were driven by decreases in total PAH. In contrast, sediment quality is declining coincident with urbanization in the three lakes in newer urban watersheds. In the newer urban watersheds decreasing trends in normalized total DDT, PCBs, and lead are more than offset by increases in PAHs, arsenic, zinc, and (or) chlordane.

INTRODUCTION

A National Water-Quality Assessment (NAWQA) Program study has been underway since 1992 that is using paleolimnological methods to define past trends in metals and hydrophobic organic compounds in river basins (Callender and Van Metre, 1997; Van Metre and others, 1997; Van Metre and Callender, 1997). Sediment cores were collected from 35 reservoirs and 6 natural lakes from 1992 to 1998 from all parts of the United States (fig. 1). Each water body was selected to represent a specific land use setting. Individual element and compound trends often can be clearly identified in these cores; however, it is not immediately clear if overall sediment quality is improving or declining. Here we attempt to answer this question by normalizing contaminant concentrations to sediment quality guidelines (Environment Canada, 1995), then evaluating trends in normalized concentrations for constituent groups and overall.

Results from four urban lakes or reservoirs are presented in this paper. Three of these are in watersheds dominated by newer (post-1960) residential and commercial development: Lake Anne (Reston, Va.), Palmer Lake (Minneapolis, Minn.), and White Rock Lake (Dallas, Tex.). The fourth water body, Lake Harriet, is in an older (mostly developed before 1940) residential and commercial neighborhood in Minneapolis.

Details of sampling, analytical, and age-dating methods are presented elsewhere (Van Metre and Callender, 1997; Van Metre and others, 1997; 1998). Briefly, cores were collected from the deepest part of the lake or in the lower part of the reservoir and sectioned into vertically discrete subsamples for analysis of major and minor elements, ^{137}Cs, ^{210}Pb (Lake Harriet only), chlorinated organic pesticides, PCBs, and PAHs. Date of deposition of sediment intervals within each core was

Figure 1. Location of 41 reservoirs and lakes sampled during 1992–98 (four reservoirs and lakes used in this paper are circled).

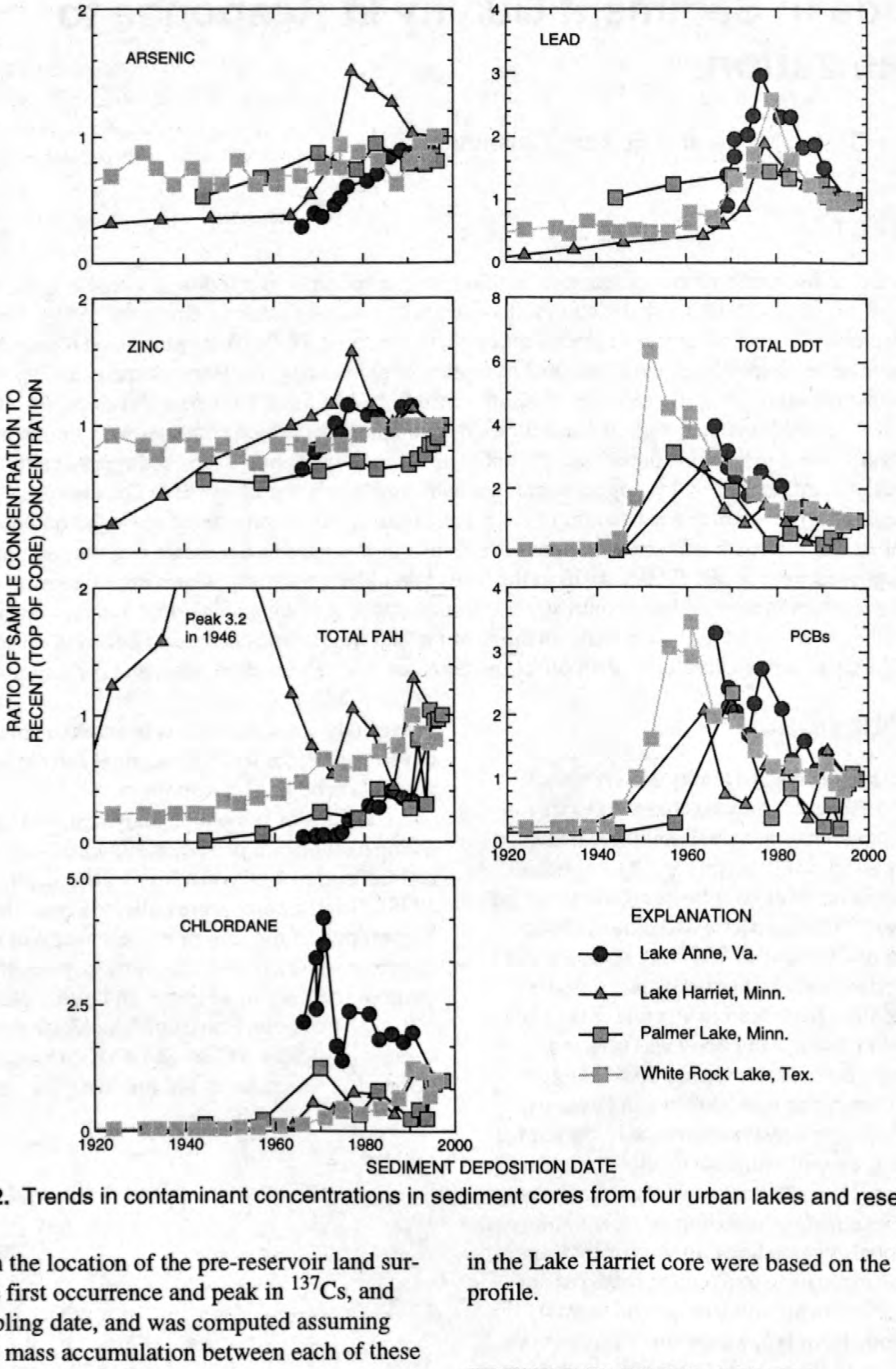

Figure 2. Trends in contaminant concentrations in sediment cores from four urban lakes and reservoirs.

based on the location of the pre-reservoir land surface, the first occurrence and peak in ^{137}Cs, and the sampling date, and was computed assuming constant mass accumulation between each of these depth-date markers. Age assignments to recent sediments were corroborated on the basis of lead peaks in the mid-1970s (Callender and Van Metre, 1997) and DDT peaks in the early 1960s (Van Metre and others, 1997). Age assignments to deeper intervals in the Lake Harriet core were based on the ^{210}Pb profile.

TRENDS IN RESPONSE TO URBANIZATION

Trends in concentrations of three metals (lead, arsenic, and zinc), total PAH (excluding perylene), total DDT, PCBs, and chlordane are shown in figure 2 for the four lakes or reservoirs. Concentrations are divided by the concentration at the top of the core, thus normalized relative to current conditions. In this way, trends in several lakes can be shown together, even if the magnitude of concentrations varies greatly.

Constituent trends among the three newer urban watersheds compare well to each other, with the exception of chlordane (fig. 2). Arsenic, zinc, and total PAH are increasing to the present; lead, total DDT, and PCBs peaked sharply coincident with maximum anthropogenic releases and are decreasing. Chlordane peaked in Lake Anne and Palmer Lake in the 1970s, consistent with the chlordane trend in an agricultural watershed in Iowa (Van Metre and others, 1997), whereas chlordane peaked in about 1990 in White Rock Lake and has a second smaller peak in the 1990s in Palmer Lake. Recent chlordane peaks in urban watersheds have been attributed to continued urban uses of chlordane as recently as 1990 (Van Metre and Callender, 1997). Trends in the older urban watershed, Lake Harriet, contrast with the newer urban watersheds for arsenic, zinc, and total PAH. Relatively large peaks in arsenic and zinc occurred in Lake Harriet in the 1970s, whereas concentrations in the newer urban watersheds are increasing to the present. A very large peak in total PAH occurred at Lake Harriet in the 1940s–50s, consistent with regional trends in PAH reported for remote northeastern lakes and the Great Lakes (Gschwend and Hites, 1981; Heit and others, 1981; Christensen and Zhang, 1993). Total PAH at Lake Harriet showed a decreasing trend from the 1950s to 1970s and a variable trend during the past 20 years.

From figure 2 it is unclear if overall sediment quality (and water quality related to the hydrophobic and particle-reactive contaminants) is improving or declining in these watersheds. Interim sediment quality guidelines developed by Environment Canada (1995) (table 1) are used here as a normalizing tool to try to determine overall directions in sediment quality and to assess the relative contributions of three major groups of constituents to sediment quality degradation: metals, organochlorine compounds, and PAHs. The guidelines were developed using the National Status and Trends Program approach (Long and Morgan, 1990; Long, 1992; Long and MacDonald 1992; MacDonald, 1994; Long and others, 1995) and the spiked-sediment toxicity test approach. Documentation of the guidelines includes a review of the sources of each chemical listed in table 1 to the aquatic environment, its distribution in Canadian sediments, its behavior and persistence in sediments, its potential to bioaccumulate, and its effects on aquatic organisms (Environment Canada, 1995).

Concentrations of each chemical listed in table 1 in each sediment-core sample were divided by the probable effects level (PEL). The PEL is the concentration above which adverse effects on biota are predicted to occur frequently (Environment Canada, 1995). The main assumption in this analysis is that guideline-normalized concentrations are

Table 1. Interim Sediment Quality Guidelines (Environment Canada, 1995)

[TEL, threshold effect level; PEL, probable effects level; mg/g, milligrams per gram; µg/kg, micrograms per kilogram]

Chemical	TEL	PEL
Metals (mg/g)		
Arsenic	5.90	17.0
Chromium	37.3	90.0
Copper	35.7	197
Lead	35.0	91.3
Mercury	.174	.486
Nickel	18.0	35.9
Zinc	123	315
Polycyclic aromatic hydrocarbons (µg/kg)		
Phenanthrene	41.9	515
Benz(*a*)anthracene	31.7	385
Benzo(*a*)pyrene	31.9	782
Chrysene	57.1	852
Fluoranthene	111	2,355
Pyrene	53.0	875
Organochlorine compounds (µg/kg)		
Chlordane	4.50	8.9
Dieldrin	2.85	6.57
DDT, total	6.98	44.5
PCBs, total	34.1	277

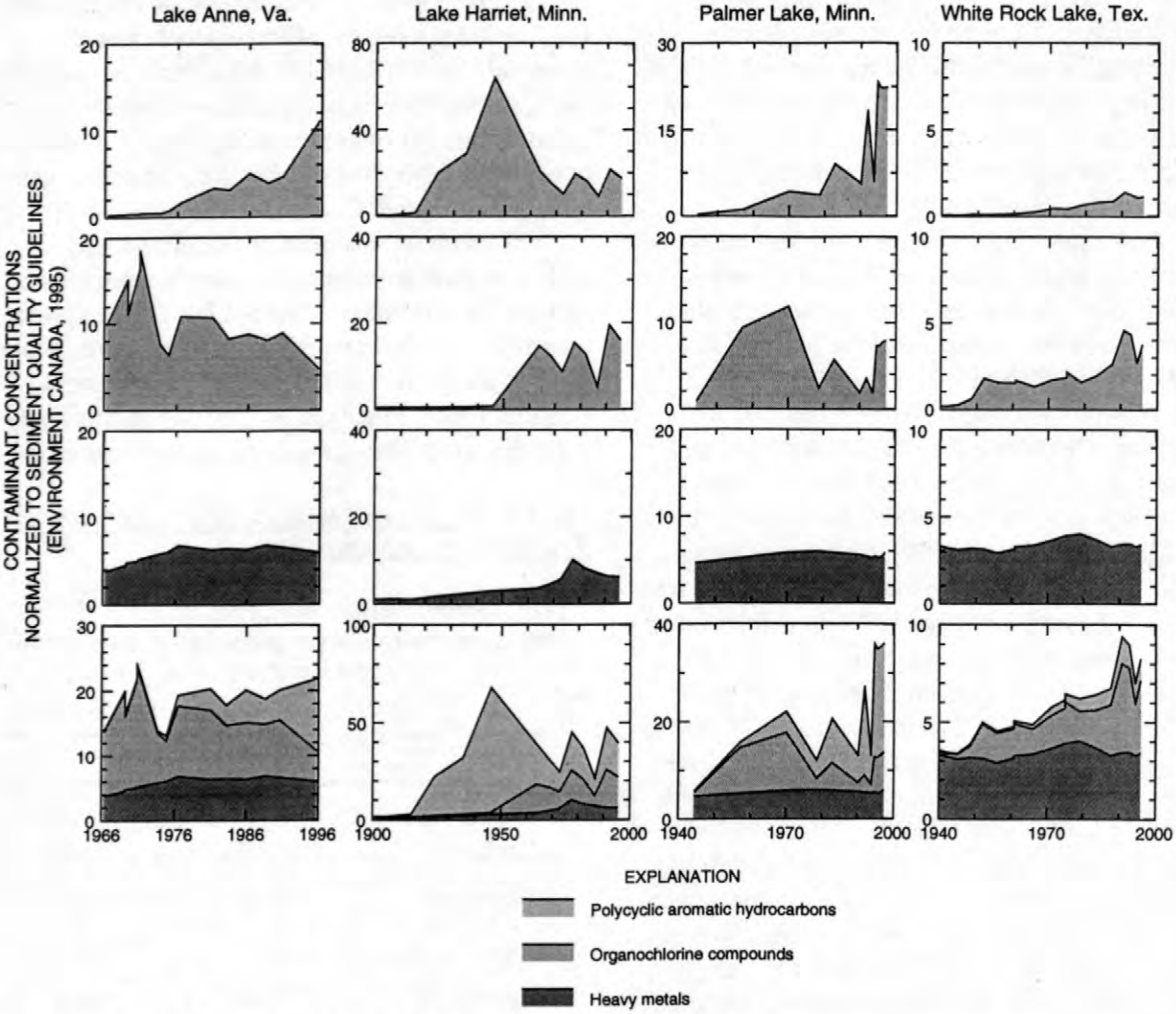

Figure 3. Trends in sediment quality in four urban reservoirs and lakes.

roughly equivalent among constituents. This assumption means that we are giving equal weight for purposes of quality-trend assessment to, for example, a mercury concentration that is three times the PEL and a benzo(*a*)pyrene concentration that is three times the PEL. Qualitative and quantitative variability could be associated with this assumption; thus, the comparison of summed trends in normalized concentrations should be viewed as qualitative.

Overall sediment quality improved from about 1950 to about 1970 in Lake Harriet but has remained approximately constant since then (fig. 3). The earlier improvement at Lake Harriet resulted from a large decline in PAH concentrations. Sediment toxicity in Lake Anne and Palmer Lake peaked around 1970 with maximum use and occurrence of organochlorine pesticides and PCBs, decreased briefly in the 1970s, then rose during the 1990s. Most of the recent rise is caused by increasing concentrations of PAHs. Sediment toxicity in White Rock Lake increased steadily from the 1940s to the present, driven mostly by increases in organochlorines and PAHs. The increase in toxicity from organochlorines since the late 1960s (fig. 3) is caused by chlordane, which peaked in White Rock Lake in about 1990 (fig. 2). At all four lakes, the continued importance of organochlorine compounds to sediment toxicity is evident. Also notable is the increasing contribution of PAHs to the overall decreases in sediment quality with urbanization in the three newer urban watersheds. In the newer urban watersheds, decreasing trends in normalized total DDT and PCBs since the 1960s are more than offset by increases in PAHs, arsenic, zinc, and (or) chlordane.

Recent declines in sediment quality at Palmer Lake on the north side of Minneapolis contrast to the relatively steady overall sediment quality at Lake Harriet since about 1970. The watershed of Palmer Lake has been rapidly urbanized since the 1970s and is characteristic of the suburban sprawl around many U.S. cities. At present, the sum of (sediment quality guideline) normalized concentrations is about 40 at both sites with the rapidly increasing PAHs at Palmer Lake accounting for a greater fraction of the sum than at Lake Harriet.

On the basis of the analysis presented here, PAHs are a major contributor to overall declining trends in sediment quality as watersheds urbanize. Had we used the TELs (threshold effect levels) instead of the PELs, the relative importance of PAHs would have been much greater. This is because the TELs are proportionally much lower than the PELs for PAHs than they are for the other constituents. Thus, PAHs appear to be an issue as watersheds urbanize.

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Estimating the Environmental Behavior of Inorganic and Organometal Contaminants: Solubilities, Bioaccumulation, and Acute Aquatic Toxicities[1]

By Dr. James P. Hickey

ABSTRACT

The estimation of environmental properties of inorganic species has been difficult. In this presentation aqueous solubility, bioconcentration and acute aquatic toxicity are estimated for inorganic compounds using existing Linear Solvation Energy Relationship (LSER) equations. Many estimations fall within an order of magnitude of the measured property. For complex solution chemistry, the accuracy of the estimations improve with the more complete description of the solution species present. The toxicities also depend on an estimation of the bioactive amount and configuration. A number of anion/cation combinations (salts) still resist accurate property estimation, and the reasons currently are not understood. These new variable values will greatly extend the application and utility of LSER for the estimation of environmental properties.

LSER ESTIMATES ENVIRONMENTAL PROPERTIES OF INORGANIC COMPOUNDS

Researchers, manufacturers, and regulating agencies must evaluate properties for chemicals that are either present in or could be released into the environment, many of these persistent and bioaccumulative. The routine use of more than 70,000 synthetic chemicals stresses the need for this information. However, minimal physical data and no toxicity data are available for about 80% of these compounds. The cost of testing these myriad present or potential chemicals is prohibitive, so researchers and managers increasingly rely on predictive models (i.e., quantitative structure-activity relationships (QSARs)) for chemical property estimation, hazard evaluation, and information to direct research and set priorities.

The Linear Solvation Energy Relationship (LSER) developed by Kamlet and others (1987, 1988) for neutral organic compounds can be a useful predictive tool, quite suitable for environmental property estimations (Hickey and others,1990 and Hickey,1996 compile most of the Kamlet and others LSER references). In LSER, the solution behavior of a substance (e.g., solubility, bioaccumulation, and toxicity) is directly related to several aspects of its chemical structure. The form of the equation used in this study is

$$Log(Property) = \underline{m}V_i/100 + \underline{s}\pi^* + \underline{b}\beta_m + \underline{a}\alpha_m \quad (1)$$

where V_i is the intrinsic (van der Waals) molecular volume,

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π^* is the solute's ability to stabilize a neighboring charge or dipole by nonspecific dielectric interactions, and

β_m, α_m are the solute's ability to accept or donate a hydrogen in a hydrogen bond, respectively.

The equation coefficients m, s, b, and a are coefficients for a particular set of conditions, determined by multiple linear regression of the LSER variable values for a series of organic chemicals against their measured values for a particular chemical property.

Estimation of environmental properties for both inorganics and organometals has presented a persistent challenge. Because LSER has proven to be a very useful QSAR for organics, LSER variable values were devised at the Great Lakes Science Center for inorganic species of the remaining periodic table elements (Hickey, in press). Proportional relationships were used for $V_i/100$, but the values for π^*, β_m and α_m were deduced heuristically (*vide infra*) from literature values for the existing group of elements.

This presentation will examine the current utility of these unorthodox but useful inorganic LSER variable values with the estimation of 1) inorganic species aqueous solubilities, 2) inorganic species bioconcentration factors in freshwater fish, and 3) acute aquatic toxicities for several species, all estimations using published LSER equations.

INORGANIC LSER VARIABLE VALUE ESTIMATION AND REFINEMENT

Values for the "steric" parameter $V_i/100$ were calculated from atomic parachors (McGowan, 1956), through a characteristic molecular volume V_x (McGowan, 1978; McGowan and others, 1979) to a corresponding intrinsic molecular volume, V_I (Abraham and McGowan, 1987). For the other variables (dipolar π^* and hydrogen bonding β and α), reasonably complete sets of appropriate elemental property values (*vide infra*) were not available to serve as a proportional reference for value calculation for the remaining periodic table elements. A heuristic development process was followed that related trends from measured physical property data with existing LSER parameter values in order to suggest parameter values for unassigned elements. Solution species and behavior were deduced from descriptive solution chemistry. The property data used were: 1)for π^*, dipole moments, polarizabilities and electronegativity and 2) for β and α, ionization potentials (basicity), electron affinities (acidity) and pK values.

The LSER values for the neutral inorganic molecule or salt were the sums of contributions for the molecule components or salt anion and cation components. The resultant component values used are listed in Table 1. These suggested values were processed through a published LSER equation (Kamlet and others,1987) for solubility estimation:

$$Log(S_w) = 0.05 - 5.85V_i/100 + 1.09\pi^* + 5.23\beta \quad (2)$$

where S_w is the aqueous molar solubility for the compound at 20°C.

Variable values for elements with the simplest solution chemistry (e.g., alkali and alkaline earth metals as halides), as well as for those elements with solution chemistry analogous to the eleven traditional LSER elements, were developed first. These parameter values were then used to develop values for more complex elements, in more complex salts or molecules. Overall agreement within an order of magnitude between observed and estimated solubilities for numerous compounds containing the element(s) in question was the criterion for parameter value "acceptance." The process was repeated until all elements were assigned LSER values. Since solubility is a fundamental factor involved in bioaccumulation and aquatic toxicity, the inorganic species LSER values generated by this process were then processed through pertinent published equations to determine general utility (*vide infra*).

The measured data used to test the accuracy of prediction using Equation 2 (aqueous solubility at 20 °C) consisted of solubility values compiled for a large list of inorganic compounds and salts from the 44th Edition of the CRC Handbook of Chemistry and Physics (Weast, 1961). For Equation 3 (bioconcentration factors or BCF), a short set of "generic" BCFs (Bysshe, 1988) were used for metal ions for "generic" freshwater fish, mindful that BCFs are highly compound-, condition- and organism /species /gender-dependent. Hawker's curvilinear equation (Hawker, 1990) accounted for the coordination energy difference between water and lipid molecules around the solute:

$$Log(K_b) = 1.125 - 0.477(V_i/100)^{\wedge}4.3] + 0.58V_i/100 + 0.384(\pi^* - 0.4 * \delta) - 4.092 * \beta \qquad (3)$$

Where K_b is the bioconcentration factor, and

δ is the polarizability correction factor to π^*

No error limits were given for the data in either solubility or bioaccumulation data sets, and each set was assumed to have been compiled from more than one source. The solution structures of the compounds were for most-likely solution species.

For acute aquatic toxicity (EC50 or LC50), several aquatic organisms were examined, ranging from eukaryotes to vertebrates. These included Microtox (as *Vibrio fischeri* Beijerinck 1889), *Daphnia magna* (Hickey and others,1990) and the Golden Orfe (*Leuciscus idus melanotus*) (Kamlet and others, 1988).

Microtox (µM/L, 15 min, 20 °C)

$$log(EC50) = 7.49 - 7.39Vi/100 - 1.38\pi^* + 3.70\beta - 1.66\alpha \qquad (4)$$

Daphnia magna (mM/L, 48hr, 20 °C)

$$log(EC50) = 4.18 - 4.73Vi/100 - 1.67\pi^* + 1.48\beta - 0.93\alpha \qquad (5)$$

Golden Orfe (M/L, 48hr, 20 °C)

$$log(LC50) = 2.90 - 5.71Vi/100 - 0.92\pi^* + 4.36\beta - 1.27\alpha \qquad (6)$$

where

EC50 is the effective concentration at which 50% of the test organisms demonstrate a particular biological endpoint, such as cessation of luminescence or movement, and

LC50 is the lethal concentration at which 50% of the test organisms die.

Estimated toxicities were compared with composite sets of literature endpoint data to determine the prediction accuracy. LSER values for compounds examined in the Microtox test, *Daphnia magna,* and the Golden Orfe data were multiplied by a "bioavailable fraction" value adapted from Newman and McClosky (Newman and McClosky, 1996).

RESULTS

From a comparison of predicted with observed aqueous solubilities for 233 inorganic and organometal compounds and salts, the estimated solubilities for most of the compounds were at or within an order of magnitude of the measured value. Solubilities for some salts and compounds (e.g., highly insoluble metal sulfides and chromates and other outlying species such as borates) were better estimated using multiple dominant solution species (e.g., 25% CrO_4^{-2} and 75% $Cr_2O_7^{-2}$ for $Na_2Cr_2O_7$) to reflect expected solution composition. The LSER values were calculated as a linear sum of the proportional contributions for each form. The difference between the the measured and the predicted values served as a non--statistical measure of prediction accuracy. Only 67 compounds had a prediction difference (Pred-Obs) greater than 1.3 log units, and 20 of these were highly insoluble salts. The outliers were primarily highly insoluble compounds including lead and barium compounds, silver halides and cyanides, aluminates, and metal acetates, oxalates and carbonates.

A short series of "generic" BCFs for metal ions was also estimated by LSER using the dominant solution species, generally the aquo ion. The inorganic ion or molecule was used without a counterion. Most estimations (Be, Cd, Cr, Co, Cu, Hg, Ni, Pb) were at or within an order of magnitude of the reported value. However, solution species for Sn, As, and Se could not be modeled well.

Acute aquatic toxicities of most nonreactive

Table 1. Metal LSER Parameter Values

Ion	V_i/100	π*	β	α
Li^{+1}	0.158	0.05	0.00	0.10
Na^{+1}	0.229	0.00	0.05	0.00
K^{+1}	0.360	0.10	0.06	0.00
Rb^{+1}	0.417	0.07	0.06	0.03
Cs^{+1}	0.533	0.10	0.10	0.03
Be^{+2}	0.144	0.00	0.00	0.00
Mg^{+2}	0.216	0.00	0.00	0.10
Ca^{+2}	0.349	0.00	0.00	0.10
Sr^{+2}	0.406	0.00	0.00	0.10
Ba^{+2}	0.529	0.00	0.00	0.10
B^{+3}	0.131	0.03	0.00	0.40
Al^{+3}	0.202	0.06	0.00	0.20
V^{+5}	0.316	0.05	0.10	0.10
$Cr^{+3/+6}$	0.305	0.05	0.05	0.20
$Mo^{+3/+6}$	0.362	0.10	0.05	0.10
Mn^{+2}	0.294	0.10	0.10	0.00
$Fe^{+2/+3}$	0.283	0.05	0.10	0.05
Co^{+2}	0.272	0.10	0.05	0.35
Ni^{+2}	0.261	0.10	0.05	0.30
Cu^{+2}	0.251	0.10	0.05	0.35
Ag^{+1}	0.307	0.15	0.05	0.25
Zn^{+2}	0.240	0.15	0.00	0.25
Cd^{+2}	0.296	0.20	0.00	0.25
Hg^{+2}	0.319	0.19	0.00	0.55
Tl^{+1}	0.311	0.03	0.00	0.15
Pt^{+2}	0.337	0.15	0.05	0.25
As^{+3}	0.207	0.23	0.13	0.05
Sb^{+3}	0.263	0.35	0.09	0.07
Bi^{+3}	0.294	0.18	0.10	0.08
Sn^{+2}	0.274	0.05	0.03	0.00
Pb^{+2}	0.302	0.00	0.00	0.00
Ce^{+3}	0.507	0.00	0.00	0.20
Yb^{+3}	0.405	0.04	0.03	0.00
Th^{+4}	0.496	0.02	0.02	0.10
U^{+6}	0.479	0.03	0.03	0.10
F^{-1} covalent	0.077	0.08	0.19	0.06
F^{-1} ionic	0.077	0.18	0.29	0.06
Cl^{-1} covalent	0.149	0.35	0.15	0.06
Cl^{-1} ionic	0.149	0.60	0.40	0.06
Br^{-1} covalent	0.185	0.43	0.17	0.05
Br^{-1} ionic	0.185	0.68	0.32	0.05
I^{-1} covalent	0.242	0.45	0.18	0.04
I^{-1} ionic	0.242	0.70	0.33	0.04
ClO_3^{-1}	0.269	0.50	0.40	0.30
ClO_4^{-1}	0.309	0.00	0.40	0.42
BrO_3^{-1}	0.458	0.60	0.30	0.41
BrO_4^{-1}	0.549	0.20	0.57	0.53
IO_3^{-1}	0.515	0.50	0.45	0.40
IO_4^{-1}	0.402	0.25	0.50	0.40
-OH ionic	0.105	0.45	0.50	0.00
-OH covalent	0.105	0.40	0.47	0.33
-SH ionic	0.176	0.25	0.20	0.00
-SH covalent	0.176	0.35	0.16	0.03
=O	0.091	0.34	0.10	0.12
=S	0.162	0.24	0.05	0.05
-O-	0.091	0.27	0.45	0.00
O^{-2} ionic	0.091	0.10	0.15	0.00
S^{-2} covalent	0.162	0.50	0.23	0.00
S^{-2} ionic	0.162	0.10	0.00	0.00
Se^{-2}	0.196	0.20	0.00	0.00
SO_3^{-2}	0.282	0.65	0.82	0.36
SO_4^{-2}	0.322	0.65	0.82	0.00
$S_2O_3^{-2}$	0.433	0.89	0.87	0.41
$-OS(=O)_2OH$	0.336	1.00	0.80	0.75
PO_4^{-3}	0.336	0.45	0.87	0.00
HPO_4^{-2}	0.350	0.95	0.80	0.75
$H_2PO_4^{-1}$	0.364	0.95	0.75	0.75
$(-O)_2(H)P(=O)$	0.310	0.75	0.75	0.00
(-O)(HO)(H)P(=O)	0.325	0.68	0.47	0.33
$P_2O_7^{-4}$	0.581	0.90	1.74	0.00
VO_3^{-1}	0.436	1.07	0.40	0.46
CrO_4^{-2}	0.465	0.80	0.87	0.44
$HCrO_4^{-1}$	0.505	1.52	0.85	0.56
$Cr_2O_7^{-2}$	0.839	1.49	1.15	0.64
MnO_4^{-1}	0.454	1.46	0.50	0.48
WO_4^{-2}	0.531	0.67	1.00	0.36
SeO_4^{-2}	0.356	1.14	0.84	0.24
SeO_3	0.316	0.80	0.74	0.12
AsO_3	0.327	1.25	0.43	0.41
AsO_4	0.367	1.59	0.53	0.53
$HAsO_4$	0.375	1.65	0.90	0.74
$B_4O_7^{-2}$	0.498	1.04	1.54	1.60
NO_2^{-1}	0.184	0.53	0.49	0.00
NO_3^{-1}	0.224	0.50	0.49	0.00
$[S=C=N]^{-1}$	0.282	0.63	0.22	0.00
$[S=N=C]^{-1}$	0.282	0.85	0.42	0.00
-OC(=O)H	0.212	0.62	0.37	0.00
$-OC(=O)CH_3$	0.308	0.65	0.80	0.06
CO_3^{-2}	0.230	0.44	0.55	0.00
-OC(=O)OH	0.252	0.55	0.48	0.55
$[-O_2CCO_2-]^{-2}$	0.345	1.10	0.45	0.24
-C#N covalent	0.171	0.45	0.11	0.22
-C#N ionic	0.171	0.70	0.30	0.22
H_20	0.119	0.45	0.45	0.45
coordinated water	0.068	0.25	0.00	0.55
NH_3	0.146	0.15	0.65	0.00
NH_4^{+1}	0.160	0.00	0.00	0.05

inorganic species were assumed to act by a baseline narcosis mechanism, and were estimated at or within an order of magnitude of the observed value. Using an additional "bioavailable fraction" of the metal in solution greatly improved the prediction accuracy of many species. Outliers in common that were more toxic than estimated for their tested species included cyanides, Pb^{+2} and Be^{+2} salts, $AgNO_3$, $CuSO_4$, and $HgCl_2$, with dominant specific toxicity mechanisms other than baseline narcosis.

DISCUSSION

Reliable property estimation is rarely successful for inorganic species. While acknowledging that this present approach is highly irregular, it appears that LSER can provide a much-needed tool for estimating solubilities for inorganic and organometal compounds, when used in the equation developed for aliphatic compounds. Since the solubility equation was developed for nonionic organic compounds, the use of the whole salt as a neutral species indirectly addresses any question of valence. In view of the lack of error limits on the solubility data used as reference, an arbitrary outer difference limit of +/- 1.3 log unit was used to define a "good fit". Use of the dominant solution species gave an accurate solubility estimation for a large number of salts.

The more difficult species to estimate accurately included the least soluble compounds, aluminates, borates, and some of the organometals. Improved solubility estimation for the difficult classes is likely as solution species are better defined. It is also likely that there is another variable needed to explain the activity of (and so estimate more accurately) the highly insoluble salts.

These inorganic LSER values must apply to all property-predictive LSER equations and to similar processes. Examination of a small series of generic BCFs using the curvilinear relationship (Equation 3) also demonstrated that proper solutionspecies were necessary to estimate bioconcentration. Most of the metal species were best modeled as the aquo ions. For two elements, Cr and Se, several structures were tested for each metal ion. No anionic chromate species was modeled well, but the observed reactivity of these species suggests that the ion would react before it accumulated. A much better estimation was achieved with the $Cr(H_2O)_6^{+3}$ ion. The elements As, Se, and Sn eluded a satisfactory approximation for reasons not apparent.

Acute aquatic toxicity estimations also demonstrated the necessity for defining proper solution species by requiring a "bioavailable fraction" factor. The active toxic species may not be the predominant solution species, and will likely resemble the species successfully transported into the cell, so use of other probable solution species along with the bioavailability factor may give more accurate toxicity approximations.

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