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* To whom correspondence should be addressed.

This issue contains no papers for which there is supplementary material in microform.

DEPARTMENTS

ES&T
LETTERS

Quality control

Dear Sir: I have read Dr. Fogel's letter in the September 1983 issue (p. 394A) in which he comments on my article, "Quality Control in Water Analysis" (*ES&T*, April 1983, p. 174A).

He criticizes me for being careless in omitting a particular reference by Glaser et al. (*ES&T*, December 1981, p. 1426) and indicates that the reviewers were remiss in not insisting on the inclusion of the reference. In fact, I believe neither was the case. I previously commented on Glaser et al.'s paper in a letter published in *ES&T*, August 1982, p. 430A. In it I clearly indicated that I did not agree with the approach of Glaser et al. It would have been inconsistent of me to turn around and reference the article. Also, my article was not a review article. It presented a certain viewpoint, which was well-referenced but not comprehensively referenced like a review article.

<div style="text-align: right">

Cliff J. Kirchmer
505 Lalor Dr.
Manchester, Mo. 63011

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Correction, quality control

With reference to "Quality Control in Water Analysis" (*ES&T*, April 1983, p. 174A), there is an error in Figures 3a, 3b, and 4. The equation $\sigma' = \sqrt{2\sigma_B}$ should read $\sigma' = \sqrt{2} \cdot \sigma_B$. On the last page of the article, ". . . coordinating laboratory and, when all standards are in satisfactory agreement, and . . ." should read ". . . coordinating laboratory and, when all standards are in satisfactory agreement . . ." The final *and* is not needed.

Correction

An incorrect order of authors appears in the August issue for the feature article on tunable diode laser systems (p. 352A). The correct order is: D. R. Hastie, G. I. Mackay, T. Iguchi, B. A. Ridley, and H. I. Schiff. The mix-up in the order of the authors is the error of this managing editor who inadvertently changed the order after the galley had been approved.

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Stan Miller
Managing Editor, *ES&T*

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GUEST EDITORIAL

Multidisciplinary research—an experiment

The National Science Foundation (NSF) is supporting long-term ecological research (LTER) at 11 national sites on a 5–10-year time scale. There are several benefits to the program: 1) interaction among scientists doing related work, 2) confirmation of trends and relationships not apparent with one-year studies, and 3) well-documented and accessible records of background and correlative data. Of these three benefits, the first is of social interest to scientists, and it is perhaps a somewhat controversial subject, known as multidisciplinary research.

For those of us who work in environmental chemistry of water, air, and soil, it is often necessary to have at least a working knowledge of several fields. Obviously, it is not possible to have an in-depth and current understanding of the literature in more than one or two disciplines. Therefore, a program of scientists working jointly, at least to some degree, seems desirable.

However, there are problems associated with multidisciplinary research. The research may be led by the strongest interest group, both scientifically and financially, which may narrow the scope of the overall project. Also, it is sometimes difficult for scientists who are motivated on an individualistic basis to work together and share results, fame, and ultimately major publications. Finally, our scientific reputations are often judged on both the quality and quantity of our publications. Second-author publications in multidisciplinary work may not be highly regarded during peer review, by either universities or government.

There are, however, positive aspects of multidisciplinary research, especially the formulation of different ideas and approaches. As an example, ecologists may view environmental problems conceptually, with hypothesis testing in mind. Chemists, on the other hand, may have an analytical or methodological view of the problem. Both may be valid points of view but different approaches would be used. Another advantage is the help of experts on each aspect of a problem. For example, a study of organic compounds in rivers is an environmental chemistry problem requiring an analytical organic chemist. Yet if a geomorphologist is consulted, new insights into the meaning of the sample, taken at a specific meander of the river, may be added. A stream ecologist may know that one part of the stream is biologically much different than another.

With some of these thoughts in mind, the NSF has given "seed" money on a long-term basis to support 11 national sites with the idea that researchers in environmental sciences would be attracted to the program and would work together. There are sites in Colorado (Niwot Ridge and Pawnee Grasslands), Kansas (Konza Prairie), Oregon (H. J. Andrews Forest), New Mexico (Jornada Desert), South Carolina (North Inlet Estuary), Georgia (Coweeta Forest and Okeefenokee Swamp), Minnesota (Cedar Creek Wetlands), Illinois (Illinois and Mississippi Rivers), and Wisconsin (Northern Lakes). Researchers are encouraged to work cooperatively and to submit other proposals for fundamental research at these sites. It's an interesting idea, and it might be called a national experiment in multidisciplinary research.

Those interested in the program should contact Dr. G. R. Marzolf, Division of Biology, Kansas State University, Manhattan, Kan. 66506. He will supply a publication that describes the national program, objectives, and research projects on air, water, plants, and soil, as well as addresses of coordinating investigators.

E. M. Thurman

E. Michael Thurman is an organic geochemist for the U.S. Geological Survey, Denver, Colo. He has worked on multidisciplinary problems in organic geochemistry, groundwater contamination, and trace-element chemistry.

INTERNATIONAL

A Canadian Supreme Court judge has ruled that a mixture of herbicides 2,4-D and 2,4,5-T can be used safely by a pulp and paper company to kill unwanted weeds in forests on Cape Breton Island, Nova Scotia. Fifteen local landowners had asked the Court to stop the company from spraying the phenoxy herbicides near their homes. Trace amounts of 2,3,7,8-TCDD are contained in 2,4,5-T. In arguing their case, the landowners cited both economics and possible health effects. Some witnesses maintained that the forests would be just as profitable with manual weeding or no weeding at all and that a diversity of species is necessary for healthy growth.

WASHINGTON

The Senate voted 63–33 to place a moratorium on the leasing of federal coal reserves for six months until a special commission and Congress

Watt: *resigned his position*

can review the leasing practices. The week prior to the vote, Interior Secretary James G. Watt defied a House Interior Committee directive by offering leases on 540 million tons of coal in North Dakota. The directive had stated that the offering should be delayed for environmental reasons. The vote marks the first time the Senate has strongly opposed Watt's policies.

Earlier the House had called for a delay in coal leasing. At press time, Watt had resigned his position but planned to remain on the job until a successor could be confirmed.

In mid-October a new regulation went into effect that expands the rights of coal companies to develop strip mines on private property in national parks. It also expands the right to mine in national wildlife refuges, national forests, and other protected areas. The Interior Department's Board of Land Appeals has issued the new rules, which allow leasing of land within national parks to be considered for the first time in 25 years. The regulation says that state officials must decide whether a company may mine in a national park or other federally protected area within a state's boundaries. Extensive private lands within 55 units of the park system are believed to have coal deposits. Applications for oil and gas leases in national wildlife refuges are now being processed.

The EPA and the Occupational Safety and Health Administration (OSHA) are preparing to regulate ethylene dibromide. As early as 1974, studies suggested that it is a powerful carcinogen. OSHA has asked the Office of Management and Budget to approve a reduction from 20 000 ppb to 100 ppb for average 8-h exposure in the workplace. EPA is preparing a report which will probably recommend that virtually all agricultural uses be phased out. Ethylene dibromide has been used as a soil fumigant for nematode control and as a grain and fruit fumigant. It has been found in drinking water supplies from Florida to California and in fresh produce, meat, and dairy products. Animals exposed to this chemical develop cancer in a relatively short period of time.

An electronic catalog that identifies the existence, location, characteris- tics, and availability of environmental data sets has been developed by the National Oceanic and Atmospheric Administration (NOAA). It is known as the National Environmental Data Referral Service. It is expected that the service will eventually become a national network of federal, state, and private organizations that will cooperate to provide access to data to anyone who needs it. The service is located at NOAA's Assessment and Information Services Center, Washington, D.C.

Recent analyses by the U.S. Geological Survey (USGS) of 10 to 15 years of water quality records for 47 headwater streams across the country show that sulfate concentrations have increased over a broad area of the continental U.S., from the Southeast to the mountain states and the Northwest, and decreased slightly in the Northeast. At the same time, acidity has decreased slightly in the Northeast, while increasing in most other regions. This pattern of changes is similar to the trends in sulfur emissions to the atmosphere during the same period. The water quality data were collected on streams that are relatively unaffected by land use. According to USGS scientists, "the results seem to indicate that the effects of relatively small changes in atmospheric emissions are observable as changes in stream quality."

STATES

The EPA has released a seven-year study showing that the Chesapeake Bay is "clearly an ecosystem in decline." It states that the blue crab yield has been decreasing since 1970 while catches of freshwater fish such as striped bass and shad have dropped by more than 50%. The ecological decline of the bay is generally attributed to runoff of nitrogen, phosphorus, heavy metals,

and toxic organic compounds from surrounding areas. The report recommends more careful land planning, extra sewage treatment facilities, new controls on runoff from paved surfaces and construction sites, and new curbs on phosphate detergents. The General Accounting Office also released a related report which states that the provisions of the Chesapeake Bay Research Coordination Act of 1980 have not been carried out.

For the first time, a state agency has mandated that scrubbers be installed on an older power plant when it is converted from oil to coal. The New York State Department of Environmental Conservation has ruled that Con Edison must install scrubbers on three of its units proposed for conversion. The ruling was made specifically to hold to a minimum the presumed major cause of acid rain—sulfur dioxide emissions. Without scrubbers, conversion would have caused SO_2 emissions to increase from 10 000 to 76 000 tons/y.

Work to restore the Kissimmee River in Florida to its original 92-mile path is beginning. This will be the first attempt to restore a major river in the U.S. to its original channel. In the 1960s the river, which empties into a major source of freshwater, Lake Okeechobee, was changed into a straight 54-mile canal. This drained 32 000 acres of wetlands. It is hoped that restoring the river will improve the wildlife population that was depleted when the canal was built and improve water quality in Lake Okeechobee. The estimated cost of the project is between $75 and $200 million.

The temporary suspension of the use of TEMIK aldicarb pesticide imposed by the state of Florida in January 1983 will be lifted in January 1984. A committee of scientists established by state Agriculture Commissioner Doyle Conner decided that TEMIK residues degrade under Florida's environmental conditions, and when used properly, pose no threat to the state's drinking water. The suspension was imposed after residues were discovered in drinking water wells at several agricultural locations. During the past year, 900 wells were tested statewide; three were found to have low levels of aldicarb residues.

Each of these wells was found to be defective in construction.

AWARDS

The Gordon Maskew Fair Award of the American Academy of Environmental Engineers was presented to Arthur C. Stern, Professor Emeritus at the University of North Carolina (Chapel Hill). This award is
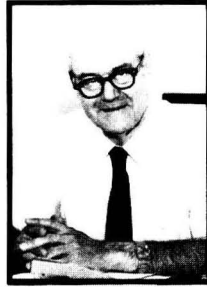
Arthur Stern: winner of Fair award

given in recognition of "achievements, leadership, and contributions to the total environmental effort." Upon receiving his award, Stern observed that he was "the second awardee . . . who did not work in the fields of water supply, sewage treatment, or solid waste disposal, in which Gordon Fair was a revered leader." Stern is widely recognized as a principal authority in the air pollution field.

SCIENCE

Will drinking-water trihalomethanes (THMs) become a thing of the past? Robert Hoehn and his colleagues at the Virginia Water Resources Research Center (Blacksburg) point out that not only humic acids, but other organic materials in water may act as THM precursors. They refer to laboratory tests which have shown that optimizing clarification processes can reduce THM precursors by as much as 75%. Hoehn hopes to use the Newport News, Va., water works as a demonstration project. He believes that proper planning and new technologies will one day help to make THMs "a buzzword of the past."

A four-year scientific study has revealed that meadow voles (field mice) living near the Love Canal toxic dump site have shortened lifespans and organ damage. John J.

Christian, professor at the State University of New York at Binghamton, who headed the research team, said that the fatty tissue of the voles living closest to the canal had large amounts of the pesticide lindane and smaller amounts of other halogenated hydrocarbons. Signs of liver damage that generally appear when the organ is exposed to a wide variety of toxic compounds were also found in the voles living near the dump site. The voles living near the inner ring (the area evacuated in 1978) had a life expectancy only half that of voles living in the control area.

A recent article in _Science_ magazine states that Americans consume 10 000 times more natural mutagens and carcinogens in their daily diet than man-made pesticides. Author Bruce N. Ames, chairman of the Department of Biochemistry at the University of California at Berkeley, also found that foods contain a range of cancer-preventing chemicals or "anti-carcinogens." He predicted that in a few decades it will be possible to "fine-tune" diets to avoid many causes of cancer. He said that a variety of vegetables contain "natural pesticides," chemicals the plants make to protect themselves against insects, fungi, and animals, and that the intake of these natural pesticides is probably several grams a day.

A colorless alga called _Prototheca_ can pollute water and infect humans and animals, say scientists at the National Animal Disease Center (Ames, Iowa). _Prototheca_ seems to be a colorless mutant of a green alga. It can barely survive in pure water, but it thrives on sewage, animal wastes, and tree wound drainage and is incapable of photosynthesis. It is the only alga known to cause disease in mammals. For example, it has been shown to cause mastitis in dairy cows. In humans, _Prototheca_ apparently can cause skin infections or infections secondary to other disease (20 cases). The alga does not survive more than 15 s in milk heated to 143 °F.

TECHNOLOGY

A new automated mutagenicity testing system uses specially designed plastic disposables, ready-to-use reagent kits, and a microcomputer-

controlled analyzer. The system was developed by Labsystems Oy (Helsinki, Finland). Test samples, bacterial strains, and metabolic factors are automatically dispensed according to preprogrammed instructions. Bacteria mutate and grow in a liquid medium. Growth patterns are measured by vertical pathway photography and are reported and stored by the microcomputer. Labsystems says that the system "produces answers in 24 h as compared with two weeks." Lab personnel are protected from exposure to test materials.

Five isobutylamide insect growth inhibitors and toxicants were recently isolated from the East African medicinal tree *Fagara macrophylla* by Isao Kubo of the University of California (Berkeley) and his associates. This tree, a member of the rue family, is relatively free from insect attack. To test this resistance, *F. macrophylla* bark extract was administered to pests such as the pink bollworm, tobacco budworm, corn earworm, and fall armyworm. The scientists were able to purify and identify the inhibitors and obtain values for effective doses for 50% growth inhibition (ED_{50}). One compound, pelloritine, was lethal against the pink bollworm.

An "important milestone" in solar photovoltaic energy was the attainment of nearly 5% efficiency for large-area amorphous silicon cells. Actually, 10.1% efficiency had been achieved last year, but the cell's area was only 1 cm^2. The cell submodule areas in this case were 127 and 350 cm^2. The submodules consisted of cells connected in series. One advantage of amorphous

solar cells is the possibility of mass-producing them at much lower cost than that of present-day single-crystal photovoltaic cells. Also cell thickness is 1 μm, rather than the 100-250 μm of single-crystal cells. RCA and Chronar Corporation developed these amorphous cell systems for the Solar Energy Research Institute (Golden, Colo.).

Determining the immediate presence of toxic chemicals in drinking water may soon be possible with a system now being completed at Gulf South Research Institute (GSRI, Baton Rouge, La.). The system will be able to monitor toxic chemicals in water on a continuous basis, which is "unprecedented" according to GSRI. Determination of these substances could be "virtually instantaneous," says James Clinton, GSRI vice-president. The key is a new type of microcalorimeter developed by Rex Lovrien of the University of Minnesota. Normally toxicity tests take 2 days; they would still be needed, but this system would point out when longer, more detailed tests are required.

INDUSTRY

A test program for methanol-fueled cars has been "kicked off" by Celanese Chemical Company, Ford Motor Company, and The Southland Corporation. Celanese and Southland will test four Ford cars in various day-to-day business and community volunteer activities. Methanol can be made from natural gas, coal, and biomass. John Lauer, vice-president of marketing for Celanese Chemical points out that "compared to gasoline or diesel, methanol is clean-burning. Sulfur emissions are eliminated, ni-

trous oxides are reduced, and ozone formation is decreased." One aim is to lessen dependence on petroleum-based fuels for transportation.

Third-party financing of solar energy systems was the topic of a conference sponsored by the Tennessee Valley Authority (TVA) and held in Nashville, Tenn., in September. David Burrows of TVA described third-party financing as "a way for businesses and industries to obtain lower-cost energy through energy lease or purchase arrangements with third-party investors." A conference aim was to show businesses how to set up third-party financing to reduce expenses for energy with essentially no capital outlay. Another was to impart information about solar investments, tax credits, and service agreements to financial and legal advisors.

A $1.5-million contract to monitor and analyze air quality in and around U.S. Air Force (USAF) and other federal facilities was awarded to Radian Corporation (Austin, Tex.). The USAF awarded the three-year contract. The task is to measure pollutants in ambient air near USAF facilities and to determine how much and what kinds of air pollutants are being released by various sources within the facilities. Field studies, laboratory analyses, and mathematical models will comprise major portions of the work. This is in keeping with the Clean Air Act, which requires federal as well as private facilities to comply with all standards.

Proposed effluent guidelines for the organic chemical industry are "seriously flawed," according to the Chemical Manufacturers Association (CMA, Washington, D.C.). CMA said the same about guidelines for the plastics and synthetic fibers industries. Geraldine Cox, technical director of CMA, said that EPA "sampled too few plants over too short a period of time, and did not take into account the uncertainty of sample analysis at low concentration." She added that "with an untested model," EPA "underestimated the success already achieved in removing toxic pollutants." Cox called for more rigorous analytical procedures and emphasis on toxicants that occur most frequently.

Amorphous solar cell system

Amorphous silicon

Metal

Transparent conducting electrode

Glass
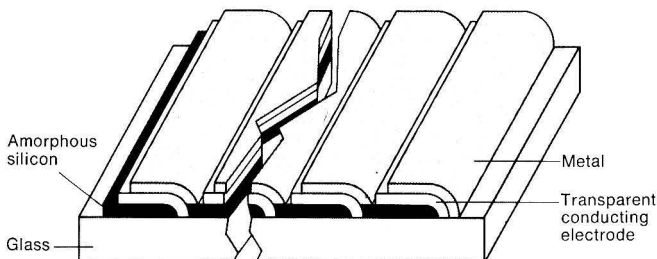
Source: Solar Energy Research Institute

Water research in Norway

An interview with Lars Overrein, the director of the Norwegian Institute of Water Research

Two and a half years ago, Lars Overrein became director of the Norwegian Institute of Water Research (NIVA), which is celebrating its 25th anniversary this year. The changes in management that Overrein has introduced have gained recognition here and abroad. This spring, he was awarded a European prize in environmental protection by the Johann Wolfgang von Goethe Foundation in Switzerland. Based on the judgment of an international jury, this prize is seen as personal recognition of a high order. More important, it recognizes the work Norway did in acid deposition and the fact that earlier in his career, Overrein managed the acid deposition study (1972–80), the largest program of this type ever conducted in Norway. In April, he was called upon to present a keynote speech to the European Parliament on the issue of acid deposition. His actual address was given in Geneva in June at the high-level conference on acidification of the environment, attended by representatives from 34 countries, including the U.S. and Canada.

The organization

ES&T. What is it that NIVA is doing today that it was not doing earlier? What are the changes at NIVA?

Overrein. Before I came, the former director and the board of directors responsible for NIVA were fully aware of the fact that certain changes had to

be initiated. They certainly supported me in the need for change, not simply change for the sake of change, but to demonstrate that the institute means business. We decided to have a much higher turnover of personnel within the institute. As part of that reorganization, all central positions at our organization are now three- or five-year appointments.

I think that any leader, including the director of NIVA, should be judged first and foremost on the basis of how he or she is able to groom younger people for bigger tasks. You can't ap-

point a leader; you have to develop one. What were perhaps good ideas in the seventies are by no means good enough in the eighties, nor will they be good enough in the nineties. We always have to be in the process of change, reviving, realigning, and doing things differently. In my way of thinking, we have a long way to go.

One day, I would like to see everybody at our institute become a leader. I find something very meaningful in that goal. When you start to dig into management practices, it is the way it must go.

ES&T. How large is NIVA? How many scientists are involved? Do you have an exchange with foreign scientists?

Overrein. NIVA is a national institute, located at the outskirt of Oslo, the capital city of Norway, that deals with water-related problems all over the country. It is important to note that as an institute NIVA is an independent, nonprofit organization of 200 people. Half are professionals, from ecologists to civil engineers; the other half are technicians and administrative personnel.

NIVA has a very wide roster of international contacts. We always have some of our scientists in other countries and other institutions. At the present time, we have top scientists from the U.S., Canada, Great Britain, Germany, and Sweden working with us. We are very pleased to say that we have more requests from people abroad wanting to come to our institute on their sabbatical leaves than we would

be able to handle.

ES&T. What is the relationship of NIVA to the Norwegian government?

Overrein. NIVA receives a quarter of its funds from the Department of the Environment. The other 75% comes from contract research through official agencies and industry—in many cases, worldwide industry. As a nation, Norway has decided to become an oil-producing country, and some of the work at the institute is steadily increasing in cooperation with international oil companies.

New directions

ES&T. What are the changes that have been instituted at NIVA since you became director?

Overrein. We have established two regional research stations; in a couple of years we would like to have four. Over the past two years, we have paid more and more attention to the fact that Norway is moving suddenly and quite rapidly in the direction of becoming an oil-producing country. This means that our institute has many challenges ahead, particularly with respect to ocean systems and the fjords.

Another aspect involves presenting NIVA as a service organization that can carry out research in developing countries. Although we have been involved in research in developing countries for more than 10 years, we are now paying more and more attention to that part of our activity.

ES&T. What are some other directions that have been taken since you became director?

Overrein. NIVA is becoming more businesslike. Efficiency is something that most research institutes hate to hear about. If you are involved in basic research, you have to react in a different way than if you are in contract research. Even if you are involved in basic research, for example, in European society, you have to understand that you have to learn efficiency, even in the process of deciding what projects to pursue.

NIVA has constructed and developed its new marine research station near Oslo. The station involves the University of Oslo along with British Petroleum, one of the biggest oil companies in Norway. Our research institute has much to learn from large companies like oil companies. We are trying to get financial support for our studies, but equally important is a transfer of knowledge from those companies into our limited reserve of resources.

We are orienting NIVA toward the international market, not only a market in which we want to compete but more importantly, a market where we are cooperating with other institutions. For example, NIVA has several research groups in the U.S. working with our people in Norway. Moreover, research is conducted in Canada by

> **It is of utmost importance that an institute in a small country work at an international level.**

NIVA. There is also research that the Canadian government has asked NIVA to do in Norway, and we are doing it for them. We have also had contracts with the U.S. EPA for many years in different areas of research.

It is of utmost importance that an institute in a small country work at an international level. This is the only way that NIVA can keep fresh, get the necessary exchange of young scientists, and it is a way to contribute to society at large.

ES&T. Tell us about the new marine research station.

Overrein. I believe in small steering groups, small boards, few people, efficiency. For the marine research station we have a three-member steering

committee. Further, we have appointed an advisory board of five people. This board includes only one representative from Norway. The others are representatives from the U.K., the U.S., Bermuda, and Canada. In this way, domestic problems do not consume the discussion.

This board has worked well. There is a great deal of mutual trust among its members. A few years ago, this station nearly came to a stop and was very close to being given up because of lack of financial support and lack of personnel. But now that has changed. Construction was only completed about four months ago.

Acid deposition

ES&T. What can you tell us of the acid deposition activity in Norway?

Overrein. Acid deposition effects on forests and fish turned out to be the largest research project of this type ever conducted in Norway. It involved the cooperation of 16 institutions and 155 scientists. I was director of this national research program from 1972–80, when the project was finished. The project was decided by the Norwegian Parliament and also funded by the Parliament, in cooperation with two of the Research Councils of Norway.

Norway is very badly hit by acid deposition. The effects have been described in many publications. Here, the aquatic effects are the most striking. Acidification of thousands and thousands of lakes and rivers has taken place. The recent acidification in freshwaters in parts of Europe and eastern North America has had profound impacts on aquatic life.

How different countries look at the acid deposition problem has changed drastically. The Scandinavian countries were certainly first on the stage. Very few people were involved with the problem until the mid-1970s. It was not until 1977 that long-range transport of air pollutants in Europe was a scientifically proven fact.

Let us jump very fast to 1983. After a week of meetings and discussion, the high-level conference in Geneva in June concluded that acid deposition is indeed a very serious international environmental problem.

NIVA has put high priority on acid deposition as a topic for years to come. The subject is ready for an international project, and I hope that NIVA will be the lead organization or the main research component to coordinate the international project. I do not want to get into the dispute between the U.S. and Canada. The new thing in

Europe is that for the first time, the damage to the forest area in the middle part of Europe, particularly in Germany, is being documented. But it will take time before all the details are clarified and publicized.

Management

ES&T. Where did the plan to change the management structure at NIVA come from?

Overrein. First, it was introduced in a very special way. I obtained the necessary guarantees from the unions and then I met with the employees in a plenary session. I told them that I was going to bring about a big change, but I did not give them details. I asked the employees as well as the board of directors if they were willing to give me absolute freedom in appointing the top layer of people at the institute. All said yes, go ahead.

Then I told everybody at NIVA that they would be given from three- to five-year contracts, as a maximum. It was clear to me that personnel at the institute needed to be renewed every so often. When I became director on July 1, 1981, many of the top leaders at the institute had been in their jobs for a long time. Obviously I didn't ask them to quit, but to act in their positions until the new organizational system was in place. I tried to act in a very open way so that everybody knew that I was going to propose something quite different.

I had the opportunity and keen interest in getting some professional training in management. As a matter of fact, I had been taking courses in your country. Incidentally, I also consider it mine. I am Norwegian and received my degree from the University of Oslo. Then I came to the U.S. for graduate studies and received degrees from Cornell University and Purdue University, and then taught at the University of California for more than two years. Then I was called upon by research organizations in Norway to come back. I have also been very interested, as a student, in the developments in Japan over the last 10 years. With extreme interest I also read what the new director of Scandinavian Airlines, SAS, accomplished and how he did it. Some ideas introduced at SAS are related to the ideas built into the NIVA system. As other people have phrased the question, "Why be a chief for a lifetime?"

ES&T. When you have a contract with someone for five years, do you go through an annual performance evaluation of that person, or does that get to be of no importance?

Overrein. People have said to me, "OK, you have managed to get these reviews out of the way. How in the world are you able to keep going? How do you find out whether you want to have this or that person to continue in the function?" I don't think it is a problem at all. It should never be allowed to develop into a problem. You have to realize that it is an extremely important part of a top director's job to keep in touch with the people acting around him. This allows me more time for thinking, watching, and planning. But the formal responsibility for judging people should be at the top executive level.

At NIVA there are decentralized responsibilities. Scientifically speak-

As other people have phrased the question [on management structure], "Why be a chief for a lifetime?"

ing, each person is actually responsible for his or her own activities and production. We need to keep the process of organization ongoing. No organizational structure should become static; it must be dynamic. The important thing in our institute today is that the main lines within the organization structure are quite firm. After

a short period, the idea of evaluating scientists at three- and five-year intervals was adopted at NIVA. The review process has been extended to cover central secretaries. All top scientists and secretaries are on five-year terms. However, we might change any position rather quickly when business comes up. Then we discuss with the person what is best for him or her and the organization.

ES&T. Do you have a monthly meeting with these top leaders or is your door open all the time?

Overrein. Physically speaking, I have a closed door. Everyone knows that the door is open. I have not gone through the whole institute yet. I have done this change in such a way that I do not call each worker into my office; I will come and meet him where he is working, if he pleases. If he likes it the opposite way, he or she can come to my office. This is each person's half hour. I am going to listen. If he or she is there to tell me things of a more personal matter, it's up to the person. There will be no limit on whatever they have to discuss. But as a top leader at NIVA, I must say that the most important results you have are the inquiries to the institute, and the second is the production package, the results that are delivered.

ES&T. How do you persuade university professors who have become tenured to consider career changes perhaps as often as every five years?

Overrein. Society in general will gradually change its attitudes. When this happens, people may think from the very beginning about the day when they will have to move to another job. Personally, I feel that it is a difficult time in this respect in many countries and institutions. When people are asked to move, they think they are going down the drain. With younger people you can get these ideas across easier. Over a time the younger scientists will create a necessary change in attitude in the society.

ES&T. When you, Mr. Overrein, some years hence tell your wife and your family that you are not going to be the director any longer, how will you face the situation?

Overrein. I will tell them that I have had my turn, and they should not feel that it will be the end of my life. I should have support from society. Someone else more fit than I will take the position. Today, people don't even think about a change like this at most research institutions and universities. But this change has to start in the educational system. It has to start with the young scientists.

Subsurface organic contaminants

Scientists are studying their chemical and biochemical behavior, fate, and transport underground—all aimed at developing a sound basis for regulations for protecting groundwater

In a 1904 decision concerning the ownership of underground water resources, the Texas Supreme Court characterized "... the existence, origin, movement, and course of such waters, and the causes which govern their movements" as "secret, occult, and concealed." During the 80 years since that ruling scientific and technical knowledge has reduced the secrecy, occultness, and concealed nature of groundwater materially.

What remain far less known are the fate, transport, and transformation of contaminants—especially organics—in or on their way to groundwater. For instance, do they react with the water or with the media through which it travels? To what extent, if any, are such organic contaminants adsorbed or changed on their way through the unsaturated zone to the aquifer, and by what means? How does one properly sample, extract, identify, and quantify trace amounts of these contaminants in a manner that can lead to a valid picture of their underground movement and behavior?

Answers to these questions are needed if a workable regulatory scheme to protect groundwater is to be devised. Laws such as the Resource Conservation and Recovery Act (RCRA) and the Safe Drinking Water Act (SDWA) require this same type of regulation. Discussion of experimental efforts aimed at providing data to help find these answers comprised a major portion of the deliberations of the Symposium on the Behavior of Organic Contaminants in the Subsurface Environment. The symposium was a part of the American Chemical Society's 186th Annual Meeting held in Washington, D.C., at the end of August. Coordinated by Paul Roberts of Stanford University (Calif.), the symposium was designed to draw together researchers active in investigating each of the processes thought to
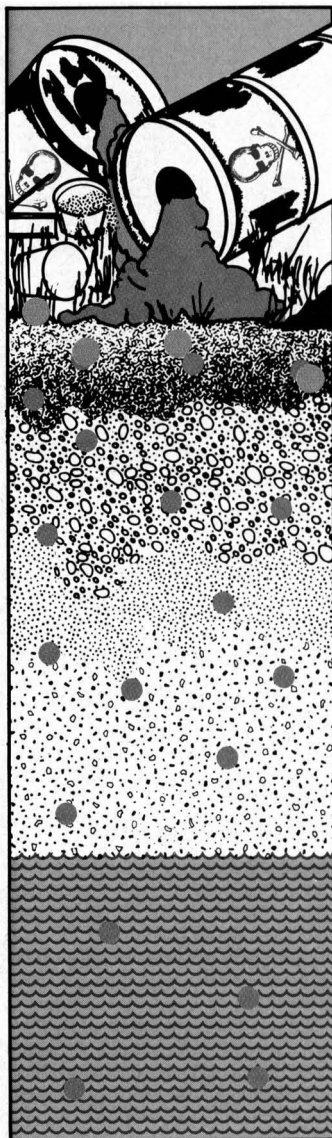
affect subsurface contaminant transport, as well as those concerned with validating the processes' effects in the field and those attempting to develop mathematical means to represent this knowledge in predictive transport models.

Sorption

One subsurface contaminant of concern is trichloroethylene (TCE); its presence in groundwater has led to closures of water supply wells on Long Island (N.Y.) and in Massachusetts. Richard Richter of Boyle Engineering Corporation (Newport Beach, Calif.) observed that these contaminants entered groundwater partly because of "industrial misuse and improper disposal." Yet the fate and transport of contaminants such as TCE are thought to depend on the sorptive nature of the soils, the geochemical conditions that may control biological or chemical transformation, and hydrodynamic characteristics of the aquifer.

Where conditions do not favor transformation, sorption of the contaminant by the soil may be the dominant process. While still at Washington State University (Pullman), Richter evaluated the strength of sorption of TCE, 1,1,1-trichloroethane, and 1,1-dichloroethane on soil constituents such as hydrous manganese oxide ($Mn_xO_yH_z$), bentonite clay, organic peat, and the fine fraction of glacial till. He performed a series of standard batch sorption experiments with initial solute concentrations of 5–200 $\mu g/L$.

Richter's work corroborates previous findings that the capacity of an organic compound to adsorb and the intensity of its adsorption vary *directly* with the octanol/water partition coefficient ($K_{O/W}$), and *inversely* with its solubility in water. He cautioned that maximum sorptive capacities were in the $\mu g/g$ ranges at equilibrium

concentrations of 100 $\mu g/L$. Still, Richter reaffirmed that soils—particularly those with appreciable organic matter content—could influence transport of organic contaminants and that where organic soil content is low, "as in many aquifers," certain mineral solids themselves may exert an important sorption effect.

Groundwater transport

A field investigation of halogenated organic solutes is being conducted by Douglas Mackay and his associates at Stanford University and the University of Waterloo (Ontario, Canada). In August 1982, they injected 12 m³ of an aqueous solution of several halogenated organics into a relatively uncontaminated portion of an unconfined sand aquifer in Ontario (above an existing landfill leachate plume). The injection concentrations were so chosen as to allow detection of solute plumes for a period of more than 1 y by ion and gas chromatographic techniques.

To monitor the solute plumes as they migrate, because of the natural groundwater flow, Mackay's team uses a dense three-dimensional network of sampling wells. Horizontal spacings are 0.5–3.0 m, and vertical spacings are 0.2–0.3 m. The network follows the direction of the natural gradient (x-axis) about 40 m; it is 15–20 m wide (y-axis) and covers an interval of 1.5–6.0 m below the ground surface (z-axis). As of September, the team had analyzed over 9000 samples, using specially designed devices to ensure sample integrity. Most samples were taken in ten 2–3-day "sessions," distributed over the year, to obtain three-dimensional "snapshots" of solute distribution.

These snapshots provided quantitative data on the size, shape, and location of solute plumes. This included estimates of total dissolved mass, location of the center of mass, and other pertinent information for each solute. Quantitative descriptions of plume advective and dispersive characteristics are in the process of being developed from such data.

Sampling at a rate of 1–5 times/wk is being conducted at several well locations. This information is used to construct "breakthrough curves" for each solute (Figure 1), which clearly illustrate the varied mobility of different solutes underground. Furthermore, Mackay and his colleagues suggest that the mobility observed in the field for three of the organic solutes is in good agreement with that expected when one assumes that sorption

FIGURE 1
Subsurface movement of some organic contaminants
Solutes used in injection experiment[a] ...

Solute	Concentration, $\mu g/L$[b]
Chloride	8.92×10^5
Bromide	3.24×10^5
Carbon tetrachloride	31
Bromoform	35
Tetrachloroethylene	30
o-Dichlorobenzene	337
Hexachloroethane	20

... and their breakthrough curves[c]
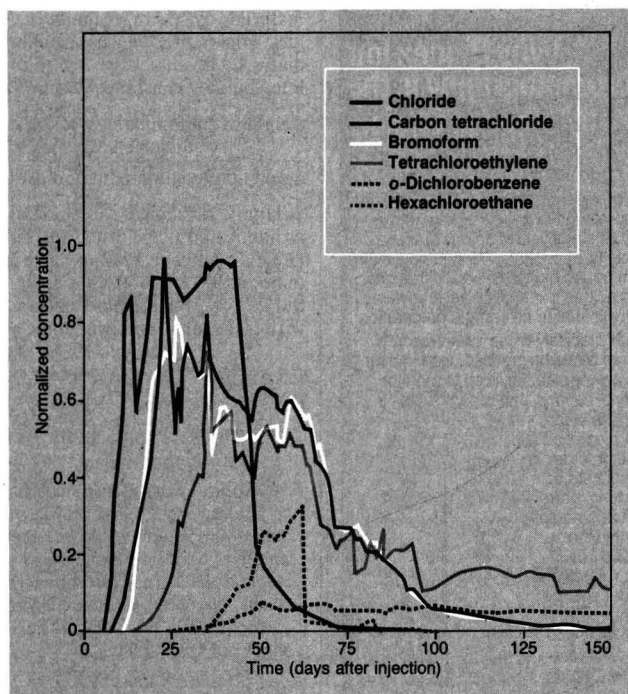
[a] Total volume injected = 12 m³.
[b] Injected concentrations.
[c] At a point 2.5 m downgradient.
Note: Bulk density of aquifer material is taken as 1.76×10^6 g/m³, porosity or void fraction as 0.38, and carbon content of aquifer solids as 0.075–0.2%; values are taken from the literature or from analysis.
Source: Symposium presentation by Mackay et al.

How sorption strength differs for three selected contaminants [a]

Contaminant	Ko/w	Solubility (mg/L)	Relative sorption strength
TCE	195	800	Strongest
1,1,1-trichloroethane	148	1350	
1,1-dichloroethane	62	4000	Weakest

[a] With regard to soil sorptive capacity, peat > bentonite > glacial till > $Mn_xO_yH_z$.
Source: Symposium presentation by Richard Richter

by the organic matter in the soil is the dominant controlling factor.

Effects of humic materials

Since groundwater and soil are normally in close contact, humic substances in the soil could affect the subsurface aqueous behavior of organic contaminants. Charles Carter of Versar Inc. (Springfield, Va.) and Irwin Suffet of Drexel University (Philadelphia, Pa.) have quantitatively measured the binding of nonpolar organic compounds to dissolved humic materials by several techniques, "the most important of which is equilibrium dialysis." Results were used to calculate "binding constants" defined as:

$$\frac{\text{weight of contaminant/g of dissolved organic carbon}}{\text{weight of contaminant/g of water}}$$

Humic substances can be classified as humic and fulvic acids, and humin (*ES&T*, January 1982, p. 20A; September 1982, p. 510A). Carter and Suffet tested DDT and anthracene with three fulvic and two humic acids

from different surface waters and soils. It turned out that binding to humic acids was much stronger than binding to fulvic acids; indeed, for one fulvic acid, the binding constant to the latter was zero for both DDT and anthracene. Moreover, there are large differences in binding constants among humic and fulvic acids from different sources.

It also appeared that the logarithm of $K_{O/W}$ of selected organics varied *directly* with the logarithm of the binding constant. Thus, for example, lindane and fluorene, with lower log $K_{O/W}$'s, showed lower log binding constants, whereas DDT and di-2-ethylhexyl phthalate, with higher log $K_{O/W}$'s, had higher log binding constants.

Although unable to attend the symposium, Shahamat Khan of Agriculture Canada suggested in his submitted abstract that "substantial evidence exists to indicate that some pesticides can form bound residues with humic substances" and that "such binding may greatly increase the persistence of these compounds in soil and aquatic environments." He added that "conventional analytical methods may not detect these types of residues; thus, the soil burden of total pesticide residues might be underestimated." To document his hypothesis, Khan tested ^{14}C-"labeled" 2-(methylthio)-4,6-*bis*-(isopropylamino)-*s*-triazine, also known as Prometryn.

Khan found that proportions of the binding of the labeled Prometryn to humin, humic acid, and fulvic acid were 57%, 11%, and 26%, respectively. To obtain data on the nature of the pesticide binding, he used thermoanalytical methods (250–400 °C), at which most of the ^{14}C was released. Thermogravimetric methods (200–400 °C) helped to release bound pesticide residues from humic material matrices for subsequent determination. Khan also found that while ultraviolet (UV) light fully decomposed Prometryn in water in about 3 h, UV light broke down humic-acid- and humin-bound Prometryn residues very poorly (6% and 1%, respectively). He queried whether the binding of certain pesticide residues to humic substances might inhibit photolytic degradation of such contaminants.

Other reactions

It is possible for certain halogenated hydrocarbons to be transformed into volatile organosulfur compounds if conditions are right. An example of favorable conditions would be an aquifer with an anaerobic "atmosphere."

"Educts" and "products" of groundwater contamination[a]

"Educts"

R—CH₂—Br

$\begin{matrix} R \\ R' \end{matrix}$CH—Br

Br—CH₂—CH₂—CH₂—Cl

Br—CH₂—CH₂—CH₂—CH₂—Br

(R, R', R" = H, CH₃, C₂H₅, C₃H₇, C₄H₉)

"Products"

R—CH₂—S—CH₂—R'

$\begin{matrix} R \\ R' \end{matrix}$CH—S—CH₂—R"

R—CH₂—S—CH₂—CH₂—CH₂—Cl

(Cl CH₂CH₂CH₂)₂S

CH₂$\overset{CH_2 - CH_2}{\underset{S\ -\ S}{}}$CH₂
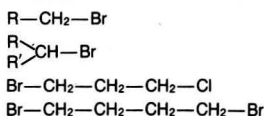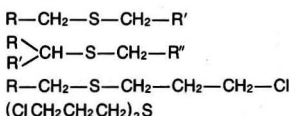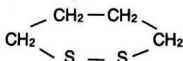
... and a suggested reaction scheme[b]

Determined in the laboratory for R = R' = C₅H₁₁

$k_h = 1.96 \times 10^{-7}\ s^{-1}$ (pH = 8)
⟶ $t_{1/2}$ (hydrolysis) ≈ 40 d
$k_{HS^-} = 8.56 \times 10^{-4}\ Lmol^{-1}\ s^{-1}$
$k_{RS^-} = 8.06 \times 10^{-3}\ Lmol^{-1}\ s^{-1}$
$pK_{RSH} = 9.44$ (T = 25 °C)
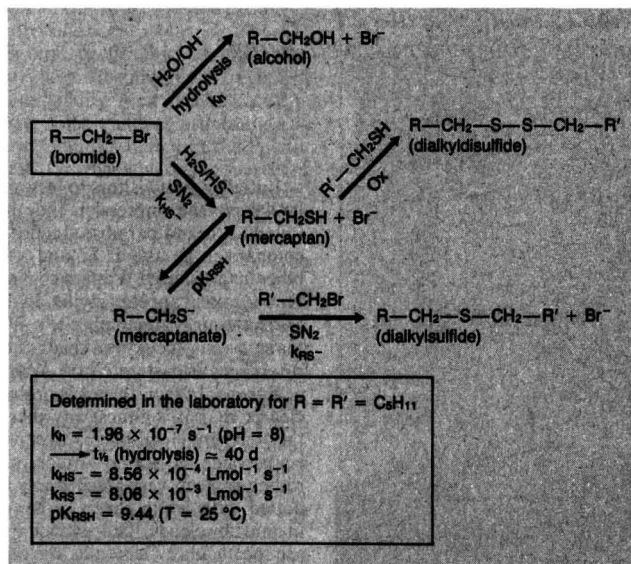
[a] Contamination with halogenated hydrocarbons.
[b] For reactions involving primary alkyl bromides.
Source: Presentation by René Schwarzenbach et al. of EAWAG, Dübendorf, Switzerland.

René Schwarzenbach and his colleagues at the Swiss Federal Institute for Water Resources and Water Pollution Control (EAWAG, Dübendorf, Switzerland) showed that certain brominated and bromochlorinated alkanes may undergo nucleophilic substitution reactions with hydrogen sulfide (H₂S) to form organosulfur products. Schwarzenbach named the halogenated compounds introduced into the ground "educts" and noted that they had been discharged over a period of many years from an industrial plant that is now closed.

In his laboratory experiments, Schwarzenbach used 1-bromohexane in water in the presence of H₂S. Simulating "natural" underground anaerobic conditions of pH = 8 and $[H_2S]_{tot} \approx 5 \times 10^{-5}$ M, he was able to demonstrate the chemical formation of sulfur-containing organics (Figure 2). Schwarzenbach pointed out that typical groundwater temperatures of 10 °C could cause such nucleophilic reactions and that at these lowered temperatures, "such nucleophilic reactions may well compete with hydrolysis," the more normal underground water reaction of many alkyl halides.

Other underground reactions of organic contaminants could include transformation or mineralization by microorganisms. These and other subsurface chemical and biochemical mechanisms are now beginning to come to light. Progress in this field is being made; nevertheless, much more work is needed to develop a sound scientific basis for groundwater protection regulatory activity.

—Julian Josephson

New Zealand at a crossroads

Policy choices being considered now
may determine its environmental well-being

Wanganui River. *There is a movement in New Zealand to reserve the northern part of this wild and scenic river as a national park.*

Initially, most visitors to New Zealand have the impression they have found an island paradise. Indeed, by comparison to the U.S. and many other industrialized Western countries, New Zealand appears idyllic. In most areas, the air is absolutely clear. When the sun is out, the sky is a shade of blue rarely seen in eastern North America or Europe. In fact, the air is probably clearer than it is almost anywhere in the Northern Hemisphere. Most of the countryside is scenic—much of it rolling or steep, very green grassy hills dotted with white sheep. When not mantled by clouds, the high mountains are spectacular. Generally, the coastline is clean and unmarred. Some of the world's most beautiful beaches seem to be enjoyed by no living creatures except birds, or sheep that graze on the slopes above the sand and surf. The visitor may come to believe that New Zealand is a country that has found an ideal way to protect its environment.

Accidental preservation

It is true that New Zealand has taken active steps to maintain environmental quality. Since World War II, the government has changed many policies, established certain institutions, and enacted legislation for the preservation of the environment. Compared to other countries, New Zealand has set aside a large portion of its land (18%) as national or forest parks. But the fact that the environment appears rather well protected is in large part an incidental result of economic and geographical factors and immigration policy mostly unrelated to the environment, rather than active efforts aimed at environmental protection.

One major element that promotes environmental preservation is the low population density. New Zealand has 3.13 million people living in an area of 269 060 km². This gives it a population density of less than 12 persons/km² compared to 23/km² in the U.S., 229/km² in the United Kingdom, and 309/km² in Japan. In addition, New Zealand's small population is concentrated mostly in the northern third of the North Island and a few other cities, while the remainder of the population is spread thinly over vast areas of the countryside.

Several geographical factors also contribute substantially to environmental quality. New Zealand consists of two major islands (North Island and South Island) situated in the Pacific Ocean, far from any foreign sources of pollution. The closest outside source, Australia, is more than 1000 miles away. In addition, New Zealand lies in the path of wet westerly winds (the "roaring forties"). That, together with the mountainous terrain, causes relatively high and generally well distributed reliable rainfall, averaging 25–60 in. annually over most of the land area. The winds across the narrow islands carry much of the air pollution out to sea. Frequent rainfalls also cleanse the air and cause the rivers to carry high volumes of water. Except in the worst cases, this substantially dilutes any contaminants that enter.

The main source of electricity generation in New Zealand, hydropower, also helps greatly in maintaining air and water quality. Seventy-five percent of this power is produced by hydroelectric dams. Although these have certain drawbacks, they create no air pollution and little water pollution compared to coal- and oil-fired generators. Because of the abundance of dependable, swiftly flowing rivers, hydropower was the natural economic choice for power generation.

Another historical accident contributing to environmental quality is that the New Zealand economy depends heavily on primary industries—farming, fishing, and forestry. Fish, wood products, and agricultural commodities comprise 72% of its exports in contrast to 15% in most industrialized nations. Compared to many others, these industries create little obvious pollution. The large, heavy industrial facilities that might produce air contaminants or hazardous waste can almost be counted on one's fingers—two steel mills, an aluminum smelter, several phosphate fertilizer plants, a new ammonia–urea plant,
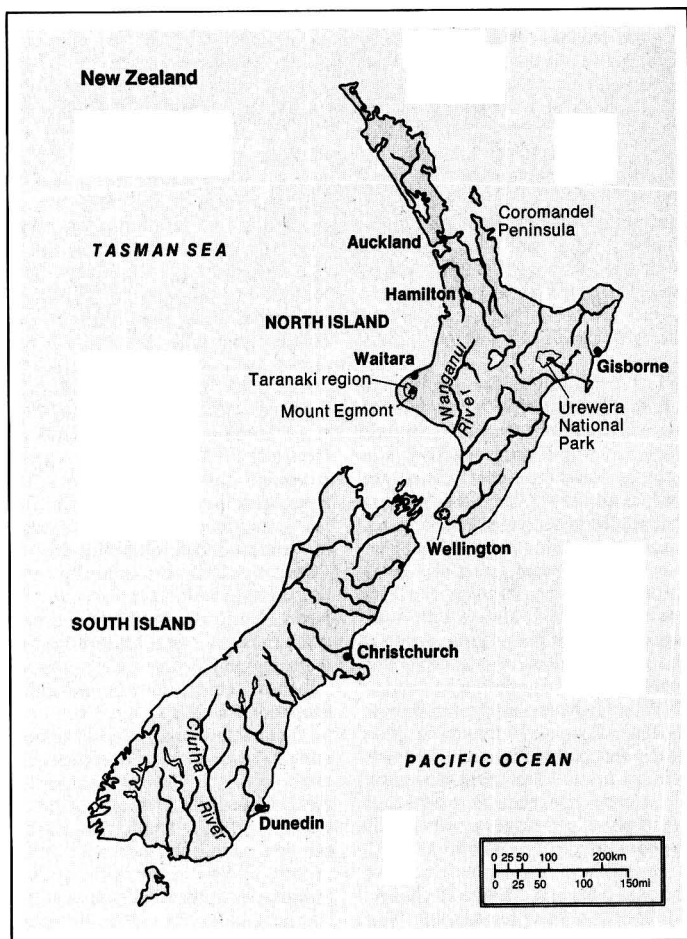
one oil refinery, and three large pulp and paper mills. There are no major petrochemical plants to generate copious quantities of toxic wastes.

For all of these reasons, New Zealand is fortunate environmentally. It is a developed country that has so far avoided many of the environmental problems of industrialized nations. But even now it has a number of serious problems that are not so obvious, and others that are looming in the eighties as the country embarks on a very large investment program in a range of energy and industrial projects.

A ruthless change

One major factor not at all obvious to the visitor who delights in seeing the ubiquitous emerald pastures dotted with sheep is that most of these pastures were originally thick forest, called "native bush." Before European settlement, native bush covered more than 70% of the land area; now it covers only 22%.

Felling the native forests in New Zealand has been called one of the most ruthless and rapid changes which man has produced in the vegetation cover of the earth. Kauri forests, one type of indigenous vegetation, have been virtually eliminated. The original 1.2 million ha of kauri have shrunk to scattered remnants of 5000 ha or less. Comparable to the Sierra sequoias as mature specimens, kauri grow to a giant size, with massively stout trunks soaring as high as a 15-story building. Because of their height and straight-grained, strong wood, young kauri trees were sought after by the British Admiralty for ship spars and older kauri by others as a bountiful source of lumber soon after their discovery by Captain Cook. The bush contains several other valuable tree species that were also highly prized.

It is easy to understand why New Zealand logged a major portion of its native forests. When the English settlers first came there, they had to slash

or burn away the bush to do any farming at all. Also, one of England's original reasons for colonizing New Zealand was to use it as a source of lumber to replace the dwindling supply in the British Isles. Logging was a primary industry for New Zealand's first 100 years after colonization and remained important until the beginning of World War II. Although the pace slowed somewhat after this, forest clearing continued until the early seventies with only sporadic public protest. In most places the native tree species did not regenerate. In effect, the vegetation of the bush was mined.

For generations, the public had minimal interest in preserving the native bush, partly because their attitude toward it was largely negative. The average New Zealander viewed the bush as a kind of man-eating medusa that needed to be chopped up. Even the casual visitor may come to feel that way about it when he or she walks through the forest. Many of the trees are massive, nearly always with vines, ferns, and other plants growing along the trunks and main branches. The vegetation is sometimes so high and so thick that light can barely penetrate to the forest floor, and it frequently poses nearly insuperable barriers to cross-country travel. Once inside the bush, all orientation is readily lost in a maze of steeply sloped ridges smothered by exuberant temperate rain forest.

By 1970, the public perception of the native bush had begun to change. At this time, what has become New Zealand's most active environmental group was formed, the Native Forests Action Council. Through local meetings and organized trips to threatened areas of the forest, the council effected a radical change in the general concept of native bush and educated the public to realize that if logging continued at its current rate, the lowland bush would soon disappear except for remnants protected by national parks and reserves. The council obtained signatures from about one-quarter of the adult population on a petition that requested protection for remaining forests. The Forest Service responded by promising that no more government land with indigenous forest would be clear felled. In 1973, the council defeated a proposal to log 320 000 ha of native bush and since then has defeated three more proposals to cut large areas.

The public's attitude toward native bush has changed, but logging continues, even on government land, although at a reduced rate. This is partly to enable isolated communities that have been historically dependent on indigenous forests to find new sources of livelihood before the logging industry is phased out. Some of the cutting on state land takes place in forest parks. National parks are the only areas that have total legal protection from logging. There is virtually no regulatory control over private indigenous forests.

Meanwhile, large tracts have been replanted with exotic tree species. The majority of these are planted with a monoculture of *Pinus radiata* (Monterey pine). This rapidly growing tree can be seen marching across the hills in straight rows as far as the eye can see on some of the world's largest tree farms. Although it can be harvested after 20–25 years in New Zealand's temperate climate, some believe that such high dependence upon *radiata* makes New Zealand forestry highly vulnerable to imported or mutated pests.

If the native forest in New Zealand could be regrown easily once it is chopped down, cutting it would not be so likely to cause irreversible damage. However, according to a government forestry expert, many species are difficult, if not impossible, to reestablish. He observed that research is needed to find ways of growing the trees and of harvesting them in such a way that the surrounding trees are not injured. Now when isolated trees are removed, even with selective logging, the adjacent trees often become unstable and subject to being blown down by the wind.

Loss of species

One of the most serious problems associated with cutting the native forests is that it depletes the habitat of many species of both plants and animals, a large portion of which are peculiar to New Zealand. A number of this country's flora and fauna are under threat, and several unique species of birds have already become extinct. The "Red Data Book" of the International Union for the Conservation of Nature and Natural Resources lists endangered birds from around the world; New Zealand has 11% of them. There are more than 250 plant species known to be threatened and four species of freshwater fish, out of 11 in the world. A high percentage (90%) of New Zealand's insect species are not found elsewhere. Actually, the knowledge of which species are peculiar to New Zealand and which threatened is quite incomplete. There is a pressing need to create a comprehensive biological resource inventory (see *ES&T*, February 1982, 94A).

Another factor further threatening species survival is that the great bulk of the parks and reserves are located on land with high relief or at a high elevation. Few parks are situated on rolling land, wetlands, or flatlands. The flatter, lower terrain provides habitat for different kinds and usually a greater variety of plant and animal life. Also, some of the birds that normally live in the mountains winter at a lower elevation.

Vanishing natural rivers

Ironically, the fact that New Zealand relies on hydropower, a relatively clean source of energy, for most of its electricity creates certain environmental problems. It has diminished the number of wild and scenic rivers in the country. Several of the large rivers have been dammed, and the government is now looking at the smaller rivers to create further generating ca-

The town of Jerusalem on the Wanganui River

pacity. Dams and canals for irrigation projects have also altered many rivers and streams. Chris Horne, secretary of the Environment and Conservation Organisations of New Zealand, Inc., said that at least 87 dams now exist and that almost all of New Zealand's rivers have been affected one way or another. The organizations he represents are particularly opposed to the damming of more rivers and streams because they believe that the few streams still in their natural state have greater intrinsic value than the power that they could produce. The structure of New Zealand's Water and Soil Conservation Act is such that it is very difficult to reserve a wild or scenic river permanently for nondevelopment purposes.

Many New Zealanders value natural rivers for recreation. Users, including canoeists, fishermen, hikers, and others, have formed a coalition to preserve rivers. Each new proposed dam now meets with opposition from this coalition.

One especially controversial project is the dam under construction at Clyde on the Clutha River. Keith Johnston of the Coalition for Open Government explained why a large fraction of the population considers this project objectionable. First, the Clutha River is particularly scenic and accessible. It has a road along its steep-walled gorge and many orchards nearby. Earthquake faults add a further complication. A substantial fault 1 km upstream from the dam site and another smaller fault directly under the site cause concern for the safety of downstream communities.

Originally, the government justified the Clutha project by saying that it would be needed to power a second aluminum smelter on the South Island. Because other large load centers for future growth in power supply are primarily on the North Island, electricity from the project may not be needed in the forseeable future unless the second smelter is built. Yet, construction plans for the smelter are indefinite owing to depressed world aluminum prices. Moreover, a number of citizen organizations believe that the smelter could never be economically profitable if it were required to pay the true marginal cost of producing power from the Clutha. Nevertheless, construction on the dam continues, and it will be completed.

Threats to water quality

It would be very easy to believe that New Zealand has no water quality problems. With its high rainfall, its

Water pollution from New Zealand's 60 million sheep is difficult to control.

lack of large-scale industrialization, and its population centers sited primarily along the coastal estuaries, it is in a very fortunate position compared to most developed countries. A number of New Zealand's aquifers, lakes, and harbors, however, show evidence of environmental stress. The main source of pollution is nonpoint source runoff in the form of phosphates and nitrates from artificial fertilizer and stock dung. As a result, some lakes are in an advanced state of eutrophication, and extensive aquifers in the Waimea, Heretaunga, and Canterbury Plains are adversely affected by high nitrate levels.

Where animals can be kept in feedlots, pollution is relatively easy to control by collecting the runoff and treating it in ponds before it enters rivers or streams. The pollution from New Zealand's millions of freely grazing animals (60 million sheep and eight million cattle) and from fertilizers is more difficult to reduce. Certain measures, however, are fairly effective such as fencing animals away from streams and planting trees and shrubs along water bodies. Measures to control erosion also help to diminish the runoff from animals and fertilizer.

Because eutrophication seems to depend on the availability of phosphorus, the Organisation for Economic Co-operation and Development (OECD) report, "Environmental Policies in New Zealand" (1981), recommends that New Zealand consider banning the polyphosphate builders in detergents as regions of the U.S., Scandinavia, and Canada have done. It also suggests that the aerial application of phosphate fertilizer be restricted in the vicinity of streams and lakes.

Roughly 80% of New Zealand's

population is served by wastewater treatment at least at the primary level. However, certain estuaries that receive large discharges of treated sewage (even when treated at the secondary level) have damaged water quality. Moreover, a 1978 survey showed that 31 towns with a total population of 630 000 (about one-fifth of New Zealand's population) dispose of essentially raw sewage via ocean outfalls. Additional problems are created by meat processing plants that release untreated ground-up wastes into the ocean via short or damaged outfalls.

Usually the municipal outfalls discharge into deep water where wind and tidal conditions normally prevent the sewage from being carried back to near-shore areas. New Zealand health authorities believe that in this situation, the ocean functions as an enormous and economical natural purification system. But for some cities, such as Wellington, the outfalls release sewage near the shore, and shellfish are contaminated. In such areas either land-based treatment or deep-sea outfalls are obviously needed. Engineering deep outfalls that are resistant to damage from strong surf and tidal forces has proved to be difficult in some regions.

It is expected that many new industries will be built in New Zealand in the next few decades. At present, it has no national standards for outfalls. A classification system for streams was set up, but it turned out to have major flaws and is undergoing revision. Molly Melhuish, one of New Zealand's leading policy analysts, believes that establishing national standards for outfalls should be an important priority before future industrial development proceeds. The OECD report recommends that the use of "best

The synfuels plant is being constructed on a beautiful section of the coastline near the town of Waitara.

practicable technology" should be considered as a requirement for industrial outfalls.

Air quality

Although air pollution is no problem over approximately 95–99% of New Zealand, parts of certain cities (and perhaps towns) suffer high levels of air pollution. An informed government official said that the two main streets in Auckland and Christchurch breach World Health Organization air pollution standards nearly all the time. In many areas no one really knows what the average contaminant levels are. At present, there are only eight monitors in the entire country—four in Auckland and four in Christchurch. This same official remarked, "There is no air pollution in New Zealand because no one bothers to monitor it."

New Zealand has no ambient air pollution standards or automobile emission standards. It has an advisory limit for lead in gasoline, which is set by agreement between the government and the oil companies, but this limit is the most permissive in the world—0.84 g/L or about 3.36 g/gal. (The U.S. allows 0.5 g/gal.) Another deficiency is that New Zealand has not yet established standard emission limits for industries. Specific limits are worked out on a case-by-case basis with the requirement that the "best practicable means" of emission control be used. The OECD report states that "this system has relied too heavily on the informal relationship established between the individual representatives of the implementing authority and the industry" and that the system is "too secretive." Moreover, the report cautions that the New Zealand system is not conducive to technological innovation and that new methods of setting emission limits will be needed in the future as industries proliferate.

But the most obvious air pollution problem in New Zealand is an urban one. When the natural ventilation system ceases to function for a day or two, photochemical pollutant levels build up in the major cities. Even though gasoline is expensive, New Zealanders rely heavily on automobiles for transport. Emission controls on these are lax by U.S. standards, thereby providing the opportunity for urban smog to develop during periods of stagnation.

Additionally, residential combustion of wood and coal produces high particulate concentrations in some communities. Christchurch, the most populous city on the South Island, is particularly troubled by coal smoke that is trapped by surrounding hills during wintertime inversions.

A controlled problem?

Erosion in New Zealand is one problem that many authorities believe has been solved fairly adequately. Alistair Graham, information officer at the Ministry of Fisheries and Agriculture, said that erosion used to be an important concern but several institutions have been set up that serve to control it reasonably well. Local catchment boards have been established throughout most of the country. They give the farmers grants for combating erosion, such as money to build what are called debris dams. To some extent, catchment boards have the power to keep farmers from clearing erosion-prone slopes.

In spite of these measures, certain steep and erosion-prone areas of the North Island are still creating a prob-

lem. Some of them are quite productive agriculturally at least in the short term, but soil loss in some of these areas far exceeds regenerative capacity and causes aggradation and high turbidity in water bodies downstream. Reforestation with native bush in these areas is thought to be the only means of halting the process. Along the coast north of Gisborne, the problem is so severe that sharply delineated turbid plumes of suspended sediments extending out into the ocean for a great distance from the mouths of rivers can be seen after heavy rainstorms. In many places, the signs of rill and gully erosion are clearly visible from the road.

Some of the government's policies with regard to erosion are contradictory. Farmers are given financial assistance to control erosion. They are also given assistance to bring marginal land into production. The funds are greater and the terms under which they are offered are more attractive for the latter than for erosion control. Another drawback is that funds for creating farmland encourage the draining of wetlands, which, it is estimated, have already been depleted by 90%.

Gold and black gold

Gold fever struck parts of the North and South Islands on several occasions in the mid-1800s. The mines on the South Island were shorter lived than the hard-rock mines that followed the quartz veins on the North Island. While these North Island mines also produced substantial amounts of silver, copper, and other metals, gold was their most valuable product. Yet, even the largest of the North Island mines closed in the third decade of this cen-

tury as the richer bearing veins were depleted.

Today there are plans for reopening one mine and speculation about reopening others on the scenic Coromandel Peninsula because the world gold price has made mining profitable once more. Such mining would be on a massive scale, seeking a fraction of an ounce of gold in every ton of rock removed, thus reopening and enlarging some of the mining scars of the last century that now are slowly being assimilated by the land.

A more ominous threat is created by the government's interest in developing a large-scale coal mining industry. Although scattered small-scale coal mines have supplied fuel for homes for more than a century, little coal has been burned to produce electricity. Now the government is vigorously attempting to produce steam coal on a large scale. These attempts are being slowed by severely faulted seams in mines in the Waiketo basin on the North Island and the watery layers of peat or swamp covering many of the coal or lignite deposits on both islands. It remains to be seen whether much of New Zealand's plentiful coal deposits can be mined with proper regard for the environment and miner safety and still compete with relatively inexpensive, high-quality Australian coal.

Energy policy

When you speak to environmentalists in New Zealand, they return again and again to the subject of energy policy and the so-called Think Big projects—developments that would rapidly expand New Zealand's industrial activity and domestic fuel supplies. Among these are projects that would increase coal production fourfold during the eighties, an expansion of the Marsden Point oil refinery, and several industries that would convert natural gas into other products. Environmentalists are openly opposed to some of them and highly critical of most of the others.

It is easy to understand why the projects were initiated. With its heavy dependence on exports of farm commodities, fish, and forest products, New Zealand's position in the world economy is highly vulnerable because the prices for these commodities fluctuate widely while prices for petroleum and industrial supplies have risen steadily over the past decade. New Zealand imports 85% of its oil and the majority of its industrial supplies. After the price of oil rose in the seventies, New Zealand's balance of payments deteriorated rapidly.

Therefore, the country has a strongly felt need to develop a more self-sufficient economy and in particular to reduce its dependence on oil imports.

The Think Big projects were planned to help solve these problems. An offshore gas field called the Maui gas field was discovered off the North Island in the late sixties. It is the only proven major field of natural gas in the country and contains 90% of New Zealand's known gas reserves. New Zealand plans to use the entire resource in 40 years with electricity generation, distribution to industry and commerce on the North Island, and three projects to convert gas to other products—an ammonia-urea plant, a methanol plant, and a synthetic gasoline plant. All three plants are located or will be located in an idyllic dairy farming area called the Taranaki region. The first facility is built and operating, the second is almost complete, and, according to current plans, the third will be finished in 1986. Thus far, only the foundations of the synfuels plant have been built.

The third project and the largest, the Mobil-Bechtel synfuels plant, has been subject to the greatest criticism for a number of reasons. It is projected to consume a substantial fraction (16%) of the Maui gas field and about 67% of the remaining Kapuni natural gas reserve. Converting natural gas to gasoline wastes 45% of the energy. The gas can instead be compressed into

Sign in the town of Waitara warns that shellfish are contaminated from municipal outfalls.

high-pressure cylinders with an energy loss of only 3%. Therefore, using a unit of compressed natural gas (CNG), rather than converting the gas to gasoline, would run a vehicle nearly twice as far. Almost 50 000 vehicles in New Zealand have been converted to CNG, and the number is rising steadily.

Consequently, environmentalists consider converting natural gas to synthetic gasoline a profligate waste of a valuable energy resource. They point out that a smaller amount of money could be invested in converting one-third of New Zealand's vehicles to CNG. In this way the natural gas resource would be more efficiently used, and the automobile fleet would be equipped to use biogas, a renewable resource, after the natural gas runs out. Biogas can be made easily from hay, farm waste, or even wood waste. Although it is technically possible to make gasoline from farm and wood wastes, the process is economically inefficient.

Basil Walker, who is in charge of energy planning at the Ministry of Energy, described the events that led to the government's decision to build the synfuels plant. Fourteen years after the Maui gas field was discovered, the price of oil rose precipitously. At the same time, the demand for electricity was increasing at a rate of about 7%/y. New Zealand's energy planners, along with most energy planners in the rest of the world, expected the demand to continue at that rate. Therefore, they believed that nearly all the Maui gas would be needed to generate electricity in the next few decades, and in 1973 signed a "take or pay" agreement with a company to use up the gas in 30 years. This required that the government pay for a certain amount of gas each year even if it is not used. If the gas is not depleted before 2008, the gas would, Walker said, revert to the ownership of the company, and the contract would have to be renegotiated.

Immediately after this, the rate of increase in electricity demand dropped to 2%/y. In 1978, the government planners realized that most of the gas would not be needed for power generation and tried to find other ways to use it. Within a short time, several projects were chosen. The synfuels plant was designed to kill two birds with one stone—consume gas and reduce the dependence on foreign oil. The synthetic gasoline would provide one-third (530 000 t/y) of the gasoline needed in New Zealand.

Walker said that the idea of converting a large fraction of New Zea-

land's automobiles to CNG was considered but dropped because the government would have to interfere too much in the citizens' private lives to effect a rapid enough conversion. The government was philosophically opposed to using large tax incentives or other strong means to effect a changeover within a short period of time. Walker mentioned several reasons why CNG is not an attractive fuel for some people. The compressed-fuel tanks use up trunk space and must be filled about three times as often. Also, the cost of conversion is not economical for the individual who drives very little or owns an old automobile.

When asked whether under the take or pay contract one aim of the government was to make sure the gas was depleted rapidly, Walker replied, "It is misleading to talk about wanting to use up the gas quickly. The drive was rather to put gas to economically beneficial uses. In doing this, a very cautious approach to depletion was adopted, one which left half the gas still in the ground at the year 2000."

An informed government official and other sources took a different view of the matter. They said that the synfuels project was chosen partly because it is one means of consuming the Maui gas rapidly, not because it is an economical means of using the fuel. They also noted that under current plans, half the gas will be gone by the year 2000, but all of it will be used by 2015, and that the take or pay contract does not specify who will own the gas after 2008. Other more cynical observers say that politicians chose the project largely so that they would be able to take credit for initiating a large development.

The synfuels plant is opposed for other reasons. It relies on a new technological process that has not been commercially proven anywhere in the world. A small demonstration plant has been built in New Jersey, but it produces only four barrels of gasoline a day. The synfuels facility is being constructed in a very scenic agricultural region of New Zealand, on a water-saturated bench above the ocean and within sight of Mount Egmont (Taranaki), one of the country's mountain national parks. Inevitably, it will alter the character of the region. The original plans called for releasing the treated wastes from the plant in a special ocean outfall over some of the last remaining unpolluted reefs in that area. Concerted opposition by the native Maori people, who use shellfish from the reefs as a major food supply for spiritual and cultural occasions,

Coromandel Peninsula. *Idyllic scenes such as this are common along the Coromandel Peninsula. Gold mining may be revived there.*

persuaded the government to use the outfall in the nearby town of Waitara instead, where the reefs are already polluted. The multiyear effort to induce this change produced a new awareness among government officials of the importance of New Zealand's seafood for the Maori way of life.

The relative importance of the various factors that were weighed in the decision to build the synfuels plant may never be known. The Coalition for Open Government and many other citizen organizations believe that New Zealand has a closed form of government. A weak form of a "freedom of information act" has been in effect for only a few months. By not allowing public scrutiny of the way in which major decisions are made, the government is likely to make itself more prone to reinforce or repeat unwise decisions of the past.

Outlook for the future

New Zealand is experiencing rapid changes, perhaps without some of the laws needed to plan these changes to prevent unnecessary adverse effects on the environment. It has no government department or ministry comparable to the U.S. Environmental Protection Agency. The Commission for the Environment is somewhat analogous to the U.S. Council on Environmental Quality before it was emasculated by the Reagan administration. It acts in an advisory capacity, but its decisions are not legally binding.

Until recently, New Zealanders may have felt little need for strict environmental laws. The supply of clean water and scenic rivers and farmland may have seemed unlimited as it seemed in U.S. frontier days. These days are now a matter of history in

New Zealand, and there is a need to proceed in the future with a rational awareness of long-term consequences.

Several factors augur well for this outcome. Because one of New Zealand's major industries is tourism, it will have to establish some kind of balance between development and the environmental quality that will continue to attract tourists. New Zealand's universities are capable of producing the professionals required by the government to carry out more enlightened environmental protection measures as the country becomes more industrially self-sufficient. The government has traditionally been involved in planning and regulating economic activities, so government intervention to ensure the attainment of environmental goals would not be contrary to accustomed political practices in New Zealand. But most important, a significant fraction of the population cares strongly about maintaining a clean, attractive environment, and in this democratic society, citizens have at times been able to exert considerable influence on the government. However, it remains to be seen whether New Zealand will develop sufficient political will to care for the long-term environmental well-being of this beautiful island country.

Monitoring toxic air pollutants

Richard M. Dowd

Congress has become increasingly concerned about human exposure to potentially toxic air pollutants. There is already legislation in place that requires EPA to control toxic air pollutants. Some members of Congress feel that EPA has been too slow in acting on these requirements. As a result, EPA is likely to receive more specific congressional direction to list and control pollutants that may be toxic to humans.

To be considered toxic, a chemical must not only have the potential to be toxic but must also be present in ambient concentrations that may cause harmful human health effects. Without knowledge of what concentrations currently exist, the existence of detrimental health effects cannot be determined. This raises several questions about the availability and quality of data on potentially hazardous pollutants.

There are two major subsets of potentially toxic air pollutants: elemental metals and metal compounds, and organics.

Monitoring metals

Elemental metals have been used as a proxy for determining concentrations of metal compounds at about 150 of the National Air Surveillance Network (NASN) sites. Samples are sent every quarter to EPA's Environmental Systems Laboratory and analyzed for about a dozen different metals. Ambient data on metals are also available from EPA's Inhalable Particulate Network (IPN). IPN samples were collected using a dichotomous sampler with two particle size cutoffs—10 and 15 μm. IPN samples were analyzed for 16 metals by X-ray fluorescence. The IPN was terminated last year; now EPA only gathers information from NASN samples.

Collecting data on organics

EPA has been investigating several different methods for monitoring organic air pollutants. A Philadelphia site has been used to monitor many organics in different ways. Data collected by the various methods will be compared for their accuracy and reproducibility. EPA is testing such methods as Tenax filter analyzed by gas chromatography/mass spectroscopy (GC/MS), photoionization with differential GC columns, cryogenic trap followed by GC/MS, and MS/MS.

Preliminary results indicate that each of these methods has advantages and disadvantages. Since the study will take some time to complete, multiple methods of analysis will probably be used to analyze each functional group of organic chemicals.

Although EPA has not established monitoring method guidelines, the agency is developing a strategy to initiate toxics monitoring programs. The program is expected to begin in 1984 with monitoring networks in three cities. The most likely locations are Boston, Chicago, Houston, St. Louis, and the New York–New Jersey area. Each city would have one site located in an industrialized area. Two consecutive 12-h samples would probably be taken once every six days. It is anticipated that a Tenax filter would be used and a dozen or more chemicals will be analyzed. The results of the program would give EPA an indication of the ambient levels of potentially toxic air pollutants in industrialized areas. By 1985, the program may be expanded to include 15 cities. Other monitoring programs are being designed to quantify the impacts of fugitive sources such as landfills and sewage treatment ponds.

Some state agency monitoring programs have been enlarged to include sampling for metals and organics. New Jersey currently has the most extensive program. More than 50 substances are being monitored daily at four residential sites for six weeks in the summer and six weeks in the winter. Twenty-four-hour IP samples are analyzed for metals, arsenic, organic matter fraction, polycyclic aromatic hydrocarbons, bacterial mutagenicity, and sulfates. Volatile organic carbon samples are analyzed for pollutants regulated under New Jersey Administrative Code 27, Subchapter 17 and alkylating agents.

Most state programs currently in operation analyze for fewer chemicals than the EPA network. Several states have invested in mobile units to supplement stationary networks and enable them to conduct special short-term projects.

Industry has been monitoring various pollutants in and around its own facilities, some for a number of years. These data, however, are not usually public information (unless they are submitted as part of a permit application). Since no guideline sampling methods have been defined for these pollutants, much of the available data is not comparable.

Future considerations

Monitoring of pollutants that are present in low concentrations (often close to detectable limits) will increase the need for stringent quality assurance and quality control. The sensitivity and application of certain measurement methods will continue to be a major concern among members of the regulated community.

Next month's Regulatory Focus will discuss EPA's regulatory agenda for toxic pollutants.

Richard M. Dowd, PhD, manages the Washington, D.C., office of Environmental Research & Technology, Inc.

Chlorinated dioxins as herbicide contaminants

The Veterans Administration and other federal agencies are conducting studies of their effects on humans, with special focus on veterans of Vietnam, where "Agent Orange" was used.

Alvin L. Young
Han K. Kang
Barclay M. Shepard
Agent Orange Projects Office
Veterans Administration
Washington, D.C. 20420

Few environmental or occupational health issues have received the sustained international attention that has been focused on the chlorinated dibenzo-*p*-dioxins—especially 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Much of the present concern centers on veterans of the Vietnam conflict, who may have been exposed to TCDD, a contaminant of the military defoliant Agent Orange. The public has become even more familiar with this dioxin because of improper disposal of toxic wastes in Missouri and New York; through reports of contaminated fish in the Great Lakes and Michigan; and as a consequence of publicity about a large PCB transformer fire in the State Office Building in Binghamton, N.Y. However, what drives the controversy is the veterans issue and the potentially larger question of whether human health has been significantly affected by the widespread use of dioxin-contaminated herbicides to enhance agricultural production.

Agent Orange in Vietnam

The use of herbicides to control vegetation has been one of the most persistent controversies arising from the Vietnam War. The U.S. Air Force applied most of these herbicides to dense jungle areas to uncover hidden enemy staging areas, to clear vegetation from the vicinity of military bases and along lines of communication, and to destroy the enemy's crops. The objectives were to provide defoliated zones, so that the number of ambushes would be reduced and enemy attacks could be disrupted. The most commonly used defoliant was Agent Orange, a mixture of the normal butyl esters of the two commercial herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). For many years, these materials had been employed for brush control in forests and rangelands and for weed control in agriculture throughout the U.S.

During the five-year period from 1965 to 1970, the U.S. Air Force applied more than 40 million L of Agent Orange in South Vietnam. About two million American military personnel served one-year tours during the same period. Recently, many veterans of that era have reported medical problems that might stem from exposure to Agent Orange during their military assignments. Their complaints have ranged from tingling sensations in the extremities to rare forms of cancer. Some veterans have fathered children with birth defects and have suggested that Agent Orange is the culprit. Nevertheless, the basis for resolving the Agent Orange controversy must, in large measure, stem from the results of scientific inquiry.

Accordingly, the Veterans Administration (VA), in cooperation with other federal agencies, has begun extensive health studies of veterans exposed to Agent Orange and its dioxin contaminant 2,3,7,8-TCDD during the Vietnam conflict. In addition, numerous studies of nonveteran populations exposed to the phenoxy herbicides and TCDD have been encouraged and funded by several agencies of the federal government.

Problems with human studies

To conduct epidemiologic studies of human populations exposed to the phenoxy herbicides and their dioxin contaminants, it is first necessary to know what health outcomes should be selected for study. Second, one must be able to identify a study cohort with a high likelihood of exposure and a control group with a low or zero probability of exposure. Finally, a sufficient number of individuals in each cohort must be located, recruited, and examined to impart statistical validity to the results.

These three requirements, so important for the conduct of valid studies, are in themselves not clearly defined with respect to either the chemicals used in Vietnam or the population at risk. Health studies of the effects of phenoxy herbicides are difficult enough under conditions of normal agricultural use, but they become much more complex when conducted with cohorts briefly exposed more than a decade ago under conditions of war in a tropical environment.

The problems inherent in conducting epidemiological studies of Vietnam veterans are similar to those encountered in conducting studies of individuals and families who live, or have lived, near dioxin-contaminated toxic waste dumps. Heath, for example,

wrestled with problems of carrying out field epidemiological studies on the residents of Love Canal (Niagara Falls, N.Y.) (*1*).

Phenoxy herbicide toxicity

TCDD toxicity in animals. Acute toxic effects induced by TCDD vary markedly among different species. For example, as little as 0.6–2.0 μg of TCDD/kg of body weight given orally to guinea pigs killed half of the male animals (*2*). However, in the hamster, TCDD is much less toxic, with an oral LD_{50} of 1157–5051 μg/kg of body weight (*3, 4*).

TCDD is teratogenic in mice and causes an increased frequency of cleft palate as well as kidney abnormalities (*4*). In rats, TCDD does not produce a teratogenic effect, but does show embryo- and fetotoxicity (*5*). Also, it is carcinogenic in rats and mice after long-term ingestion at levels that induce toxic effects (*6, 7*).

Toxicity in humans. Precise information concerning adverse effects of 2,4-D, 2,4,5-T, and 2,3,7,8-TCDD is lacking. Acute and subchronic effects are reported quite uniformly following accidental and industrial exposures and suicide attempts, but there remains considerable scientific debate about the nature of delayed and long-term effects. Much of the medical knowledge of the effects of 2,4-D and 2,4,5-T exposure of humans is derived from case histories. Since many of the individuals described in such reports were exposed to multiple chemical agents, it is difficult to determine which chemical(s) produced the specific symptoms or health problems. Of the vast array of symptoms attributed to 2,4-D, the most consistently reported complaints involve behavioral, nervous system, liver, and intestinal problems.

Medical data associated with exposure to 2,4-D come primarily from spraying incidents, while data for 2,4,5-T and TCDD come from industrial exposures. Since the beginning of commercial production of 2,4,5-T, numerous industrial incidents have involved exposure to trichlorophenol, 2,4,5-T, and TCDD. Fifteen of the 23 episodes recorded in the literature apparently resulted from occupational exposures during industrial production of chlorinated phenols. However, on eight occasions, personnel were exposed either during cleanup operations or by working in improperly decontaminated workshops (*8*). The effects of 2,4,5-T could not be clearly distinguished from the possible effects of TCDD. Symptoms attributed to

The military use of herbicides in South Vietnam

The first shipment of Herbicides Purple and Blue was received at Tân Son Nhứt Air Base, Republic of Vietnam, on Jan. 9, 1962. These were the first military herbicides used in Operation RANCH HAND, the tactical military project for the aerial spraying of herbicides in South Vietnam. Two additional phenoxy herbicide formulations were received in limited quantities in South Vietnam and evaluated during the first two years of Operation RANCH HAND. These were code-named Orange and White, and had been evaluated and subsequently brought into the spray program. The use of Herbicide Orange replaced all uses of Purple, Pink, and Green, and it eventually became the most widely used military herbicide in South Vietnam. The composition and quantities of the herbicides used in South Vietnam are shown below.

Number of liters of military herbicide procured by the U.S. Department of Defense and sprayed in South Vietnam from January 1962 to October 1971

Code name	Herbicide	Liters	Period of use
Orange	2,4-D: 2,4,5-T	40 299 005	1965–70[a]
White	2,4-D: picloram	21 322 790	1965–71[b]
Blue	Cacodylic acid	4 353 005	1962–71[b]
Purple	2,4-D: 2,4,5-T	548 833	1962–65
Pink	2,4,5-T	464 820	1962–65
Green	2,4,5-T	31 070	1962–65

[a] Last fixed-wing mission of Orange, April 16, 1970; last helicopter mission of Orange, June 6, 1970.
[b] Last fixed-wing mission, Jan. 9, 1971; all herbicides under U.S. control stopped Oct. 31, 1971.

Estimated quantities of herbicides and TCDD disseminated in South Vietnam from January 1962 to February 1971

Chemical	(kg)
2,4-D[a]	25 374 450
2,4,5-T[a]	20 063 910
TCDD[b]	167
Picloram	1 379 760
Cacodylic acid	1 609 695
Total herbicides	48 427 982

[a] Expressed as the acid equivalents.
[b] The mean TCDD concentration in Herbicide Purple was estimated at 32.8 ppm. The mean TCDD concentration in Herbicides Pink and Green was estimated at 65.6 ppm. The mean TCDD concentration in Herbicide Orange was estimated at 1.98 ppm.

Source: Young, A. L. et al. "The Toxicology, Environmental Fate, and Human Risk of Herbicide Orange and Its Associated Dioxin," Technical Report OEHL-TR-78-92; USAF Occupational and Environmental Health Laboratory, Brooks AFB, Tex. 78235; 1978. Available from National Technical Information Service, document AD-A062-143.

2,4,5-T and TCDD exposure include all of the symptoms of 2,4-D exposure, plus a skin disease, chloracne.

Many scientists believe that chloracne is the "hallmark" of exposure to dibenzo-*p*-dioxins, especially 2,3,7,8-TCDD (9). Chloracne is a skin reaction characterized by an acniform dermatitis with comedones (blackheads) and inclusion cysts or papules and frequently pustules so severe that they cause permanent scarring. Morphologically, it is similar to adolescent acne, but it is usually more severe, particularly on the upper face, ears, and neck. Active chloracne lesions have been reported many years after exposure to TCDD, but the condition usually clears up spontaneously in a few months. Premature aging of involved skin areas has been reported in some instances, as well as the appearance of hyperpigmentation (10).

Mortality experience

Recently, a number of epidemiological studies have reported on mortality experiences in industrial settings. In January 1980, Zack and Suskind (12) published the results of a 30-year follow-up study of 121 chemical workers who had developed chloracne following exposure to TCDD in an industrial accident at Nitro, W.Va. The plant involved the manufacture of trichlorophenol. Although they observed no apparent excess in total mortality from cancer or cardiovascular disease, they could not consider the results conclusive because of the small cohort and the relatively small number of deaths observed.

In a 1983 report, Zack and Gaffey (13) expanded this study to include 884 men, of whom 721 were still alive and 163 had died. Analyses of these data showed no excess in total deaths or in deaths caused by cancer or other diseases of the nervous, circulatory, respiratory, or digestive systems. Although most of the men in this larger population were employed in the trichlorophenol plant, and thus were potentially exposed to TCDD, they did not develop chloracne.

In August 1980, Ott et al. (14) examined the mortality experience of 204 persons employed by Dow Chemical Company who were exposed to 2,4,5-T during its manufacture from 1950 to 1971. Among the 11 deaths observed, (20.3 expected), one was caused by a respiratory malignancy (3.6 malignant neoplasms expected). Thus, within the scope of this mortality survey, no adverse effects were observed with respect to occupational exposure to 2,4,5-T or its feedstock, 2,4,5-trichlorophenol.

In a subsequent report, Cook et al. (15) detailed a study of 61 males involved in a chloracne incident at Midland, Mich., in 1964. Of these men, 49 developed chloracne while working in a trichlorophenol manufacturing plant operated by Dow Chemical Company. Within the limits imposed by the size of the cohort and the length of the follow-up, TCDD apparently had no adverse effect on mortality experience, and deaths from cardiovascular disease or cancer were statistically insignificant.

Soft-tissue sarcoma

Soft-tissue sarcomas (STS) are a complex and diverse group of malignant neoplasms that usually originate in muscle, fat, and fibrous connective tissue, such as tendons or ligaments; they are found less frequently in blood vessels and nerves that serve these tissues. They account for about 1% of all malignant neoplasms and about 2% of all cancer deaths. The average annual age-adjusted incidence rate is 3.89/100 000; it is estimated that 8000 new STS cases are diagnosed in the U.S. each year (16). The most common histologic types are malignant fibrous histiocytoma, leimyosarcoma, sarcoma not otherwise specified, lipo-

sarcoma, and fibrosarcoma, in that order (17). Little is known about the etiology of STS. The epidemiologic study of STS has been especially difficult because of uncertainties in the morphologic classification of this diverse group of neoplasms. In addition, the International Classification of Disease (ICD), which is anatomic site-oriented, fails to categorize these neoplasms as to cell type and other morphologic characteristics.

The possibility that occupational exposure to phenoxy herbicides and chlorophenols may induce rare forms of cancer in humans, such as STS, has been suggested from recent case control studies in Sweden. The Swedish studies have shown that persons reporting exposure to the chemicals have a five- to sixfold higher risk of developing STS, compared to unexposed persons (18, 19). In consideration of possible recall bias among cancer patients regarding their past exposure to certain chemicals, a subsequent study included controls consisting of colon cancer patients. This latter study still indicated a fivefold increase in the risk of developing STS among persons reporting exposure to the herbicides (20).

Other studies of workers, however, have not yet replicated these significant observations. In a recent Finnish study of a cohort of 1926 men who had sprayed phenoxy herbicides from 1955 to 1971, no cases of death from STS or lymphomas were reported (21). But as the authors indicated, these results should be interpreted with caution because of study limitations, such as the small size of the cohort, a short follow-up period, and brief and low exposure to herbicides or TCDD.

Similarly, a case control study of STS by New Zealand investigators failed to show higher risk in the occupational groups involving agriculture and forestry (22). Phenoxy herbicides have been used extensively in New Zealand in both industries. Since the occupation at the time of diagnosis might not have contributed directly to the development of STS, further investigations into the individuals' exposures to the herbicides are warranted. Indeed, even the Swedish investigators stated that if "agriculture" and "forestry" are taken as a crude measure of exposure to the herbicides, no significant increase in the risk of STS or malignant lymphoma was found (23).

Several cases of STS have been re-

Defoliation. *This area in Vietnam was sprayed with Agent Orange.*

ported among American workers involved in the manufacture of herbicides (24–26). These industrial workers, in contrast to those who apply herbicides, are believed to be exposed to relatively high levels of TCDD. On the other hand, no STS cases have been reported in studies of workers involved in the manufacture of herbicides in either West Germany (27) or Czechoslovakia (28).

Except for mesothelioma, which is known to result from asbestos exposure, and angiosarcoma of the liver, which is known to be caused by vinyl chloride, no cancers of this type have an etiology related to chemical exposure. A small fraction of STS may be induced by heavy external radiation therapy for various benign disorders or malignant tumors (29).

Birth defects

Outside the U.S. The first reports of human birth defects attributed to Agent Orange appeared in Vietnamese newspapers in June 1969. As a result of expressions of public and scientific concern arising from these reports, Cutting et al. (30) and Meselson et al. (31) conducted two independent surveys of South Vietnamese hospital records. Although neither report reached definite conclusions on the validity of the accusations, both reports acknowledged that searches of the records probably would have revealed any marked increase in birth defects or introduction of a striking defect, such as the defects produced by thalidomide. Subsequent reports by Tung et al. (32) in 1971 and Rose and Rose (33) in 1972 centered on clinical ob-

servations and interviews conducted in Hanoi with refugees who claimed that they were repeatedly sprayed with defoliants in South Vietnam. The authors reported unusually high rates of abortions and severe birth defects in both humans and domestic animals.

Perhaps the most thorough assessment of South Vietnamese birth records was conducted at the request of the National Academy of Science by Kunstadter (34) between April 1972 and January 1975. Kunstadter's research relied on U.S. Department of Defense records of geographic and temporal distribution of herbicides. It was also based on the unpublished and published statistical reports of births in Tu Du and Húng Vuong hospital maternity facilities in Saigon and detailed examination of patient records. Interviews with patients' mothers at the Barsky Unit, Cho Rây Hospital, Saigon, which drew patients from all over the country for surgical repair of birth defects and other injuries, were also included in his evaluation.

Records of birth defects and perinatal mortality from the hospitals showed few consistent statistically significant results to support the hypothesis that there was a positive association between the military use of herbicides in Vietnam and the incidence of birth defects in children born to mothers exposed during pregnancy. Cleft lip increased as a proportion of all malformations during the period of heavy spraying. However, this increase continued after spraying had stopped, suggesting a trend unrelated to herbicide exposure. An increase in the stillbirth rate following the close of the

heavy spraying period was also inconsistent with the hypothesis, unless the teratogenic effects were persistent and cumulative.

Analysis of the records of Barsky Unit patients suggested that, with regard to the distribution of patients by diagnosis and sex over time and by geographic region, there was no consistent, statistically significant support for the hypothesis. Within the analysis of distribution of diagnoses and known risk factors, only the tabulation of variations in proportion of cleft lip patients with *relatives* who also have cleft lip was statistically significant. In that case, one might infer that if there

et al. (*36*), in a recent survey of professional sprayers and a control group of exposed agricultural contractors, determined the number of births among the families of 989 study participants. The sprayers exposed to 2,4,5-T had a 1.19 times greater likelihood of fathering children with birth defects. The wives on the other hand had a lesser risk of having miscarriages, by a factor of 0.89. The differences are not statistically significant. Exposure of the wives also had no other detectable reproductive effect.

In the U.S. The U.S. EPA, the U.S. Department of Agriculture (USDA), and the National Institute for Occu-

rice acreage (indicating low exposure to 2,4,5-T) and for regions with high production of rice (indicating high 2,4,5-T exposure) increased over the years studied. This trend was attributed to better detection of cleft palate, and not to herbicide usage (*38*).

The USDA sponsored a study designed to evaluate the relationship between exposure of human males to 2,4-D and the incidence of spontaneous abortions in their wives. In this study reported by Carmelli et al. (*39*), questionnaires pertaining to these two factors were mailed to almost 15 000 people who were employed in occupations involving the manufacture or use of herbicides. From the respondents, a group of 134 cases of miscarriages was selected and compared to a control group of 311 cases of live births. The cases were analyzed to determine the incidence of parental exposure to 2,4-D prior to conception and the presence of other relevant confounding factors, such as smoking, illnesses, and drug use. The data were obtained or verified by telephone interviews.

No positive association was established between 2,4-D exposure and abortions for the entire study population. For a group of 21 cases of wives of young forestry and commercial workers, an association was suggested, but was not found for any other subgroup. Further investigations were suggested, in order to clarify whether this relationship resulted from biases in study design or reflected a real increase among this small subset.

In November 1979, NIOSH received a request to determine whether an excess number of birth defects occurred among the children of maintenance employees working for the Long Island Railroad, N.Y., and whether these defects may have been caused by exposure to 2,4,5-T. Honchar (*40*) investigated 170 live births observed from among the study population of approximately 1400 employees. It was found that all major birth defects combined, as well as inguinal hernia, were underrepresented in the study population: 3 observed vs. 3.81 expected for major birth defects; 2 observed vs. 2.3 expected for inguinal hernia. However, minor defects such as the congenital foot deformity *metatarsus adductus* (8 observed vs. 3.47 expected) and tear duct obstruction (2 observed vs. 0.22 expected) were both significantly overrepresented in the study population. Honchar concluded that these minor defects were probably overrepresented in the study population because of diagnostic bias.

In summary, it would appear that

There was no evidence that service in Vietnam increased the risk of fathering a child with a birth defect.

was an association between herbicides and cleft lip, there was a positive interaction between herbicides and *other causes* of cleft lip.

Donovan et al. (*35*) recently completed a case control study of the effect of Vietnam service on the development of congenital anomalies. This Australian birth defects study examined records from 34 hospitals and four cytogenetic laboratories to identify infants born with birth defects. Matched healthy infants born in the same hospitals served as controls. The fathers of 8517 cases and an equal number of controls were identified, and the nature of their total service in the army was determined, as well as that of their duty in Vietnam. In all, 127 of the fathers of children with birth defects were Vietnam veterans, while 123 of the fathers of normal, healthy, children were Vietnam veterans. There was no evidence that service in Vietnam increased the risk of fathering a child with a birth defect.

In New Zealand, a number of workers have been exposed to phenoxy herbicides during their manufacture and spraying. Both sprayers and their wives are exposed during the handling and field spraying of chemicals. Smith

pational Safety and Health (NIOSH) have all completed limited studies on the potential associations of phenoxy herbicides with human spontaneous abortions and birth defects.

Two studies were conducted by EPA to evaluate the relationship between herbicide spraying and reproductive errors. In 1979, Johnson (*37*) released the data used by the agency in the decision to order the suspension of registrations of 2,4,5-T for uses in forest, rights-of-way, and pastures. He reported that the incidence of spontaneous abortions over a six-year period in Alsea, Ore., was found to be higher than the rates in two other regions of Oregon that had lower rates of 2,4,5-T usage. Although a cyclic pattern in the incidence of spontaneous abortions in the Alsea group correlated positively with the pattern of annual 2,4,5-T spray usage, Johnson acknowledged that EPA did not consider this as proof of a cause-and-effect relationship.
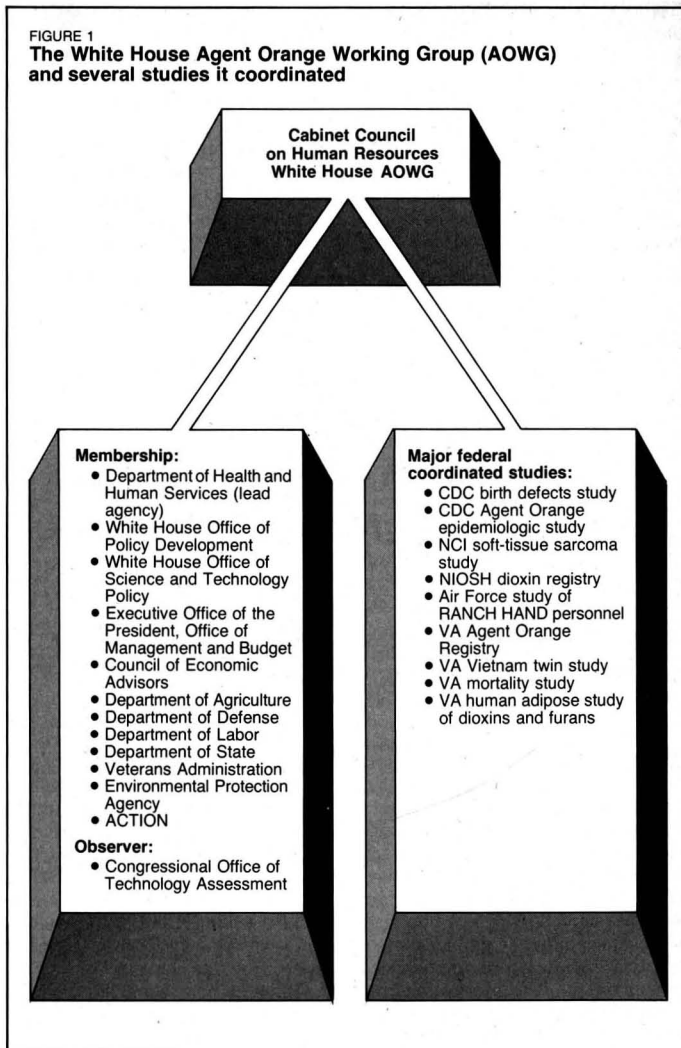
One of the major uses of 2,4,5-T is for weed control in rice fields. In another study sponsored by EPA, the incidence of cleft palate over a period of 32 years in Arkansas was shown not to be related to the usage of 2,4,5-T. The incidence of cleft palate for both of the regions with a low proportion of

based on the studies to date, there is very little epidemiologic data associating TCDD or the phenoxy herbicides with any long-term health effects in humans other than chloracne. Conversely, as noted by Wolfe (41), neither is there strong evidence to validate the absence of such effects. Most studies have not included sufficient numbers of subjects to detect an increased risk of an uncommon condition. Also, the period of observation in many studies has been inadequate to detect an illness with a long latency period between exposure and illness.

Health status of Vietnam veterans

As of May 1983, more than 17 000 Vietnam veterans had submitted claims to the VA for disabilities that they believe are the result of exposure to herbicides. These claims describe more than 130 different effects in five major categories: psychiatric, dermatological, reproductive, neurologic, and carcinogenic. The scientific data validate specific links between exposure to Agent Orange and TCDD in the sense that some symptoms reported by the veterans have also been documented in other cases of exposure to the herbicides or to TCDD. But most of these symptoms, such as peripheral neuropathy, fatigue, weight loss, and some psychological disturbances, are acute symptoms that manifest themselves shortly after exposure. Such symptoms arising years after the last known exposure are most likely not caused by 2,4-D and 2,4,5-T or TCDD. A large number of veterans have claimed dermatological problems, and many believed they had chloracne. To date, however, only a few cases of chloracne have been confirmed by physical examination in this group of veterans.

Despite the lack of solid scientific evidence to support the veterans' allegations that Agent Orange and its dioxin contaminant are the cause of their difficulties, one recognizes that some of the veterans have experienced serious health problems. Therefore, the purpose of many of the government studies is to determine whether Agent Orange is responsible for these health problems. If Agent Orange is not the causative agent, other factors associated with the Vietnam War may be responsible. Consequently, the goals of other research efforts are to determine whether Vietnam veterans as a group are experiencing more or different health problems than their counterparts who did not serve in that part of the world. In such a complex situation, no single study can provide all of the

FIGURE 1
The White House Agent Orange Working Group (AOWG) and several studies it coordinated

Cabinet Council on Human Resources White House AOWG

Membership:
- Department of Health and Human Services (lead agency)
- White House Office of Policy Development
- White House Office of Science and Technology Policy
- Executive Office of the President, Office of Management and Budget
- Council of Economic Advisors
- Department of Agriculture
- Department of Defense
- Department of Labor
- Department of State
- Veterans Administration
- Environmental Protection Agency
- ACTION

Observer:
- Congressional Office of Technology Assessment

Major federal coordinated studies:
- CDC birth defects study
- CDC Agent Orange epidemiologic study
- NCI soft-tissue sarcoma study
- NIOSH dioxin registry
- Air Force study of RANCH HAND personnel
- VA Agent Orange Registry
- VA Vietnam twin study
- VA mortality study
- VA human adipose study of dioxins and furans

answers. Thus, there is a need for a number of different approaches to examining the health of the Vietnam veteran.

Current federal investigations

What has been the response of the federal government to the critical issues raised by Agent Orange and dioxin exposure? In 1981, the President established the White House Agent Orange Working Group (AOWG) of the Cabinet Council on Human Resources. While AOWG does not conduct any research, it is charged with being the overall coordinator, clearinghouse, and evaluator of the federal research effort. Under the umbrella of AOWG, all of the federal agencies involved in research

and policy related to Agent Orange and other herbicides used in Vietnam are drawn together and work together. Conclusions reached by federal projects are provided to the Secretary of the Department of Health and Human Services so that the administration and Congress can develop policy based on scientifically supportable facts. Figure 1 shows the structure and membership of AOWG and some of the major research projects currently being coordinated and monitored.

The VA initiated its involvement with Agent Orange through the development of a communication process for gathering scientific and medical information. In early 1978, it established the Agent Orange Registry in order to accomplish four objectives:

• to identify all Vietnam veterans expressing a concern about the possible adverse health effects of their exposure to Agent Orange;

• to provide a mechanism for Vietnam veterans to voice their concerns to a physician, receive a physical examination, and obtain responsible answers to some of their questions;

• to serve as a mechanism for follow-up of these veterans if, at a later

ometry Branch of the National Cancer Institute collected data on cancer incidence and mortality in the U.S. for a five-year period (1973–1977) through 11 SEER program centers. The total number of subjects in the SEER program represents about 10% of the U.S. population and is fairly representative with respect to age.

Data on a total of 84 456 veterans were computerized in the Agent Or-

11.7%. Some differences, however, were found for cancer of the buccal cavity and pharynx, and lymphomas.

The 95% confidence limits for differences in proportions for these two sites were 1.2–5.7% and 1.6–8.4%, respectively. In other words, proportions for these sites in the registry were higher than expected from the reference population. Whether these marginal but statistical differences are artifacts or something of importance is not clear at this time. A recent study by Hardell et al. (42) also implicated an association of phenoxy acid or chlorophenol exposure with nasal and nasopharyngeal cancer. We will continue to monitor the registry data.

The VA in cooperation with EPA agreed to study levels of 2,3,7,8-TCDD in adipose tissue from a select group of U.S. males.

Assay of human adipose tissue

Since TCDD is known to accumulate preferentially in the adipose tissue of certain species of laboratory animals, it was suggested early in the history of the Agent Orange issue that the analysis of human fat for TCDD might provide a way to determine earlier Agent Orange exposure. A relation between the presence of this substance and health problems had also been suggested (43). Although methods for TCDD analysis had improved in recent years, no such study was carried out in humans with known exposure to herbicides containing this toxic contaminant. Consequently the VA embarked on a small feasibility study to test the methodology and to determine whether conclusions might be drawn regarding the significance of the results. The study was carried out with three groups of adult males:

• twenty Vietnam veterans, all but one of whom claimed health problems related to Agent Orange exposure, who volunteered for the fat biopsy;

• three U.S. Air Force officers with known heavy and relatively recent exposure in connection with herbicide disposal operations, including one who served in Vietnam; and

• ten veterans with no service in Vietnam and no known exposure to herbicides who were undergoing elective abdominal surgery and volunteered to serve as controls.

The procedure called for the removal of 10–30 g of subcutaneous adipose tissue from the abdominal wall. This was accomplished surgically under local anesthesia. Precautions were taken before, during, and after the procedure to avoid contamination by compounds such as hexachlorophene that could contain TCDD. Specimens were collected in glass containers previously rinsed with acetone and dried before use. All tissues

date, new information develops as a result of the various research efforts under way or being planned; and

• to obtain, as a by-product, some preliminary information on the current health status of the veterans who have participated in the registry.

The Agent Orange Registry provides a listing of names and addresses, together with some background data on a self-reported group of veterans with service in Vietnam. A complete physical examination and a group of baseline laboratory tests are provided to each veteran. The information from this effort is currently being computerized. The agency is in the process of collating the data to determine whether or not there are any significant health trends in these individuals that might suggest areas for further investigation. Although we have collected information on a large number of veterans, great caution must be exercised to analyze and interpret the data properly because of the self-selection of the sample, lack of precision in the reporting of exposure, and possible selective recall of symptoms and exposure.

With these limitations in mind, we have attempted to compare distribution of malignant neoplasm cases in the Agent Orange Registry with that in a reference population. These data are presented in Table 1. Subjects in the SEER (Surveillance Epidemiology End Results) program were selected for the reference population. The Bi-

ange Registry as of May 17, 1982. Among these veterans, 720 were diagnosed as having malignant neoplasms (ICD 140-208); 263 had previous personal histories of malignant neoplasms (ICD V 10.0-V 10.9); 2 had in situ carcinoma of the skin; and 18 had neoplasms of uncertain behavior or nonspecific nature (ICD 236-239). To be comparable with the SEER data, 136 cases of nonmelanoma cancer of the skin (ICD 173) reported in the Agent Orange Registry were excluded from the analysis.

Although nonmelanoma cancer of the skin is the most common malignant neoplasm in the white population of the U.S., statistics on skin cancer are usually incomplete, because most skin cancer patients are seen and treated in physicians' offices and are not hospitalized. The primary source of data for cancer registries including the SEER program is the hospital patient file.

The distribution of malignant neoplasm cases in the SEER program was calculated using the number of malignant cases diagnosed from 1973 to 1977 among U.S. males aged 25–39. This age group should include most of the Vietnam era veterans and, therefore, would serve as a reasonable comparison group. In general, no significant disparity in the proportion of cancer of various sites was noted between the two groups. Proportions of STS and skin cancer in the registry were not different from the SEER population: 1.9% vs. 2.6%; 9.1% vs.

TABLE 1

Number and percent distribution of malignant neoplasm cases [a]

Primary site (ICD)	Number of cases	Percent distribution Registry	Percent distribution SEER [b]
Buccal cavity and pharynx (140–149)	46	7.9 [c]	4.5
Digestive system (150–159)	68	11.6	12.2
Respiratory system (160–169)	60	10.3	8.5
Bones and joints (170)	8	1.4	1.1
Soft tissue (171)	11	1.9	2.6
Skin (172) [d]	53	9.1	11.7
Breast (174, 175)	3	0.5	0.06
Male genital system (185, 186, 187)	80	13.7	16.0
Urinary system (188, 189)	32	5.5	6.1
Eye (190)	4	0.7	0.5
Brain and other nervous system (191, 192)	28	4.8	6.0
Endocrine system (193, 194)	20	3.4	6.5
Lymphomas (200, 201, 202)	117	20.0 [c]	15.0
Multiple myeloma (203)	7	1.2	0.4
Leukemia (204–208)	30	5.1	6.0
Others and ill-defined sites (195–199)	17	2.9	3.0
Total	584	100	100

[a] Among 84 456 veterans recorded in the Agent Orange Registry and compared with a reference population.

[b] SEER (Surveillance Epidemiology End Results): percent distribution of malignant neoplasm cases diagnosed from 1973 to 1977 by primary site, males age 25–39, all races and all areas excluding Puerto Rico.

[c] The 95% confidence limits for differences in proportions do not include zero.

[d] Excluding basal and squamous cell carcinomas.

were refrigerated during shipment to the assay laboratory. Each of the volunteers had a medical history, physical examination, and routine clinical chemistry. The details of military service in Vietnam from the volunteer's report and his service record were examined to evaluate his potential exposure to herbicides using the dates, location, and nature of his service. From these a rough estimate of the likelihood of exposure to TCDD was made without knowledge of the assay results.

The extraction and assay of all samples for TCDD were conducted at the University of Nebraska, Midwest Center for Mass Spectrometry, Lincoln, Neb. Gas chromatography/high resolution mass spectrometry was employed for quantification of 2,3,7,8-TCDD and coeluting isomers. Extracts that contained materials giving signals greater than 2.5 times noise at the exact mass of TCDD (that is, m/z 321.8936 $\pm$ 0.0020) over the integration period were reanalyzed.

For the second analysis, signal profiles of m/z 321.8936 and m/z 319.8965 were monitored over the elution period of 2,3,7,8-TCDD (determined by injection of standard solutions). A positive detection was reported if signals were observed above the detection limit and if their intensity ratio was consistent with the presence of four chlorine atoms in the molecule. Samples meeting all criteria except the isotope intensity ratio were considered to contain "not detectable" levels of TCDD. For these samples, it was judged that the presence of TCDD is not disproved by the observation of an incorrect isotope ratio at these low concentrations; rather, the presence of TCDD is not confirmed.

The analysis revealed that of the 20 veterans who served in Vietnam, seven had no detectable TCDD with the limit of detection at 2–6 parts per trillion (ppt). Another two had detectable material that could not be validated as TCDD, and the results for one were considered equivocal because

the measured value was only questionably above the detection limit. Five of the 10 remaining Vietnam veterans had TCDD in amounts from 5 to 7 ppt. Three Vietnam veterans had TCDD in amounts from 9 to 13 ppt; one had 63 and 99 ppt; and another had 23 and 35 ppt.

Of the 10 control (unexposed) veterans, four had TCDD identified in their fat (6, 7, 7, and 14 ppt). Two other veterans had values low enough to be considered equivocal, and in three instances, the detected material was not validated as TCDD. The remaining veteran had no detectable TCDD.

One of the three Air Force officers with known heaviest exposure had no identified TCDD in his fat. The unidentified substance in his case and the TCDD measured in the other two officers was never more than 3 ppt above the limit of detection.

Among the 20 Vietnam veterans there was no uniformity of symptoms, either immediately after exposure, at the time of biopsy, or during the intervening period. No one symptom or group of symptoms was common to veterans with detectable TCDD in their fat. The presence of TCDD did not mean ill health, nor did its absence indicate good health. No detailed statistical analysis of this small pilot series was attempted.

In summary, the results of this very complex and technically difficult analysis indicated that very low levels of TCDD, believed to be 2,3,7,8-TCDD, could be detected in human adipose tissue in the range 3–99 ppt. The levels, however, did not correlate well with known exposure and nonexposure, and there was no correlation with health status. The study results, however, did indicate that the assay method was feasible, but would serve no clinically or administratively useful purpose until additional data were available on background levels of TCDD in the general U.S. population. Accordingly, the VA in cooperation with EPA, agreed to study levels of 2,3,7,8-TCDD in adipose tissue from a selected group of U.S. males.

EPA has been collecting adipose tissue from the U.S. general population by region, age, sex, and race. This National Adipose Tissue Bank was started in 1968 and now contains specimens from over 12 000 individuals. Adipose tissue from approximately 550 males born between 1937 and 1952 is available from this bank. Many of these men served in the military during the Vietnam era, and some served in Vietnam during the period of Agent Orange use. A retrospective

study of their adipose tissue may establish data on background levels of 2,3,7,8-TCDD in the U.S. male population, as well as whether service in the military and especially in Vietnam has had an effect on the levels of TCDD in adipose tissue.

The study will be conducted in three phases. Phase I will obtain the name and social security number for the approximately 550 males noted above. This information will be used to determine military service status. Phase II will be the development of analytic methods for the determination of selected dioxins (especially 2,3,7,8-

frequency or specific cause of death. Nonetheless, the study will provide a large amount of potentially useful information on Vietnam veteran mortality. In addition, it will provide ideas for future research. The results are to be reported in late 1984.

Vietnam Experience Twin Study

The Vietnam Experience Twin Study was begun by a group of research staff members at the VA Medical Center, St. Louis, Mo. The study will identify pairs of identical twin veterans of which one twin served in Vietnam during the period of Agent

registry, approximately 7500 had significant anatomical defects at birth. The investigators will locate and interview both parents of approximately 5400 of the children in this group. In addition, the parents of 3000 matched control normal babies born during the same time period will be interviewed.

Since the major objective of this study will be to determine whether an unusually high proportion of fathers of babies born with defects served in Vietnam, information will be gathered about Vietnam service, as well as other factors that may be associated with occurrence of birth defects. If results demonstrate that a Vietnam veteran has an increased risk of fathering a child with a defect, it may be desirable to attempt to determine if the increase is associated with Agent Orange exposure or with some other factor(s). The study is scheduled to be completed by the end of 1983.

Neither the government nor the scientific community has resolved the . . . controversies involving the use of Agent Orange . . . or the use of 2,4,5-T in U.S. agriculture.

TCDD) and furans in human adipose tissue. The method will be subjected to rigorous interlaboratory validation by an independent analytic referee, such as the Association of Official Analytical Chemists. Phase III will be the analysis of the adipose tissue and the preparation of a final report. Phases I and II should be completed within calendar year 1983, and the report from Phase III should be available in early 1985.

Vietnam veteran mortality study

The VA initiated a large mortality study that will compare cause-of-death profiles between veterans with service in Vietnam and comparable veterans with no service in Vietnam. Because there are no estimates of the population at risk, the study will provide only proportionate mortality ratio (PMR) data. The study will use existing records to identify a group of approximately 60 000 deceased veterans and determine cause of death and Vietnam service status. The study will also include independent validation of the computer data base used to identify deaths.

It is important to note that the mortality study will not provide information specifically related to a reason for greater or lesser relative

Orange spraying and the other served in an area other than Southeast Asia. Approximately 600 pairs of twins will be examined using a series of psychologic, physiologic, and biochemical tests.

The development of a protocol for the study and the establishment of a Twin Registry from which to solicit participants is now under way. The study will focus primarily on the total Vietnam-experience rather than any single factor such as Agent Orange. If a sufficient number of identical twin pairs are identified, there may be an opportunity to draw some conclusions regarding the effects of specific aspects of the Vietnam experience such as combat, herbicide exposure, or use of insecticides. Following formal review of the protocol, the study is to begin in January 1984. A report on the results is planned for early 1986.

Birth defects

In 1981, the Centers for Disease Control (CDC) in Atlanta, Ga., began a study designed to determine if veterans are at an increased risk of having children with birth defects. Since 1968, CDC has maintained a registry of all babies born with defects in the greater metropolitan Atlanta area. Of the more than 15 000 children in this

The Air Force health study

In 1979, the Air Force established the protocol for a comprehensive epidemiologic study of RANCH HAND personnel, a group of approximately 1260 men who conducted the fixed-wing aerial herbicide spraying missions in Vietnam from 1962 through 1971. The study is designed around the question, "Have there been, are there now, or will there be in the reasonably forseeable future, any adverse health effects among RANCH HAND personnel caused by repeated exposure to Herbicide Orange?"

The investigation is composed of three integrated elements—a mortality study of those individuals who have died since their exposure, a morbidity study to examine the current health status of the study subjects, and a follow-up study to look for delayed effects over the next 20 years. The mortality and morbidity study elements are being conducted simultaneously on RANCH HAND personnel and on a group of very carefully matched controls using personnel tracking procedures coupled with an extensive review of military medical personnel records; detailed face-to-face questionnaires to ascertain current and past health events as well as occupational and family data; and comprehensive physical examinations, psychological testing, and diagnostic laboratory studies to determine exact health status. Additional questionnaires and physical examinations are to be administered periodically during the follow-up phase.

In June 1983, a report was released comparing the mortality experience of

1247 RANCH HAND personnel and 6171 comparison subjects (43). The comparison subjects were individuals assigned to selected Air Force units with the mission of flying cargo to, from, and in Vietnam during the same period. As of Dec. 31, 1982, 50 RANCH HAND and 250 comparison subjects had died. The mortality experience, including deaths caused by malignant neoplasms, of the RANCH HAND population was almost the same as that of the comparison group. No STS deaths were detected in either group. Although there was no indication that the RANCH HAND subjects suffered from any increased mortality or any unusual patterns of death, the study results should not be regarded as final because of the small sample consisting of young, healthy individuals and the relatively short follow-up period. The results of the initial questionnaire, current health status, and reproductive histories are to be released in late 1983.

Study of ground troops

Public Law 96-151 charged the VA to "design a protocol for and conduct an epidemiological study of persons, who while serving in the Armed Forces of the United States during the period of the Vietnam conflict, were exposed to any of the class of chemicals known as 'the dioxins' produced during the manufacture of the various phenoxy herbicides (including the herbicide known as 'Agent Orange') to determine if there may be long-term adverse health effects in such persons from such exposure." Efforts by CDC (through an interagency agreement with the VA) are under way to complete a protocol and to identify suitable cohorts. This will be a huge research effort that will include interviews and examinations involving several thousand veterans.

A major problem in the design of such a study is that there are very few records maintained that would link specific ground troops to herbicide exposure. Another problem encountered is that it is difficult to design data-collecting instruments and techniques when the health outcome variables are so ill-defined. However, much design work has been accomplished, and it is hoped that the study will begin early in 1984 and be completed by 1987.

Soft-tissue sarcoma studies

In view of concern raised by many veterans that their contact with phenoxy herbicides and TCDD during Vietnam service may increase the risk

of developing STS, and because of the conflicting research findings in the scientific literature regarding association between exposure to phenoxy herbicides and STS, many epidemiological studies have been initiated in the U.S. to determine host and environmental risk factors for STS. The National Cancer Institute is conducting a case control study in the state of Kansas to evaluate the effects of occupational and environmental risk factors including herbicide exposure on the development of STS, non-Hodgkin's lymphoma, and Hodgkin's disease. A minimum of 100 white male cases of each cancer drawn from the University of Kansas Medical Center Cancer Data Service will be compared to controls matched to each case (3:1) by age, race, sex, and area of residence. Results of this study are due in mid-1984.

The VA, in collaboration with the Armed Forces Institute of Pathology (AFIP), has started a case control study of STS. A minimum of 500 STS cases selected from the AFIP registries will be compared to controls matched (2:1) to each case by age (±5 years), race, sex, and place of initial diagnosis. Phase I of the study will investigate whether military service in Vietnam increases the risk of developing STS. Phase II of the study will investigate other host and environmental risk factors for STS based on information obtained from interviews with subjects or their next of kin. Phase I of the study is scheduled for completion in the fall of 1984; Phase II will be completed in late 1985.

Since 1979, NIOSH has begun to develop a registry of U.S. workers involved in production of herbicides possibly contaminated with dioxin. As of May 1, 1983, about 4000 workers have been included in the registry. The enrollment of an expected number of 6000 workers will be completed by the end of 1983, and their mortality pattern, including deaths caused by STS, will be available in early 1985.

Unresolved controversies

Neither the government nor the scientific community has resolved the numerous environmental, medical, and political controversies involving the use of Agent Orange in Vietnam from 1962 to 1970 or the use of 2,4,5-T in U.S. agriculture. A report by the National Academy of Sciences in 1974 documented some of the environmental impacts of Agent Orange, but the arrangements that terminated the Vietnam conflict precluded additional scientific studies in that area. Such

studies might have clarified current medical concerns about herbicide exposure. Moreover, to date, federal agency positions on environmental hazards associated with TCDD and 2,4,5-T are either not well defined or not uniformly accepted, thus perpetuating the controversy.

The scientific community must continue to conduct valid research on pertinent environmental and health-related issues to provide a reliable basis for appropriate decision making. The VA stands firmly committed to working closely with other agencies of the federal government, as well as with the private sector, to obtain as many answers as quickly as possible, consistent with sound scientific principles, to resolve this perplexing issue. The resolution of the controversy, however, will be achieved only following the public's acceptance of the outcome of scientific investigations. To that end, scientists must accept the responsibility for not only conducting quality research, but also for translating the results of their efforts for legislators, the courts, the media, and ultimately, the public at large.

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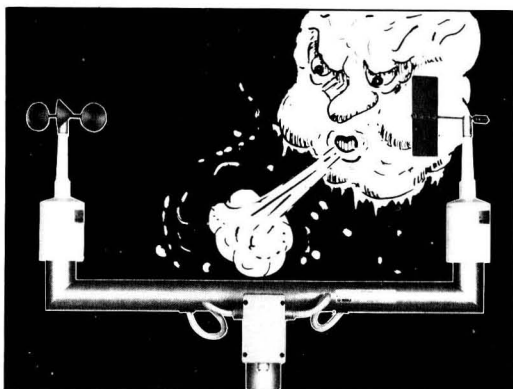
Alvin L. Young is a lieutenant colonel in the U.S. Air Force on special detail to the VA as special assistant for environmental sciences. Since receiving his PhD in herbicide physiology from Kansas University in 1968, he has been associated with all facets of the Agent Orange Program. His primary research interest is in the environmental fate and toxicology of the chlorinated dibenzo-p-dioxins.

Han K. Kang (l.) received a doctorate in public health in 1976 from the University of California at Los Angeles. As a senior health scientist with OSHA he had considerable experience in evaluating health hazards from chemical exposure in the workplace. Currently, he is chief of Research Section of the VA's Agent Orange Projects Office and is responsible for managing the technical aspects of Agent Orange-related research.

Barclay M. Shepard (r.), a 1958 graduate of Tufts University School of Medicine, diplomate of the American Board of Surgery and American Board of Thoracic Surgery, spent most of his professional career in the Navy. For the past three and a half years, he has been director of the Agent Orange Projects Office and a member of the federal government's interagency work group monitoring dioxin-related research.

Freshwater acidification from atmospheric deposition of sulfuric acid: A conceptual model

A new way of addressing the complex temporal relationships between changes in sulfur deposition and changes in surface water chemistry

James N. Galloway
University of Virginia
Charlottesville, Va. 22903

Stephen A. Norton
University of Maine at Orono
*Orono, Maine 94469*

M. Robbins Church
University of Virginia
Charlottesville, Va. 22903

Existing models of the acidification of freshwaters by acid deposition span a wide spectrum of approaches. At one extreme are empirical models that relate changes in one variable, such as the acidity of precipitation or the deposition of SO_4^{2-}, to changes in another variable (the acidity or the SO_4^{2-} concentration of freshwaters) (*1–3*). These empirical models, especially those of Henriksen, have been very useful in providing an initial understanding of the relationship between acid deposition and freshwater composition as a function of sulfur and H^+ deposition (*2*). Such models are based on the concepts of electrical neutrality and alkalinity but do not reflect biogeochemical processes or the temporal variations in the response of freshwaters to acid deposition.

At the other extreme are dynamic mechanistic models. These are much more data intensive and site specific than the empirical models and may require an extraordinary amount of effort to verify and use (*4*).
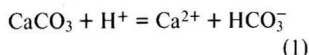
We present here a simple conceptual model that incorporates only the few key processes that we believe determine how aquatic ecosystems respond
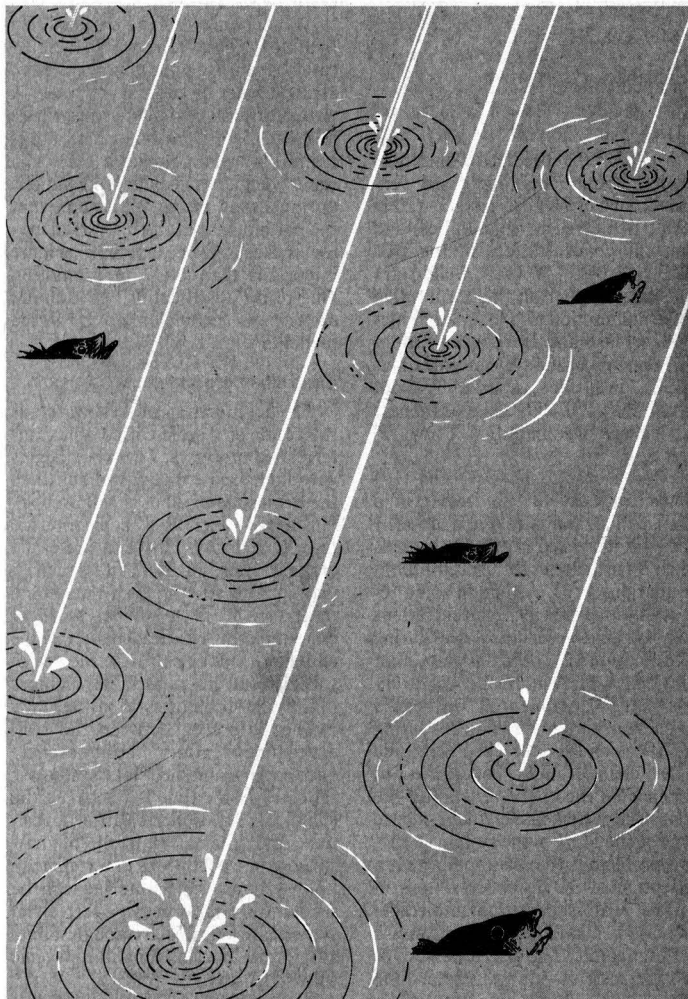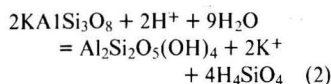
to acid deposition with respect to time. This model is based on the assumption that if the rate of acid deposition changes, a variety of constituents (such as SO_4^{2-}, base cations [BCs], and alkalinity) in both the terrestrial and aquatic systems will respond.

From a stoichiometric viewpoint, we view all the chemical reactions as capable of shifting equilibrium in response to changes in concentrations of H^+ or SO_4^{2-}. For example:

Congruent solution
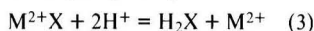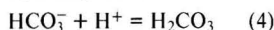
$$CaCO_3 + H^+ = Ca^{2+} + HCO_3^- \tag{1}$$

Incongruent solution

$$2KAlSi_3O_8 + 2H^+ + 9H_2O$$
$$= Al_2Si_2O_5(OH)_4 + 2K^+$$
$$+ 4H_4SiO_4 \tag{2}$$

Absorption/desorption

$$M^{2+}X + 2H^+ = H_2X + M^{2+} \tag{3}$$

Changes in speciation

$$HCO_3^- + H^+ = H_2CO_3 \tag{4}$$

Most of the predictive empirical models assume that increased concentrations of SO_4^{2-} in surface waters result in decreased alkalinities, and that the concentrations of the BCs $(Ca^{2+} + Mg^{2+} + Na^+ + K^+)$ are constant. Implicit in this assumption is the assumption also that alkalinities will increase and BC concentrations will remain constant if concentrations of SO_4^{2-} in surface waters are reduced by the control of SO_2 emissions. These models are oversimplified in two respects.

First, a change in SO_4^{2-} (and H^+) concentrations will not result in a change in alkalinity (e.g., Equations 1 and 4) if increased H^+ concentrations cause an increase in BC concentrations (Equations 1–3). Although some reports assume that BC concentrations do not change in surface waters during acidification (2), others assume that they do (5–8). A number of researchers have documented increased leaching of soils and sediments during acidification (9, 10). The magnitude of the changes in BC concentrations depends on the characteristics of the terrestrial system and on the residence time of the SO_4^{2-} in the system. A second problem is the assumption that a reduction of SO_4^{2-} concentrations in surface waters cause an immediate increase in alkalinity. This response is not immediate. The characteristics of the terrestrial system determine whether the lag in time is weeks, decades, or centuries.

This article represents a new way of addressing the complex temporal relationships between changes in sulfur deposition and changes in surface water chemistry by creating a conceptual model that includes

• the stages of acidification of aquatic ecosystems,
• the stages of recovery,
• the time lags in acidification and recovery, and
• the processes that control the time lags.

The first two points of the model provide a conceptual framework for the last two, which must be addressed before we can state the time scale over which aquatic systems respond to changes in sulfur deposition.

Our model is applicable to those lakes where terrestrial processes mediate the lake water acidification process. It does not apply to those unique hydrologic situations where acidification results from the dilution of more alkaline water by less alkaline water (a virtual titration and dilution) or where more acidic water, unaltered by terrestrial processes, replaces less acidic water, volume by volume (for example, during a snow melt). We assume that the net biomass in the terrestrial system is constant. Because SO_4^{2-} is more important than NO_3^- in promoting long-term acidification of aquatic systems, we include only SO_4^{2-} in the model (8).

Controlling processes

The acidification of freshwaters is the result of a series of complex, interrelated processes. The series begins with increased emissions of sulfur to the atmosphere. This is followed by "instantly" increased deposition to terrestrial ecosystems and increased concentrations of SO_4^{2-} in terrestrial and aquatic ecosystems. We assume that, on the geographical scale of eastern North America, changes in the emission rates of sulfur to the atmosphere result in proportional changes in the deposition rates of sulfur (11).

The difference between the residence time of sulfur in the atmosphere and that in watershed–lake systems is reflected in the time lag between the change in atmospheric sulfur deposition and the attainment of new steady-state conditions in watershed–lake systems. This time lag can be substantial largely because terrestrial systems are able to act, through sulfate adsorption, as a sink for anthropogenic sulfur (12, 18).

Increased concentrations of sulfur (as SO_4^{2-}) in the aquatic system also must result in decreased alkalinity, increased BC concentrations, or a combination of the two. Both, however, may not change at the same rate. An initial increase in SO_4^{2-} concentrations may result in proportionally large increases in BC concentrations relative to decreases in alkalinity in the aquatic system. This continues for some time until the easily weathered or exchangeable reservoirs of BCs in the soils associated with the hydrologic pathway through the terrestrial system are depleted (that is, the percent base saturation [%BS] approaches zero). Then, the concentration of H^+ (and possibly aluminum species) increases more rapidly with a concurrent decrease in alkalinity (or an increase in strong acidity). Consequently, a time lag also will exist between the decreased sulfur deposition resulting from emission controls and significantly increased lake alkalinity.
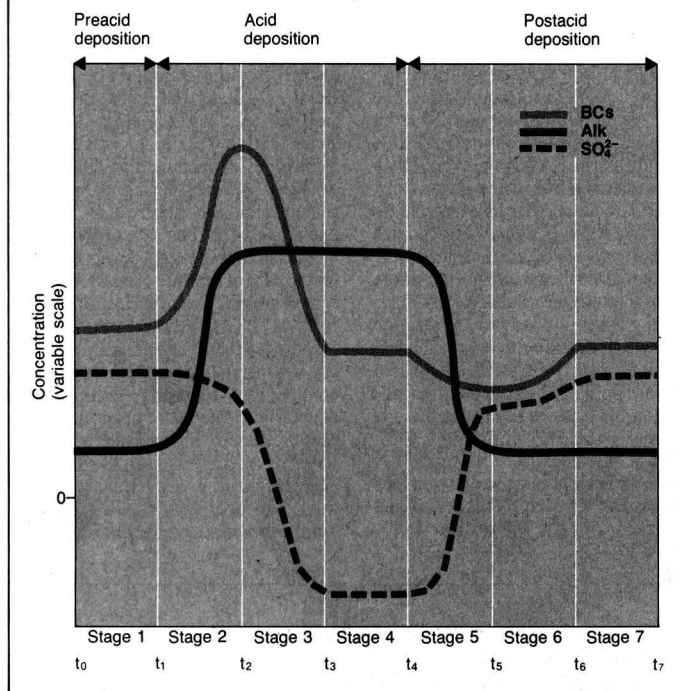
The conceptual model

The model has seven stages. The effect of each stage on the water composition of an oligotrophic lake is shown in Figure 1.

Stage 1. *Preacidification stage* $(t_1–t_0)$. This is the steady state prior to significant emissions of anthropogenic sulfur. The concentrations of SO_4^{2-}, alkalinity, and BCs are relatively constant over time, and the solution chemistry is dominated by BCs and HCO_3^- formed by soil solution neutralization during the weathering of primary minerals.

Stage 2. *Undersaturated sulfate adsorption capacity (SAC)* $(t_2–t_1)$. Increased sulfur emissions result in immediately increased sulfur deposition. All of the deposited sulfur, however, does not enter the aquatic systems until the SAC of the soil is saturated. As the increased sulfur deposition partially accumulates in the terrestrial system, the BCs gradually increase in the aquatic system and alkalinity gradually decreases (Figure 1).

Stage 3. *Saturated SAC in soil* $(t_3–t_2)$. The soil system is now saturated with respect to the new level of SO_4^{2-} deposition. This allows an amount of SO_4^{2-} equivalent to the sulfur deposition from the atmosphere to be discharged to the aquatic system. Because the concentration of SO_4^{2-} in the aquatic system has increased, the anion (HCO_3^-) concentration must decrease or the cation (BC, H^+, or Al^{n+}) concentration must increase. The exact proportions depend on the

Stage 6. *Recovery of %BS* (t_6-t_5). This stage is one in which the sulfur budget of the terrestrial and aquatic systems is in a steady state with respect to the constant rates of SO_4^{2-} deposition. The changes occurring during this stage are an increase in the %BS of the terrestrial system and a recovery of BCs in surface waters because the BCs supplied by primary weathering now exceed adsorption demands.

If the hydrologic pathway through the terrestrial system is such that it bypasses the zone of primary weathering, the %BS of the soils along the hydrologic pathway recovers only if the supply of BCs from atmospheric deposition and litterfall decomposition exceeds that lost by the decreased SO_4^{2-} flux.

Stage 7. *Stable period of lake recovery* (t_7-t_6). The systems are now in steady state with the lower levels of SO_4^{2-} deposition.

Use of conceptual model

The model can be used by defining the pertinent characteristics of each stage and identifying geographical regions that have those characteristics. Because sulfur deposition has not yet begun to decrease significantly, if at all, the pertinent characteristics of Stages 1–4 only are listed below.

- Stage 1: Systems are not receiving acid deposition.
- Stage 2: Systems are receiving acid deposition, soil is unsaturated with SO_4^{2-}, and the BC concentrations are increasing.
- Stage 3: Systems are receiving acid deposition, soil is saturated with SO_4^{2-}, alkalinity is decreasing, and the BC concentrations are decreasing.
- Stage 4: Systems are receiving acid deposition, soil is saturated with sulfur, alkalinity is constant but lower than initially, and the BC concentrations are constant.

Examples of low-alkalinity aquatic ecosystems in Stage 1 are those in the Rocky Mountains (*13*) and Sierra Nevada Mountains (*14*). These are not receiving the significantly elevated sulfur deposition that accompanies acid deposition. (Figure 2, Area I). For most of the high-altitude lakes in these areas, alkalinities are ≤ 100 μeq/L and SO_4^{2-} concentrations are ≤ 20 μeq/L.

Aquatic ecosystems in Stage 2 receive acid deposition and are associated with terrestrial systems having soils unsaturated with SO_4^{2-} (soils are rich in sesquioxides) (Figure 2, Area II). Regions containing such systems are large and are located primarily in

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FIGURE 1

The temporal variation of the concentrations (μeq/L) of SO_4^{2-}, BCs and alkalinity (alk) during the seven stages of acidification

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rate of primary weathering, the %BS and the cation exchange capacity of the soil along the hydrologic pathway of the precipitation, the cation content of the precipitation, and adsorption characteristics of the ion exchange surfaces.

Because the BCs are held much less tightly to ion exchange surfaces than are H+ ions, *initially* a greater proportion of BCs accompanies the SO_4^{2-} into the lake. Therefore, as Figure 1 shows, the BC concentration increases substantially and H+ concentration increases only slightly as the SAC of the soil becomes saturated (t_2) in Stage 2. The ratio of [BCs] to [H+] and the %BS of the soil along the hydrologic pathway decrease slowly unless BCs are resupplied from primary weathering or if primary weathering is increased by acid deposition. As the %BS approaches a lower equilibrium value, the [BCs]:[H+] ratio then decreases relatively quickly until it reaches the new steady-state value (that is, at t_3 on Figure 1). At this time, the BCs are supplied mostly from primary weathering of minerals in the hydrologic pathway and only secondarily from depletion of BCs on the ion exchange surfaces. Note that as [BC] decreases

during t_3-t_2, [H+] also increases. This prevents the formation of HCO_3^- from primary weathering and results in a negative alkalinity (strong acidity).

Stage 4. *Steady-state period of lake acidification* (t_4-t_3). During this stage both the terrestrial and aquatic systems are in a new steady state as a result of a higher level of steady-state deposition of SO_4^{2-}. Aquatic alkalinities remain low and %BS values in terrestrial systems are near zero.

Stage 5. *Supersaturated SAC* (t_5-t_4). This stage begins with decreases in the deposition of SO_4^{2-} (and H+) resulting from decreases in sulfur emissions. Concurrently, the higher pH shifts the adsorption reactions, and both the terrestrial and aquatic systems (soil and sediment) scavenge cations, causing a decrease in BCs. The SO_4^{2-} concentration is lowered in surface waters, although the soils release SO_4^{2-} for some time if the SO_4^{2-} in the soil is reversibly adsorbed. If the SO_4^{2-} is irreversibly adsorbed, the decrease of [SO_4^{2-}] in the lake or stream approximately parallels the SO_4^{2-} decrease in atmospheric deposition.

Stage 5 ends at t_5, when the terrestrial system reaches a new steady state as a result of lower rates of SO_4^{2-} and

the southern U.S. Documented examples are Shenandoah National Park, Va. (15), Camp Branch, Tenn. (16), Coweeta Experimental Forest, N.C. (17), and Walker Branch Watershed, Tenn. (18).

Aquatic ecosystems in Stages 3 and 4 are associated with soils saturated with SO_4^{2-} (Figure 2, Areas III and IV). The lack of reliable temporal data for alkalinity, however, makes it difficult to state which systems have reached a new steady state (19). Only aquatic ecosystems that have experienced heavy loadings of SO_4^{2-} for long periods of time (such as the Sudbury and Wawa regions in Ontario, Canada) or aquatic ecosystems with an original low %BS (such as Woods Lake, in the Adirondacks [8]) may have reached new steady states. Studies of New England lake sediments suggest that the BCs have not stabilized in that area (20). This may result from one of two causes: Deposition may be becoming increasingly acidic or equilibrium may not yet have been reached with respect to a constant acid loading.

Time scales

Quantifying the time scale for each stage of the conceptual model is a more difficult task. The following section discusses the approximate duration of each stage.

Stage 1. Stage 1 lasts as long as sulfur deposition does not increase substantially. It represents a steady state.

Stage 2. The length of Stage 2 depends on the SAC of and sulfate loading to the terrestrial ecosystem as well as the cation exchange capacity of the soils. Systems located in the southeastern U.S. are generally the only ones that have characteristics of Stage 2 (Figure 2). Johnson indicates that the length of (t_2-t_1) may depend on latitude (18).

In the northeastern U.S. and Canada, t_2-t_1 may be as short as a few years at present SO_4^{2-} loading rates. Thus, these areas have reached Stage 3 and perhaps Stage 4. The southern U.S. is probably still in Stage 2; thus SO_4^{2-} will increase (and alkalinity will decrease if the %BS is low enough) in aquatic systems in this region even if sulfur deposition remains unchanged.

Stage 3. The temporal extent of this stage (wth SAC saturated) depends on the rates of desorption and primary chemical weathering, the size of the soil reservoir associated with the hydrologic pathway, and the cationic composition of the precipitation. Be-

cause most systems in the northeastern U.S. and southeastern Canada are probably in Stage 3, further alkalinity decreases can be expected as BCs and the %BS continue to decrease.

Stage 4. Inasmuch as this is a new steady state, it lasts as long as sulfur deposition remains contant.

Stage 5. Decreases in sulfate deposition will result in a gradual loss of the sulfate accumulated in the soil during Stage 2. The time required for this depends on the SAC, the reversibility of the adsorbed SO_4^{2-}, and the amount of soil associated with the hydrologic pathway. As in Stage 2, the thinner podzolic soils of the northeastern U.S. and Canada will have a shorter Stage 5 than the southern soils. We estimate that the length of Stage 5 may range from years to many decades. Therefore, it is important to realize that a decrease in sulfur emissions and deposition may not result in significant increases in lake alkalinity for several years, if not decades. Concurrently, with a higher pH in precipitation and a decreased flux of SO_4^{2-} in the soil, soil and lake sediment will adsorb an in-

creasing amount of cations, decreasing the BC concentration.

Stage 6. During Stage 6 the %BS of the terrestrial systems recovers. The %BS determines, in effect, the size of the pool from which vegetation acquires its cationic nutrients for growth. Unfortunately, the recovery of the soil (%BS) is more complex and less understood then the recovery of the aquatic part of the system. The pre-acidification (Stage 1) value of the %BS is determined by many thousands of years of weathering of primary minerals and the capture of BCs on ion exchange sites. The rate of recreation of the %BS to Stage 1 levels depends on the rate of organic productivity, the rate of primary weathering, and other factors. It may take centuries for heavily leached deep soils and the organisms that live in them to recover completely. Generally, lakes should deacidify in the same order at which they acidified. Those lakes with watersheds having essentially no soil to adsorb cations and release SO_4^{2-} should recover very quickly, for example, in a matter of years, if the res-

FIGURE 2
Areas of different susceptibility to acid deposition[a]
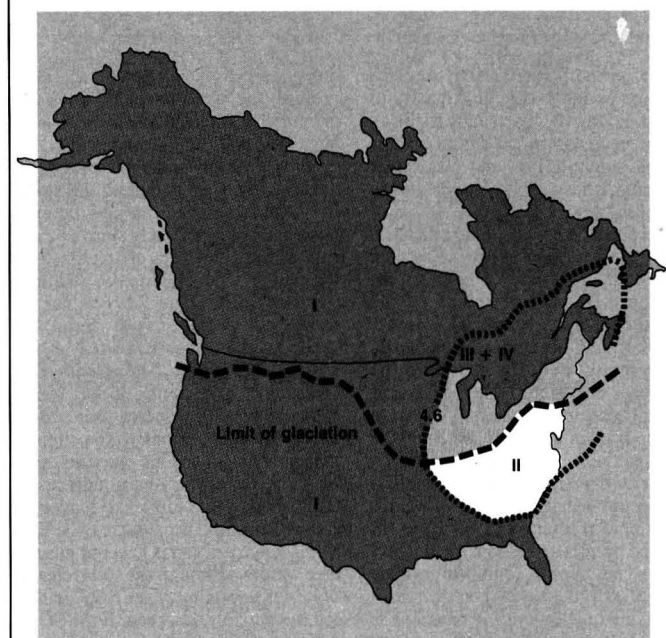
[a]Area I is not receiving acidic deposition and is defined by the area outside of the 4.6 pH precipitation isopleth. The precipitation data are taken from the National Atmospheric Deposition Program and the Canadian Precipitation Monitoring Network. Area II contains those soils that are receiving acidic deposition, but the soils are unsaturated with respect to sulfur. The boundary between soils that are unsaturated (II) and saturated with sulfur (III and IV) is defined by the maximum southerly extent of Pleistocene glaciation. Nonglaciated soils are highly leached, sesquioxide, and clay rich, with high SAC. Area designations (I, II, III, and IV) correspond to the stages of acidification on Figure 1.

idence time of water in the lake is short.

Summary

We have presented a simple conceptual model that we feel describes the dynamic response of surface water chemistry as a function of rates of acid deposition (notably H_2SO_4) and a few key soil processes. Work continues on the use of temporal water chemistry data as a means of qualitatively verifying the concepts underlying these seven stages of acidification.

Acknowledgment

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James N. Galloway is associate professor of environmental sciences at the University of Virginia. His research interests include the investigation of the long-range transport of atmospheric materials, the acidification of freshwaters, and the processes that control the composition of precipitation.
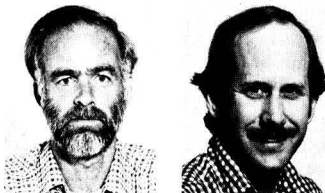
Stephen A. Norton (l.) is professor and chairman of the Department of Geological Sciences at the University of Maine at Orono. His research is in the area of low temperature–low pressure aqueous geochemistry and limnology.

M. Robbins Church (r.) received his PhD from the University of Virginia in 1980. He is currently employed as a program manager in the Aquatic Effects Task Group of the National Acid Precipitation Assessment Program, CERL, U.S. EPA, Corvallis, Ore. 97330.

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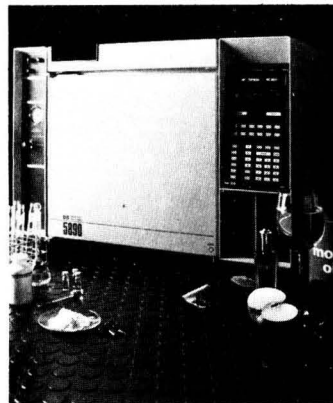

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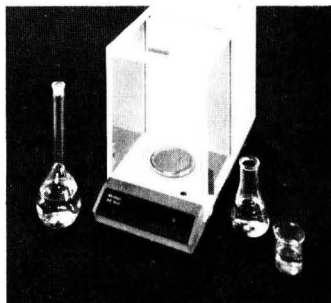
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numerous gases and sounds an alarm when a gas concentration exceeds the preset limit. Universal Sensors and Devices **108**

Residual chlorine monitor

This device has automatic ranging for operation from 1 ppb to 10 ppm and a new self-cleaning, flow-through filter system that allows sampling of "dirty" waters. The solid-state electronics provide stable operation, and the new electrode system requires minimum maintenance. The internal calibration system eliminates the need for standard solutions and allows the instrument to be calibrated in the field. Epco **109**
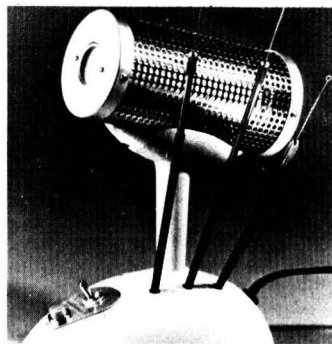
Sterilizer

This uses IR heat to sterilize loops, needles, and culture tube mouths. It does not require oxygen and can be used in anaerobic chambers. The temperature in the heating element cavity is never less than 1500 °F. Monoject Scientific **110**

Infrared gas analyzer

Analyzer determines changes in CO_2 and H_2O vapor levels in plant chambers that are used to monitor photosynthesis. The instrument measures absolute and differential CO_2 as well as absolute and differential H_2O. The unit is portable and works on 12 V dc or 120 V ac. Both analog and digital

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displays of the measured components are available. Tekmar **111**

Sampling nozzle
Isokinetic sampling nozzle has an $1/8$-in.-diameter inlet and is designed for use with the Pilat Source Test Mark 3 and Mark 5 cascade impactors. Two types of nozzles are available: a standard straight nozzle and a right-angle nozzle. Pollution Control Systems **112**

Dual-purpose columns
These fused-silica columns can be used in both capillary and packed-column gas chromatograph analyses. They have a stationary-phase film thickness of 1.5 to 3 μm, providing adequate capacity for 1-μL injections of solutions that normally are analyzed on a 3–5% packed column. When operated under packed-column conditions, the new columns allow the chromatographer to use a technique previously restricted to capillary operation. Hewlett-Packard **113**

Fish activity meter
This includes an Apple II computer, video digitizer, and a TV vidicon camera. The computer calculates in real time the distance the fish travels and plots its movement patterns as well. Periodically, the computer's Epson printer prints this information along with the time of day. The same system can be used to measure the activity of rodents or insects, provided the subjects are moving on a contrasting background. Columbus Instruments **114**

Ambient gas sampler
Wet-chemical system meets or exceeds EPA reference method specifications for sampling sulfur dioxide in ambient air. A solid-state thermoelectric cooling–heating system maintains the SO_2-collecting reagent between 5 and 25 °C at ambient temperatures ranging from −25 to 50 °C. The sampler also collects NO_2 and an optional third gas. Andersen Samplers **115**

Automated pH meter
Designed especially for the quality control laboratory by Beckman instruments, this meter simplifies accurate pH measurement and makes data available automatically. It has a large memory and automatically computes and prints out electrode slope. The instrument also flags questionable slope readings. VWR Scientific **116**

Plasma spectrometer
This instrument has a full-color graphics display and printout. It is a 1-m spectrometer with a holographic grating that provides the resolution needed for most experiments. It can replace several atomic adsorption spectrophotometers. Bausch & Lomb **117**

Preseparator for cascade impactors
This device collects particles larger than 10 μm, thus eliminating the problems of particle bouncing, reentrainment, and wall losses. It holds gram quantities of particulate without overloading and attaches directly upstream of the cascade impactor with no additional adapters required. Andersen Samplers **118**

Annunciator
Compact unit measures $7 \times 10 \times 13^{1}/_{2}$ in. and has unusual text and graphics features. It has standard size and four-times-larger (double height and double width) characters, bold writing, reverse display and blinking, real-time bar graph presentation of analog variables, and optional bit-mapped graphics. ADAC **119**

Inconel crucibles
These crucibles are made of a nickel–chromium–iron alloy and can replace platinum crucibles in many high-temperature applications. Inconel crucibles maintain excellent weight stability at high temperatures and cost as little as 3.5% of the cost of platinum crucibles. They are available in a wide range of sizes from 5 mL to 1000 mL. B-J Scientific Products **120**

Air sampler pump
The pump operates in any of three selectable flow ranges: ultrahigh,

6–7 L/min; medium, 4–5 L/min; or low, 1.5–2 L/min. The compact unit is operated by self-contained nickel-cadmium batteries and includes an ac adapter for charging. It can be used for "grab sampling" and for personal sampling programs. Gilian Instrument **121**

x-y recorders
The pens in these recorders respond with high speed and high acceleration. All models include a built-in time base, electronic limiter, and remote control functions. Writing speeds up to 1500 mm/s (x-axis) and 2000 mm/s (y-axis) are available. SOLTEC **122**
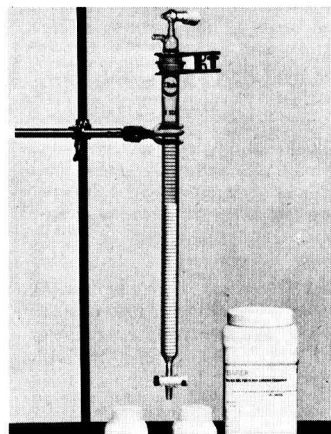
Silica gel for flash chromatography
The gel is quality controlled for the following parameters: average particle size, particle size distribution, mean pore diameter, moisture, pore volume, specific surface area, and pH. J. T. Baker Research Products **123**

Aerosol generator
Using a single instrument setup, three different aerosols can be produced, either sequentially or simultaneously. Constructed with all controls located on the front panel, this model can be used for calibrating particle sizing instruments and generating aerosols for filter testing and research chambers. TSI **124**

Computer-aided titrator
The system performs titrations to fixed endpoint targets, titrations with derivative detection of equivalence points, computational methods such as equilibrium and incremental titrations, as well as direct potentiometry in pH, millivolts, or activity with ion-selective electrodes. Titration rate control is automatic, as are electrode standardization and electrode efficiency computation. Fisher Scientific **125**

ES&T
LITERATURE

"Pollution Liability." Booklet shows how companies using or producing hazardous materials can evaluate their risk of pollution liability. Legislative and judicial trends are discussed. Environmental Technology (U.S.) **151**

Condensate polishing. Technical bulletin addresses condensate polishing equipment, including process selection, distribution, and collection systems, and other data. Infilco Degremont **152**

Baghouses. Folder describes firm's inventorying and marketing of large baghouses, precipitators, dust collectors, and other air pollution control equipment. APEX **153**

Data acquisition. Brochure describes Model IMP-831 digital data processor for low-cost, remote data acquisition, with special emphasis on air pollution measurements and similar tasks. Climatronics **154**

Laboratory products. 1984 catalog lists many lines of laboratory products, equipment, and safety devices. Wheaton Scientific **155**

Conversion factors. Booklet gives conversions from hogsheads to gallons, newtons to dynes, and many other measurements of length, area, volume, and weight. Innovative Sensors **156**

Gas detection. Brochure PGD-80-0282 describes detector for combustible and toxic gases and oxygen deficiency in confined spaces. Meets NIOSH recommendations and OSHA requirements. ENMET **157**

Temperature control. Brochure GPB-1 lists large line of instruments and accessories for temperature control. Burling Instrument **158**

Spin-on filters. Brochure 2320 (use No. FLD-054 to inquire) lists line of 45-gal/min, 150-psi-rated spin-on filters. Parker Hannifin **159**

Air monitoring. Data sheet PSS 6-5B1 A describes ambient air monitoring

system that can detect up to five gases or vapors at 24 remote locations. Object is personnel protection. Foxboro **160**

Tissue processing. Wall chart, "Problems in Processing," is an instructional guide for processing histological/cytological samples. Monoject Scientific **161**

Permeation tubes. Catalog lists Trace Source permeation tubes and instruments for generating precise gas standards. Rate data are given for more than 100 compounds. Kin-Tek Laboratories **162**

Volatile organic analysis. Application note, "Volatile Organic Analysis," describes use of EPA Methods 601 and 602 for detection of purgeable halocarbons and aromatics by gas chromatography. Tracor **163**

AA spectrophotometers. Brochure describes Video 11, Video 12, and Video 22 atomic absorption (AA) spectrophotometers for analysis, with background correction features. Instrumentation Laboratory **164**

Histology reagents. Brochure describes histology and cytology reagents, decalcifiers, embedding media, dyes, mounting media, and laboratory equipment. Polysciences **165**

Titration. Brochure describes Metrohm 636 titrator. Features include fast, reliable operation and printed output. Sybron/Brinkmann **166**

Hazardous materials compliance. Catalog lists placards, placard holders, warning labels and signs, bills of lading, and other items necessary to ensure compliance with hazardous materials regulations. J.J. Keller & Associates **167**

Mist eliminator. Bulletin TR81-9000130 describes tortuous-path mist eliminator for liquid–gas separation applications. Andersen 2000 **168**

Air pollution samplers. Bulletin 2300-R1 lists and describes air pollu-

tion samplers and monitors, especially for automatic, sequential sampling of airborne particles or hydrogen sulfide gas. Research Appliance **169**

2,3,7,8-TCDD separation. Separation of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD) is one topic discussed in the August 1983 issue (Vol. 2, No. 4) of the *Supelco Reporter*. Supelco **170**

Dust and erosion control. Literature describes soil stabilization product used for erosion and dust control, land reclamation, and similar applications. The product is first mixed with water. Soil Seal **171**

Computer courses. Brochure lists technical computer courses with tuition prices ranging from $695 to $895. Integrated Computer Systems **172**

Chemicals, materials. 1983–84 catalog lists more than 11 000 chemicals and materials, including organics, analytical standards, research-grade gases, and more. Alfa Products **173**

Pollutant tests. Article in Summer 1983 issue of *Analytical Control* (Vol. 8, No. 2) explains how EPA allows alternate procedures for pollutant tests. NUS **174**

Peristaltic pumps. Catalog lists a broad range of modular peristaltic pumps for many industrial purposes. Flow rates from 0.003 mL/min to as high as 120 L/min. New Brunswick Scientific **175**

Activated carbon. August 1983 issue of *Calgon air/water report* tells about various applications of granular activated carbon for water treatment and other needs. Calgon **176**

Toxic waste scrubbing. Bulletin TR82-9000145 tells how thermal oxidizers "scrub" toxic and hazardous materials from toxic waste incinerators. Liquid and solid materials are destroyed. Andersen 2000 **177**

Microprocessor-controlled analysis. Issue of *GT Technology Newsletter* (Vol. 2, No. 1) tells how AutoAnalyzer

GT continuous-flow technology facilitates sampling and analysis of trace environmental chemicals and performs other needed functions. Technicon **178**

Laboratory safety. Catalog lists more than 3600 products for laboratory safety, including signs, respirators, books, eye protection, and many other needs. Lab Safety Supply **179**

Intermittent cooling. Bulletin TIB-125 sets forth step-by-step procedures for maintaining intermittent cooling system operation or scheduling long-term cooling system shutdown. Mogul **180**

Soot cleanup. Brochure describes sonic cleaning systems for cleanup or removal of soot, fly ash, and dust in baghouses, precipitators, and other such devices. Works up to 2000 °F. Analytec **181**

Infectious wastes. Brochure defines closed contamination containment collection system for infectious waste management. Assures proper waste handling. M.D. Industries **182**

Ambient air analysis. Data sheet PSS 6-5A5 A describes a microprocessor-controlled analyzer for multicomponent gases in ambient air. More than 300 gases can be analyzed in keeping with OSHA rules, and other gases are also analyzed. Foxboro **183**

Flow monitoring. Bulletin 683 lists and details a line of instruments and primary elements for microprocessor-controlled open-channel flow monitoring. NB Instruments **184**

Nonasbestos gasketing. Reprint of "Performance and Reliability of Non-Asbestos Gasketing Materials" discusses nonasbestos materials available since 1979. Rogers **185**

Air pollution control. Catalog highlights engineered air systems approach to air pollution control and details major components. Zurn Industries **186**

Metering pumps. Bulletin DM1 describes highly accurate metering pumps, which are especially useful when proportional control of fluids is of importance. Up to 28.5 gal/h per feed; can work to 2500 psig and viscosities to 1500 cp. Duriron **187**

Mass spectral search. Announcement discusses mass spectrum searches available for more than 40 000 compounds from data in company's library. The firm developed the NIH/EPA Chemical Information System. Fein-Marquart Associates **188**

Safety training. Releases outline hazardous chemical safety training program and seminars on management and disposal of hazardous and chemical wastes. J.T. Baker Chemical **189**

Nonaqueous titration. Booklet explains how highly accurate titrations can be run in nonaqueous solutions, and what materials and conditions are necessary. Crescent Chemical **190**

Solids-handling pump standards. Single copies of "Submersible Solids-Handling Pumps Standard" are available. Used for several wastewater applications. Submersible Wastewater Pump Association, 221 N. LaSalle St., Chicago, Ill. 60601 (write direct)

Worker health. Catalog lists more than 200 articles and reports about worker health. American Occupational Medical Association, 2340 S. Arlington Heights Rd., Arlington Heights, Ill. 60005 (write direct)

Energy projects. List of technical papers on energy and other large-scale engineering projects by company authors. Jan Dividson, Bechtel Power Corp., P.O. Box 3965, San Francisco, Calif. 94119 (write direct)

Waste sites. Hazardous Waste Sites National Priority List. Gives order of ranking and other information. HW-7.1 Office of Public Affairs (A-107), U.S. Environmental Protection Agency, Washington, D.C. 20460 (write direct)

Industrial hygiene. Free subscriptions to *Industrial Hygiene News.* Write the publication at 8650 Babcock Blvd., Pittsburgh, Pa. 15237-9978 (write direct)

Solar energy research. How does solar perform in New York State? Programs are described in *Next Generation,* a new quarterly journal. Niagara Mohawk Power Corp., 300 Erie Blvd. W., Syracuse, N.Y. 13202 (write direct)

Environmental jobs. "Jobs and the Environment: A Selected and Annotated Bibliography," by Richard Kazis. $6. CPL Bibliographies, 1313 E. 60th St., Chicago, Ill. 60637 (write direct)

ES&T BOOKS

The Hazardous Waste Consultant. Periodical, bimonthly. McCoy & Associates, 13131 W. Cedar Dr., Lakewood, Colo. 80228. $350/year.

This periodical covers economic, engineering, and regulatory aspects of hazardous waste management. The first issue (July) examines dioxins, permits, industry liability, breakdown of clay liners, and other topics. A bibliographic listing of articles of interest is presented.

Ionizing Radiation: Tumorigenic and Tumoricidal Effects. Charles L. Sanders, Ronald L. Kathren. 352 pages. Battelle Press, 505 King Ave., Columbus, Ohio 43201. 1983. $49.95, hardcover.

Radiation is often blamed for causing cancer, but it is used as a cure in some cases. This book examines radiation's role in the formation and breakdown of tumors and discusses basic concepts, bone marrow, the skeleton, body organs, and carcinogenic mechanisms.

Acid Rain: A Review of the Phenomenon in the EEC and Europe. Graham & Trotman Ltd., Sterling House, 66 Wilton Rd., London SW1V 1DE, U.K. 1983. £12.50 (about $27.50).

This book examines the possible effects of acid rain on trees, crops, other terrestrial flora and fauna, aquatic flora and fauna, buildings, and human health. Sources and mechanisms of adverse effects are also discussed.

Pesticides and Neurological Diseases. Donald J. Ecobichon. 296 pages. CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Fla. 33431. 1983. $78 ($89 outside U.S.), hardcover.

The toxicology of various pesticide classes is discussed, as is their ability to damage the central and peripheral nervous systems. Topics covered include toxicokinetics; chlorinated, phosphorous, and carbamic ester insecticides; and mercurial fungicides.

Fugitive Dust Issues in the Coal Use Cycle. 272 pages. Publications Department, Air Pollution Control Association, P.O. Box 2861, Pittsburgh, Pa. 15230. 1983. $25 ($20 for APCA members), hardcover.

Topics include measurements and characterizations of fugitive emissions, methods of control and their effectiveness, and redefinition of and perspectives on ambient particulates. The book draws its material from a recent meeting on the subject.

Handbook of Hazardous Materials. 2nd ed. 248 pages. Alliance of American Insurers, Suite 400 West, 1501 Woodfield Rd., Schaumburg, Ill. 60195-4980. 1983. $8 (add $1.40 for UPS shipment or $2.24 for first-class mail).

This publication lists and discusses physical properties, flammability characteristics, toxicity specifics, and threshold limit values of more than 300 substances. It includes an outline of the principles of recognition, evaluation, and control of workplace contaminants as they are related to occupational disease.

Dictionary of Water and Wastewater Disposal (Wörterbuch für das Wasser und Abwasserfach). Revised ed. F. Meinck, H. Möhle. R. Oldenburg Verlag GmbH, Rosenheimerstrasse 145, D-8000 München 80, West Germany. 1983. About $80.

This dictionary is published in English, French, German, and Italian. The revised edition contains 12 018 entries, up from 8844 in the previous edition.

Implementing Tradable Permits for Sulfur Oxides Emissions. Glen R. Cass et al. Vols. I, II, III. 26, 215, and 412 pages. Publications Secretary, EQL 314-40, Caltech, Pasadena, Calif. 91125. 1983. $25 for the set (California residents add $1.63 sales tax).

This work deals with a system of transferable permits to emit air pollutants as an alternative to setting pollution limits on a source-by-source basis. It examines the viability of such an approach and the possible technical and economic advantages and shortcomings of permit trading.

Agricultural Management and Water Quality. Frank W. Schaller, George W. Bailey, Eds. 472 pages. Iowa State University Press, 2121 S. State Ave., Ames, Iowa 50010. 1983. $39.95 (add $1 for airmail), hardcover.

This book reviews the latest research and experience in agricultural non-point-source water pollution and its management. It contains material from a conference on the subject and discusses topics such as fate and transport of agricultural chemicals, water quality impact, nonpoint source control, and economic and social aspects.

The Acid Rain Resources Directory. Periodical, semiannual. The Acid Rain Foundation, Inc., 1630 Blackhawk Hills, St. Paul, Minn. 55122. 1983. $6.

This directory lists public, private, and educational resources currently available on the subject of acid rain. Sections include legislation, conferences, basic references on acid rain, current books, and many other important sources of information.

EARS Energy Catalog No. 15. 31 pages. Environmental Action Resource Service, Box 8, Farisita, Colo. 81037. 1983. $1, paper.

This catalog lists publications, hardware, systems, and do-it-yourself kits among the many items devoted to renewable resources and environmental protection.

The Great Extinction. Michael Allaby, James Lovelock. ix + 182 pages. Doubleday & Co., Inc., 245 Park Ave., New York, N.Y. 10167. 1983. $13.95, hardcover.

One of the greatest mysteries of natural history involves the complete disappearance of dinosaurs of the Mesozoic Era, the subsequent proliferation of mammalian species in the Cenozoic Era, and no apparent link between the two events. What environmental change (or cataclysm) brought about this seemingly unconnected change and essentially erased records of what went on as the Mesozoic Era became the Cenozoic? The authors offer an explanation.

ES&T
MEETINGS

Nov. 17–18 Arlington, Va.
Toxics in the Environment: The Regulatory, Legislative, and Enforcement Outlook. Center for Energy and Environmental Management
  Write: Brooks Cook, CEEM, P.O. Box 536, Fairfax, Va. 22030; 800-424-9068 or 703-250-5900

Nov. 21–22 Washington, D.C.
Hazardous Waste: Regulation, Litigation, and Liability Briefing. Executive Enterprises, Inc.
  Meeting is Package No. E4044. Fee: $650 ($575 for additional registrations). *Write:* Executive Enterprises, Inc., 33 W. 60th St., New York, N.Y. 10023; 212-489-2680

Nov. 28–30 Chicago, Ill.
Workshop on New Technologies for Recovering Energy from Garbage and Other Municipal Wastes. Argonne National Laboratory and others
  Fee: $125. *Write:* Miriam Holden, Director of Conference Services, Argonne National Laboratory, 9700 S. Cass Ave., Argonne, Ill. 60439; 312-972-5587

Nov. 29–30 Toronto, Ontario
Technology Transfer Conference No. 4. Ontario Ministry of the Environment
  Write: M. Moselhy, MOE Policy and Planning Branch, 135 St. Clair Ave. W., Toronto, Ontario M4V 1P5, Canada; 416-965-5788

Nov. 29–Dec. 2 Atlanta, Ga.
6th World Energy Engineering Congress. Association of Energy Engineers
  Write: 6th World Energy Engineering Congress, 4025 Pleasantdale Rd., Suite 340, Atlanta, Ga. 30340; 404-447-5083

Dec. 4–7 Norfolk, Va.
11th Annual Water Quality Technology Conference. American Water Works Association
  Fee: $225. *Write:* American Water Works Association, 6666 W. Quincy Ave., Denver, Colo. 80235; 303-794-7711

Dec. 5–6 Portland, Ore.
Seminar on Continuous Emission Monitoring Systems for Total Reduced Sulfur (TRS) Emissions. Applied Technology Consultants
  Fee: $275. *Write:* Merry Burt, ATC, Inc., 1800-B Airport Rd., Opelika, Ala. 36801; 205-749-6366

Dec. 5–6 Madison, Wis.
Sanitary Landfill Operations. University of Wisconsin—Extension
  Fee: $310. *Write:* Philip R. O'Leary, Department of Engineering and Applied Science, University of Wisconsin—Extension, 432 N. Lake St., Madison, Wis. 53706; 608-262-0493

Dec. 6–8 Atlanta, Ga.
EPRI PCB Seminar, Electric Power Research Institute
  Write: Gil Addis or Ralph Komai. EPRI, P.O. Box 10412, Palo Alto, Calif. 94303; 415-855-2286 or 415-855-2463

Dec. 6–9 East Lansing, Mich.
Dioxins in the Environment. Michigan State University and other sponsors
  Fee: $50. *Write:* R. W. Leader or Michael Kamrin, C-231 Holden Hall, Michigan State University, East Lansing, Mich. 48824; 517-353-6469

Dec. 12–14 Miami Beach, Fla.
6th Miami International Conference on Alternative Energy Sources. University of Miami
  Write: T. Nejat Veziroğlu, Clean Energy Research Institute, University of Miami, P.O. Box 248294, Coral Gables, Fla. 33124; 305-284-4666

Jan. 9–13, 1984 Tarpon Springs, Fla.
Chapman Conference on Natural Variations in Carbon Dioxide and the Carbon Cycle. American Geophysical Union
  Write: American Geophysical Union, 2000 Florida Ave., N.W., Washington, D.C. 20009; 202-462-6903; TWX: 710-822-9300

Jan. 16–17 Columbus, Ohio
Groundwater Investigations at Hazardous Materials Sites: Safety and Liability Considerations. National Water Well Association
  Write: NWWA Education Foundation, 500 W. Wilson Bridge Rd., Worthington, Ohio 43085

Jan. 24–25 Philadelphia, Pa.
The Fate of "Toxics" in Surface and Groundwaters. The Academy of Natural Sciences of Philadelphia
  Write: James Wilson, The Academy of Natural Sciences, 19th and Parkway, Logan Square, Philadelphia, Pa. 19103; 215-299-1107

Jan. 30–Feb. 3 Lake Buena Vista, Fla.
8th Annual Meeting on Energy from Biomass and Wastes. Institute of Gas Technology
  Write: Maryann Manrot, Institute of Gas Technology, 3424 S. State St., Chicago, Ill. 60616; 312-567-3881; Telex: 25-6189

March 5–9 Atlantic City, N.J.
1984 Pittsburgh Conference and Exposition on Analytical Chemistry and Applied Spectroscopy
  Write: The Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Inc., 437 Donald Rd., Pittsburgh, Pa. 15235

March 26–27 Columbus, Ohio
4th Ohio Environmental Engineering Conference. Ohio State University and others
  Write: Thomas D. Davis, Burgess & Niple, Limited, 5085 Reed Rd., Columbus, Ohio 43220; 614-459-2050

COURSES

Dec. 1–2 Houston, Tex.
Stack Sampling and Particle Sizing Seminar. Andersen Samplers Inc.
  Write: Ralph J. Bulger, Andersen Samplers Inc., 4215 Wendell Dr., Atlanta, Ga. 30336; 800-241-6898

Dec. 6–8 Jacksonville, Fla.
Visible Emissions Evaluation Certification. Eastern Technical Associates
  Write: David B. Savage, Jr., Eastern Technical Associates, P.O. Box 58495, Raleigh, N.C. 27658-8495; 919-834-2970

Dec. 12–15 East Brunswick, N.J.
Industrial Hygiene Practice. Center for Professional Advancement
  Fee: $895. *Write:* Center for Professional Advancement, P.O. Box H, East Brunswick, N.J. 08816-0257; 201-249-1400; Telex: 139303 CENPRO EBRW

Dec. 12–16 Madison, Wis.
Wastewater Treatment and Disposal—Part II with CAPDET. University of Wisconsin—Extension
  Fee: $450 for three days; $790 for five days. *Write:* John T. Quigley, Department of Engineering, University of Wisconsin—Extension, 432 N. Lake St., Madison, Wis. 53706; 608-262-0820

The Annual ACS LabGuide— the Definitive Source for

- Laboratory supply houses
- Sources for chemicals and instrumentation
- Meeting information
- Research and analytical services
- Trade names and new books

Jan. 9–10, 1984   Cleveland, Ohio
Sample Preparation for Trace Inorganic Analysis. Finnigan MAT Institute
   Fee: $300. *Write:* Nancy Kranpitz, Finnigan MAT Institute, 11 Triangle Park Dr., Cincinnati, Ohio 45246; 513-772-5500; Telex: 241482

Jan. 12–15   Park City, Utah
Occupational Toxicology and Respiratory Disease. University of Utah
   Write: Katharine C. Blosch, University of Utah, Bldg. 512, Salt Lake City, Utah 84112; 801-581-5710

Jan. 16–20   Madison, Wis.
Groundwater Computer Models. University of Wisconsin—Extension
   Write: Fletcher G. Driscoll, University of Wisconsin—Extension, 432 N. Lake St., Madison, Wis. 53706; 608-263-7756

INTERNATIONAL

Nov. 23–25   Noordwijkerhout, The Netherlands
Anaerobic Wastewater Treatment. European Symposium
   Write: Symposium Secretariat, Congress Bureau of TNO Communication Department, P.O. Box 297, 2501 BD, The Hague, The Netherlands; Phone: (070) 81 44 81, ext. 411 or 458; Telex: 31660 TNOGV NL

Dec. 7–8   London, U.K.
Symposium and Exhibition on Deterioration of Underground Assets. Institution of Water Engineers and Scientists
   Fee: £175.55 (£126.50 for IWES members; includes value-added tax; £1 = about $1.65). *Write:* B. J. Dangerfield, Institution of Water Engineers and Scientists, 31–33 High Holborn, London WC1V 6AX, U.K.; Phone: 01-831-6578

Feb. 8–12, 1984   Bombay, India
ENVIROTECH '84 Conference and Exhibition. CHEMTECH Secretariat
   Fee: $75. *Write:* Indra Mohan, Secretary General, CHEMTECH Secretariat, 3rd Floor, 210, Dr. D. N. Rd., Bombay 400 001, India; Phone: 262044; Cable: CHEMTECH; Telex: 011-3757 JSGR

Feb. 29   London, UK.
Information Retrieval of Environmental Chemicals. Royal Society of Chemistry
   Write: C. S. Collis, 46, Lyndhurst Rd., London N22 5AT, U.K.

CALL FOR PAPERS

Nov. 15 deadline (abstracts)
Symposium on Biological Monitoring of Exposure to Organic Chemicals in the Workplace. American Chemical Society
   The symposium will be April 8–13, 1984, in St. Louis, Mo. *Write:* H. Kenneth Dillon, Industrial Hygiene Chemistry Institute, P.O. Box 55305, Birmingham, Ala. 35255

Jan. 12, 1984 deadline (abstracts)
4th Annual National Symposium on Recent Advances in the Measurement of Pollutants from Ambient Air and Stationary Sources. EPA Environmental Monitoring Systems Laboratory
   The symposium will be May 8–11 in Raleigh, N.C. *Write:* Seymour Hochheiser, MD 75, EPA Environmental Monitoring Systems Laboratory, Research Triangle Park, N.C. 27711

Jan. 15 deadline (abstracts)
Symposium on Impacts of Acid Rain and Deposition on Aquatic Biological Systems. American Society for Testing and Materials, ASTM Committee D-19
   The symposium will be Oct. 28–29 in Fort Lauderdale, Fla. *Write:* Kathy Greene, ASTM Publications Division, 1916 Race St., Philadelphia, Pa. 19103; 215-299-5414

Feb. 17 deadline (abstracts)
13th Space Simulation Conference: "The Payload—Testing for Success." Institute of Environmental Sciences and others
   The conference will be Oct. 9–11 in Orlando, Fla. *Write:* Robert P. Parrish, Jr., Martin Marietta Corp., P.O. Box 179, MS-S-0435, Denver, Colo. 80201

March 1 deadline (abstracts)
Concentration Techniques for Collection and Analysis of Organic Chemicals for Biological Testing of Environmental Samples. American Chemical Society
   The symposium will be Aug. 26–31 in Philadelphia, Pa. *Write:* Mel Suffet, Department of Chemistry, Drexel University, Philadelphia, Pa. 19104; 215-895-2270

March 31 deadline (abstracts)
International Symposium on Industrial and Hazardous Waste. ASTM Committee D-34 and others
   The symposium will be June 24–27, 1985, in Alexandria, Egypt. *Write:* Kathy Greene, ASTM Publications Division, 1916 Race St., Philadelphia, Pa. 19103; 215-199-5414

professional consulting services directory

CLASSIFIED SECTION ■ POSITIONS OPEN

INDEX TO THE ADVERTISERS IN THIS ISSUE

Prediction of Buffer Catalysis in Field and Laboratory Studies of Pollutant Hydrolysis Reactions

Edward M. Perdue[*][†]

Environmental Science/Chemistry Department, Portland State University, Portland, Oregon 97207

N. Lee Wolfe

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

■ A mathematical model, based on application of the Brönsted equations for general acid–base catalysis, has been developed for use in forecasting the maximum contribution of buffer catalysis in pollutant hydrolysis reactions. The predictive capacity of the model is utilized to examine the potential significance of buffer catalysis in selected buffers and in a nutrient medium, both typical of laboratory studies, as well as in natural waters of varying chemical composition. Buffer catalysis is predicted to be potentially significant in laboratory studies that use buffers at concentrations above 0.001 M to maintain constant pH; however, at the very low concentrations of Brönsted acids and bases in most aquatic environments, this phenomenon is probably insignificant.

Introduction

Many aquatic pollutants may be degraded through a variety of abiotic, dark reaction pathways, of which hydrolysis is probably the most important. It is imperative, therefore, that the parameters affecting the rate of hydrolysis of any aquatic pollutant be identified and quantified, to assess the significance of hydrolysis as a degradation pathway.

Mabey and Mill (1) have critically reviewed the hydrolysis literature and have tabulated kinetic parameters for a great many organic pollutants. Hydrolysis reaction rates are often a function not only of pH but also of the nature of the acid–base system used as a buffer and its concentration. This phenomenon is generally referred to as buffer catalysis. Natural waters contain a wide variety of weak acids and bases, e.g., carbonates, phosphates, silicates, amines, sulfides, and humic and fulvic acids. It is conceivable, therefore, that buffer catalysis may be significant in selected types of aquatic environments, its relative importance varying with the chemical composition of each type of environment (fresh water, seawater, interstitial waters of anaerobic sediments, etc.).

At present, there is no rigorous method for predicting the potential significance of buffer catalysis in simple laboratory experiments where rate constants are determined, much less in aquatic ecosystems where a wide variety of acid–base catalysts are found. Each pollutant and

each aquatic system must be treated as a separate case and subjected to experimental investigation. Therefore, a theoretically based procedure that permits the a priori estimation of the maximum contribution of buffer catalysis in hydrolytic degradation reactions is warranted. Such a procedure would make it possible to design laboratory experiments to minimize buffer catalysis and would identify those natural environments where buffer catalysis is potentially important.

The objective of this paper is to obtain a suitable mathematical model to permit a priori estimation of the maximum contribution of buffer catalysis in aqueous systems. The validity of this approach depends primarily on the applicability of the Brönsted equation, the oldest and best established of the so-called linear free energy relationships (2–5). To quote from Bell (2), "...the validity of the Brönsted relation has been established for very many examples of general acid–base catalysis. In fact, any considerable deviations would now be regarded as casting doubt on the reaction mechanism, or reflecting some abnormal properties of the species concerned".

Brönsted Equation

Brönsted and Pedersen (6) noted that, in a wide variety of acid–base-catalyzed reactions, the *kinetic* reactivity of an acidic or basic catalyst is related to the *thermodynamic* acidity or basicity of the catalyst. These empirical relationships are mathematically expressed as the Brönsted catalysis laws for acid and base catalysis (eq 1 and 2, respectively):

$$k_{HB} = G_A(K_{HB})^\alpha \tag{1}$$

$$k_B = G_B(K_{HB})^{-\beta} \tag{2}$$

where k_{HB} and k_B are second-order rate constants for acid catalysis by HB and base catalysis by B, K_{HB} is the acid dissociation constant of HB, G_A and G_B are constants for a given substrate, and α and β are constants that express the sensitivity of the reacting substrate to the *strength* of the acidic or basic catalyst. It should be noted that the symmetry corrections commonly included in the Brönsted equation are omitted from eq 1 and 2 and throughout this paper to simplify the subsequent derivations.

Experimentally, acid–base-catalyzed reactions have been subdivided into two categories: (a) those reactions that are catalyzed by all Brönsted acids and/or bases present

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†Present address: School of Geophysical Sciences, Georgia Institute of Technology, Atlanta, GA 30332.

in a reaction mixture (*general* acid–base catalysis) and (b) those reactions that are catalyzed only by H_3O^+ and/or OH^- (*specific* acid–base catalysis). *Buffer catalysis*, a term often encountered in the literature, is observed only in general acid–base-catalyzed reactions.

In the most general case, all acidic and/or basic species in a solution can act as catalysts. Consider, for example, the acid–base-catalyzed hydrolysis of a pollutant (P) that obeys pseudo-first-order kinetics at constant pH:

$$d[P]/dt = -k_{obsd}[P] \qquad (3)$$

where

$$k_{obsd} = k_{H_2O}[H_2O] + k_{H_3O^+}[H_3O^+] + k_{OH^-}[OH^-] + \sum_i (k_{HB_i}[HB_i] + k_{B_i}[B_i]) \quad (4)$$

and HB_i and B_i are the *i*th Brönsted acid–base pair in solution. The overall contribution of a particular catalyst to k_{obsd} is expressed as the *product* of a second-order catalysis rate constant and the concentration of the catalyst. Furthermore, the effects of multiple catalysts are clearly additive, i.e., the catalytic contribution of the *i*th acid–base catalyst is independent of the contributions of all other catalysts in the solution.

The first three terms of eq 4 can contribute to k_{obsd} in all aqueous solutions, their contribution being predictable if the second-order rate constants (k_{H_2O}, $k_{H_3O^+}$, and k_{OH^-}) and pH are known. The first term is generally more important near pH 7, when the concentrations and, hence, the catalytic contributions of H_3O^+ and OH^- are both small. At constant pH, the combined contributions of H_2O, H_3O^+, and OH^- to k_{obsd} are constant and can be represented by a pseudo-first-order rate constant (k_w):

$$k_w = k_{H_2O}[H_2O] + k_{H_3O^+}[H_3O^+] + k_{OH^-}[OH^-] \quad (5)$$

Thus, the observed pseudo-first-order rate constant (k_{obsd}) equals the pseudo-first-order rate constant for catalysis by solvent species (k_w) plus the *buffer catalysis* contribution (the last two terms of eq 4).

Mathematical Prediction of Maximum Buffer Catalysis

Recognizing at the outset of this derivation that the relative contribution of buffer catalysis to k_{obsd} is a function of pH, catalyst, and substrate, we developed the following calculations for predicting the *maximum* contribution of buffer catalysis for a particular catalyst and pH. The calculated results, therefore, describe the behavior of a *hypothetical* substrate whose sensitivity to the strengths of both acidic and basic catalysts results in the largest possible relative contribution of buffer catalysis to k_{obsd}. Buffer catalysis would be somewhat less important for any real substrate. The rationale for this approach lies in the fact that, in practice, the actual α and β values of a real substrate (see eq 1–2) are less likely to be known (and are much more difficult to determine experimentally) than are the solution pH and the acid dissociation constant of a weak acid–base catalyst. Thus, a priori estimates of the maximum relative contribution of buffer catalysis to k_{obsd} are most readily obtained from a process that is ultimately compound independent.

If both sides of eq 4 are divided by the first three terms on the right side of the equation, eq 6 is obtained (with

$$\frac{k_{obsd}}{k_w} = 1 + \left(\frac{k_{HB}[HB] + k_B[B^-]}{k_{H_2O}[H_2O] + k_{H_3O^+}[H_3O^+] + k_{OH^-}[OH^-]} \right) \quad (6)$$

only one weak acid–base catalyst (HB) in solution), where k_w is defined in eq 5. The left side of eq 6 is an expression of the extent to which k_{obsd} exceeds the contributions of H_2O, H_3O^+, and OH^-, three species common to all aqueous solutions. Clearly, in the absence of additional catalysts (HB and B), k_{obsd}/k_w equals 1. Likewise, if HB and B are present, but contribute negligibly to k_{obsd}, k_{obsd}/k_w approaches a value of 1. Because k_{obsd} and k_w are pseudo-first-order rate constants, they are easily related to the respective hydrolysis half-lives.

$$k_{obsd}/k_w = (t_{1/2}^{obsd}/t_{1/2}^w)^{-1} \quad (7)$$

From this reciprocal relationship, it is easily shown that the hydrolysis half-life is strongly dependent on k_{obsd}/k_w when k_{obsd}/k_w is in the range 1–5.

To facilitate direct comparisons between the components of the buffer and water (H_3O^+, H_2O, and OH^-), the following acid dissociation constants are defined.

$$HB + H_2O = H_3O^+ + B^- \qquad K_{HB} = \frac{[H_3O^+][B^-]}{[HB]} \quad (8)$$

$$H_3O^+ + H_2O = H_3O^+ + H_2O \qquad K_{H_3O^+} = \frac{[H_3O^+][H_2O]}{[H_3O^+]} = C_w \quad (9)$$

$$H_2O + H_2O = H_3O^+ + OH^- \qquad K_{H_2O} = \frac{[H_3O^+][OH^-]}{[H_2O]} = \frac{K_w}{C_w} \quad (10)$$
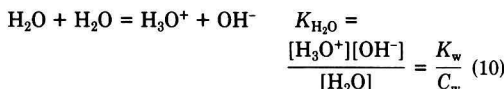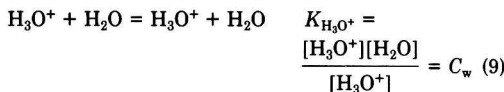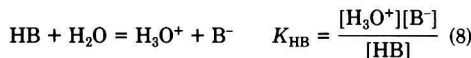
Here C_w is the molar concentration of H_2O (55.34 M at 298 K) and K_w is the ion product of H_2O (1.00×10^{-14} at 298 K).

Although H_3O^+, H_2O, and OH^- are the most commonly encountered deviants from the Brönsted catalysis laws, they are generally assumed to obey these relations. The kinetic contribution of H_2O, usually referred to as the neutral hydrolysis reaction, is, in fact, often in agreement with the predictions of the Brönsted equations, suggesting that H_2O may act as an acid–base catalyst in the neutral hydrolysis reaction (7). The second-order rate constant for catalysis by H_2O, therefore, can be considered, in the most general case, to be the sum of an acid catalysis component (θk_{H_2O}) and a base catalysis component [$(1 - \theta)k_{H_2O}$], where θ is the fraction of k_{H_2O} that is attributable to acid catalysis. Only the overall rate constant (k_{H_2O}) is experimentally measurable, so this separation of terms is introduced here mainly for convenience in the subsequent derivation. The Brönsted equations for acid catalysis by H_2O, base catalysis by H_2O, acid catalysis by H_3O^+, and base catalysis by OH^- are given in eq 11–14.

$$\theta k_{H_2O} = G_A(K_{H_2O})^\alpha = G_A(K_w/C_w)^\alpha \quad (11)$$

$$(1 - \theta)k_{H_2O} = G_B(K_{H_3O^+})^{-\beta} = G_B(C_w)^{-\beta} \quad (12)$$

$$k_{H_3O^+} = G_A(K_{H_3O^+})^\alpha = G_A(C_w)^\alpha \quad (13)$$

$$k_{OH^-} = G_B(K_{H_2O})^{-\beta} = G_B(K_w/C_w)^{-\beta} \quad (14)$$

When these equations are combined with those of the acid–base solute (eq 1–2), the G_A and G_B values can be eliminated. All rate constants are therefore expressed in terms of k_{H_2O}, the neutral hydrolysis rate constant.

$$k_{HB} = \theta k_{H_2O}(K_{HB}C_w/K_w)^\alpha \quad (15)$$

$$k_{H_3O^+} = \theta k_{H_2O}(C_w^2/K_w)^\alpha \quad (16)$$

$$k_B = (1 - \theta)k_{H_2O}(C_w/K_{HB})^\beta \quad (17)$$

$$k_{OH^-} = (1 - \theta)k_{H_2O}(C_w{}^2/K_w)^\beta \qquad (18)$$

Returning to eq 6, k_{H_2O} can be cancelled on the right side of the equation, yielding eq 19.

$$k_{obsd}/k_w = 1 +$$
$$\frac{[\theta(K_{HB}C_w/K_w)^\alpha][HB] + [(1 - \theta)(C_w/K_{HB})^\beta][B]}{C_w + [\theta(C_w{}^2/K_w)^\alpha][H_3O^+] + [(1 - \theta)(C_w{}^2/K_w)^\beta][OH^-]} \qquad (19)$$

The introduction of the θ parameter to fractionate k_{H_2O} into its acidic and basic components has the added advantage of making eq 19 generally applicable to all hydrolysis reactions, including those that are susceptible only to acid catalysis ($\theta = 1$), those subject to acid and base catalysis ($0 < \theta < 1$), and those that are susceptible only to base catalysis ($\theta = 0$). Before proceeding further with the derivation, it should also be noted that k_{obsd}/k_w can be calculated from eq 19 *without knowledge of any rate constants*.

Thus far in the derivation, it has been implicitly assumed that HB is a *monoprotic* acid. In view of the fact, however, that many potentially important acid–base catalysts are polyprotic (H_3PO_4, H_2CO_3, H_2S, etc.), the extension of this derivation to the more general catalyst H_nB seems desirable. This modification affects only the numerator of eq 19, which becomes

$$\sum_{i=1}^{n}[\theta(K_iC_w/K_w)^\alpha[H_{n+1-i}B] + (1 - \theta)(C_w/K_i)^\beta[H_{n-i}B]] \qquad (20)$$

where K_i is the ith acid dissociation constant of H_nB. As an illustration, for H_2B the numerator is

$$\theta(K_1C_w/K_w)^\alpha[H_2B] + (1 - \theta)(C_w/K_1)^\beta[HB] +$$
$$\theta(K_2C_w/K_w)^\alpha[HB] + (1 - \theta)(C_w/K_2)^\beta[B] \qquad (21)$$

It is important to note that any species other than H_nB and B is amphoteric and can potentially be an acid catalyst and/or a base catalyst. These contributions must be separately treated in terms of the Brönsted equation even though only the sum of acidic and basic contributions of an amphoteric species can be measured experimentally. This point does not appear to have been widely recognized in the literature on the Brönsted equation.

One final substitution makes it possible to express the k_{obsd}/k_w in terms of C_B, the stoichiometric concentration of the buffer catalyst, where

$$C_B = \sum_{i=0}^{n}[H_{n-i}B] \qquad (22)$$

When the mass balance (eq 22) is combined with the acid dissociation constants of H_nB, the following general expressions are readily obtained.

$$[H_{n+1-i}B] = C_B\left[\frac{([H_3O^+]/K_i)\prod_{m=1}^{i}(K_m/[H_3O^+])}{1 + \sum_{i=1}^{n}[\prod_{m=1}^{i}(K_m/[H_3O^+])]}\right] = C_Ba_i \qquad (23)$$

$$[H_{n-i}B] = C_B\left[\frac{\prod_{m=1}^{i}(K_m/[H_3O^+])}{1 + \sum_{i=1}^{n}[\prod_{m=1}^{i}(K_m/[H_3O^+])]}\right] = C_Bb_i \qquad (24)$$

Table I. Selected Acid Dissociation Constants

catalyst	pK₁	pK₂	pK₃
acetate	4.75		
ammonia	9.24		
borate	9.24		
carbonate	3.92[a]	10.31	
citrate	3.13	4.76	6.40
cysteine	1.96	8.18	10.28
phosphate	2.13	7.20	12.24
phthalate	2.95	5.41	
silicate	9.71		
sulfide	6.96	12.90	

[a] The pK_1 value usually tabulated for H_2CO_3 (6.36) does not make a distinction between $CO_2(aq)$ and $H_2CO_3(aq)$. No distinction is required for equilibrium calculations, but only $H_2CO_3(aq)$ is a potential Brönsted acid catalyst.

where a_i and b_i are solute mole fractions of the ith acid and its conjugate base, respectively. When these results are combined with eq 20, the k_{obsd}/k_w is given by

$$k_{obsd}/k_w = 1 + C_B \times$$
$$\left[\frac{\sum_{i=1}^{n}[\theta a_i(K_iC_w/K_w)^\alpha + (1 - \theta)b_i(C_w/K_i)^\beta]}{C_w + \theta[H_3O^+](C_w{}^2/K_w)^\alpha + (1 - \theta)[OH^-](C_w{}^2/K_w)^\beta}\right] \qquad (25)$$

This equation clearly shows that, for a given substrate, buffer catalyst, and pH, k_{obsd}/k_w is a linear function of C_B.

Earlier it was stated that these calculations would be directed toward prediction of the *maximum* kinetic contribution of buffer catalysis for a particular catalyst and pH. In essence, then, only α and β, the parameters that characterize the sensitivity of the *substrate* to acid or base strength, remain as variables for maximizing k_{obsd}/k_w. Only the bracketed term in eq 25, defined here as the *buffer catalysis factor* (BCF), is dependent on α and β. At constant pH, for a particular catalyst, the BCF can be represented by

$$BCF = \frac{\sum_{i=1}^{n}[\theta a_i A_i{}^\alpha + (1 - \theta)b_i B_i{}^\beta]}{C + \theta dD^\alpha + (1 - \theta)eD^\beta} \qquad (26)$$

where A_i, B_i, C, d, D, and e are constants that represent more complex expressions in eq 25. It remains simply to determine the set of α and β values that maximize BCF in eq 26 (and, hence, k_{obsd}/k_w in eq 25).

Numerical methods that use iterative techniques to locate maxima of surfaces are well suited to the problem at hand. For the moment, a value of 0.5 will be used for θ in eq 26. (It will subsequently be shown that the results are independent of θ). BCF values were numerically maximized at pH 7 for acetate, ammonia, and phosphate. The acid dissociation constants for these and other catalysts are given in Table I. The results are given in Figure 1. Despite the wide variations in charge type, number of acidic hydrogens in H_nA, and the K_i values, all three catalysts yielded qualitatively similar dependence of BCF on the values of α and β. The observed quantitative difference in BCF values will be subsequently explained.

These calculations were extended to other pH values, and the values of α and β that correspond to a maximum BCF were found to be linearly related to pH. Furthermore, the sum of optimum α and β values *exactly* equals one for the monoprotic catalysts and is very close to one for H_3PO_4
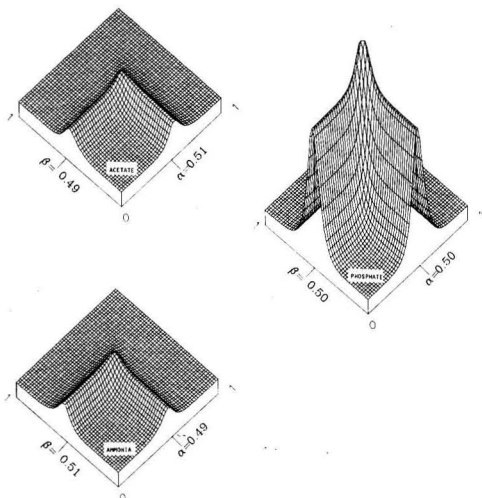
Figure 1. Numerical maximization of BCF values for acetate, ammonia, and phosphate at pH 7.

Figure 2. Variation of optimum α and β with pK of catalyst at pH 0.

(to within ±0.01% at all pH values). Letting the slope of α vs. pH be defined from the values at pH 0 and pH 14, the slopes and intercepts required to calculate optimum α values for these catalysts and for other buffer catalysts are summarized in Table II. The corresponding optimum β values are obtained from the relationship $\beta = 1 - \alpha$. The results in Table II show that the set of α and β values corresponding to the maximum BCF (and, hence, to the hypothetical substrate that would be most susceptible to buffer catalysis) is relatively independent of the nature of the catalyst but is strongly pH dependent.

The numerically derived results, although quite interesting, do not provide a mathematical basis for the relative insensitivity of optimum α and β values to the nature of the catalyst. Mathematically, the α and β values that correspond to a maximum BCF value in eq 26 are those for which $\partial(\text{BCF})/\partial\alpha$ and $\partial(\text{BCF})/\partial\beta$ equal zero. The second derivative test indicates that this procedure will locate the maximum BCF value if $K_{H_2O} < K_i < K_{H_3O^+}$ for all acid dissociation constants of H_nB, a constraint that is met for any acid or base that is commonly encountered as a significant chemical species in aqueous solution (due to the leveling effect of H_2O on acid and base strength).

Partial differentiation of eq 26 with respect to α and β eventually leads to eq 27 and 28 when both derivatives equal zero. (Throughout this derivation, it is useful to recognize that $D = A_iB_i$ for all i values).

$$B_i^\alpha = A_i^\beta[ea_i \ln A_i/(db_i \ln B_i)]Z_i \quad (27)$$

$$Z_i = [1 + \sum_{j \neq i}(b_j/b_i) \times$$
$$[A_i^\beta \ln B_j/(A_j^\beta \ln B_i) - eB_jA_i^\beta \ln A_j/(CB_j^\alpha \ln B_i)]]^{-1} \quad (28)$$

Resubstitution of eq 27 into one of the partial derivative expressions (not shown), followed by extensive algebraic manipulation, ultimately yields

$$\sum_{i=1}^{n}(a_iA_i^\alpha \ln A_i)[1 - \theta Z_iA_i^{\alpha+\beta-1} - (1-\theta)B_i^{\alpha+\beta-1}] = 0 \quad (29)$$

The α and β values that satisfy this equation are those that correspond to the maximum BCF in eq 26 for a particular catalyst and pH. The general analytical solution to eq 29 is not immediately obvious.

In the particular case of monoprotic catalysts (H_nB, $n = 1$), $Z_i = 1$ in eq 27–29. The single term in the summation of eq 29 reduces to

$$1 - \theta A_i^{\alpha+\beta-1} - (1 - \theta)B_i^{\alpha+\beta-1} = 0 \quad (30)$$

By inspection, eq 30 is satisfied whenever $\alpha + \beta$ equals 1, regardless of the numerical values of θ, A_i, and B_i. Letting $\beta = 1 - \alpha$ in eq 27, replacing A_i and B_i by the original composite variables in eq 25, and converting to logarithmic equations, eq 31 is obtained.

$$\alpha = \left[\frac{1}{\log (C_w^2/K_w)}\right]\left[\log C_w + \right.$$
$$\left. \log\left[\frac{\log (K_iC_w/K_w)}{\log (C_w/K_i)}\right]\right] + \left[\frac{1}{\log (C_w^2/K_w)}\right]\text{pH} \quad (31)$$

Thus, α is a linear function of pH with a constant slope of 0.05719 and an intercept (α_0) that is dependent on the K_i of the catalyst. Of course, because $\beta = 1 - \alpha$, the β-intercept (β_0) is given by $\beta_0 = 1 - \alpha_0$. The variations of α_0 and β_0 with the pK_i of the monoprotic catalyst are given in Figure 2. It should be noted that the predictions of eq 31 agree perfectly with the numerically derived results for the monoprotic acid–base catalysts listed in Table II and that they are independent of θ (which was arbitrarily assigned a value of 0.5 in the previously described numerical procedures). The relative insensitivity of optimum α and β values to the nature of the catalyst, previously observed in the numerical solutions, is substantiated by eq 31 and the results given in Figure 2.

When H_nB is a polyprotic catalyst ($n > 1$), eq 29 does not readily yield an analytical solution. At a pH value
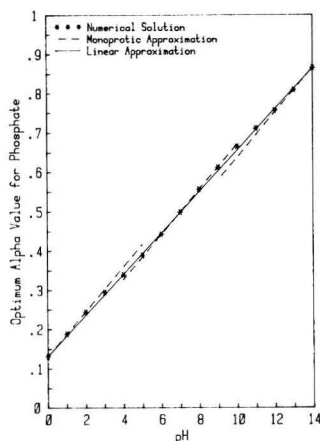
Figure 3. Approximate pH dependence of optimum α for H_3PO_4.

Figure 4. Dependence of BCF on pH and pK values.

where one acid–base pair (a_i, b_i) is very dominant, however, the ith term dominates the summation in eq 29, Z_i approximately equals 1 $(b_j/b_i \simeq 0$ for all $j \neq i)$, and the solution is identical with that obtained for a monoprotic catalyst (eq 31). These conditions generally exist when pH $< pK_1$ or pH $> pK_n$, and possibly at some intermediate pH values. The general prediction of the variation of α with pH for a polyprotic catalyst is illustrated for H_3PO_4 in Figure 3. The asterisks represent the computer-derived numerical solution at each pH value. The dashed lines are "monoprotic" solutions (eq 31) at pH values close to pK_1, pK_2, and pK_3 of H_3PO_4. The solid line is the approximate solution for this system obtained by using the monoprotic solutions to obtain α values at pH 0 and pH 14 and assuming a linear variation of α with pH between these extreme values. This procedure correctly reproduces the numerically derived results in Table II for all the polyprotic catalysts that were examined.

Having finally arrived at equations that can be used to calculate optimum α values and β values for a particular catalyst and pH, we can calculate the corresponding *maximum* BCF values from eq 26. Before particular catalysts are examined, it is of interest to see how BCF values vary as a function of catalyst pK_i and solution pH. By use of eq 31 and 26, BCF values were thus calculated for monoprotic catalysts for $0 \leq pK_i \leq 14$ and $0 \leq pH \leq 14$. The results of these calculations are summarized in Figure 4 in the form of BCF isopleths at BCF values of 10, 50, and 100. The most striking aspect of the calculated BCF values is the very restricted pK_i–pH domain for which BCF > 100. For all practical purposes, a BCF value of 100 represents a limiting maximum value (the actual maximum value of 106.3 occurs at pK_i = pH = 7.00).

For purposes of assessing the potential significance of buffer catalysis in aqueous solutions (our original objective), we might arbitrarily define "significant" to mean those instances where $k_{obsd}/k_w > 1.10$. In other words, a buffer catalysis contribution that equals or exceeds 10% of the combined kinetic contributions of H_2O, H_3O^+, and OH^- is regarded as significant. With reference to eq 4, this definition of significant implies that a 10% increase in k_{obsd} resulting from buffer catalysis is experimentally measurable. Recalling from eq 25 that

$$k_{obsd}/k_w = 1 + C_B(\text{BCF}) \tag{32}$$

with an upper limit of approximately 100 for BCF, it follows that buffer catalysis can be significant only for
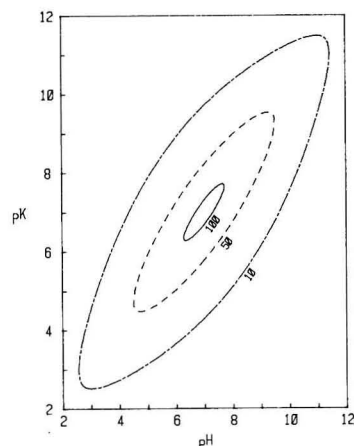
catalysts with $C_B > 0.001$ M. Such concentrations are often encountered in laboratory kinetic studies in which pH buffers are employed, and indeed, buffer catalysis is frequently observed in such studies. In contrast, most potential acid–base catalysts in aquatic environments are present at much lower concentrations. Accordingly, buffer catalysis by monoprotic catalysts is not likely to be important in the aquatic environment. Because a polyprotic catalyst can, in fact, be adequately described by the equations derived for monoprotic catalysts, it seems highly unlikely that general acid–base catalysis is significant in most aquatic environments. This question will be addressed directly in the following section of the paper.

Applications

Having developed the theoretical basis for assessing a maximum contribution of general acid–base catalysis (buffer catalysis) in hydrolysis reactions, we applied the previously derived equations to several types of solutions that typify either natural waters or laboratory degradation experiments. Before the individual cases are examined, it is useful to characterize each buffer catalyst of interest. Accordingly, BCF values were calculated for each buffer listed in Table I in the pH 0–14 range by using the results from Table II to obtain optimum α and β values and eq 26 to calculate maximum BCF values. The results are given in Figures 5 and 6.

The effectiveness of a buffer to maintain constant pH in an aqueous solution is quantitatively expressed by the buffer intensity (also known as buffer index or buffer capacity). Modifying the procedures described by Butler (8), we calculated "corrected" buffer intensities for the buffers in Table I for the pH 0–14 range.

$$\text{BI}^c = \frac{\text{BI} - ([H_3O^+] + [OH^-]) \ln 10}{C_B} \tag{33}$$

The BI values in eq 33 are buffer intensities. (We have used BI rather than β to represent buffer intensity in order to avoid confusion with the Brönsted β parameter used elsewhere in this paper.) The corrected buffer intensity (BI^c) is that part of the overall buffer intensity (BI) attributable to the weak acid–base buffer at a buffer concentration of C_B = 1.0 M. These results are also plotted in Figures 5 and 6.

One fairly common feature of these figures is the approximate coincidence of the BCF and BI^c plots, particularly near neutral pH. It is virtually impossible to use a
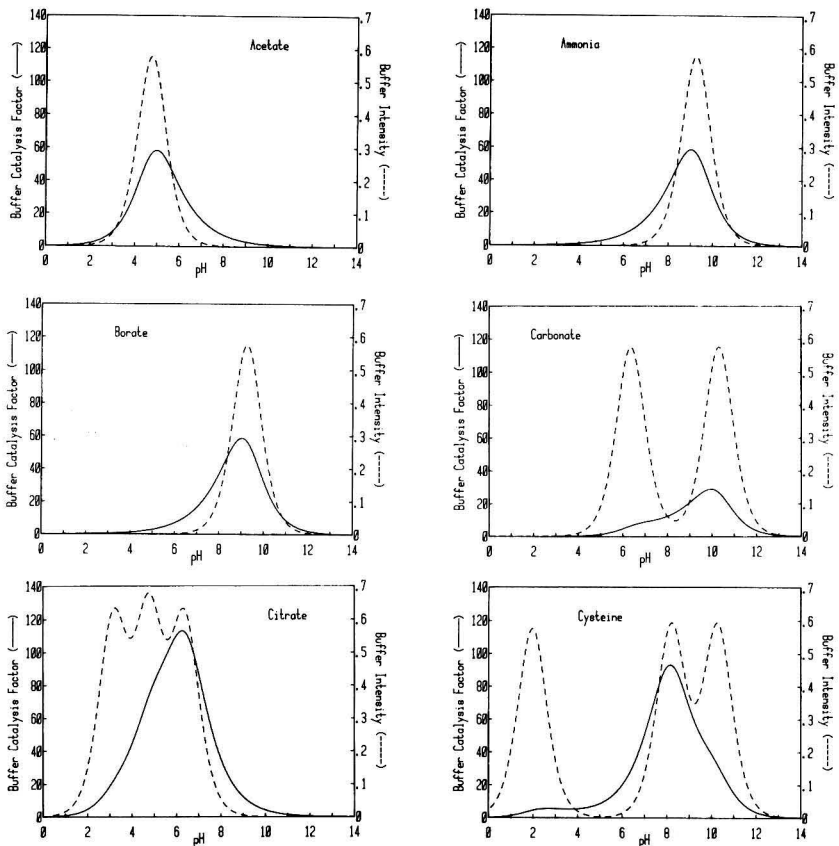
Figure 5. pH dependence of BCF and BI^c for acetate, ammonia, borate, carbonate, citrate, and cysteine buffers.
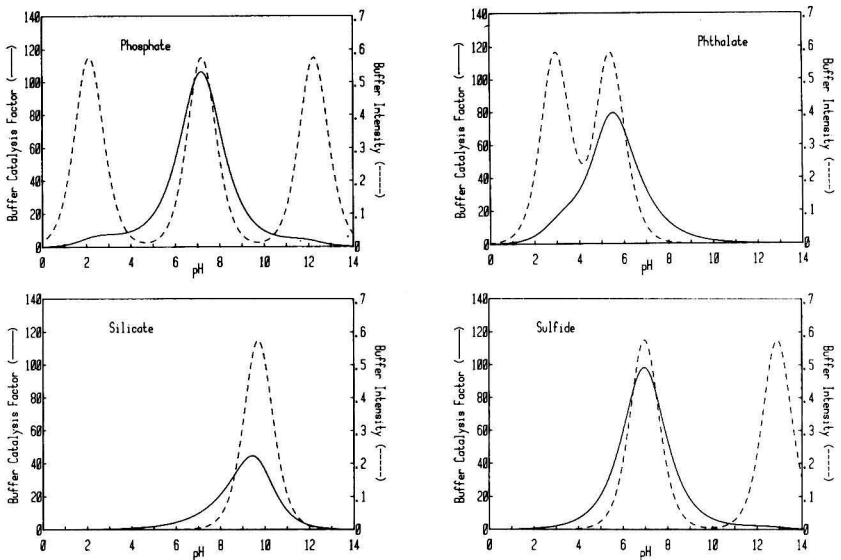
Figure 6. pH dependence of BCF and BI^c for phosphate, phthalate, silicate, and sulfide buffers.

buffer at pH 6–8 without simultaneously maximizing the potential significance of buffer catalysis. A notable exception is the carbonic acid system, which, because of the kinetic inertness of $CO_2(aq)$ and the very low equilibrium concentration of $H_2CO_3(aq)$, is capable of acting as a good pH buffer with minimal catalytic activity (3). A major
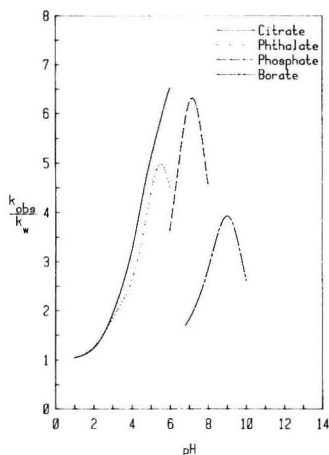
Figure 7. pH dependence of k_{obsd}/k_w for selected 0.05 M buffers.

disadvantage of this buffer is the exchange of CO_2(aq) with atmospheric CO_2(g).

Another common feature of these figures is the tendency of the BCF plots to be skewed toward pH 7 whereas the BI^c plots are symmetrical around the pK_i value. The catalytic contribution of the buffer is simply relatively more important at lower concentrations of H_3O^+ and OH^-. If a buffer has a pK_i value of approximately 7, the BCF plot becomes essentially symmetrical around pH 7, as would be expected (see, e.g., H_3PO_4 and H_2S).

Finally, it should be noted that some polyprotic catalysts will exhibit much lower kinetic activity when used as buffers near those pK_i values farthest removed from pK_i = 7. For example, citric acid is as good a buffer in the pH 3–4 range as in the pH 6–7 range. Its catalytic activity, however, is only about 20–30% as high in the lower pH range. This theoretical advantage is admittedly offset to some extent by the greater practical need to use pH buffers in the neutral pH range.

Laboratory Studies. In laboratory studies of hydrolysis, photolysis, and biodegradation of aquatic pollutants, conditions of constant pH are desired to simplify kinetic interpretations. In hydrolysis reactions, for example, pseudo-first-order kinetics are usually observed only at constant pH, as can be seen in eq 3 and 4. For this reason, pH buffers are commonly employed as recommended in the OECD Guidelines for testing the hydrolysis of chemicals as a function of pH (9). These include 0.05 M phthalate buffers (1.0 ≤ pH ≤ 6.0), 0.05 M citrate buffers (2.2 ≤ pH ≤ 6.0), 0.05 M phosphate buffers (6.0 ≤ pH ≤ 8.0), and 0.05 M borate buffers (6.8 ≤ pH ≤ 10.0). By use of BCF values at selected pH values and eq 32, k_{obsd}/k_w values were calculated for these buffers at selected pH values. The results are summarized in Figure 7 for the recommended pH range of each buffer.

Virtually all buffer solutions represented in Figure 7 exceed 10% catalytic contribution. For that matter, most k_{obsd}/k_w values lie above 2.0, the point at which the buffer catalysis contribution potentially equals that of H_2O, H_3O^+, and OH^-. Clearly, the potential for significant buffer catalysis is quite high in all these buffers at C_B values of 0.05 M, where hydrolysis half-life can potentially be reduced by a factor of 5 (see eq 7). The only apparent remedy to this situation would be the use of less concentrated buffers. At C_B values of 0.005 M, for example, all the curves in Figure 7 would lie between k_{obsd}/k_w values of 1.00 and 1.55. This decrease in potential buffer catalysis, of course, is

Table III. Buffer Catalysis in an Anaerobic Basal Salts Medium

catalyst	C_B, mol/L	BCF^a	C_B(BCF)
ammonia	2.33×10^{-3}	19.60	0.046
carbonate	8.93×10^{-2}	9.37	0.837
cysteine	3.42×10^{-3}	59.99	0.205
phosphate	1.10×10^{-3}	106.33	0.117
sulfide	2.50×10^{-3}	94.27	0.236

$$k_{obsd}/k_w = 2.441$$

^{a} Calculated at pH 7.2.

gained at the expense of buffer capacity, which also decreases with decreasing C_B. Reasonable pH control, nevertheless, should be possible with C_B values of 0.005 M in systems closed to the atmosphere and at pollutant concentrations below 10^{-4} M.

Another laboratory situation where buffer catalysis is a likely problem is a biological culture medium, exemplified in this case by a basal salts medium used for culturing anaerobic methanogenic bacteria (10). The analytical concentrations of the major weak acid–base solutes in this medium, the calculated BCF value of each buffer catalyst at the pH of the medium (pH 7.2), the total contribution of each buffer catalyst to k_{obsd}/k_w (C_B(BCF)), and the overall k_{obsd}/k_w value of the medium are given in Table III. Because the BCF value of a buffer depends only on pH, the catalytic contributions of multiple buffers are additive. Thus, in a mixture of buffers

$$k_{obsd}/k_w = 1 + \sum_{i=1}^{z} C_{B_i}(BCF)_i \qquad (34)$$

where C_{B_i} and $(BCF)_i$ are the stoichiometric concentration and BCF of the ith buffer and z is the total number of buffers in solution (in this example, $z = 5$). The results in Table III clearly demonstrate the potential for buffer catalysis in this particular culture medium, in which hydrolysis half-life could be reduced by a factor of about 2.5 (see eq 7).

Environmental Studies. To assess the role of buffer catalysis in aquatic ecosystems, three systems were chosen: (a) the world average river, for which the inorganic composition of Livingstone (11) and the organic carbon content of Schlesinger and Melack (12) were used; (b) the world average seawater, for which the composition of Turekian (13) was used; and (c) the interstitial waters of an anaerobic lake sediment (14). Dissolved organic carbon, assumed to be fulvic acid that is 50% (w/w) carbon with an acidic functional group content of 5.0 mmol/g, was tabulated as the molar concentration of acidic functional groups. Because fulvic acids are so poorly defined with respect to acidic functional groups, they have not been incorporated into the buffer catalysis model. As a first approximation, therefore, the contribution of fulvic acid functional groups was represented in this paper by the contribution of an equal concentration of acetic acid (an admittedly simple approximation). A proposed model for the kinetic effects of humic substances was recently published by Perdue (15). Only those inorganic buffer catalysts for which concentrations were tabulated or readily calculable from other tabulated data were included. A pH of 8.3 was estimated for world average river water assuming equilibrium with atmospheric CO_2(g). The results of all these calculations are summarized in Tables IV–VI. As previously described, eq 34 was used to compute the overall k_{obsd}/k_w values.

In the world average river, for which carbonic acid is the major buffer catalyst, a maximum of 2% enhancement of k_{obsd} values by buffer catalysis is predicted. Although some of the more powerful catalysts such as phosphoric acid are

Table IV. Buffer Catalysis in World Average River Water

catalyst	C_B, mol/L	BCF[a]	$C_B(BCF)$
carbonate	9.78×10^{-4}	13.9	0.014
fulvic acid[b]	1.00×10^{-4}	5.4	0.001
silicate	2.18×10^{-4}	21.2	0.005

$$k_{obsd}/k_w = 1.020$$

[a] Calculated at pH 8.3.   [b] See text.

Table V. Buffer Catalysis in World Average Seawater

catalyst	C_B, mol/L	BCF[a]	$C_B(BCF)$
borate	4.10×10^{-4}	37.1	0.015
carbonate	2.33×10^{-3}	12.1	0.028
fulvic acid[b]	5.00×10^{-6}	7.0	0.000
phosphate	2.84×10^{-6}	71.4	0.000
silicate	1.03×10^{-4}	17.0	0.002

$$k_{obsd}/k_w = 1.045$$

[a] Calculated at pH 8.0.   [b] See text.

Table VI. Buffer Catalysis in Anaerobic Interstitial Waters

catalyst	C_B, mol/L	BCF[a]	$C_B(BCF)$
ammonia	2.00×10^{-4}	16.9	0.003
carbonate	9.09×10^{-3}	8.9	0.081
fulvic acid[b]	2.00×10^{-4}	15.7	0.003
phosphate	1.80×10^{-5}	105.2	0.002
sulfide	3.96×10^{-7}	97.9	0.000

$$k_{obsd}/k_w = 1.089$$

[a] Calculated at pH 7.05.   [b] See text.

not included in the estimate of k_{obsd}/k_w, their concentrations are so low ($10^{-6}–10^{-8}$ M) that such contributions to k_{obsd}/k_w are negligible.

The results for seawater are quite approximate because no effort was made to make ionic strength corrections to the acid dissociation constants of the buffer catalysts. In view of the rather approximate nature of this entire analysis, such corrections were considered to be superfluous. Generally, the results for seawater are comparable to those for river water. A notable exception is the kinetic contribtion of boric acid, which accounts for one-third of all buffer catalysis. Even at the much higher concentrations of most solutes in seawater, however, buffer catalysis is not likely to be significant.

The interstitial waters of an anaerobic lake sediment (Lake Greifensee, Switzeland) provide a somewhat different kind of environment, one that includes NH_3 and H_2S, neither of which was present in the aerobic water types examined thus far. Nevertheless, the major potential catalyst is still carbonic acid, and buffer catalysis, although predicted to be somewhat larger, is still probably not significant. It should be noted, however, that on the basis of eq 7, a small change in k_{obsd}/k_w would significantly offset the half-life of hydrolysis.

Summary and Conclusions

The model proposed in this paper permits the a priori estimation of the potential significance of buffer catalysis of hydrolysis reactions in aqueous solutions, the potential rate enhancement being expressed as k_{obsd}/k_w, the ratio of pseudo-first-order hydrolysis rate constants at constant pH in the presence and absence of a buffer. The calculated k_{obsd}/k_w values represent the theoretical maximum rate

enhancement regardless of the nature of the pollutant. These calculations indicate that buffer catalysis is a possibility in laboratory studies in which high concentrations of pH buffers are used. It is possible to determine the maximum concentration of buffer that can be used without significant catalytic activity, the upper limit being near 0.001 M for most common buffers. Because the potential buffer concentrations seldom exceed 0.001 M in most aquatic environments, buffer catalysis is not expected to be important.

Because of the predictive capacity of this model, natural environments in which buffer catalysis might be significant can be identified. The model is expected to be useful in planning laboratory experiments and in identifying those types of buffers that have minimal catalytic activity. Experimental results from studies in which buffer catalysis was not taken into consideration can be quickly screened to determine whether buffer catalysis was potentially significant.

Because there are known exceptions to the Brönsted equation on which this model is based, actual k_{obsd}/k_w values may occasionally exceed the theoretical values predicted from the model. To echo the quote from R. P. Bell under Introduction, those exceptions will suggest more complex mechanisms (e.g., bifunctional catalysis or nucleophilic catalysis) and will have to be studied more fully to elucidate the reaction mechanisms involved.

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Membrane-Filter, Direct-Transfer Technique for the Analysis of Asbestos Fibers or Other Inorganic Particles by Transmission Electron Microscopy

Garry J. Burdett* and Anthony P. Rood

Occupational Medicine and Hygiene Laboratory, Health and Safety Executive, London NW2 6LN, United Kingdom

■ A new method to transfer inorganic particulate material collected on a membrane filter directly onto an evaporated carbon film is described. This allows the advantages of membrane-filter collection to be combined with the analytical capabilities of the transmission electron microscope. The method forms part of a suite of new techniques that allow optical, scanning electron, and transmission electron microscopy to be carried out on the same sample. Special attention has been given to the evaluation of asbestos fibers. A series of controls showed that there was negligible loss of even the finest chrysotile fibrils, when a Millipore (0.1-μm nominal pore size) membrane filter was used to filter waterborne suspensions of asbestos. Airborne samples of fibers on 0.8- and 1.2-μm nominal pore size membrane filters also gave negligible losses provided that the surface of the collapsed filter was etched for 6 min in an oxygen plasma. Quantitative calibrations gave mass transfer efficiencies equivalent to, or greater than, 100%, but poor analytical precision was obtained, due to the unequal distribution of fiber mass in a small number of relatively large fibers. A direct comparison of the fiber count between 0.1-μm pore size Nuclepore and Millipore filters showed that the Millipore filters retained a higher number of chrysotile fibrils.

Introduction

The high percentage of suboptical asbestos fibers in lung sections (*1*, *2*) and the experimental relationship between fiber dimensions and tumour induction (*3–5*) have given increased importance to the development of electron microscopical methods for the monitoring of airborne and waterborne asbestos levels. Only an analytical transmission electron microscope (TEM) is capable of performing accurate sizing and identification down to the smallest fibers encountered (0.01 μm wide). However, the problems of taking a representative filter sample and presenting the fibers on a suitable electron-transparent substrate for TEM analysis have led to variable success.

A number of techniques, based on Nuclepore polycarbonate and the Millipore cellulose ester membrane filters, have been used to prepare samples for analysis in the TEM. The Nuclepore filters have a flat surface dotted with circular pores, and particles lying on the surface can be trapped in a thin carbon coat (10–50 nm thick) while the filter is dissolved in chloroform. A modified Jaffe washer, which takes 8–72 h to dissolve the filter is frequently used (*6–10*), although several more rapid solvent extraction methods, condensation washing (*10*, *11*), dripping chloroform onto the filter (*12*), and washing in chloroform (*13*), have been described. The method that appears least prone to losses collects the particulates on a carbon-coated filter, and then the filter is coated again, so as to trap the material between two layers of carbon (*12*). When a single coat of carbon is used efficiencies of 100% have been suggested (*6*), but details or accuracy of the measurements are poorly defined. When an uncoated filter is dissolved in contact with a carbon film supported on an EM grid, relative fiber losses of 21% for chrysotile, 33% for crocidolite (*8*), and 30% for amphiboles (*14*) have been reported.

Due to the shortcomings of Nuclepore filters for air sampling (*15*, *16*), several attempts have been made to carry out transfer preparations with Millipore cellulose ester membrane filters. This type of filter has a matted structure which gives extremely high filtration efficiency, even for particles smaller than the nominal pore size (*16*). However, the structure is replicated when an evaporated carbon film is applied, which makes it difficult to see the fibers. Also particles may penetrate into the filter structure and not be held in the carbon film. Initial attempts to transfer material used uncoated filters, which were dissolved in acetone or tetrahydrofuran onto carbon film supported on EM grids (*14*). By use of a quantitative atomic absorption method, a Jaffe-wick extraction gave losses of <10% (average 6%), and a condensation washing extraction gave losses of up to 60% for chrysotile and amphibole asbestos (*14*). A TEM fiber count gave relative losses of between 50 and 80% for Jaffe-wick and condensation washing preparations of 0.22- and 0.45- μm pore size Millipore filters loaded with chrysotile, crocidolite, and taconite, when compared to a 0.1-μm pore size carbon-coated Nuclepore filter. Methods that use coated membrane filters have first fused the surface of the filter by exposure to acetone vapor to form a smooth surface, and the filter is then coated and placed in a Jaffe washer (*17*, *18*). Fiber losses for this method have been assessed at <5% (*17*).

Recent work at this laboratory has led to the development of methods for collapsing membrane filters by chemical means for optical (*19*) and scanning electron microscopy (SEM) (*20*) analysis. This allowed the advantages of membrane filter collection (low pressure drop, high filter efficiency, and particle retention) to be combined with the analysis of the particles on a smooth, flat, featureless substrate. The methodology has now been extended to allow analysis by transmission electron microscopy (TEM), by the transfer of particulates onto an electron-transparent carbon substrate. An important feature of this method is to etch the collapsed filter in an oxygen plasma to expose fibers on the filter surface. The methods are applicable to all inorganic particulates, but much of the work has been directly involved with the measurement of airborne asbestos fibers (*21*).

In the present work, the aim of the direct-transfer technique was to obtain quantitative TEM determination of asbestos fiber size, number, and mass distributions from membrane filter samples. It was intended that the following criteria would be met: (a) It should be possible to perform TEM analysis on samples taken in a way that is simple and compatible with existing methods used in occupational hygiene. (b) The sample preparation should be relatively quick and easily adaptable for routine use. (c) The same filter sample should be available for optical, SEM and TEM analysis, and ideally all three methods should be applicable to the same field of view. (d) There should be minimal disturbance of the fibers as sampled on the filter, and the fiber size distribution should be retained. (e) The asbestos fibers should be readily identifiable by using optical methods, as well as by energy dispersive X-ray analysis (EDXA) and selected area

Table I. Conditions for the Preparation of Different Types of Membrane Filters (Airborne Samples)

type of membrane filter	recommended collapsing	time of standing, min	optimum time of etching, min	time and temp for oven drying
Millipore MF				
0.1-μm pore size	35% dimethylformamide, 15% acetic acid, 50% water	0	not required	10 min at 65–70 °C
>0.1–1.2 μm	35% dimethylformamide, 15% acetic acid, 50% water	0	6	10 min at 65–70 °C
GELMAN Metricel 0.45- and 0.8-μm pore size	66% cyclohexanone, 33% 1,4-dioxane	3–5	6	10 min at 65–70 °C

electron diffraction (SAED) used during electron microscopy. (f) A quantitative estimate of losses (if any) should be obtainable.

The technique that has been developed has been applied to a variety of filters: e.g., Millipore-MF (mixed esters of cellulose) of 0.1-, 0.8-, and 1.2-μm nominal pore size and Gelman DM Metricel [a copolymer of acrylonitrile and poly(vinyl chloride)], 0.45 and 0.8 μm nominal pore size. Other types of membrane filters could be used if required. The methodology described is for Millipore-MF filters; the necessary modifications to the technique for use with Gelman DM Metricel filters are summarized in Table I. A series of quantitative controls and calibrations has been carried out on factory- and laboratory-prepared samples.

Generation of Calibration Samples

A series of three stock suspensions of UICC Canadian chrysotile "B" and two suspensions of UICC amosite were prepared by weighing (to an accuracy of ±1 μg) on a Sartorius 4401 microbalance amounts ranging between 130 and 205 μg.

The asbestos samples were washed off the scale pans into separate 50-mL, flat-bottomed, conical flasks and diluted to 50 mL with distilled water that had been filtered through a 0.2-μm membrane. A few drops of Decon 90 were added to act as a surfactant, before ultrasonically treating each suspension for 20 min with a Dawes 7532A ultrasonic probe, operating at 75–80 W metered power. This separates individual fibers and produces a homogeneous suspension. The suspensions were then further diluted by using successive 1 to 10 dilutions into 50-mL flasks. Each new dilution was ultrasonically treated for 5 min to ensure good mixing.

Immediately after preparation, aliquots were withdrawn for filtration onto prewetted Millipore VC (0.1-μm pore size) and Gelman DM (0.45-μm pore size) membrane filters. The filters were held in a 25-mm, stainless steel, Sartorius, pressure, filter holder with a backing filter (0.45-μm pore size; Gelman DM) positioned between the coarse mesh support and the top filter. Approximately 3 mL of filtered, distilled water was placed into the filter holders to aid even dispersion, before the aliquot was slowly dripped onto the water surface and vacuum filtration applied.

The mass of asbestos on the filter was calculated from the weighed amount and the subsequent dilution and pipetting factors.

Experimental Procedures

The Millipore membrane filters were first prepared as for optical microscopic analysis (*19*) by the following method: 60–80 μL of clearing solution, made up of 35% dimethylformamide (DMF), 15% glacial acetic acid, and 50% water (all proportions by volume), was placed on a clean microscope slide with a push botton micropipet and smeared out over an area about the size of the filter. The filter, sample face upward, was then carefully laid on top

of the solution, and the filter and solution were brought together with an angle of about 20° between them to help exclude air bubbles. Any solution not absorbed by the filter was removed with a tissue, before the slide was placed into a thermostatically controlled oven at 65–70 °C for 10 min. As the water evaporated the dimethylformamide gently dissolved the filter material, causing it to collapse to about 15% (20 μm) of its original thickness. This left a permanent, thin, transparent plastic film in the surface of which the fibers were embedded.

The cleared filter on the slide was etched in a plasma oven (Nanotech, Plasmaprep 100) for 3–4 min with an oxygen flow rate of 8 cm³/min and forward and reflected radio-frequency power of 100 and 2 W, respectively. The etched filter was then placed in an Edwards 12E6 carbon evaporation unit. A converted rotating table mount, positioned on the top plate of the chamber, was used at 0° tilt, to rotate the sample while carbon was vaporized upward from the Edwards gun. A thickness of approximately 30–50 nm of carbon was deposited by using about six 5-s bursts.

After being coated, the filter was placed on a clean air bench, and a scalpel blade was used to cut a square of sides 2–3 mm from the center of the filter. The square was placed with the carbon coating face upward onto a 3-mm diameter, 300-mesh, nickel EM grid and transferred into a DMF washer. The washer was a variation on the Jaffe–Wick method and was constructed by placing a stainless steel support mesh on top of a tissue pad, held in a Petri dish. The liquid level was controlled by the capillary action of the tissue pad, with only enough DMF being introduced into the bottom of the Petri dish so that menisci just begin to appear in the support mesh. Too much liquid can cause the collapsed filter to float off the grid.

After about 2–3 h the membrane-filter material was completely dissolved, leaving a thin carbon film containing the particles originally collected on the filter. The grid and film, once removed from the washer, dried in about 5 min and were then ready for TEM. DMF is a relatively toxic solvent and should be handled in a fume cupboard using covered Petri dishes. An alternative solvent, dimethyl sulfoxide (DMSO), has also been used successfully but requires 4–5 h to dissolve the plastic from a collapsed Millipore-MF filter and approximately 40 h for a Gelman DM filter.

Quantification

The fiber count and distribution on the samples were assessed by examining at least two EM grids for each sample in a Phillips EM400T TEM. The grids were first scanned at a magnification of 800× to check that the deposit was even over the whole grid. Then fibers in five grid openings on each grid, one opening from the top, bottom, and the side edges and one from the center, were counted at 17000× magnification. The mean, standard deviation, and the 95% confidence limits of the mean of the fiber

counts were computed, as well as testing sample uniformity with a X^2 test. The counting and statistical procedures were similar to those described by Chatfield (10). Samples with high loadings of chrysotile (>200 filbers/grid opening) were not screen counted due to the time of analysis required.

Quantitative mass evaluation of the chrysotile and amosite controls was made from TEM plates taken at 6000× and 1300× magnification, respectively. The plates were projected at 9.5× magnification onto the measuring tablet of a Reichert Jung "Videoplan" semiautomatic measuring analyzer, to give a sizing magnification of 12300× for amosite and 57000× for chrysotile. Two different magnifications were required because of the large difference in the size distribution of the chrysotile and amosite fibers. The length and diameter of each fiber were measured by accurately locating two pairs of perpendicularly opposite points on the edges of the fiber by using a nonparallax, cross-wire cursor and a 7× magnification eyepiece. The Videoplan automatically measures the shortest distance between the two points and stores the result; frequency distributions etc. can be constructed by using the supplied software. The mass of each individual fiber was calculated by approximating them to uniform cylinders, with densities of 2500 kg/m^3 for chrysotile and 3000 kg/m^3 for amosite. Then when the values were summed over a known area, a multiple of a 300-mesh, EM grid opening, an estimate of the total amount of asbestos on the filter was obtained.

The Videoplan measuring operation was calibrated against TEM plates of a replicated cross-grating with 2160 lines/mm.

Controls

Several additional controls were applied to the samples. The penetration of chrysotile and amosite fibers into the filter was monitored by subjecting sections of the same filter to different periods of etching. This would expose more fibers on the filter surface with time of etching, if penetration was occurring.

A fiber count comparison between Millipore VC and Nuclepore (0.1-μm pore sizes) filters was carried out by using equal loadings of chrysotile fibrils. The Nuclepore filters were prepared by the method desribed by Chatfield and Dillon (10), except for the difference in the filtration apparatus.

A check on the losses of optical–sized fibers was made by comparing same fields of view by phase-contrast optical microscopy and TEM. Samples for optical microscopy were prepared after the filters were chemically collapsed. A temporary mount was formed by placing a coverslip on the filter and allowing cyclohexane (RI 1.43) to run between them. The sample was examined under phase-contrast illumination on a Reichert Zetopan microscope with an optical magnification of 500×; 35-mm photographs were taken on Kodak SO 115 film with a green filter (Wratten 58). After examination the coverslip was removed and the cyclohexane allowed to evaporate. Same fields of view were identified by first marking a 3-mm-sided square on the collapsed filter with a scalpel blade. Optical micrographs were then taken within this area choosing fields of view containing easily identifiable large fibers. The sample was then prepared for TEM analysis as described previously and the EM grid examined for the identifiable features.

Results and Discussion

Fibers that are sampled onto membrane filters are captured in the matlike structure of the filter. The depth
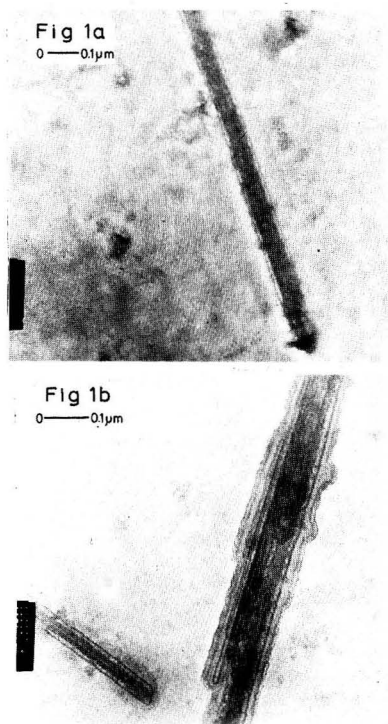
Figure 1. High-magnification TEM micrographs of chrysotile fibers on a Gelman DM-450 membrane filter with (a) 4- and (b) 15-min etching.

to which the fibers penetrate into the filter will depend on the fiber size, the nomimal filter pore size, and the filtration medium (air or water). With small pore size filters, fibers are held in the surface region, and when the filter is collapsed under controlled conditions, the fibers lie embedded in the surface. The etching process attacks the surface of the collapsed filter, removing successive layers of filter material and other organic particulates, unless protected by an overlying inorganic particle. Therefore, as etching proceeds, the fibers are exposed on the surface of the filter free from any overlying plastic material. When exposed in this way, particles can be easily trapped in an evaporated carbon film. This is shown by a high magnification electron micrograph of a chrysotile fiber (Figure 1a).

With a longer etching period a particle will be left proud of the filter surface, supported on a small pedestal of plastic or, in the case of fibers, a ridge. The carbon film will replicate the surface of the ridge and appear at high magnifications as a fringe (Figure 1b), usually up to about 20 nm wide. Therefore, if accurate fiber width measurements are of particular importance, higher magnifications must be used to locate the true edges of the fiber. No change to the sensitivity of the EDXA and SAED analytical methods was found on the etched samples.

Comparisons of the same field of view on Millipore-MF filters using phase-contrast (500× magnification) and transmission electron microscopy (for example, Figure 2) have demonstrated that there is negligible loss or movement of optical fibers in the samples. A fiber that moves or is lost is immediately apparent on the TEM image as a regularly shaped area of high electron transmission. This is due to the carbon film rupturing around the edges of
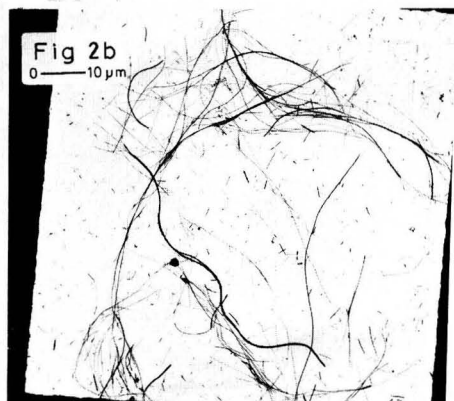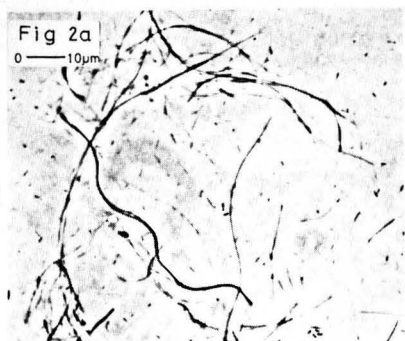
Figure 2. UICC chrysotile fibers with no ultrasonic dispersion on a Millipore VC membrane filter: same field of view by (a) phase-contrast and (b) transmission electron microscopy.

Table II. TEM Fiber Count as a Function of Time of Etching for Filtered Suspensions of Ultrasonically Treated UICC Chrysotile and Amosite

	mean fiber[a] count per grid opening			
time of etching in oxygen plasma, min	18 ng of chrysotile on a 0.1-μm pore size Millipore filter	18 ng of chrysotile on a 0.45-μm pore size Gelman filter	1230 ng of amosite on a 0.1-μm pore size Millipore filter	1230 ng of amosite on a 0.45-μm pore size Gelman filter
0	34.6	0.2	22.8	4.4
3	27.4	1.4	24.6	5.0
6	32.4	2.0	23.5	7.9
9	26.8	1.6	20.5	9.8

[a] Mean fiber size: chrysotile, 0.04-μm width, 1.07-μm length; amosite, 0.24-μm width, 3.00-μm length.

the fiber resulting in the loss of both the fiber and the film. The carbon film is, however, remarkably robust and can deal with filter loadings much denser than would be considered suitable for optical analysis.

Unrecorded losses from this technique can occur if the fibers are lost before the carbon-coating stage or if parts of the carbon film are damaged. Under the assumption that samples are handled carefully, fiber losses before carbon coating will only occur if either the smaller fibers are buried too deeply to be etched out of the collapsed membrane or if the larger fibers fall off due to overetching of the sample.

Table III. TEM Fiber Count as a Function of Time of Etching for Airborne Samples from Factories

	mean fiber[a] count per grid opening		
time of etching in oxygen plasma, min	chrysotile on a 0.8-μm pore size Gelman filter	chrysotile on a 1.2-μm pore size Millipore filter	microquartz on a 0.8-μm pore size Gelman filter
0	12.4	7.7	17.8
3	11.3	10.9	23.6
6	13.4	14.6	27.0
12	7.7	12.5	27.4

[a] Mean fiber size: chrysotile, 0.06-μm diameter, 1.7-μm length; microquartz, 0.32-μm diameter, 7.1-μm length.

Table IV. TEM Fiber Counts and Statistics for Pipetted Mass Loadings of Ultrasonically Treated UICC Chrysotile on Millipore 0.1-μm Pore Size Filters

mass on filter, ng	mean fiber count per grid opening	SD	95% confidence limits		calcd X^2 statistic
			upper	lower	
594	1126				
200	613				
68	179	18	192	166	21
30	68	8	74	67	11
27	54	4	57	51	3
18	41	5	45	37	7
9	18	2	20	16	3
3	5	1	6	4	2
0	1				

Fiber losses due to penetration into the membrane filter were monitored by etching the filters for increasing periods of time, to expose fibers below the filter surface. Results in Table II show that with water suspensions of chrysotile (mean diameter 0.04 μm and mean length 1.07 μm) and amosite (mean width 0.24 μm and mean length 3.0 μm) there was no penetration into a 0.1-μm nominal pore size Millipore filter. However, when a larger (0.45 μm) nominal pore size filter was used, appreciable penetration occurred, and after 9-min etching only 45% of the expected amosite count and <10% of the chrysotile count were recorded.

Airborne samples (Table III) of chrysotile (mean diameter 0.06 μm and mean length 1.70 μm) and microquartz fibers (mean diameter 0.32 μm and mean length 7.1 μm) on 0.8- and 1.2-μm nominal pore size filters showed some evidence of penetration into the surface region. The fibers were sufficiently buried in the collapsed filter that if no etching was used, losses of up to 50% were indicated for chrysotile. However, after 6-min etching there was no further increase in the fiber counts and all the fibers were exposed sufficiently to be held in the carbon coat.

Losses due to overetching have been checked by photographing the same field of view under phase-contrast illumination, after successive periods of etching. Even after a total of 45-min etching time, no loss or movement of fibers was observed.

Quantitative measurements of asbestos fiber mass concentrations will have low statistical precision, as the result depends on the number of fibers counted, the distribution of fibers on the filter, and the fiber size/mass distribution. Results of the fiber concentration and distribution for a range of filter loadings of chrysotile and amosite are given in Tables IV and V. There was a proportional increase in fiber number with the mass loading with some 2-3 fibers/300-mesh grid opening for each nanogram of chry-

Table V. TEM Fiber Counts and Statistics for Pipetted Mass Loadings of Ultrasonically Treated UICC Amosite on Millipore 0.1-μm Pore Size Filters

mass on filter, ng	mean fiber count per grid opening	SD	95% confidence limits upper	95% confidence limits lower	calcd X^2 statistic
4100	49	6	55	43	4
1230	24	5	28	21	9
1230	20	6	25	16	11
322	8	3	10	6	6
0	<1				

Table VI. Mass Recovery Efficiencies for UICC Chrysotile and UICC Amosite on 0.1-μm Pore Size Millipore Membrane Filters

type and mass of asbestos loading, ng	no. of fibers sized in the analysis	calcd mass recovery efficiency	% contribution to the measured mass by the largest single fiber	% contribution to the measured mass by the largest 4% of fiber
chrysotile				
594	507	100	5	33
200	613	96	3.2	28
30	156	170	13	33
18	122	141	17	43
amosite				
4100	244	360	44	75
1230	204	170	18	66
1230	244	150	7	44
322	148	270	18	42

sotile fibrils and 12–25 fiber/300-mesh grid opening for each microgram of amosite fibers. As several stock suspensions were used, the consistency of the fiber counts demonstrates the overall uniformity of the preparation method, rather than just the accuracy of pipetting from a single stock solution. The low standard deviations and the corresponding narrow 95% confidence limits about the means show that the fibers were evenly distributed over the filter. Ideally the fibers should have a random and uniform (Poisson) distribution on the filter. The degree of deviation from a Poisson distribution was measured by using a χ^2 test. A measured significance level of <0.1% (corresponds to a χ^2 statistic of 27.9 for 10 grid openings) was used to reject samples as non-Poisson. However, nearly all counts in Tables IV and V had significance levels of >1%.

The efficiencies for the mass recovery from the calibration samples were equivalent to, or greater than, 100% (Table VI). A cylindrical morphology was assumed in the calculations, and although this is a reasonable assumption for the scroll structure (22) of chrysotile fibrils, it will overestimate the mass of the rectangular cross-sectioned amosite fibers, by a factor of approximately 1.8 (13). The precision of the mass analysis is controlled by the larger fibers. Even with chrysotile fibrils a factor of 2 variation in the mass (±33%) is controlled by 4% of the fibers, and a single amosite fiber can account for nearly half of the measured mass (Table VI). Increasing the number of fibers counted will improve precision, as much depends on the chance encounter with a large fiber. For this reason relatively high filter loadings were used in the mass determination, which also avoids the use of large numbers of photographic plates. A method that uses the Videoplan system for direct screen sizing from the TEM is at present being evaluated for low density samples which are common in environmental sampling.

As no precise measure of efficiency can be obtained, a fiber count comparison between Millipore and Nuclepore (0.1-μm pore size) filters was carried out with identical loadings of chrysotile fibrils from the same stock suspension. The Nuclepore filters consistently gave low counts (Table VII), which were shown to be statistically significant at the 1% level when a two-sided t test was applied. The measured size distributions showed that the mean fiber size was smaller on the Millipore filters, which is consistent with smaller fibrils being lost from the Nuclepore filter. This is an interesting observation that merits further work on the efficiency of Nuclepore filters for the filtration of waterborne suspensions of asbestos.

It was noted that when other workers have compared fiber count distributions, 0.45- or 0.22-μm Millipore filters and 0.1-μm Nuclepore filters were used (8, 14). The relatively higher penetration of the smaller fibers into and through the 0.45- and 0.22-μm membrane filter during water filtration may, in part, account for the lower efficiencies accorded to the transfer method. The present work suggests that other filter types and pore sizes should be compared with a 0.1-μm pore size Millipore filter.

All but one of the sampling and analysis criteria set out for the method have been fulfilled. The collapsed Millipore filters are not stable in an electron beam, and Gelman DM filters are used for SEM evaluations (20). Even then the SEM beam will degrade the filter plastic over prolonged exposures (during photographic recording), making it insoluble in the DMF washer so that the same fields of view cannot be compared. However, if the plastic is dissolved first, the carbon film containing the particles can

Table VII. Fiber Count Comparisons between 0.1-μm Pore Size Millipore and Nuclepore Filters with Equal Loadings of Chrysotile Fibrils

Millipore mean count per grid opening	SD	95% confidence limit upper	95% confidence limit lower	calcd X^2 statistic	Nuclepore mean count per grid opening	SD	95% confidence limit upper	95% confidence limit lower	calcd X^2 statistic
11	3	13	8	9	8[a]	2	11	6	5
42	3	44	40	2	24[a]	4	26	21	5
82	8	92	72	8	72	8	77	67	4
172	10	179	165	6	121	7	126	116	3
353	32	376	330	26	241	20	255	227	16

measured fibril size: mean diameter = 0.046 μm; mean length = 1.07 μm   measured fibril size: mean diameter = 0.06 μm; mean length = 1.37 μm

[a] Sample prepared by using Millipore apparatus specified in ref 10 and corrected for the difference between the areas of deposit obtained with the Sartorius apparatus.

be examined on the same area by SEM and TEM.

Conclusion

The membrane-filter, direct-transfer technique allows routine TEM analysis of asbestos fibers or other inorganic materials without disturbance to the sample. This allows the advantages of membrane-filter collection to be combined with the full analytical and resolution capabilities of the TEM. The sample preparation forms an integral part of existing methods for optical (phase contrast) and SEM analysis and can be combined to allow optical, SEM, and TEM analysis on the same sample and optical and TEM analysis on the same field of view.

Fiber count estimates have shown that there is negligible penetration of chrysotile fibrils into Millipore 0.1-μm pore size filters and little or no etching is necessary to expose the fibrils. With airborne samples on 0.8- and 1.2-μm pore size membrane filters, small fibers did penetrate into the upper region, but 6-min etching in an oxygen plasma was sufficient to expose the fibers on the surface of the collapsed filter so that they could be held in a carbon film.

When 0.45-μm pore size membrane filters were used to filter waterborne suspensions, losses of 55% for amosite fibers and >90% for chrysotile fibrils were recorded, after prolonged etching of the filter surface.

Quantitative calibrations gave mass transfer efficiencies equivalent to, or greater than, 100%, but poor analytical precision was obtained, due to the unequal distribution of fiber mass in a small number of relatively large fibers. A direct comparison of the fiber count between 0.1-μm pore size Nuclepore and 0.1-μm pore size Millipore filters showed that the Millipore filters retained a higher number of chrysotile fibrils.

Registry No. Water, 7732-18-5.

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Effects of Metal Chelates on Wet Flue Gas Scrubbing Chemistry

Shih-Ger Chang, * **David Littlejohn, and Scott Lynn**[†]

Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720

■ Addition of metal chelates to the liquor in a wet stack gas scrubber to improve removal efficiency of both NO and SO_2 may provide a new environmental control technology without requiring major capital modification to existing power plants. Criteria for an effective metal chelate additive include large absorption capacity and rapid binding rate for NO and rapid regeneration of metal chelates from nitrosyl metal chelates by reaction with absorbed SO_2 in aqueous solutions. Data on the thermodynamics and kinetics of a number of the chemical reactions involved have been obtained. Laser Raman spectroscopy has been employed to identify, directly and unambiguously, some of the reaction intermediates and products. These results have been used to predict optimum scrubber design and performance for desulfurization and denitrification of flue gases.

Introduction

Power plant flue gas frequently contains several hundred ppm of NO_x and up to several thousand ppm of SO_2. Most of the NO_x is in the form of relatively insoluble NO. Since NO_2 is a minor constituent, its effect on scrubbing chemistry will not be considered. Several simultaneous desulfurization and denitrification processes (1–3), still in the development stage, are based on the addition of metal chelates such as Fe^{2+}(EDTA) (EDTA = ethylenediaminetetraacetate) to the aqueous scrubbing liquor to promote absorption of NO. These metal chelates can bind NO to form nitrosyl metal chelates, which can in turn react with absorbed SO_2 to produce reduced nitrogen species such as N_2O, N_2, and sulfate while metal chelates are regenerated. Identification of an optimum metal chelate for better removal efficiency of NO and SO_2 in a wet scrubber requires knowledge of the thermodynamics and kinetics of the coordination of NO to various metal chelates. Knowledge is also needed of the kinetics and mechanisms of the reactions between nitrosyl metal chelates and absorbed SO_2 to calculate the regeneration rate of metal chelates and to control the products of reaction by adjusting the operating conditions. Not much of this information is available in the literature, although several ferrous and cobalt chelates have been used as additives for testing in bench-scale wet stack gas scrubbers. This paper discusses some important factors that should be considered in identifying an optimum metal chelate catalyst and in developing an efficient scrubber for the simultaneous desulfurization and denitrification of a power plant stack gas. The kinetics and products of the reaction of nitrosyl metal chelates with aqueous SO_2 have been investigated. The effect of this reaction on scrubber operation is also discussed.

Thermodynamic Equilibrium and Kinetics of NO Absorption

(1) Thermodynamics. Nitric oxide is nonreactive in water (in the absence of oxygen), and the solubility of NO in aqueous solutions is very small. The solubility coefficient is 1.93×10^{-3} mol/(L·atm) at 25 °C and zero ionic

strength (4). The solubility decreases with increasing temperature; the enthalpy of solution is $\Delta H° = -2.94$ kcal/mol. The solubility of NO decreases with increasing ionic strength (μ); this decrease amounts to approximately 8% for $\mu = 0.1$ mol/L. The solubility of NO in aqueous solution was found to be independent of pH over the range 2–13. For 1000 ppm of NO in equilibrium with aqueous scrubbing solution at 50 °C and $\mu = 0.1$ mol/L, the concentration of NO in the aqueous phase is only 1.2×10^{-6} mol/L.

The absorption of NO is enhanced by some water-soluble metal chelate compounds which form complexes with NO (5). Many studies have been published on the structure of these chelates, yet few have been done to determine their thermodynamic properties and reaction rates. Hishinuma et al. (6) and Lin et al. (7) have recently determined the equilibrium constants, enthalpy, and entropy for the coordination of NO to Fe^{2+}(EDTA) and Fe^{2+}(NTA) (NTA = nitrilotriacetate), respectively. Both these groups performed their experiments by bubbling a known mixture of NO and N_2 through a metal chelate solution and then measuring the NO concentration in the outlet gas with a NO_x analyzer (Figure 1). NO absorption was carried out until the NO concentration in the outlet gas became equal to that in the inlet gas, i.e., until equilibrium was reached.

The binding of NO to Fe^{2+}(L) can be expressed by the following reaction:

$$n\text{NO} + Fe^{2+}(\text{L}) \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Fe^{2+}(\text{L})(\text{NO})_n \qquad (1)$$
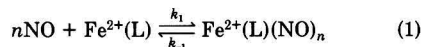
where L is a ligand. The equilibrium constant of this reaction can be written as

$$K = \frac{k_1}{k_{-1}} = \frac{[Fe^{2+}(\text{L})(\text{NO})_n]}{[\text{NO}]^n[Fe^{2+}(\text{L})]} =$$
$$\frac{[Fe^{2+}(\text{L})(\text{NO})_n]}{[\text{NO}]^n[[Fe^{2+}(\text{L})]_0 - [Fe^{2+}(\text{L})(\text{NO})_n]]} \qquad (2)$$

When $H^n p_{NO}{}^n$ is substituted for $[\text{NO}]^n$

$$\frac{[Fe^{2+}(\text{L})]_0}{[Fe^{2+}(\text{L})(\text{NO})_n]} - 1 = \frac{1}{KH^n p_{NO}{}^n} \qquad (3)$$

where $[Fe^{2+}(\text{L})]_0$ is the initial concentration of Fe^{2+}(L), $[Fe^{2+}(\text{L})(\text{NO})_n]$ is the concentration of the NO adduct obtained by graphically integrating the outlet NO concentration, p_{NO} is the partial pressure of NO in the gas, and H is the constant for solubility of NO in water. The experiments were performed at several p_{NO} to calculate n and K and at several temperatures to evaluate enthalpy and entropy of the reaction (Table I). It was found that the ability of Fe^{2+}(NTA) and Fe^{2+}(EDTA) solutions to absorb NO was affected by solution pH at low pH conditions. This may be due to the inability of the ligands to bind Fe^{2+} at low pH.

Littlejohn and Chang (5) have recently determined the equilibrium constant for the coordination of NO to $Fe^{2+}(H_2O)_6$, Fe^{2+}(citrate), and Fe^{2+}(acac)$_2$ (acac = acetylacetonate) using a temperature-jump apparatus (Table I and Figure 2) which is similar to that described by Czerlinski and Eigen (8). NO displaces one of the water molecules on Fe^{2+} to form $Fe^{2+}(H_2O)_5$NO. The source of

Table I. Kinetic and Thermodynamic Data for Reversible NO Coordination to Ferrous Chelates

ferrous chelates	k_1,[a] mol/(L·s)	k_{-1},[a] s^{-1}	K, L/mol at 298 K	$\Delta H°$, kcal/mol	$\Delta S°$, eu
$Fe^{2+}(H_2O)_5(NO)$	$(7.1 \pm 1.0) \times 10^5$	$(1.5 \pm 0.6) \times 10^3$	$(4.7 \pm 2.0) \times 10^2$ [a]		
$Fe^{2+}(citrate)(NO)$	$(4.4 \pm 0.8) \times 10^5$	$(6.6 \pm 2.4) \times 10^2$	$(6.7 \pm 2.0) \times 10^2$ [a]		
$Fe^{2+}(acac)_2(NO)$	$(4.0 \pm 3.0) \times 10^2$	24 ± 2	17 ± 14 [a]		
$Fe^{2+}(NTA)(NO)$	$\geqslant 7 \times 10^7$	$\geqslant 35$	2.14×10^6 [b]	-11.94 [b]	-11.0 [b]
$Fe^{2+}(EDTA)(NO)$	$\geqslant 6 \times 10^7$	$\geqslant 6$	1.15×10^7 [c]	-15.8 [c]	-20.7 [c]

[a] Ref 5. [b] Ref 7. [c] Ref 6.
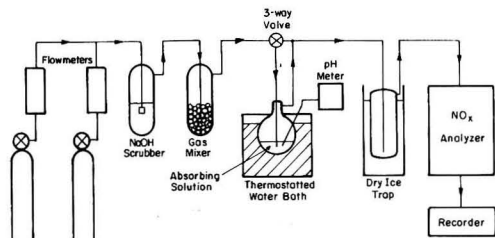
Figure 1. NO absorption apparatus. This setup is used for determining the enthalpy, entropy, and equilibrium constants for the reversible binding of NO to various metal chelates in aqueous solution.
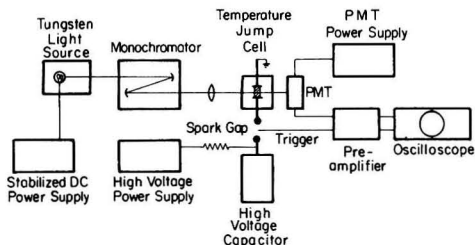
Figure 2. Temperature-jump apparatus. This setup is used for determining the formation and dissociation rate constants and equilibrium constants for the reversible binding of NO to various metal chelates in aqueous solutions.

the energy for the temperature jump is a high-voltage dc power supply connected to a capacitor through a solenoid switch. After the capacitor is charged, the switch is disconnected. When a variable spark gap is closed, the energy stored in the capacitor can be discharged through a cell containing the reaction under study. A temperature jump of about 8 °C was created by discharging a 0.25-mF capacitor, charged to 20 kV, through platinum-coated electrodes in the cell, whose volume is approximately 1.4 cm^3. The discharge time is dependent on the ionic strength of the solution but in general less that 10 μs. The temperature jump induces a change in the concentrations of reactants and products as the reaction shifts to a new equilibrium. The shift is monitored by a photomultiplier tube and displayed on an oscilloscope.

The reciprocal of the relaxation time equals the forward rate constant times the sum of the final equilibrium concentration of $Fe^{2+}(L)$ and NO plus the backward rate constant (8). When the reciprocal of the relaxation time is plotted against the final concentrations of $Fe^{2+}(L)$ plus NO, the slope of the curve gives the forward rate constant (k_1), and the point of interception gives the backward rate constant (k_{-1}).

When the equilibrium constants of reactions listed in Table I are compared, it is obvious that $Fe^{2+}(EDTA)$ and $Fe^{2+}(NTA)$ have much larger absorption capacities for NO than do $Fe^{2+}(H_2O)_6$, $Fe^{2+}(citrate)$, or $Fe^{2+}(acac)_2$. For an aqueous scrubbing solution initially containing 0.1 mol/L $Fe^{2+}(NTA)$ at 50 °C, $\mu = 0.1$ mol/L, the fraction of the iron

chelate that is converted to $Fe^{2+}(NTA)(NO)$ is about 36% when the solution is in equilibrium with a gas containing 1000 ppm of NO at 1 atm. For a gas containing 100 ppm of NO the conversion is about 5%. Thus, the presence of the iron chelate increases the capacity of the scrubbing solution for NO by a factor of 30 000 or more.

(2) Kinetics. With the temperature-jump technique, Littlejohn and Chang (5) directly measured the formation and dissociation rate constants of $Fe^{2+}(H_2O)_5(NO)$, $Fe^{2+}(citrate)(NO)$, $Fe^{2+}(acac)_2(NO)$, $Fe^{2+}(EDTA)(NO)$, and $Fe^{2+}(NTA)(NO)$ (Table I). The forward and reverse rate constants for the formation of $Fe^{2+}(citrate)(NO)$ are somewhat smaller than the values for the $Fe^{2+}(H_2O)_5(NO)$ complex, while the equilibrium constant is larger. The kinetics for the formation and dissociation of the Fe^{2+}-$(acac)_2(NO)$ complex are much slower than any other complex studied.

For both $Fe^{2+}(EDTA)(NO)$ and $Fe^{2+}(NTA)(NO)$, the relaxation times due to the temperature jump were too fast to be measured. However, an upper limit of 10 μs was established for the relaxation times for both complexes. By use of this value with the equilibrium constants determined for $Fe^{2+}(EDTA)(NO)$ and $Fe^{2+}(NTA)(NO)$ by Hishinuma et al. (6) and Lin et al. (7), respectively, the lower limits of forward and reverse rate constants were calculated (Table I).

Thermodynamic Equilibrium and Kinetics of Absorption of SO_2

(1) Thermodynamics. Sulfur dioxide is moderately soluble in water; four major S^{4+} species are produced after dissolution of SO_2 in aqueous solutions: $SO_2(aq)$, HSO_3^-, SO_3^{2-}, and $S_2O_5^{2-}$. The equilibrium concentrations of these S^{4+} species depend on both the partial pressure of SO_2 and the pH of the solution. The solubility coefficient of SO_2 is 1.24 mol/(L·atm) at 25 °C. Hydrated SO_2 can ionize to form bisulfite ion, HSO_3^-, which can undergo further ionization to produce sulfite ion, SO_3^{2-}.

$$SO_2(g) + H_2O \rightleftharpoons SO_2(aq) \tag{4}$$

$$SO_2(aq) \rightleftharpoons H^+ + HSO_3^- \quad K = \\ 1.27 \times 10^{-2} \text{ mol/L at 25 °C} \tag{5}$$

$$HSO_3^- \rightleftharpoons H^+ + SO_3^{2-} \quad K = \\ 6.24 \times 10^{-8} \text{ mol/L at 25 °C} \tag{6}$$
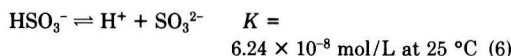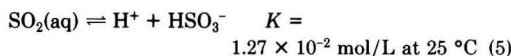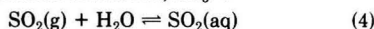
Bisulfite ion is also in equilibrium with the disulfite ion, $S_2O_5^{2-}$:

$$2HSO_3^- \rightleftharpoons S_2O_5^{2-} + H_2O \quad K = \\ 7 \times 10^{-2} \text{ L/mol at 25 °C} \tag{7}$$
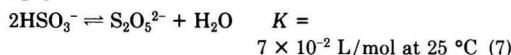
Using Henry's constant for SO_2 (9) and the equilibrium constants for eq 5–7, one can calculate the amounts of each specie in solution. Table II lists the concentrations of four S^{4+} species in a solution in equilibrium with a gas containing 1000 ppm of SO_2, at various pHs and at 25 °C. The equilibrium concentration of total aqueous S^{4+} species increases with increasing solution pH at a given partial pressure of SO_2; i.e., the capacity of an aqueous solution for absorbing SO_2 is larger at a high solution pH.

Table II. Equilibrium Concentration of S^{4+} Species (mol/L) at $p_{SO_2} = 1000$ ppm at 25 °C

S^{4+} species	pH 7	pH 6	pH 5	pH 4	pH 3
$SO_2(aq)$	1.24×10^{-3}	1.24×10^{-3}	1.24×10^{-3}	1.24×10^{-3}	1.24×10^{-3}
HSO_3^-	1.58×10^2	1.58×10^1	1.58	1.58×10^{-1}	1.58×10^{-2}
SO_3^{2-}	9.82×10^1	9.82×10^{-1}	9.82×10^{-5}	9.82×10^{-5}	9.82×10^{-7}
$S_2O_5^{2-}$	1.75×10^3	1.75×10^1	1.75×10^{-1}	1.75×10^{-3}	1.75×10^{-5}
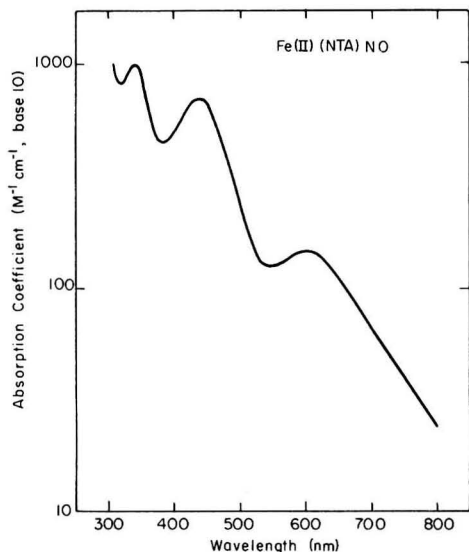
Figure 3. Absorption spectrum of $Fe^{II}(NTA)(NO)$ from 800 to 300 nm.

(2) Kinetics. Dissolution of SO_2 can be expressed by reactions 4–7. Reaction 5 proceeds very fast as can be seen from the rate constant (k_3) and reverse rate constant (K_{-3}) measured by Eigen et al. (10) by using a relaxation technique: $k_3 = 3.4 \times 10^6$ s^{-1} and $k_{-3} = 2.0 \times 10^8$ mol/(L·s) at 20 °C.

Regeneration Rate of Metal Chelate Catalysts

We have started to investigate the reaction kinetics between $Fe^{2+}(NTA)(NO)$ and sodium sulfite/bisulfite as a model for the reactions of SO_2–NO–metal chelates in aqueous solutions. The reaction can be expressed as

$$aNO + aFe^{2+}(NTA) \rightleftharpoons aFe^{2+}(NTA)(NO) \qquad (8)$$

$$aFe^{2+}(NTA)(NO) + bSO_3^{2-} \rightleftharpoons aFe^{2+}(NTA) + cN_2O + dN_2 + e\text{N-S complexes} + fSO_4^{2-} \qquad (9)$$
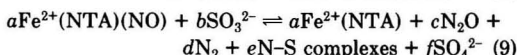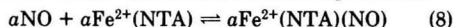
The experiments were performed under O_2-free conditions to avoid oxidation of Fe^{2+}, NO, and S^{4+}. The concentration of $Fe^{2+}(NTA)(NO)$ was monitored by visible–absorption spectrometry during the course of the reaction. A spectrum of $Fe^{2+}(NTA)(NO)$ complexes is shown in Figure 3 and has been found to obey Beer's law over the concentration range (10^{-4}–10^{-3} mol/L) employed in the experiments. The rate of disappearance of $Fe^{2+}(NTA)(NO)$ in the presence of SO_3^{2-} or HSO_3^- has been found to have a second-order dependence on the concentration of Fe^{2+}-(NTA)(NO) and a first-order dependence on the concentration of SO_3^{2-} or HSO_3^-. The rates can be expressed as

$$\frac{-d[Fe^{2+}(NTA)(NO)]}{dt} = [k_7[SO_3^{2-}] + k'_7[HSO_3^-]][Fe^{2+}(NTA)(NO)]^2 \qquad (10)$$

Tentative values for k_7 and k'_7 have been obtained. At pH 8.0, $k_7 = 3.16 \times 10^3$ (L/mol)^2/s, and at pH 3.5, $k'_7 = 4.3 \times 10^2$ (L/mol)^2/s. These values were obtained at 20 °C.

Table III. Raman Shifts and Scattering Efficiencies of Some Species Relative to Sulfate Ion

compound	Raman shift, cm^{-1}	rel scattering efficiency ($SO_4^{2-} = 1.00$)
SO_4^{2-}	985	1.00
SO_3^{2-}	~970	0.12
NO_2^-	818/1332	0.053/0.125
NO_3^-	1050	0.95
N_2O	1285	~0.18
$HON(SO_3)_2^{2-}$ (HADS)	1087	1.43
$H_2NSO_3^-$ (SFA)	1052	0.41

The dependence of the reaction rate on ionic strength was investigated at conditions near neutral pH and was found to be negligible. Since the reactions between the nitrosyl ferrous complex and the dissolved SO_2 species is so rapid, recovery of the NO before reaction does not appear feasible in this system. It does not appear that the product compounds can be easily regenerated into NO.

Identification of Reaction Products

The intermediate and final products that can be formed from the reaction of SO_2, NO, and ferrous chelates in aqueous solutions have not been well characterized as yet. A few compounds such as SO_4^{2-}, N_2O, and some N–S complexes (sulfamate (11) and aminedisulfonate (1, 2)) have been found. A tedious wet chemical analytical method for determining N–S compounds has recently been described (12). However, the mass balance between products and reactants of this reaction has not been made. Because it is necessary to identify intermediate and final products so that reaction kinetics and mechanisms can be characterized and an optimum stack gas scrubber developed, we have begun to develop analytical techniques so that species can be directly, rapidly, and quantitatively determined and the mass balances of the reactions between nitrosyl metal chelates and SO_2 in aqueous solutions can be performed. Wet analytical techniques are not sufficiently rapid or specific to identify all the species present in chemical systems as complex as the ones under study. Laser Raman spectroscopy appears to have the potential to identify the species present in mixtures of SO_2, NO, and metal chelates efficiently and unambiguously.

The first step in the identification process is to acquire Raman spectra (13) of potential reaction products so that the relative scattering efficiencies and Raman shifts will be known. The species studied to date, along with their Raman shifts and scattering efficiencies relative to sulfate ion, are shown in Table III. The limiting factors in Raman spectroscopy are the need for fairly high concentrations and the attenuation of the spectra by colored solutions. Since some of the reaction products are present in fairly low concentrations and some of the solutions used are colored, it has been necessary to use fairly long data acquisition times to obtain useful spectra. NO has a low concentration in aqueous solution because of its small Henry's constant and has not been detected.

Laser Raman spectroscopy has been used to detect the products of the reactions of $Fe^{2+}(NTA)(NO)$ with sulfite ion and $Fe^{2+}(H_2O)_5(NO)$ with bisulfite ion. Solutions of
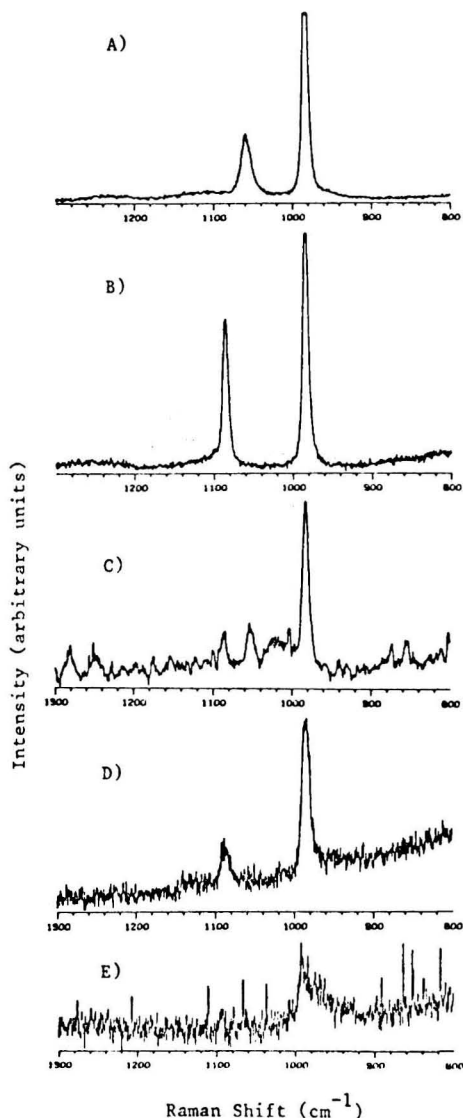
Figure 4. Raman spectra of (A) sulfamates (SFA), (B) hydroxylaminedisulfonates (HADS) and reaction mixtures of (C) $Fe^{II}(H_2O)_5(NO)$ with HSO_3^- and (D) and (E) $Fe^{II}(NTA)(NO)$ with SO_3^{2-}/HSO_3^-.

potential reaction products, including nitrogen–sulfur compounds, were prepared to obtain Raman shifts and relative scattering efficiencies. Parts A and B of Figure 4 are Raman spectra of sulfamate (SFA) and hydroxylaminedisulfonate (HADS), respectively. Sulfate ion was added to the solutions as a reference so that the relative scattering efficiencies could be obtained. The HADS peak is at 1084 cm^{-1}, the SFA peak is at 1049 cm^{-1}, and the SO_4^{2-} peak is at 981 cm^{-1}.

Figure 4C is a spectrum of a solution with initial concentrations of 0.037 mol/L $Fe^{2+}(H_2O)_5(NO)$ and 0.19 mol/L bisulfite ion at pH ~4. The 985-cm^{-1} sulfate peak is from the ferrous sulfate used to prepare the solution and the sulfate created as a reaction product. The peak due to HADS is apparent at 1087 cm^{-1}. The peak at 1052 cm^{-1} could be from both SFA and disulfite ions. The species

responsible for some of the other peaks present such as the 1245- and 1280-cm^{-1} peaks have not yet been identified. The solution is colorless after the completion of the reaction. The 488-nm Ar$^+$ laser line was used to obtain the spectra.

Figure 4D is a spectrum of $Fe^{2+}(NTA)(NO)$ and sulfite ion at pH 8.3, with initial concentrations of 0.043 and 0.100 mol/L, respectively. Additional sulfate ion was added to the solution at the time of mixing, making a total of 0.091 mol/L sulfate added to the solution. The peak due to HADS is prominent at 1087 cm^{-1}. The reacted solution absorbs strongly in the blue region of the spectrum, so the 647-nm Kr$^+$ laser line was used for this spectrum.

Figure 4E is a spectrum of an initial concentration of 0.012 mol/L $Fe^{2+}(NTA)(NO)$ and 0.100 mol/L sulfite ion at pH ~7. The 985-cm^{-1} sulfate peak overlaps with the broader sulfite peak centered at 970 cm^{-1}. The peaks ascribed to HADS and SFA are weak but visible at 1087 and 1052 cm^{-1}. While the reacted solution does absorb in the blue region of the spectrum, the absorption is sufficiently weak to allow the 488-nm Ar$^+$ laser line to be used. The sharp spikes in the spectrum are due to noise in the laser output.

Work is currently in progress to determine quantitatively the amounts of the observed products that are produced by the reactions of ferrous nitrosyl complexes with sulfite and bisulfite ions.

Implications for Scrubber Design

The results of the studies cited above show the technical feasibility of designing and operating a single scrubber capable of removing both NO$_x$ and SO$_x$ well enough to meet national air quality standards. The solution leaving such a scrubber must be regenerated before being recycled to the scrubber. As will be indicated below, regeneration can potentially be done by several different methods. The study of the chemistry of the reactions involved is still under way, and hence the optimal regeneration process has not yet been selected.

(1) **NO Removal.** The data given in Table I show that Fe^{2+} chelated by either NTA or EDTA reacts rapidly with NO under scrubber conditions. A large fraction of the iron chelate is converted to the nitrosyl complex when equilibrated with NO at concentrations commonly found in stack gases. The rate constant for forming complexed NO is so large that for either chelating agent the reaction is effectively instantaneous. Since NTA costs less per pound than EDTA, and also has a lower molecular weight, it appears to be the agent of choice at this time.

The calculation above showed that about 5% of the Fe^{2+} in an 0.1 mol/L solution is complexed with NO when in equilibrium with a gas containing only 100 ppm of NO. Hence only one equilibrium stage would be required to reduce the NO content of a flue gas to that level in a scrubber operating with about 7 times the minimum liquid flow needed for an inlet NO content of 1000 ppm. With two or more equilibrium stages comparable NO removal could be achieved with a substantially lower liquid flow rate.

(2) **SO₂ Removal.** The reaction between the complexed NO and HSO_3^- is too slow to affect the rate of absorption of SO$_2$. One must, instead, depend upon the buffering capacity of the solution for absorbing SO$_2$ and upon subsequent reaction for removing it from the solution. As is shown in Table II, the pH of the solution in the scrubber is thus advantageously kept in the range 5–7. However, examination of the acid constants of NTA (10) (pK_{a_1} = 1.97, pK_{a_2} = 2.57, and pK_{a_3} = 9.81) shows that the NTA cannot provide buffering in this pH range. The buffering

must therefore be provided by adding some other soluble base to the scrubbing solution. Ammonia, sodium carbonate, sodium citrate, and sodium phosphate are examples of bases that may be useful.

There has been substantial commercial experience with scrubbing SO_2 from stack gas with a solution containing a soluble base. With only two or three equilibrium stages in the scrubber it is possible to achieve a high degree of SO_2 removal with a relatively low liquid flow rate.

(3) Solution Regeneration. To be economically attractive, a scrubbing solution must be regenerable with little or no loss of active ingredients. For a scrubbing solution containing $Fe^{2+}(NTA)$ together with an appropriate buffer, regeneration will be a complex process. Neumann and Lynn (14) have shown that $Fe^{2+}(NTA)$ reacts rapidly with oxygen to form $Fe^{3+}(NTA)$. The fraction of Fe^{2+} oxidized will depend on the O_2 content of the flue gas. In addition to removing the absorbed NO and SO_2, the regeneration must then reduce this Fe^{3+} back to Fe^{2+}. Lynn and Dubs (15) found that H_2S reacts stoichiometrically with $Fe^{3+}(NTA)$ to form $Fe^{2+}(NTA)$ and elemental sulfur in the pH range 3.5–4.5. When the pH of the solution is above 7.0, some thiosulfate and polythionates may be formed. Neumann and Lynn (14) showed that the rate of absorption of H_2S by $Fe^{3+}(NTA)$ solution indicated the rate of reaction to be effectively instantaneous in the pH range 3.5–4.5. Absorption rates and reaction products in the pH range 5–7 must still be determined.

As noted above, NO complexes with $Fe^{2+}(NTA)$ undergoes a complex series of reactions with HSO_3^- and SO_3^{2-}. In these reactions the nitrogen-containing compounds are reduced in successive steps, and the sulfur compounds are oxidized to SO_4^{2-}. The study of the chemistry of these reactions is still under way. Still to be determined is whether H_2S or HS^- will react with $Fe^{2+}(NTA)(NO)$ in a similar series of reactions.

Several methods are available for removing the absorbed SO_2 from the scrubber liquid during regeneration. One approach is to add lime and precipitate a mixture of $CaSO_3$ and $CaSO_4$. This would require technology similar to that used in the various double-alkali processes for SO_2 scrubbing. Thorough washing to the precipitate to avoid excessive loss of scrubber-liquid chemicals would be required. A second approach is to react dissolved SO_2 with H_2S to form elemental sulfur. This type of technology has been demonstrated in the Bureau of Mines' citrate process and Stauffer Chemical's AquaClaus process. With this approach a saleable form of sulfur is produced, and costs may be reduced as a result. The reaction of H_2S and HSO_3^- in the presence of chelated iron compounds has not yet been studied.

Conclusions

The technical feasibility of removing NO_x and SO_x simultaneously from power plant flue gas has been established. Both the reaction rates and the chemical equilibria allow the use of a single, conventional scrubber for the scrubbing operation. A technique using laser Raman spectroscopy has been developed for the direct, rapid, and quantitative identification of the intermediate and final products of the reactions involved. The determination of the best method of regenerating the scrubbing liquor must be completed before a process based on this chemistry can be evaluated.

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Distribution Model for Binding of Protons and Metal Ions by Humic Substances

Edward M. Perdue*[†] and Charles R. Lytle

Environmental Science/Chemistry Department, Portland State University, Portland, Oregon 97207

■ A mathematical model is proposed for the binding of protons and metal ions by humic substances. The totally empirical nature of simple, discrete models that treat humic substances as a mixture of two or three ligands (often erroneously referred to as "classes" of ligands) is clearly demonstrated. The extreme complexity of the mixture of ligands in humic substances suggests that a continuous ligand distribution model would be more appropriate, i.e., a model in which a continuum of log K values for proton or metal binding could be treated. In the proposed model, it is assumed that ligand concentrations are normally distributed with respect to their log K values. A "class" of ligands can then be characterized by its mean log K value (μ) and the standard deviation (σ) of log K values about the mean. This model has been successfully applied to the complexation of copper(II) by aquatic humus and by sewage sludge derived fulvic acids. Proton binding by aquatic humus is efficiently described by a similar model that contains two classes of functional groups.

Introduction

Chemical equilibrium models (*1*) can provide an excellent description of chemical speciation in aquatic systems whenever the pertinent reactions are rapid relative to mixing processes (*2–4*). The binding of protons and metal ions to simple inorganic ligands is generally adequately described by such models. To a lesser extent, the binding of protons and metal ions to well-defined solids such as metal oxides and clays can be described by chemical equilibrium models (*5, 6*). Westall and Hohl (*7*) have recently shown that experimental data from such studies can be described by several different models, so there is some ambiguity yet to be removed in studies involving solid phase ligands.

Perhaps the most perplexing chemical equilibrium problem that is yet to be resolved for aquatic systems is the thermodynamic description of the binding of protons and metal ions to the complex mixture of organic acids known as humic substances (*8*). Humic substances are ubiquitous in soil and aquatic environments, being formed through random reactions that presumably occur during microbial degradation of biomass. Despite the fact that elemental analyses, functional group analyses, number-average molecular weights, etc. indicate that some bulk properties of humic substances are relatively invariant, all attempts to fractionate humic substances into simpler subfractions have been unsuccessful. For example, Reuter and Perdue (*9*) have found that the infrared spectra of molecular size fractions of aquatic humus do not decrease in complexity with decreasing molecular weight, even for fractions with number-average molecular weights as low as 340 g/mol. Curtis et al. (*10*) have found that pH gradient chromatography of humic substances on XAD-8 resins yields essentially a continuous elution profile.

The complexity of the mixture of organic ligands in humic substances is significantly greater than the complexity of ligand sites on pure solids (metal oxides and clays), and this fact alone would be sufficient to explain the greater difficulty in characterizing the ligands in humic substances. When the inherent complexity of the ligand mixture is combined with the reported tendency of humic substances to undergo aggregation reactions (*11*) and configurational changes (*12*) and with the electrostatic problems that arise from interactions between two or more ligand sites on the same molecule, it is reasonable to conclude that the problem of totally defining this ligand mixture is probably *intractable*.

As a ligand mixture becomes increasingly complex, titration curves for either proton or metal binding would be expected to become more and more featureless, with no sharply defined equivalence points. This expectation is clearly met in titrations of humic substances (*13, 14*). The smooth, featureless titration curves of humic substances lead to an interesting, and potentially confusing, situation. Almost any function with several adjustable parameters will fit most or possibly all of the data points in a typical titration, thereby making it impossible to use goodness-of-fit to test the appropriateness of a given chemical model. The multitude of chemical models that have been proposed to describe proton and metal binding by humic substances amply illustrates this point.

In this paper, we will critically examine some current chemical models, with particular concern for the chemical significance of the fitting parameters of each model. An alternative approach to the description of proton and metal binding by humic substances, based on a statistical distribution model, will be developed and applied to titration data.

Chemical Equilibria in Multiligand Mixtures

The fact that metal complexation by humic substances results in the release of protons indicates that, to some extent, the same ligands are involved in proton and metal binding. While proton binding can be studied in the absence of competitive metal binding in metal-free solutions, metal complexation must inevitably by studied in competition with proton binding. It is therefore highly advantageous to use *conditional* stability constants to describe metal binding at constant pH. In the following equations, all charges are omitted and only 1:1 metal–ligand complexes are explicitly considered. For complexation of a metal (M) by the *i*th *deprotonated* ligand or binding site in a multiligand mixture at constant pH

$$M + L_i \rightleftharpoons ML_i \qquad (1)$$

and

$$K_i = \frac{[ML_i]}{[M][L_i]} \qquad (2)$$

It is more convenient to define a conditional stability constant

$$K_i' = \frac{[ML_i]}{[M][H_{X_i}L_i]} = K_i\left(\frac{[L_i]}{[H_{X_i}L_i]}\right) \qquad (3)$$

where $[H_{X_i}L_i]$ is the concentration of all forms of the *i*th

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†Present address: School of Geophysical Sciences, Georgia Institute of Technology, Atlanta, GA 30332.

ligand that are not bound to M. The conditional stability constant (K_i') is thus equal to the thermodynamic stability constant (K_i) times the fraction of [$H_{X_i}L_i$] that is not protonated. This fraction is constant at constant pH.

In a complex multiligand mixture, both an average stability "constant" ($\bar{K}$) and a *conditional* average stability "constant" ($\bar{K}'$) can be defined, the latter being readily calculated from experimental data.

$$\bar{K} = \frac{\sum_i [ML_i]}{[M]\sum_i [L_i]} = \frac{\sum_i K_i[L_i]}{\sum_i [L_i]} \quad (4)$$

$$\bar{K}' = \frac{\sum_i [ML_i]}{[M]\sum_i [H_{X_i}L_i]} = \frac{\sum_i K_i'[H_{X_i}L_i]}{\sum_i [H_{X_i}L_i]} = $$
$$\frac{C_M - [M]}{[M](C_L - C_M + [M])} \quad (5)$$

C_M and C_L are the stoichiometric concentrations of metal and total ligand, respectively. When the numerator and denominator of eq 5 are divided by the activity of all uncomplexed forms of an arbitrarily selected reference ligand [$H_{X_r}L_r$], eq 6 is obtained. This particular form for

$$\bar{K}' = \sum_i K_i' \frac{[H_{X_i}L_i]}{[H_{X_r}L_r]} \Big/ \sum_i \frac{[H_{X_i}L_i]}{[H_{X_r}L_r]} \quad (6)$$

$\bar{K}'$ is introduced here simply to more amply illustrate the fact that $\bar{K}'$ is not a constant, but rather a function whose value changes continuously as metal is added to a multiligand mixture. For purposes of discussion let us assume that $H_{X_r}L_r$ is the weakest metal-binding ligand in the mixture. Consider then the behavior of $\bar{K}'$ as metal ions are added to this ligand mixture. The form of $\bar{K}'$ is that of a weighted average. The weighting factor ([$H_{X_i}L_i$]/[$H_{X_r}L_r$]) of the ligand with the largest K_i' value will be greatest at the lowest levels of added metal and will decrease steadily as C_M increases. The inevitable result is that $\bar{K}'$ must decrease as C_M increases and cannot be regarded as a constant at all. The functional nature of $\bar{K}'$ has been clearly recognized by previous researchers (14, 15).

For reasons that will become more apparent in subsequent sections of the paper, it is useful to examine the general equilibrium description of a multiligand mixture that contains two distinct *classes* of ligands (classes I and II). In this case, equations analogous to eq 5 can be derived for each class of ligands.

$$\bar{K}_I' = \frac{(\sum_i K_i'[H_{X_i}L_i])_I}{(\sum_i [H_{X_i}L_i])_I} \qquad \bar{K}_{II}' = \frac{(\sum_i K_i'[H_{X_i}L_i])_{II}}{(\sum_i [H_{X_i}L_i])_{II}} \quad (7)$$

Unfortunately, only the overall $\bar{K}'$ given by eq 5 can be calculated from experimental data. The experimental $\bar{K}'$ is a weighted average of $\bar{K}_I'$ and $\bar{K}_{II}'$.

$$\bar{K}' = \frac{\bar{K}_I'(\sum_i [H_{X_i}L_i])_I + \bar{K}_{II}'(\sum_i [H_{X_i}L_i])_{II}}{(\sum_i [H_{X_i}L_i])_I + (\sum_i [H_{X_i}L_i])_{II}} \quad (8)$$

More importantly, it must be recognized that, like $\bar{K}'$, $\bar{K}_I'$ and $\bar{K}_{II}'$ are functions that change continuously as the ligands within each class react sequentially with added metal. Any type of *average* stability "constant" will have a fixed value only at a single C_M value during the titration of metal into a multiligand mixture. We will return to this

point in the next section of the paper.

The third general subject that should be addressed in this section of the paper is the treatment of complexes with other than 1:1 stoichiometry. This subject has been rigorously examined by MacCarthy and co-workers (15, 16). The conclusions reached above with regard to 1:1 metal–ligand complexes also apply to other possible stoichiometries. Thus, while average "constants" for 1:1 and 1:2 complexes can be defined mathematically, those "constants" will change continuously as metal is added to a multiligand mixture.

Discrete Multiligand Models

Multiligand models or the analogous multiple binding site models usually assume 1:1 stoichiometry for all metal–ligand complexes. It is convenient to combine eq 3 with the ligand mass balance equation for the ith ligand or binding site

$$C_i = [H_{X_i}L_i] + [ML_i] \quad (9)$$

where C_i is the stoichiometric concentration of the ith ligand or binding site, to obtain

$$\nu_i \equiv [ML_i]/C_i = \left(\frac{K_i'[M]}{1 + K_i'[M]}\right) \quad (10)$$

When summed for all ligands, eq 11 is obtained.

$$\bar{\nu} \equiv \frac{\sum_i \nu_i C_i}{\sum_i C_i} = \sum_i \left(\frac{K_i'[M]}{1 + K_i'[M]}\right)\left(\frac{C_i}{C_L}\right) = \frac{C_M - [M]}{C_L} \quad (11)$$

Experimental $\bar{\nu}$ values, calculable from C_M, C_L, and [M] via eq 11, can be used to calculate $\bar{K}'$.

$$\bar{K}' = \frac{1}{[M]}\left(\frac{\bar{\nu}}{1 - \bar{\nu}}\right) \quad (12)$$

As indicated previously, $\bar{K}'$ values are not constant in multiligand mixtures. The only real constants in such systems are the K_i' values of the individual ligands and binding sites, and then only at constant pH. There is no direct method by which those K_i' values can be extracted from experimental $\bar{K}'$ values for multiligand mixtures such as humic substances, simply because there are potentially so many different metal binding groups to be characterized.

In several recent studies of metal complexation by humic substances (17–20), a serious conceptual error has resulted from attempts to use the Scatchard equation (21) or other related equations to extract equilibrium constants and ligand concentrations from experimental data. In these studies, it is assumed that the K_i' values for discrete, individual ligands in eq 11 can alternatively be assumed to represent average stability constants for distinct classes of ligands. The mathematical fallacy in this assumption is best illustrated by examining the pertinent equations. The first term of eq 11

$$\bar{\nu} = \left(\frac{K_1[M]}{1 + K_1[M]}\right)\left(\frac{C_1}{C_L}\right) \quad (13)$$

is easily rearranged to yield the one-component Scatchard equation (eq 14).

$$\frac{\bar{\nu}}{[M]} = K_1\left(\frac{C_1}{C_L}\right) - K_1\bar{\nu} \quad (14)$$

This simple equation, which predicts a linear relationship

between $\bar{\nu}/[M]$ and $\bar{\nu}$, never adequately describes the curvilinear plots of $\bar{\nu}/[M]$ vs. $\bar{\nu}$ that are always obtained in studies of metal–humus complexation equilibria. Almost without exception, those researchers who use the Scatchard equation to analyze experimental data respond to the failure of the linear one-component model (eq 14) by including the second term from eq 11, yielding

$$\bar{\nu} = \left(\frac{K_1[M]}{1 + K_1[M]} \right) \left(\frac{C_1}{C_L} \right) + \left(\frac{K_2[M]}{1 + K_2[M]} \right) \left(\frac{C_2}{C_L} \right) \quad (15)$$

The addition of the second term (which increases the number of curve-fitting parameters from two to four) generally results in a greatly improved fit of the experimental data, leading to the conclusion that humic substances contain two "classes" of metal complexing ligands. The curve-fitting constants K_1, C_1/C_L, K_2, and C_2/C_L are treated as *average stability constants* and *ligand concentrations* for the two presumed classes of ligands. The best values of K_1, C_1/C_L, K_2, and C_2/C_L are necessarily obtained by curve-fitting experimental data over a range of $\bar{\nu}$ values. So C_M and/or C_L is varied considerably, usually by titration of metal into a humic substance, to generate the data set. In the previous section of the paper, the functional nature of average stability "constants" was demonstrated. Even if humic substances did contain two distinct classes of ligands, $\bar{K}_I'$ and $\bar{K}_{II}'$ would vary continuously during a titration. Thus, $\bar{K}_I'$ and $\bar{K}_{II}'$ cannot be equated with K_1 and K_2, both of which are implicitly assumed to be independent of C_L and C_M in eq 15. The whole idea of extracting average stability constants from eq 15 is clearly erroneous. The four constants that are obtained from the two-component Scatchard equation must therefore be regarded as empirical curve-fitting parameters with no chemical significance. Statements to the effect that humic substances contain two classes of metal complexing ligands are therefore potentially misleading, and any speculation about the chemical "structures" of these presumed ligands is clearly unwarranted. In a previous paper, it was demonstrated that the two-component Scatchard equation functions quite well as an *empirical* equation even when a continuous distribution of ligands is involved in metal complexation (22).

The conclusions given in the preceding paragraph are not intended as a criticism of the Scatchard equation per se, but rather the erroneous assumption that K_i' could represent a *class* of related ligands rather than one discrete ligand. The Scatchard equation has been widely and successfully applied to biomolecules such as proteins and nucleic acids. It is not at all unreasonable to expect that a purified protein molecule might have only one or two distinct sites for binding of a metal ion or other substrate. Even in biochemistry, however, many examples of inappropriate use of the Scatchard equation can be found. This problem was succinctly described recently by Klotz (23).

The same conceptual error has also been made in studies where other types of equations have been used to analyze experimental data. For example, if eq 13 is inverted and rearranged, eq 16 is obtained. When $1/\bar{\nu}$ vs $1/[M]$ is

$$\frac{1}{\nu} = \left(\frac{C_L}{C_1} \right) + \left(\frac{C_L}{C_1} \right) \left(\frac{1}{K_1[M]} \right) \quad (16)$$

plotted, C_1/C_L and K_1 can be obtained for a system that contains only one discrete ligand. This equation has been in use for as long as the Scatchard equation for describing equilibria in biochemical systems (24). More recently, eq 16 has been used in an attempt to describe metal binding by humic substances (25) with the erroneous assumption

that K_1 could represent the average stability constant $(\bar{K}_I')$ for a class of ligands. In this case, the failure of eq 16 to describe metal complexation by humic substances was attributed to the formation of both 1:1 and 1:2 metal–ligand complexes. The addition of this new curve-fitting parameter (the average stability constant for 1:2 complexes) resulted in a greatly improved fit of the experimental data. As in the case of the two-component Scatchard equation, however, the curve-fitting parameters of the two-stoichiometry model are simply empirical constants. While it is indeed possible that 1:1 and 1:2 complexes are formed, it is not possible to determine the relevant average equilibrium constants because those "constants" change continuously as C_L and/or C_M is changed.

The discussion in the preceding paragraphs may have given the impression that there is no rigorous method by which metal complexation by humic substances can be efficiently described. However, MacCarthy and Smith (15) have shown that, under severely limiting conditions of excess ligand that may not be attainable in laboratory studies or in the environment, the average stability constants for 1:1, 1:2, and higher complexes in multiligand systems approach constant limiting values. While this conclusion is of theoretical interest, it is of little practical importance, given the analytically imposed limitations on C_M and C_L values in laboratory studies.

Continuous Multiligand Models

Many authors (26–29) have realized that a wide range of stability constants for proton and metal binding must exist in humic substances and have adapted models that were originally developed for synthetic and natural polymers (30–32). The modified Henderson–Hasselbalch equation typifies these models. All the so-called "intrinsic log K" models attribute the observed range of log K values for a class of structurally related binding sites to the electrostatic effects of charged groups in the humus "polymer". Statistical effects, delocalization effects, and, most importantly, the electrostatic effects of dipolar functional groups in close proximity to a binding site are totally ignored. With that major assumption, it follows that all binding sites are identical when there are no charged groups in the polymer. By proper extrapolations of experimental data to the point of zero charge, so-called intrinsic log K values can be obtained. While these models are an improvement over the discrete models that were previously discussed, the assumption that all binding sites are inherently identical is untenable and leaves us once again with a more-or-less empirical model of proton and metal binding.

The most rigorous method for describing proton and metal binding by humic substances has been developed by Gamble and co-workers (14, 33–35), who have clearly recognized the functional nature of $\bar{K}'$ values. They have suggested that humic substances contain a continuous distribution of nonidentical functional groups that can bind protons or metal ions. Even when they have subdivided proton binding sites into two classes, the $\bar{K}_I'$ and $\bar{K}_{II}'$ values have been consistently treated as functions rather than constants. In some instances, they have used polynomial equations to empirically describe the variation of $\bar{K}'$ values with solution composition. While Gamble and co-workers have not calculated discrete K_i' values from experimental data, they have demonstrated that the instantaneous K value for the group that is reacting at a particular set of C_L and C_M values can be estimated mathematically by partial differentiation of appropriate polynomial equations.

Despite its rigorous approach, the method of Gamble has not been widely accepted, more for psychological than technical reasons. There is no *efficient* method for describing the variation of $\bar{K}'$ with solution composition, so the $\bar{K}'$ results must be either tabulated or presented graphically. A simple set of thermodynamically defined equilibrium constants that could be used to reconstruct the data set is not obtainable by Gamble's method.

One quite different approach that can be used to model the binding of protons and metal ions by humus is the affinity spectrum technique that was used by Hunston (36–38) and has been recently applied to humus by Shuman et al. (39). This method assumes the existence of a continuous distribution of binding sites whose concentrations are functionally related to their individual log K values. The distribution function that best fits experimental data can be approximated by an equation that is obtained by formal analogy with an equation that is used to describe the dynamic response of viscoelastic materials. The affinity spectrum technique avoids the types of assumptions that have undermined the discrete and intrinsic log K models and is theoretically superior to those models. Accordingly, this approach may eventually gain acceptance in the field of humus research.

Posner (40, 41) showed that proton binding by humic substances was efficiently described by a continuous multiligand distribution model, the relative concentration of each discrete ligand being normally distributed relative to the pK_i of the ligand. In a normal distribution of ligands

$$\frac{C_i}{C_L} = \frac{1}{\sigma(2\pi)^{1/2}} \exp\left[-\frac{1}{2}\left(\frac{\mu - pK_i}{\sigma} \right)^2 \right] dpK \quad (17)$$

where C_i/C_L is the mole fraction of ligands in the interval dpK whose acid dissociation constant is expressed as a negative logarithm (pK_i), and σ is the standard deviation for the distribution of pK_i values about the mean pK value (μ) for the mixture of ligands. Similar models have been used to describe the binding of anions to proteins (24) and the adsorption of gases on catalytic surfaces (42). This general approach seems to be an excellent alternative to Gamble's method in that an entire titration curve can be summarized by the μ and σ values for the multiligand mixture. The somewhat objectionable procedure of assuming a normal distribution can be overcome by the use of more sophisticated statistical methods that actually determine the shape of the best ligand distribution curve from experimental data (43).

In the present paper, only the normal distribution model will be examined. This distribution model is easily combined with eq 11 to yield

$$\bar{\nu}_{calcd} = 1/[\sigma(2\pi)^{1/2}] \int_{-\infty}^{\infty} [[M]10^{\log K'}/(1 + [M]10^{\log K'})] \exp[-\frac{1}{2}[(\mu - \log K')/\sigma]^2] \, d\log K' \quad (18)$$

Numerical methods (e.g., Gaussian quadrature, Simpson's method) can be used to evaluate the integral in eq 18 for any value of [M], given a set of μ and σ values. Given a set of experimental $\bar{\nu}$ and [M] values, nonlinear regression techniques can be used to determine the best values of μ and σ for the set of data. Either $\bar{\nu}$ or corresponding log $\bar{K}'$ values can be used for regression.

In proton binding by humic substances, both carboxylic acid and phenol functional groups must be considered. Likewise, the possibility of two classes of metal binding ligands should be considered. Accordingly, a bimodal normal distribution model will be used in some of the applications in the following section of the paper. The
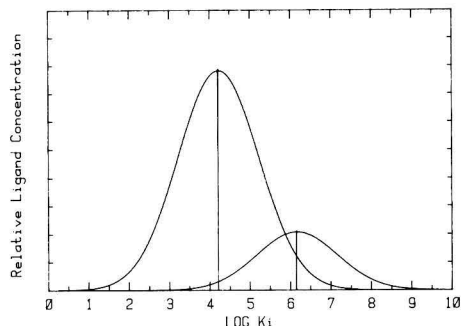
Figure 1. Comparison of discrete and continuous ligand mixtures.

extension from eq 18 to a bimodal model is easily accomplished. Equations that are analogous to eq 18 can be written for $\bar{\nu}_I$ (given μ_I and σ_I) and for $\bar{\nu}_{II}$ (given μ_{II} and σ_{II}). Then, for $C_L = C_I + C_{II}$, where C_I and C_{II} are stoichiometric concentrations of two classes of ligands (e.g., carboxyl and phenolic groups)

$$\bar{\nu} = \theta\bar{\nu}_I + (1 - \theta)\bar{\nu}_{II} \quad (19)$$

where $\theta = C_I/C_L$ and $1 - \theta = C_{II}/C_L$. The bimodal distribution model thus has five curve-fitting parameters (θ, μ_I, σ_I, μ_{II}, and σ_{II}) that must be determined by nonlinear regression.

Applications

In this section of the paper, the normal distribution model will be used to generate data sets for hypothetical multiligand mixtures containing either one or two classes of ligands, so that the empirical nature of results obtained from the discrete two-component Scatchard equation may be clearly illustrated. Experimental data for complexation of H^+ and Cu^{2+} by humic substances will be analyzed by the normal distribution model to determine how well this simple distribution model actually works.

Hypothetical Multiligand Mixtures. The mathematical case against the use of the two-component Scatchard model (eq 15) to describe metal binding in multiligand mixtures was presented earlier in the paper. A more applied demonstration may be somewhat more convincing. Can this equation, which is rigorous for *two* ligands only, be used to obtain meaningful $\bar{K}_i$ and C_i/C_L values for systems that contain *two classes* of ligands? To address this question, it is useful to represent a "class" of ligands as a normal distribution of structurally related binding sites. A system containing two discrete ligands ($C_1/C_L = 0.21$, log $K_1 = 6.16$, $C_2/C_L = 0.79$, and log $K_2 = 4.22$) and a comparable system containing two normally distributed classes of ligands ($\theta = 0.21$, $\mu_1 = 6.16$, $1 - \theta = 0.79$, $\mu_2 = 4.22$, and $\sigma_1 = \sigma_2 = 1.0$) are represented in Figure 1, in which the distribution of ligands with respect to log K values is given. From this figure, it is apparent that the discrete two-ligand system (which is accurately described by the two-component Scatchard equation) becomes equivalent to the bimodal Gaussian distribution system as σ_1 and σ_2 approach zero. Accordingly, the two-component Scatchard equation is expected to more accurately describe metal binding in the bimodal Gaussian system as both σ_1 and σ_2 approach zero.

This expectation was confirmed by generating bimodal Gaussian data sets with variable σ values and fitting those data to the two-component Scatchard equation. Input parameters for the Gaussian model that was used to generate data sets were $\theta = 0.21$, $\mu_1 = 6.16$, $\mu_2 = 4.22$, −11.0
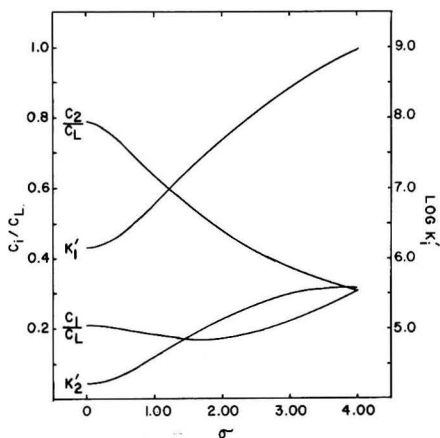
Figure 2. Variation of Scatchard parameters with the standard deviation of a continuous distribution of ligands.

Figure 3. Computed values of $\bar{K}'$, $\bar{K}_I'$, and $\bar{K}_{II}'$ in a continuous distribution of ligands.

$\leq \log [M] \leq -3.0$, and $0.1 \leq \sigma_1 = \sigma_2 \leq 4.0$. The Scatchard fitting parameters for each data set were determined by using a nonlinear regression method to minimize a weighted residual sum of squares (RSS):

$$\text{RSS} = \sum_{j=1}^{n} \frac{(\bar{\nu}_{\text{calcd}} - \bar{\nu}_{\text{exptl}})^2}{\bar{\nu}_{\text{exptl}}} \qquad (20)$$

where n is the number of generated data pairs used in the regression analysis. If the weighting factor $(1/\bar{\nu}_{\text{exptl}})$ is not used, only larger $\bar{\nu}_{\text{exptl}}$ values are important in determining the values of the fitting parameters. This problem is apparent when the $\bar{\nu}_{\text{calcd}}$ values are subsequently used to calculate $\log \bar{K}'$ or $\bar{\nu}/[\text{M}]$, neither of which agrees well with experimental values at very low $\bar{\nu}$ values.

The best Scatchard fitting parameters for each of the mathematically generated data sets are given as a function of σ in Figure 2. The actual results showed minor random deviations from the smooth curves given in Figure 2, probably as a result of using fairly coarse convergence conditions to minimize computer time in the nonlinear regression program. At very low σ values, the calculated Scatchard fitting parameters (C_1/C_L, $\log K_1$, C_2/C_L, and $\log K_2$) are very close to the actual parameters used to generate the bimodal Gaussian data sets (θ, μ_1, $1 - \theta$, and μ_2). As σ values increase, the Scatchard fitting parameters $\log K_1$ and C_2/C_L deviate dramatically from μ_1 and $1 - \theta$. Relatively smaller deviations are observed for $\log K_2$ and C_1/C_L. By definition, the sum of the relative abundances of classes of ligands equals one. Likewise, in the two-component system that is the basis of the Scatchard equation (eq 15), the sum of C_1/C_L and C_2/C_L theoretically equals 1, if C_L is defined as the total concentration of binding sites. From Figure 2, the sum of C_1/C_L and C_2/C_L is observed to gradually decrease from 1.0 to 0.6 as σ increases from 0.1 to 4.0. Thus, these fitting parameters cannot possibly represent actual abundances of binding sites. It was demonstrated earlier that there is no mathematical justification for treating $\log K_1$ and $\log K_2$ as the "average constants" for binding of metal ions to distinct *classes* of ligands. The results in Figure 2 further illustrate this point, with $\log K_1$ increasing from 6.16 (the value of μ_1) to 8.80 as σ increases from 0.1 to 4.0. Even $\log K_2$ increases by over 1 order of magnitude (4.22–5.34) as σ increases from 0.1 to 4.0.

It can be concluded that the Scatchard equation is inappropriate for description of metal complexation in
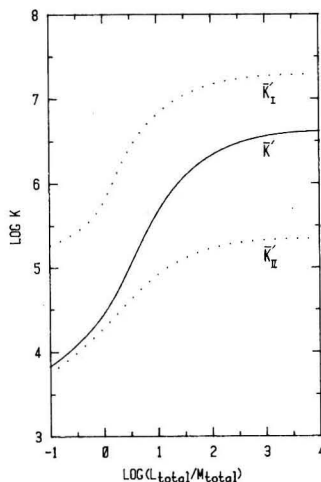
multiligand mixtures. Even though experimental data are easily fit to the two-component Scatchard equation, the four fitting parameters must be regarded as empirical curve-fitting parameters with little or no chemical significance.

Finally, to illustrate the nonconstancy of the so-called average equilibrium constants for metal complexation in a multiligand mixture, the titration of Cu(II) into a 1×10^{-4} M solution containing two classes of normally distributed ligands was simulated. The Gaussian parameters that define this ligand set are $\theta = 0.21$, $\mu_1 = 6.16$, $\sigma_1 = 1.00$, $\mu_2 = 4.22$, and $\sigma_2 = 1.00$. The values of $\bar{\nu}$, $\bar{K}'$, $\bar{K}_I'$, and $\bar{K}_{II}'$ were calculated for 10^{-11} M $\leq [\text{Cu}^{2+}] \leq 10^{-3}$ M, and the results are given in Figure 3. Clearly, $\bar{K}_I'$ and $\bar{K}_{II}'$ vary continuously and cannot be considered as constants except under extreme excess ligand conditions, as described by MacCarthy and Smith (15). The $\log K_1$ and $\log K_2$ values from the Scatchard analysis of this data set are 6.77 and 4.60 (see Figure 2) and are simply *not* equivalent to $\log \bar{K}_I'$ and $\log \bar{K}_{II}'$ or to μ_1 and μ_2, the *mean* $\log K$ values of the two classes of ligands.

Proton Binding by Aquatic Humus. The existing evidence strongly suggests that the acidic properties of humic substances are attributable primarily to carboxyl and phenolic hydroxyl groups. The total acidities of most humic substances can be determined reasonably well by the barium hydroxide method described by Schnitzer and Khan (44). Although several methods have been used to resolve total acidity into carboxyl and phenolic components, all such methods yield only operationally defined results (13).

The bimodal Gaussian distribution model has been used to fit the titration data of Perdue et al. (13) for titration of Satilla River aquatic humus in 0.1 M NaCl with NaOH. From the electroneutrality condition and the definition of $\bar{\nu}$ as the fraction of binding sites that are protonated, experimental $\bar{\nu}$ values were calculated as

$$\bar{\nu} = \frac{(C_L - [\text{H}^+]) - (C_B - [\text{OH}^-])}{C_L} \qquad (21)$$

where C_B is the stoichiometric concentration of added base (e.g., NaOH). From the $(\bar{\nu}, [\text{H}^+])$ data for this titration and the total acidity of the sample (10.4 mequiv/g) (9), the following Gaussian fitting parameters were estimated
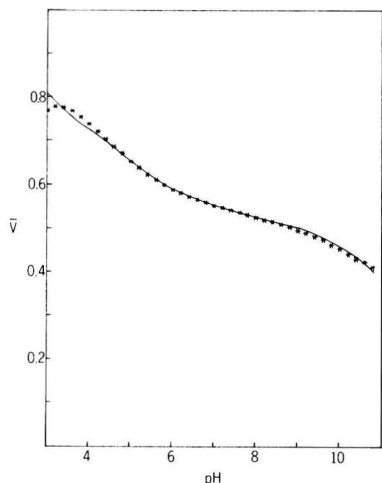
Figure 4. Proton binding by aquatic humus: (asterisks) experimental and (—) Gaussian model.

Figure 5. Cu(II) binding by aquatic humus: (asterisks) experimental and (—) Gaussian model.

Figure 6. Cu(II) binding by sludge-derived fulvic acid: (asterisks) generated from Scatchard model and (—) Gaussian model.

by using eq 18 and 19: $\theta = 0.48$, $\mu_1 = 3.62$, $\sigma_1 = 2.31$, $\mu_2 = 12.3$, and $\sigma_2 = 1.68$. The experimental data and the calculated curve are given in Figure 4. While the fit is less than perfect, the value of $\bar{\nu}$ can be predicted with a relative error of less than 3.8% above pH 4.0. The anomalous behavior of experimental $\bar{\nu}$ values at lower pH values has been previously noted (13) and cannot be described by a simple proton binding model. Considering the fact that the Gaussian function may not be the most appropriate description of the distribution of proton binding sites in aquatic humus, this fit was considered to be quite good.

Examination of the Gaussian parameters themselves further substantiates the general utility of the model. The mean pK_a values of the two classes of functional groups are consistent with the known properties of carboxyl groups ($\mu_1 = 3.62$) and phenolic hydroxyl groups ($\mu_2 = 12.3$). Actually, the mean pK_a of the "phenolic hydroxyl groups" is higher than would be expected for neutral phenols, suggesting that either the phenolic hydroxyl groups are primarily located on highly charged benzene-carboxylate ions or other types of functional groups are being ionized. Monosaccharides have pK_a values in the 12–13 range, so it is possible that part of the weak acidity is attributable to alcoholic hydroxyl groups in sugarlike structural components of the humus sample. It is difficult to assess the significance of σ_1 and σ_2, except that they are indicative of fairly wide ranges of pK_a values in both structural classes. Finally, if the total acidity of the sample is multiplied by θ, a carboxyl content of 5.0 mmol/g is estimated. This value is in reasonable agreement with the estimate of 4.4 mmol/g obtained by titration calorimetry (45) and the value of 4.4–4.5 mmol/g obtained by various indirect titrations of this sample (13).

Copper Binding by Humic Substances. The applicability of the Gaussian distribution model was evaluated by using two data sets for titration of humus with Cu(II) at pH 5.0 and $I = 0.1$ M NaClO$_4$. The first data set was obtained in this laboratory by using aquatic humus from the Williamson River, Oregon. The second data set was constructed from the Scatchard fitting parameters published by Sposito et al. (19) for sewage sludge derived fulvic acids (titration 1 in their paper). Both data sets were fit reasonably well by a single-mode Gaussian distribution model (one class of ligands), although minor improvement
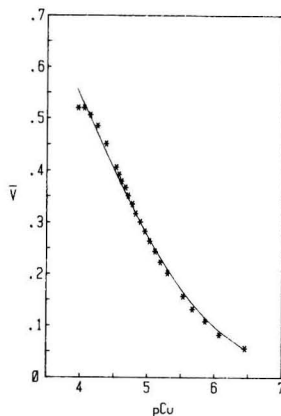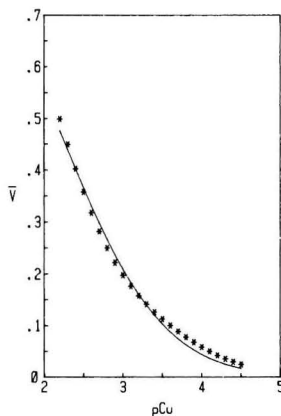
in the degree of fit was obtained with the bimodal distribution model.

The single-mode results are given in Figure 5 and Figure 6 for our titration ($\mu = 4.15$, $\sigma = 1.20$) and that of Sposito et al. ($\mu = 2.14$, $\sigma = 0.76$), respectively. In both cases, the agreement between calculated and experimental $\bar{\nu}$ values is quite good. It is particularly gratifying to note that the "experimental" points in Figure 6, which were generated by using *four* fitting parameters in the two-component Scatchard equation, can be described quite well with the *two* fitting parameters of the Gaussian distribution model. The μ value for aquatic humus is much higher than that for sewage sludge derived fulvic acid. The relatively weaker affinity of the latter material for Cu(II) is also indicated if the Scatchard parameters of Sposito et al. (19) are compared with results of other workers on soil and water fulvic acids [e.g., Bresnahan et al. (18)]. In both cases, the σ values were relatively small, indicating that most Cu(II)-binding ligands are roughly comparable in Cu(II)-binding strength.

Conclusions

The exceedingly complex mixture of ligands that are involved in proton binding and metal binding by aquatic humus cannot be unambiguously described by any type of chemical model that is currently available. It is nevertheless possible to approximate the variation of $\bar{\nu}$ with

[H$^+$] or [M] by using a variety of chemical models, none of which is totally appropriate. Given the complexity of the ligand mixture, there can be no doubt that discrete models that postulate the existence of two or three distinct binding sites with unique equilibrium constants for proton or metal binding are fundamentally incorrect from both a chemical and a mathematical point of view. In contrast, continuous distribution models that postulate the existence of one or two classes of ligands, each class consisting of a very complex mixture of ligands whose individual concentrations are a function of binding strength, are at least consistent with the known complexity of the ligand mixture.

Both proton and metal binding by aquatic humus are efficiently described by the Gaussian distribution model. Even data that were generated from the two-component Scatchard equation (with four fitting parameters) were successfully modeled by using a Gaussian distribution with two fitting parameters. In all likelihood, this approach toward modeling the behavior of complex ligand mixtures in homogeneous solutions should also find application in studies of proton and metal binding in complex heterogeneous systems such as aqueous suspensions of particulate humic substances, amorphous metal oxides, etc.

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Interspecies Correlations of Acute Aquatic Median Lethal Concentration for Four Standard Testing Species

Francis G. Doherty[†]

Betz Laboratories, Trevose, Pennsylvania 19047

■ Correlation and regression analyses were conducted on acute median lethal concentration (LC50) data for various compounds comparing the species rainbow trout (*Salmo gairdneri*), bluegill sunfish (*Lepomis macrochirus*), fathead minnow (*Pimephales promelas*), and water flea (*Daphnia magna*). Predictive equations were developed on data extracted from the literature and generated internally at Betz Laboratories, Inc., for the period 1965–1982. Results indicate there is a high level of correlation ($r > 88\%$) among these four species in all combinations. The degree of demonstrated predictability is sufficient to allow the use of *D. magna* as the first species screened in primary level hazard assessment schemes, permitting a greater latitude of choice in the second species subjected to acute toxicity testing.

Introduction

Environmental testing guidelines are expected to be promulgated in the near future under the United States Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). A large amount of aquatic testing is already necessary for providing data to set point source discharge limits under the Clean Water Act (CWA) and in support of the National Pollutant Discharge Elimination System (NPDES). Considering the number of chemicals in commerce in the United States (50 000 plus materials on the TSCA inventory) and the 1000 new chemicals entering the marketplace each year (1), comprehensive toxicity testing presents itself as an awesome undertaking.

This volume of materials demands a priority testing framework within which progressively more sophisticated tests can be structured so as to minimize laboratory resource expenditures and maximize gains in knowledge. The testing framework which currently enjoys broad acceptance is a tiered system in which individual tests increase in cost, complexity, and information returned. As the data at each level are generated, decision points are reached that determine the need to proceed to the next battery of tests.

The most commonly encountered primary toxicity test in any hazard evaluation scheme is the static acute aquatic toxicity test. An acceptable risk decision may be made on the basis of a single acute test (in conjunction with the chemical/physical properties of a material) or may require augmentation by additional acute tests incorporating alternate conditions, methods, or organisms. An attempt to minimize the number of acute tests needed to develop an appropriate data base for responsible decision making was presented by Kenaga (2). Kenaga sought to illustrate potential interspecies correlations in acute toxicity test results, thereby permitting predictive interpretations between standard species with a subsequent reduction in the number of necessary studies.

This paper attempts to corroborate and expand the aquatic portion of the Kenaga toxicity study by exhibiting the correlative properties of acute toxic action existing

[†] Present address: Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

Table I. Base Ingredients of Proprietary Product Formulations Tested

(A) Base Ingredients of Proprietary Product Formulations Tested at Betz' Aquatic Toxicology Laboratory

acids (organic and inorganic)	metal hydroxides
	metal oxides
alcohols	naphthas
amides	nitrates
amines	nitrites
bases	organic solvents
carbonates	phosphates
chelants	silicates
chromates	sulfates
ethers	sulfites
glycols	
high molecular weight polymers	

(B) Base Ingredients of Proprietary Biocide Agents Tested

acrolein	disodium ethylenebis-(dithiocarbamate)
bis(trichloromethyl) sulfone	dodecylguanidine hydrochloride
chlorinated phenols	2-mercaptobenzothiazole
chlorophenates	methylenebis(thiocyanate)
dibromonitrilopropionamide	N-alkyldimethylbenzyl-ammonium chloride
dichloroacetone oxime acetate	sodium dichloroisocyanurate
dichlorodimethylhydantoin	sodium dimethyldithiocarbamate
3,5-dimethyltetrahydro-1,3,5-thiadiazine-2-thione	bis(tributyltin) oxide

between three fish species and one aquatic invertebrate and assessing the feasibility of interspecies predictability for the ultimate purpose of data substitution at early levels of testing.

Materials and Methods

Test Methodology. The development of interspecies predictive equations utilized toxicity tests generated in accordance with currently approved, published methodologies (3) except where indicated for variables such as temperature and loading. All tests contributing to the generation of Betz regression equations were static acute toxicity tests internally conducted at the Trevose, PA, facility during the period 1965–1982. All products tested were Betz Laboratories, Inc., proprietary formulations generally consisting of two or more pure ingredients. No tests were run on effluent samples. Reagent-grade materials were occasionally tested and have been included in the analysis. All concentrations reported are based on concentrations of product, not active ingredients. A partial generic listing of the base ingredients of these products is presented in Table I.

All tests utilized the median lethal concentration (LC50) as the end point, with test durations spanning 96 h for fish and 48 h for the invertebrate *Daphnia magna*. Initial test temperatures were permitted to range from 10 to 14 °C for rainbow trout (*Salmo gairdneri*), 20 to 24 °C for bluegill sunfish (*Lepomis macrochirus*) and fathead minnow (*Pimephales promelas*), and 18 to 22 °C for *D. magna*.

Table II. Representative Diluent Water Quality Characteristics of Supply Available at Betz', Trevose, PA, Facility

pH	6.5–7.5
conductivity, $\mu\Omega^{-1}$	300–330
alkalinity, as $CaCO_3$ mg/L	0
hardness, total as $CaCO_3$ mg/L	105–120
phosphate, total PO_4 mg/L	0.4
chloride, mg/L	50–60
sulfate, mg/L	20–30
silica, mg/L	18.0
chromate, as CrO_4 mg/L	<0.05[a]
copper, mg/L	<0.05[a]
iron, mg/L	<0.05[a]
zinc, mg/L	<0.05[a]

[a] Lowest limit of sensitivity for techniques commonly employed.

Temperatures did not vary by more than ±1 °C for the duration of the test. All fish tests exposed a minimum of 10 fish per concentration with a minimum of 5 fish per test container. The bulk of the Betz tests used 20 fish per concentration with 10 fish per test container. Test fish length ranged from 2 to 5 cm. Test loading densities rarely exceeded 1 g/L. Only first instar daphnids (12 ± 12 h) were used in the definitive toxicity tests with 30 animals exposed per concentration (10 daphnids in each of three beakers per concentration).

All product concentrations reported are based on the volume or weight of product introduced to the test vessel immediately prior to the start of the test. Analytical verification of product concentration were not undertaken at any time during the test. While some Betz products possessed similar formulations, no one product tested was a direct dilution of another. The addition of toxicant to fish testing vessels was not accompanied by prior removal of equivalent amounts of diluent. Test results were discarded if there were 10% or greater mortality in the control vessels. Test results were also discarded if dissolved oxygen levels at the end of a test fell below 60% of initial for *D. magna* or 40% of initial for fish.

Some earlier protocols advocated the practices of solvent addition, pH adjustment, and test vessel aeration. Only those test pairs were used in which the application of these alternatives were equivalent.

Dilution water used in these studies was a mixture of varying amounts of dechlorinated municipal water and local well water. On a few occasions, daphnid toxicity tests were conducted in reconstituted water (3). Table II delineates a representative diluent water quality analysis.

Data pairs gleaned from the literature were chosen on the basis of test variable compatability with Betz data (test temperature, end point, duration, and method of test). Literature data pairs were used only if generated by an individual or a team of investigators and reported in a single source. Two variations from this general scheme were permitted in order to increase sample size: concurrent dynamic tests for fish and extensions of the initial temperature limitations for warm water fish.

A listing of materials reported in the literature and utilized in this study is presented in Table III.

Data Reduction. All internally generated toxicity test concentration–mortality data were analyzed through a computer program providing four statistical methodologies: the binomial method for generating 95% confidence limits and moving average, probit, and linear interpolation methods for LC50 computations (33). The moving average estimate of LC50 was utilized in the analyses reported in this study. For those instances in which the moving average or probit estimate was inappropriate, the linearly

Table III. List of Compounds and Data Sources Utilized from Published Literature

compound	data source
amines	Dave et al. (4)
chlorinated biphenyls	Dill et al. (5)
4-chloroaniline	Julin and Sanders (6)
diammonium phosphate	Johnson and Sanders (7)
diflubenzuron	Julin and Sanders (6)
fungicides	Hermanutz et al. (8); Bills et al. (9, 10)
heavy metals	Pickering and Henderson (11)
herbicide	Folmar et al. (12)
hydrogen cyanide	Smith et al. (13)
hydrogen sulfide	Smith and Oseid (14)
insecticides	Allison and Hermanutz (15); Cardwell et al. (16); Henderson et al. (17); Julin and Sanders (18); Macek and McAllister (19); Macek and Sanders (20); Marking (21); Mauck et al. (22)
lampricides	Marking and Olson (23)
nitriles	Henderson et al. (24)
pesticides	Macek et al. (25); Hobbs et al. (26)
phosphate esters	Nevins and Johnson (27)
potassium permanganate	Marking and Bills (28)
quaternary ammonium compounds	Dave et al. (4)
selenium dioxide	Cardwell et al. (29)
surfactants	Thatcher and Santner (30); Maki and Bishop (31); Folmar et al. (12)
trisodium nitrilotriacetate	Sturm and Payne (32)

interpolated value was utilized.

All LC50 values were logarithmically transformed through the function X or $Y = \log (\text{LC50} \times 10^4)$, allowing the span under study to be free of negative logarithms. Lines of best fit were constructed through least-squares estimation. All other calculations were conducted in accordance with accepted regression analysis methodology (34). The process by which species were assigned to a particular axis for the regression analyses was based on either the most easily generated test or on organism sensitivity. *D. magna* LC50's were always positioned on the horizontal axis due to the simplistic nature of the test in comparison with fish toxicity tests. Fathead minnow LC50's were always assigned a position on the vertical axis based on generally accepted findings that this species is among the least sensitive used for testing. All decision points were determined at the 0.05 significance level.

Results

Regression analyses were performed on the following species pairs as X vs. Y: rainbow trout vs. bluegill sunfish (R×S) (Figure 1a), rainbow trout vs. fathead minnow (R×F), *D. magna* vs. rainbow trout (D×R) (Figure 1b), bluegill sunfish vs. fathead minnow (S×F) (Figure 1c), *D. magna* vs. bluegill sunfish (D×S), and *D. magna* vs. fathead minnow (D×F) (Figure 1d). The total number of data sets reviewed included 261 pairs; 52% of the tests were generated at Betz, and 48% were obtained through published literature sources. Individual sample sizes for each species pairing set are listed in Table IV.

The analysis effort was initiated by generation of correlation statistics (Table IV) for which all data sets show exceedingly high levels of correlation (r). The subsequent regression analysis (Table IV) substantiates this initial finding, with consistent values for the coefficient of determination (R^2) and the precision estimate (g), a reflection
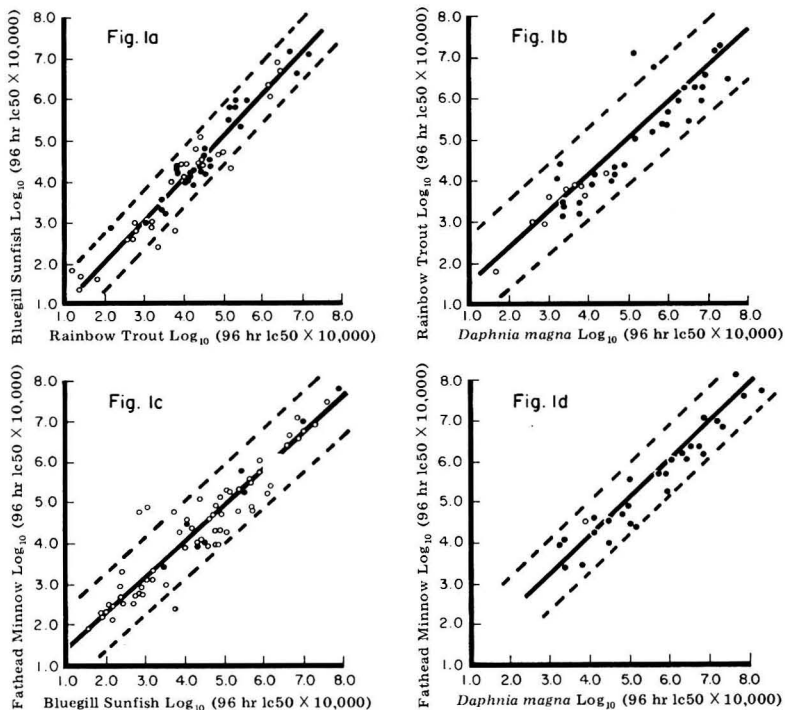
Figure 1. Interspecies regressions for acute aquatic LC50's from four possible species combinations. All values are in milligrams per liter concentrations based on the transformation on each graph axis. (O) Literature data set; (●) Betz data set; (—) line of best fit; (---) 95% prediction limits.

Table IV. Tabular Summary of Correlation and Statistical Analyses Performed[a]

data set $(X \times Y)$	data source	n	r, %	$\bar{x}$	$(x - \bar{x})^2$	s	g	$(Y - \bar{\bar{Y}})$	$Y = b_1 X + b_0$	R^2, %
R×S	B	31	96.9	4.48	33.69	0.287	0.009	±0.233	$Y = 1.04X + 0.02$	93.9
R×S	L	29	96.4	3.71	66.73	0.443	0.012	±0.313	$Y = 1.02X - 0.01$	92.9
R×S	P	60	96.8	4.10	109.26	0.369	0.005	±0.265	$Y = 1.04X - 0.02$	93.8
R×F	B	18	93.2	4.30	14.43	0.332	0.042	±0.274	$Y = 0.901X + 0.66$	86.9
R×F	L	13	90.6	4.86	19.00	0.510	0.096	±0.400	$Y = 0.829X + 1.13$	82.1
R×F	P	31	92.2	4.54	35.79	0.407	0.025	±0.323	$Y = 0.877X + 0.82$	85.1
D×R	B	31	89.7	5.13	56.53	0.569	0.033	±0.376	$Y = 0.843X + 0.81$	80.5
D×R	L	9	93.4	3.20	5.46	0.278	0.115	±0.209	$Y = 0.829X + 0.84$	87.4
D×R	P	40	92.7	4.70	88.02	0.520	0.015	±0.338	$Y = 0.845X + 0.80$	86.0
S×F	B	9	97.7	5.30	16.96	0.344	0.037	±0.264	$Y = 1.024X - 0.13$	95.5
S×F	L	62	93.5	4.23	162.05	0.538	0.008	±0.370	$Y = 0.865X + 0.62$	87.5
S×F	P	71	94.2	4.36	188.14	0.520	0.007	±0.357	$Y = 0.883X + 0.55$	88.7
D×S	B	18	95.1	5.41	50.96	0.505	0.030	±0.368	$Y = 0.866X + 0.79$	90.4
D×S	L	12	88.8	4.00	8.90	0.364	0.133	±0.285	$Y = 0.745X + 1.13$	78.9
D×S	P	30	95.3	4.85	74.19	0.451	0.015	±0.342	$Y = 0.871X + 0.70$	90.8
D×F	B	28	96.1	5.52	55.18	0.394	0.013	±0.289	$Y = 0.941X + 0.36$	92.4
D×F	L	1								
D×F	P	29	95.9	5.47	57.83	0.402	0.014	±0.295	$Y = 0.925X + 0.47$	91.9

[a] X or $Y = \log(\mathrm{LC50} \times 10^4)$. All numbers reported are in log form, milligrams per liter concentrations. R = rainbow trout, S = bluegill sunfish, F = fathead minnow, D = *D. magna*, B = Betz, L = literature, P = pooled, n = sample size, r = correlation coefficient, $\bar{x}$ = mean x, $(x - \bar{x})^2$ = sums of squares of x, s = standard deviation, g = precision estimate, R^2 = coefficient of determination, and $Y - \bar{Y}$ = mean absolute deviation.

on the ratio of the regression coefficient to its standard error (35). A majority of the data sets possess an $R^2 > 80\%$ and g values < 0.05. Other measures of dispersion providing evidence of consistency in the paired comparisons include the standard deviations (s) and the mean absolute deviations ($Y - \bar{Y}$). The spread of these parameters is generally minimal, with (s) ranging from 0.281 to 0.538 (log units) and ($Y - \bar{Y}$) from ±0.209 to ±0.400.

The best measure of usefulness of these data in future applications is provided by the 95% prediction limits in-

cluded in each graph of Figure 1. These limits are not only a function of the observed data of this study but take into account sample size (number of tests) of future studies. While the average width of these limits in Figure 1b is approximately 2 orders of magnitude, the predictive ability of a *Daphnia* test for a trout test can be narrowed to $1^1/_2$ orders of magnitude if two *Daphnia* tests are conducted on the same product rather than one. Calculation of the prediction limits in Figure 1 was based on a future test sample size of one.

The results of the regression exercises also provide evidence (slope, Y intercept) for which generalizations concerning relative species sensitivies may be inferred. Slopes approximating a value of 1 mean an increase in one variable is matched by an identical increase in the other variable. A Y intercept approximating zero means that both variables are essentially the same. The combination of these two factors proves the equivalency of the two variables. Only one species pairing satisified these criteria and can be considered equisensitive species, rainbow trout and bluegill sunfish. All other pairings presented nonzero Y intercepts and slopes not equal to one, thereby necessitating the use of the equations presented in Table IV. Generally speaking D. magna is more sensitive than trout or sunfish at or below 1 mg/L and is more sensitive than minnows at or below 10 mg/L. D. magna is equisensitive to trout and sunfish from 1 to 10 mg/L and to minnows at 10–100 mg/L. D. magna is less sensitive than trout or sunfish above 10 mg/L and less sensitive than minnows above 100 mg/L. Minnows are less sensitive than sunfish at less than 1 mg/L but more sensitive at greater than 10 mg/L; they are less sensitive than trout at less than 100 mg/L but more sensitive at greater than 1000 mg/L.

One apparent deviation from the general test results pattern concerns the sensitivity of D. magna vs. any of the fish species to chromium (Figure 1b, upper left portion of graph). In general, daphnids are more than 50–100 times more sensitive than expected. Insufficient sample size of unique products containing chromium (n = 2) precluded an independent regression analysis.

One last point supportive of interspecies correlations originates in comparisons of results from data bases compiled by different investigators. Side by side comparisons of Betz and literature statistics for each species pairing present highly compatible values. In addition, Betz intralaboratory test reproducibility is measurably consistent. Of 12 toxicity tests repeated with the same species, 64% did not differ statistically from the one previously conducted, while the remainder differed by less than an absolute factor of 3×.

Discussion

The number of materials requiring hazard assessment analysis is currently beyond the capacity of government and private sector laboratories. Fortunately modifications in hazard assessment testing methods are being developed to accommodate this volume. Streamlining approaches proposed include the use of comparably sensitive tests of shorter duration, use of organisms of greater sensitivity, and interspecies correlations for predictive purposes.

McKim (36) attempted to illustrate the sufficiency of embryo–larval and early juvenile (critical) life stage tests in lieu of complete life cycle tests with fish for elucidating chronic toxic effects of chemicals. The advantages of the former over the latter test include reduced exposure time (3 months vs. 2 years), reduced cost per test, and increased number of tests which could be conducted. McKim reported that 82% of the time results obtained for a unique material by both tests were identical. The remainder generally differed by less than a factor of 2. Macek and Sleight (37) generated compatible conclusions in a similar study.

Maki (38) generated regression analyses for chronic toxicity tests on fathead minnow vs. D. magna and found a marginally good relationship ($R^2 = 62.4\%$) for a sample set which included metals, PCB isomers, and pesticides (n = 35 total number of data pairs). The additional advantages for use of an invertebrate as opposed to critical life stage tests with fish include shorter life cycles and

subsequently shorter test duration (3 weeks), ease in culture and maintenance, reduced culture space, and water and test volume requirements. Kenaga (2, 39) and Kenaga and Moolenaar (40) developed a series of papers on potential interspecies toxicity relationships for acute and chronic applications. Although the data base utilized a narrow range of materials (pesticides, metals) whose data was generated through divergent test standards, they conclude that acute LC50's of one species can be used to predict that of another species with the appropriate mathematical function.

These studies have demonstrated that toxicity data generated for one species can be used in lieu of data generated with another species. Unfortunately these studies have restricted emphasis to relatively toxic materials in a limited number of chemical categories. If these interspecies data substitutions are to inspire confidence in their predictive capacity for hazard assessment schemes, many more classes of chemical compounds encompassing the entire toxic spectrum must be considered. It must be kept in mind that predictive equations generated for toxic substances may not be valid when extrapolated to less toxic substances for which the data base is inadequate. There is also the possibility that subgroups of generic materials adhere to a modified function. This was partly demonstrated in this study through tests conducted on products containing chromium. The graph of Figure 1b exhibiting the comparison of rainbow trout vs. D. magna has two points at the upper left of the graph which clearly do not coincide with the general pattern. Both points represent products which contain chromium. Obviously in both cases, D. magna was much more sensitive than rainbow trout.

Despite the existence of precedence for the use of regression analysis for comparison of toxicity levels, the approach is not satistically correct. The use of a parameter as the independent variable which is not "fixed" (measured without error) places the exercise into the model II category for which an alternate analyses is deemed appropriate. Generally though, predictive equations arising from the use of either model I (fixed independent variable, e.g., time) or model II will be compatible and generate similar results. Correlation analyses for which this type of data is most suitable are presented along with the regression analysis results in Table IV.

The four species evaluated comprise the core of freshwater testing species generally utilized in acute aquatic toxicity tests conducted for TSCA, FIFRA, or CWA for NPDES permits. One cold water fish, one warm water fish, and an inverterbrace are normally required as minimum data for submittal. These results suggest this practice may be redundant. The study clearly portrays the equisensitivity of rainbow trout and bluegill sunfish and the feasibility of predicting toxicity to one species based on an acute test using one of the other species (Figure 1). While there is evidence that one group of materials (chromates) may not comply with the expected response, this does not invalidate the interspecies predictive concept assuming the deviant materials can be identified.

Considering the advantages inherent in short-term invertebrate toxicity tests as compared with comparable fish studies, this author advocates the use of D. magna as the species of choice in initial hazard assessment testing tiers. Since the hazard assessment process generally results in estimates of probability of the presence or absence of hazard, and not a certainty, the use of D. magna should not perceptibly detract from confidence in the results. The

increased ease and reduced costs of *Daphnia* testing would then permit multiple *Daphnia* tests on the same material. This would result in narrower predictive ranges for effects expected on the fish species. Considering the success Betz has achieved with same product–same species repetitive testing, as mentioned earlier, this approach could prove highly useful.

In summary, this study has illustrated a number of key points with regard to interspecies predictive toxicology pertaining to acute aquatic testing. There does appear to be a linear relationship for toxic effects between species over a broader range of compounds than previously reported. The correlation between species over the entire range is high, generally greater than 90%. Daphnids appear to be much more sensitive to chromates than the fish species but would provide conservative protection levels for a waterway if *Daphnia* were used as the primary level testing organisms. Finally, the effort behind testing fish species at primary hazard evaluation levels could be better channeled into repetitive *Daphnia* tests on the same material to increase the probability of success in predicting the potential hazards to other aquatic species.

The rapidity of acceptance of interspecies correlative attempts will most likely be linked to the rate of growth and quality of the data base. Predictive toxicology may eventually provide substantial input to the hazard assessment process but must comply with all the pertinent statistical requirements in order to be effective. Raw data points analyzed in this study are available upon a specific request to augment similar efforts in substantiating predictive approaches.

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Polycyclic Aromatic Ketones in Environmental Samples

Thomas Ramdahl

Central Institute for Industrial Research, Blindern, Oslo 3, Norway

■ Various environmental samples (wood and coal combustion, municipal incineration, diesel emissions, aluminum plant work atmosphere, and urban ambient air) have been fractionated by high-performance liquid chromatography. The polycyclic aromatic ketones (PAK) in the moderately polar fractions were characterized by gas chromatography electron impact and negative ion chemical ionization mass spectrometry. PAK with molecular weight from 180 to 330 were identified. The theoretical distribution of the number of PAK compounds vs. molecular weight is very well reflected in the environmental samples.

Introduction

Polycyclic aromatic hydrocarbons (PAH) have been shown to be ubiquitous in our environment, resulting from incomplete combustion of organic materials. Several of these compounds have been shown to be carcinogenic in animal experiments and therefore associated with the occurrence of various types of cancer in man. There are numerous reports describing the analysis and identification of PAH in environmental samples (*1*). However, relatively little work has focused on derivatives and transformation products of PAH.

Polycyclic aromatic ketones (PAK) compose one such class of compounds. PAK have been identified in a number of samples such as diesel exhaust (*2–7*), biomass combustion emissions (*8–10*), carbon black (*11–13*), and urban air (*14–17*). However, no systematic efforts have been made to study these compounds in environmental samples, and little is known about their possible biological effects. The mutagenic activity using the Ames *Salmonella* mutagenicity assay of the fraction of environmental samples containing PAK has been reported by several workers (*2, 3, 5*), but the contribution to mutagenic activity from the PAK has not been estimated.

Due to the ketone group in the molecule, PAK are more polar than PAH and may therefore be separated from PAH by normal phase silica high-performance liquid chromatography (HPLC) (*3, 16, 18*). We have fractionated a number of environmental samples by HPLC and characterized the PAK fraction by gas chromatography/mass spectrometry (GC/MS) using different ionization techniques.

Experimental Section

Sample Preparation. Emission samples from wood and coal combustion, municipal incineration, and a diesel engine were sampled with a quartz glass probe. The particles in the gas were collected on Gelman glass fiber filters type A–E, diameter 142 mm, held at 125 °C. The sampling rate was 3 m³/h. Work atmosphere samples from an aluminum plant and ambient urban air samples were collected on 20 × 25 cm Gelman glass fiber filters type A–E by using high-volume samplers. The filters were Soxhlet extracted with dichloromethane for 24 h. The extracts were fractionated on a Waters HPLC system equipped with a 300 mm × 7.8 mm i.d. semipreparative μPorasil column (Waters Associates) (*16*). The extracts were injected into the column (injection volume 100–200 μL) by using 5% dichloromethane in hexane as mobile phase.

After 10 min under isocratic conditions a linear gradient was started with 5% dichloromethane/min to 100% dichloromethane. This was held for 30 min. The PAK were sampled manually from the column effluent between 24 and 50 min.

Gas Chromatography/Mass Spectrometry. All spectra were recorded on a Finnigan Model 4021 quadrupole mass spectrometer, equipped with an INCOS 2100 data system, and a Finnigan 9610 gas chromatograph. The mass spectrometer was operated at 70 eV. In the negative ion chemical ionization mode methane was used as the reagent gas at an ion source pressure of 0.20 torr. Masses from 35 to 400 amu were scanned every second. The samples were chromatographed on a 30 m × 0.25 mm i.d. DB-5 fused silica capillary column (J & W Scientific, Inc.), film thickness 0.25 μm, directly interfaced to the ion source, programmed from 100 (3 min) to 325 °C at 5 °C/min.

Chemicals. 9*H*-Fluoren-9-one and 7*H*-benz[*de*]-anthracen-7-one were purchased from Koch Light Laboratories Ltd. and Aldrich Chemical Co., respectively. The benzofluorenones were synthesized at Risö Chemical Laboratory, Denmark. 6*H*-Cyclopenta[*cd*]pyren-6-one was a gift from U. Stenberg, University of Stockholm, Sweden. All solvents were HPLC grade from Rathburne Chemical Ltd., Scotland.

Results and Discussion

The PAK fraction of environmental samples may be enriched by a fractionation of the sample by liquid chromatography (*3, 5*). PAK are eluted with other compounds of similar polarity, and the use of GC/MS is necessary to characterize the PAK in this fraction because of the complexity of the sample. Figure 1 shows the reconstructed ion chromatogram of the HPLC fraction containing PAK of ambient urban air from a small city (Elverum) in Norway (*19*). Here, the mass spectrometer was operated in the electron impact (EI) mode which shows no selectivity for any compound. In Figure 2 the reconstructed ion chromatogram with the mass spectrometer in the negative ion chemical ionization (NICI) mode is reported. Most of the interfering aliphatic compounds from Figure 1 do not undergo electron capture (*16*) and hence are not detected.

The two ionization techniques are complementary to each other in the determination of PAK. EI lacks the selectivity but provides mass spectra with structural information. NICI shows high selectivity and sensitivity but gives only the molecular ion and no mass spectra of PAK. The determination of PAK by NICI must therefore be in combination with GC retention times.

Figure 3 gives the mass chromatograms of PAK in the emission from a municipal incinerator. There is a range of more than 40 PAK present in the sample with molecular weights from 180 to 330. Corresponding sets of mass chromatograms from samples of wood and coal combustion, diesel emission, and work atmosphere of aluminum plants are nearly identical, demonstrating the ubiquitous distribution of PAK in environmental samples.

EI Mass Spectra of PAK. Figure 4 gives representative mass spectra of PAK indicated in Figure 3. All spectra
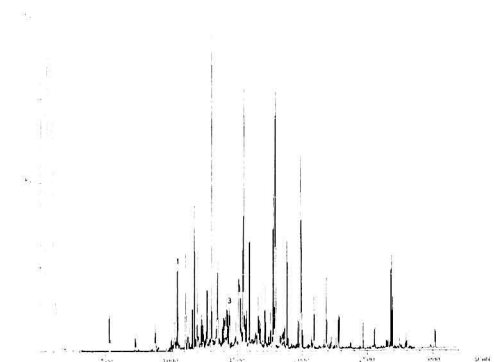
Figure 1. Reconstructed ion chromatogram (EI) of the PAK fraction of urban ambient air. The larger peaks are terpenoid and resin compounds from wood combustion (*19*). For peak assignment, see Table I.
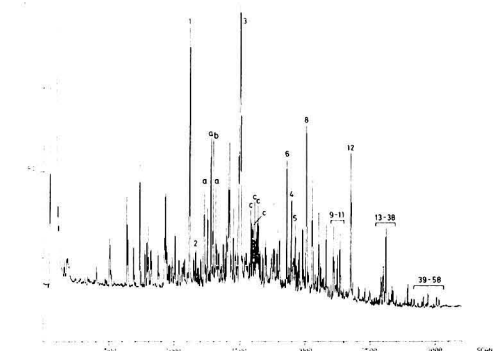
Figure 2. Reconstructed ion chromatogram (NICI) of the PAK fraction of urban ambient air. (a) Methylfluorenones, (b) 2-methylfluorenone, and (c) methylcyclopenta[*def*]phenanthrones. For other peak assignments, see Table I.

Figure 3. Mass chromatograms (extracted ion current profile, electron impact) of PAK in an extract of emissions from municipal incineration. The upper mass chromatogram is for mass 180, and the others are for masses 204, 230, 254, 278, 280, 302, 304, 328, and 330, respectively.

show the molecular ion as the most abundant ion. Characteristic of PAK is the $(M - 28)^+$ ion, loss of CO. This fragment ion is the second most important for the low molecular weight PAK. With increasing molecular weight the additional loss of hydrogen atoms with CO giving rise to the $(M - 29)^+$ and $(M - 30)^+$ ions is more apparent. After the loss of CO the resulting hydrocarbons lose C_2H_2 as also observed in the mass spectra of PAH (*20*). As for PAH (*20*) the doubly charged ions are also important. The M^{2+} ion shows low abundance; the most abundant ions of this type are the $(M - 28)^{2+}$, $(M - 29)^{2+}$, and $(M - 30)^{2+}$ ions, which always dominate the lower part of the mass spectra.

These doubly charged ions are the second most abundant ions for the high molecular weight PAK. For a PAK with molecular weight 328 the $(M - 28)^+$ ion is not detected, $(M - 30)^+$ shows 10% abundance, and the $(M - 30)^{2+}$ ion is 40%. As for PAH (*20*) there are very little differences in the mass spectra of various PAK isomers, and hence, standards must be used to make positive identifications by GC retention times.

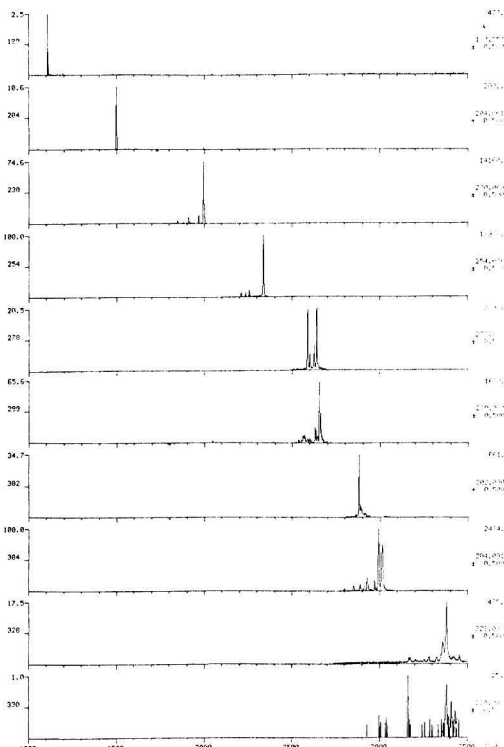**Isomeric Distribution of PAK.** As can be seen from Figure 3, for most of the PAK there exist several isomers. There are two different families of PAK, those containing a fluorenone and those containing a phenalenone substructure, the keto group being situated in a five-membered and a six-membered ring, respectively. In Figure 5 most of the possible isomers of PAK with molecular weight up to 304 are given. Examples of PAK with M_r 328 and 330

are also included. Table I lists their corresponding names after IUPAC nomenclature (*21*). Other possible isomers of benz[*de*]anthracenones than those given (compounds 7 and 8), i.e., 3H-benz[*de*]anthracen-3-one, 4H-benz[*de*]-anthracen-4-one, and 6H-benz[*de*]anthracen-6-one, are not included, because their corresponding parent PAH isomerize extremely rapidly to 7H-benz[*de*]anthracene (*22*), and hence the possible formation of these ketones should be minimal. This applies to a lesser extent also to 1H-benz[*de*]anthracene, which also easily isomerizes (*23*). This is taken into account with the high molecular PAK in the phenalenone family in Figure 5; only benzo analogues of 7H-benz[*de*]anthracen-7-one are given. There is a very good agreement between the theoretical number of isomers of PAK and their occurrence in environmental samples. Only one isomer each with M_r 204 and 302 is found, and two isomers with M_r 180, few isomers with M_r 230, 254, and 278, and many isomers with M_r 280, 304, 328, and 330 are found. Only a few PAK have been positively identified by comparison with standards, as indicated in Table I.

From the few standards available it can be concluded that within each group of isomers the PAK belonging to the phenalenone family have longer GC retention times on a nonpolar stationary phase than do the PAK from the fluorenone family. 1H-Phenalen-1-one, 7H-benz[*de*]-anthracen-7-one, and 6H-benzo[*cd*]pyren-6-one are eluted last of the PAK with M_r 180, 230, and 254, respectively. This also applies to the HPLC retention times for the two families of PAK on silica columns. The phenalenone family is more retained than the fluorenone family (*3*). From the mass chromatograms in Figure 3 it is apparent
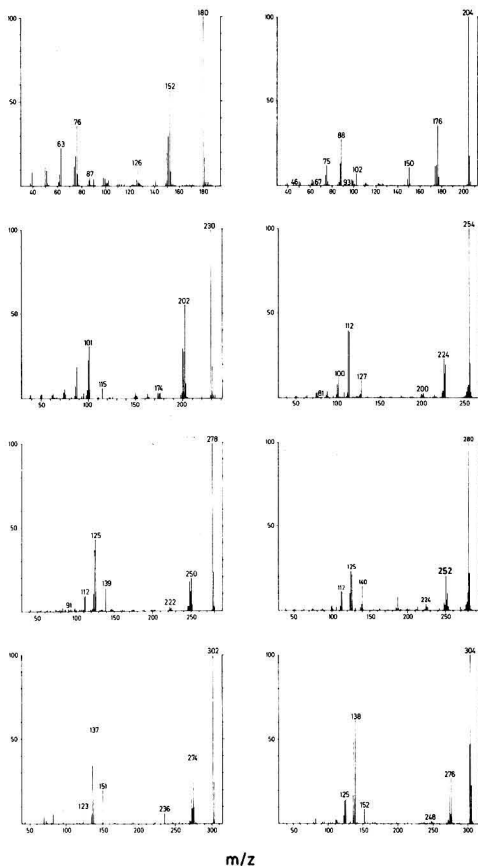
Figure 4. Representative mass spectra of polycyclic aromatic ketones (EI). The mass spectra of various isomers are nearly identical.

Figure 5. Molecular structures of polycyclic aromatic ketones. See Table I for names.

that PAK with M_r 230 or higher belonging to the phenalenone family are more abundant than those of the fluorenone family, and this is a general phenomenon for all the samples investigated.

So far only unsubstituted PAK have been discussed. There are a number of alkylated PAK possible, and several workers have reported on alkyl-PAK in diesel emissions (2–7) and biomass combustion effluents (10). The fractionation scheme for PAK described also includes the alkylated PAK; the four possible methylfluorenones and methylcyclopenta[def]phenanthrones are indicated in Figure 2.

Formation of PAK. PAK are formed by oxidation of PAH which contain a single-bonded carbon atom attached to the aromatic ring. The hydrogen atoms in the methylene group of fluorene show considerable reactivity, and fluorene and benzofluoroenes are known to undergo very rapid nonphotochemical oxidation to their respective ketones (24). 1H-Phenalene, 7H-benzo[de]anthracene, and 6H-benzo[cd]pyrene are known to be acidic and very reactive hydrocarbons (25), very easily oxidized.

Many PAH with the methylene group in a five-membered ring are found in environmental samples. Grimmer et al. (26) identified among others 11H-indeno[2,1,7-cde]pyrene, 4H-benzo[def]cyclopenta[mno]chrysene, and 11H-benzo[ghi]cyclopenta[pqr]perylene in automobile exhaust, representing high molecular weight reactive methylene hydrocarbons. PAH with the methylene group

in a six-membered ring have not been reported in environmental samples, indicating their rapid oxidation.

The occurrence of PAK in emission samples may be explained in two ways: (a) Methylene PAH formed in the combustion process are so reactive that they are oxidized immediately after leaving the combustion zone, and PAK are actually emitted. (b) Methylene PAH trapped in the sampling system are oxidized by the stack gas passing by. In ambient air PAK originates from PAK in emissions and also by further atmospheric oxidation of methylene PAH (24).

Environmental Consequences. The emission of PAK from combustion processes is not much less than the

Table I. Polycyclic Aromatic Ketones, Molecular Weights, and IUPAC Names, in Environmental Samples[a]

compd no.	M_r	compound	compd no.	M_r	compound
1	180	9H-fluoren-9-one[b]	36	280	8H-dibenzo[b,mn]phenanthren-8-one
2	180	1H-phenalen-1-one[c]	37	280	9H-dibenz[b,de]anthracen-9-one
3	204	4H-cyclopenta[def]phenanthren-4-one[b]	38	280	9H-dibenz[a,de]anthracen-9-one
4	230	7H-benzo[c]fluoren-7-one[b]	39	302	11H-benzo[ghi]cyclopenta[pqr]perylen-11-one[c]
5	230	11H-benzo[b]fluoren-11-one[b]	40	304	5H-benzo[b]cyclopenta[def]chrysen-5-one[e]
6	230	11H-benzo[a]fluoren-11-one[b]	41	304	4H-benzo[b]cyclopenta[mno]chrysen-4-one
7	230	1H-benz[de]anthracen-1-one[c]	42	304	4H-benzo[c]cyclopenta[mno]chrysen-4-one
8	230	7H-benz[de]anthracen-7-one[b]	43	304	8H-benzo[g]cyclopenta[mno]chrysen-8-one
9	254	11H-benz[bc]aceanthrylen-11-one[d]	44	304	13H-benzo[b]cyclopenta[def]triphenylen-13-one
10	254	4H-cyclopenta[def]triphenylen-4-one[d]	45	304	4H-cyclopenta[cde]benzo[m]triphenylen-4-one
11	254	4H-cyclopenta[def]chrysen-4-one[d]	46	304	4H-cyclopenta[cde]benzo[l]triphenylen-4-one
12	254	6H-benzo[cd]pyren-6-one[b]	47	304	13H-cyclopenta[rst]pentaphen-13-one
13	278	11H-indeno[2,1,7-cde]pyren-11-one[d]	48	304	13H-cyclopenta[pqr]picen-13-one
14	278	4H-benzo[def]cyclopenta[mno]chrysen-4-one[d]	49	304	6H-cyclopenta[ghi]picen-6-one
15	278	11H-cyclopenta[ghi]perylen-11-one[d]	50	304	13H-dibenz[bc,j]aceanthrylen-13-one
16	280	9H-indeno[2,1-c]phenanthren-9-one[e]	51	304	13H-dibenz[bc,l]aceanthrylen-13-one
17	280	8H-indeno[2,1-a]anthracen-8-one	52	304	13H-indeno[2,1,7-qra]naphthacen-13-one
18	280	7H-indeno[2,1-a]phenanthren-7-one	53	304	7H-indeno[1,2-a]pyren-7-one
19	280	13H-indeno[1,2-l]phenanthren-13-one	54	304	11H-indeno[2,1-a]pyren-11-one
20	280	7H-dibenzo[c,g]phenanthren-7-one	55	304	9H-indeno[1,2-e]pyren-9-one
21	280	8H-indeno[2,1-b]phenanthren-8-one	56	304	6H-dibenzo[a,fg]pyren-6-one
22	280	13H-indeno[1,2-b]anthracen-13-one	57	304	7H-dibenzo[cd,l]pyren-7-one
23	280	12H-indeno[1,2-b]phenanthren-12-one	58	304	6H-dibenzo[b,fg]pyren-6-one
24	280	13H-dibenzo[a,i]fluoren-13-one	59	328	13H-dibenzo[b,jkl]cyclopenta[def]triphenylen-13-one[e,f]
25	280	13H-dibenzo[a,g]fluoren-13-one	60	330	7H-naphtho[1,2-a]benzo[h]fluoren-7-one[e,f]
26	280	7H-dibenzo[b,g]fluoren-7-one			
27	280	12H-dibenzo[b,h]fluoren-12-one			
28	280	13H-dibenzo[a,h]fluoren-13-one			
29	280	13H-indeno[1,2-c]phenanthren-13-one			
30	280	13H-indeno[2,1-a]anthracen-13-one			
31	280	11H-indeno[2,1-a]phenanthren-11-one			
32	280	7H-dibenz[de,j]anthracen-7-one			
33	280	7H-dibenz[de,i]anthracen-7-one			
34	280	7H-dibenz[de,h]anthracen-7-one			
35	280	9H-dibenz[c,mn]phenanthren-9-one			

[a] A theoretical list based on the most probable structures. See text for further discussion. See Figure 5 for structures and Figures 2 and 3 for chromatograms. [b] Structural assignment confirmed by authentic standard compounds, corresponding retention data, and EI and NICI mass spectra. [c] Confident structural assignment by retention data, and EI and NICI mass spectra. [d] Tentative structural assignment by EI and NICI mass spectra. [e] Several compounds with this molecular weight have been tentatively identified. [f] Example of PAK with this molecular weight.

emission of PAH. The most abundant ketones in the emissions are 9H-fluoren-9-one (1), 4H-cyclopenta[def]phenanthren-4-one (3), 7H-benz[de]anthracen-7-one (8), and 6H-benzo[cd]pyren-6-one (12). We have determined the emissions of these compounds from wood combustion to be 6400, 3550, 500, and 600 μg/kg of dry wood, respectively (10). The emission of the PAK with molecular weights from 278 to 304 varied in the range from 50 to 500 μg/kg. The emissions of some abundant PAH like phenanthrene, pyrene, benz[a]anthracene, and benzo[a]pyrene were 5800, 2700, 480, and 530 μg/kg of dry wood, respectively. König et al. (17) have also demonstrated comparable amounts of 8 and 12 to benz[a]anthracene and benzo[a]pyrene in ambient air. It is therefore important to assess their environmental significance.

In the most used fractionation schemes nitro-PAH are eluted with PAK (3, 16). Although nitro-PAH are known to be very mutagenic, their content only explains a part of the mutagenicity (27). 9H-Fluoren-9-one and several methyl derivatives have been shown to be nonmutagenic in the Ames test (2); however, 1H-phenalen-1-one is toxic to microalgae (28), and 7H-benz[de]anthracen-7-one is very potent by using a photodynamic bioassay with *Paramecium caudatum* (29). Photodynamic potency affords an indirect index of caricinogenicity. There is little other information on the biological activity of other PAK. Analogous to PAH the high molecular PAK may be suspected to be mutagenic and carcinogenic. Work is under

way to synthesize some of these compounds for further studies of biological effect.

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Acute Toxicity of Butyl Benzyl Phthalate to the Saltwater Fish English Sole, *Parophrys vetulus*

Robert C. Randall, * **Robert J. Ozretich, and Bruce L. Boese**

U.S. Environmental Protection Agency, Corvallis Environmental Research Laboratory, Marine Division, Marine Science Center, Newport, Oregon 97365

■ Butyl benzyl phthalate (BBP) is a commerically important phthalate ester identified as a priority pollutant by the Environmental Protection Agency. The Clean Water Act of 1977 (PL 95-217) requires the Administrator of the EPA to publish criteria for water quality that accurately reflect current scientific knowledge of the extent and effects of pollutants present in water bodies. Section 304(a)(1) of the Act provides for the nonregulatory, scientific assessment of ecological effects. This work was conducted to provide data for Section 304 on the toxicity of BBP to saltwater fish. The 96-h LC_{50} values found for English sole, *Parophrys vetulus*, were 0.66 (static replenish exposure) and 0.55 mg/L (flow-through exposure) with lethal threshold concentrations of 0.45 and 0.30 mg/L, respectively. Sublethal effects were noted at all BBP exposures, the lowest of which was 0.1 ppm.

Introduction

The EPA has identified phthalate esters both as important industrial chemicals and as priority pollutants (*1*, *2*). Due to their high production volumes (*3*) and widespread use in the plastics industry, they are detected in water (*4–8*), soil and sediment (*5, 8–10*), fish (*5, 8, 10–13*), animal and human tissue (*14–17*), air (*18*), vegetation and minerals (*18, 19*), and remote areas (*20*). Environmentally, they have relatively slow photolysis, oxidation, and hydrolysis rates, but sorption to suspended solids and sediment, bioaccumulation, and biotransformation/biodegradation are important fate processes (*21*).

Butyl benzyl phthalate (BBP) is one of the important phthalate esters. Its 2.9 mg/L water solubility is relatively low, with a high octanol/water partition coefficient (log P = 4.8–5.8) (*21, 22*), suggesting that it would be lipophilic, tend to partition into aquatic life, and adsorb on particulates high in organic content. For this reason, the sorption mechanisms of BBP are anticipated to be relatively important.

Guidelines for deriving water quality criteria are published in the Federal Register (*23*). The minimum data base needed to produce a criterion for saltwater aquatic life consists of acute toxicity levels on at least eight different families, provided that at least two different fish familes are included. Acute toxicity data for BBP exist for one marine fish, *Cyprinodon variegatus*, one invertebrate, *Mysidopsis bahia*, and two alga species (*2*). Reported toxicity values range from 0.9 to 445 mg/L.

LC_{50} values for BBP have been reported far in excess of its solubility (2.9 mg/L), for example, 43 mg/L (*24*) for the bluegill, *Lepomis macrochirus*. Since most available toxicity data for BBP are based on unmeasured concentrations (nominal doses), it is not surprising that investigators have reported unrealistically high concentrations at which lethality occurred. For this reason, at least part of the exposures reported here are documented with measured concentrations. Both static (renewed) and flow-through toxicity tests of the phthalate ester butyl benzyl phthalate (BBP) were conducted with juvenile English sole (*Parophrys vetulus*).

test	dissolved oxygen, mg/L	pH	temp, °C	salinity, ‰	mean fish weight, g	mean fish length, cm	fish/ tank	fish/L, g
static	7.04 (0.98)	7.3 (0.16)	11.9 (0.5)	24.9 (0.2)	10.7 (2.8)	9.56 (0.68)	10	0.81 (0.22)
flow through	6.61 (0.85)	7.3 (0.14)	12.3 (0.2)	31.4 (0.5)	3.90 (1.8)	6.47 (0.99)	20	3.90 (1.8)

Table I. Summary of Experimental Data Given as Means with Standard Deviations (in Parentheses)

Materials and Methods

Young of the year English sole, *Parophrys vetulus*, were collected by beach seine from the Yaquina Bay Estuary at Newport, OR. They were transported within a few minutes, in an aerated container, to holding tanks with aerated, flowing seawater (25–32‰). After 1 week of quarantine observation, the fish were treated with 0.25% formalin solution for 1 h to remove *Gyrodactylus* parasites and placed into 270-L glass aquaria with aerated seawater flow rates of at least three turnovers per day. Pior to testing, the fish were maintained on an artificial diet, for a minimum of 3 months, composed of 25% squid, 22.5% spinach, 25% beef liver, 2% cod liver oil, 25% wheat germ, and 0.5% Nopstress vitamin mix available from Diamond Shamrock, Fresno, CA.

Except for the absence of bottom substrate, the toxicity tests were conducted in accordance with procedures outlined in American Society for Testing and Materials (25). Butyl benzyl phthalate (product code B-27750) was obtained from Pfaltz & Bauer, Inc., Stamford, CT.

Static (renewed) tests were conducted in 132-L, gel-coated, fiberglass tanks at 12 ± 1 °C with a controlled lighting cycle (16:8 L:D). Five dosage levels plus control were run simultaneously. Ten fish were added to each of the six test tanks for acclimation 1 day prior to the start of the test. Fish were not fed during the test. Eighty percent of the test solution in each test chamber was renewed daily. Water circulation was obtained in each tank by the use of a small submersible pump that was oriented to take water from the surface of each chamber and discharge it at the bottom. The BBP was dissolved in 95% EtOH and added directly to the tank, via the circulation pump intake, to obtain nominal concentrations of 1.5, 1.0, 0.6, 0.216, and 0.130 mg/L. Alcohol concentrations in the test solutions and solvent control never exceeded 0.5 mL/L.

Flow-through tests were conducted in glass, 20-L chambers with 10 volume exchanges per day. Gravity flow of the dilution water from a head tank was controlled by a screw pinchcock to obtain the desired 140 mL/min flow rates. A solution of BBP in 95% EtOH was pumped into each of six dilution water streams at a flow rate of 0.05 mL/min to obtain nominal exposure concentrations of 1.0, 0.60, 0.36, 0.216, 0.130, and 0.00 ppm. The BBP streams were pumped by a Technicon AutoAnalyzer pump equipped with solvent flexible tubing. The bioassay temperature was maintained at 12 ± 1 °C. Alcohol concentrations never exceeded 0.5 mL/L. At the start of the test, 20 fish were added to the already flowing and equilibrated chambers. Fish were not fed during the test.

The seawater used in all tests was obtained from Yaquina Bay, Newport, OR, UV sterilized, and sand filtered prior to use. This water supply has historically been nontoxic in oyster and mussel bioassay and culture (26–28).

Toxicant exposure levels were measured daily in all systems. Toxicant concentrations were determined by a Hewlett-Packard 5880A gas chromatograph equipped with a 15-M fused silica column and flame ionization detector.

The mean analytical precision of the BBP method was 98 ± 3% ($n = 10$) on standards in the concentration range 0.1–2.6 ppm. The mean recovery of BBP standard addi-tions to filtered seawater and seawater from bioassays was 98 ± 7.1% ($n = 32$) within a concentration range of 0.1–2.6 ppm.

Fish behavior was observed frequently during the daylight hours and dead fish were promptly removed. Estimations of LC_{50} values (concentrations causing 50% mortality in a specific time period) were calculated by a probit computer program by Finney (29). Wastewater was passed through carbon filters to remove toxicant prior to discharge, and the used carbon was trucked to an approved disposal site.

Results and Discussion

Preliminary exeriments showed that loss of BBP in static tests was significant; 12 h after dosing, toxicant concentrations were 73 ± 6% of the starting concentrations, and after 24 h toxicant concentrations were further reduced to 48 ± 11% of the starting value. Therefore, 80% of all exposure and control water was replenished each day during the tests, and the LC_{50} value is based on nominal concentrations.

We observed a very rapid sublethal effect of BBP on exposed English sole. Within 1 h, fish exposed to toxicant concentrations of 1.0 and 0.6 ppm had significantly slower movements with some loss of equilibrium. After 24 h, fish at all exposure levels were less active than the controls. As exposure time increased, more severe loss of equilibrium occurred. Mouths gaped, and mobility loss was complete near death.

The results of Timofievskaya's (30) work on the effect of phthalate esters, including BBP, on mice, rats, guinea pigs, and rabbits may provide insight to the toxicological mechanism responsible for effects noted on English sole. Clinical poisoning by phthalates was characterized by marked decreases in the summation of subliminal impulses with paralysis evident in poisoned test animals. The most serious phthalogical effect was with central and peripheral nervous system disruptions. Severe paralysis was noted in our English sole test populations that were BBP exposed and intoxicated.

A no-effect level of BBP to English sole was indeterminable since sublethal effects were seen at 0.1 ppm (lowest dose). No acute mortality was noted that static or flow-through BBP exposures of 0.3 ppm or less within 96 h; however, 80% of the test population died at this concentration (0.3 ppm) after a flow-through exposure of 192 h. The lethal threshold concentrations for English sole exposed to BBP were 0.45 (static) and 0.30 ppm (flow through) as determined by graphical extrapolation of the lethality data for 50% and 80% of the test animals (Figure 1).

The nominal static exposure 96-h LC_{50} value for BBP was 0.66 (95% CI 0.53–0.84) mg/L. The measured flow-through exposure LC_{50} value was 0.55 (95% CI 0.48–0.64) mg/L. Water quality parameters listed in Table I were stable and within ASTM guidelines (26).

Other investigators (4, 5, 24) have reported the toxicity of BBP in fish species to be orders of magnitude lower (3–445 ppm) than our data. This difference could be accounted for by species sensitivity. However, since the solubility of BBP is 2.9 mg/L and since most of the pre-
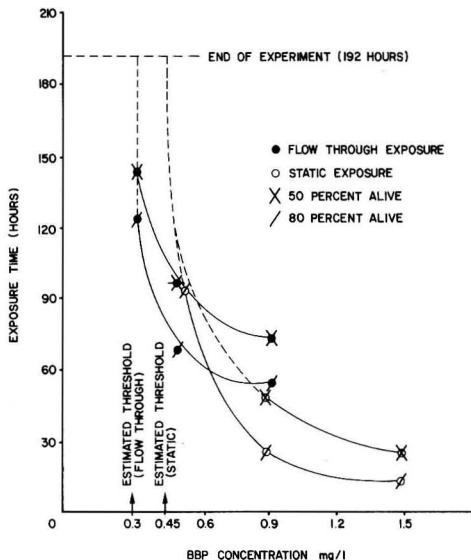
Figure 1. Lethal threshold concentration for English sole, *Parophyrs vetulus*, to (BBP).

vious work has been static, nominal exposure tests, we suspect that the toxicant concentrations actually experienced by those fish might have been orders of magnitude less than reported.

Our results indicate that BBP is acutely lethal to English sole at toxicant concentrations well within its water solubility.

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Interactions of Polycyclic Aromatic Hydrocarbons with Atmospheric Pollutants

Daniel Grosjean,* **Kochy Fung, and Jeffrey Harrison**

Environmental Research & Technology, Inc., Westlake Village, California 91361

■ The polycyclic aromatic hydrocarbons (PAH) benzo[a]pyrene, perylene, and 1-nitropyrene deposited on glass and Teflon filters (0.05, 0.25, and 4.1 μg cm^{-2}, respectively) as well as on glass and Teflon filters loaded with fly ash, diesel exhaust, and ambient particles were exposed in the dark to pure humid air, 100 ppb of ozone, 100 ppb of SO$_2$, 100 ppb of nitric acid free NO$_2$, and particle-free ambient air. All exposures were conducted for 3 h at sampling face velocities equivalent to those typical of high volume sampling. Under these conditions, there was no evidence for chemical transformations of the PAH irrespective of filter type and substrate. The environmental implications of these results are discussed in the context of existing literature data concerning interactions of PAH and their nitro derivatives and pollutant gases, with emphasis on PAH nitration.

Introduction

Chemical reactions and phototransformations of airborne benzo[a]pyrene (BaP) and other polycyclic aromatic hydrocarbons (PAH) have been investigated for nearly three decades. In their early studies demonstrating the presence of BaP and other PAH in the Los Angeles atmosphere and the carcinogenicity of atmospheric extracts in mice, Kotin et al. (1) also investigated the "effects of urban air pollution (smog) on the survival of 3,4-benzo-pyrene". They observed that, upon exposure to NO$_2$, only 40% of the initial amount of BaP exposed was recovered. Subsequently, Kotin and co-workers (2) extended their study to 10 PAH, including BaP, deposited on filters or engine exhaust soot and exposed to clean air and to synthetic smog prepared by the reaction of ozone with gasoline. Again, significant losses were observed for several PAH upon exposure to synthetic smog.

Reactions of PAH with gaseous pollutants in the absence of light have recently received renewed attention. For example, Gundel et al. (3) identified nitro derivatives formed by reaction of NO$_2$ with anthracene and perylene, as did Pitts et al. (4) upon exposure of BaP and perylene to NO$_2$ and nitric acid. Pitts and co-workers (4) also investigated interactions between BaP and ozone, peroxyacetyl nitrate (PAN), and polluted ambient air. In all cases, pure BaP deposited on glass fiber filters reacted readily with the pollutant gases. In the past few years, more than 50 other studies have been directed at some aspects of the issue of atmospheric transformations of PAH (5).

The above studies indicate that reactions of airborne particulate PAH with gaseous pollutants may be of importance in the atmosphere and/or during sampling. However, as pointed out by Kotin et al. (1) and subsequently by other investigators (6, 7), the behavior of PAH adsorbed on soot or other carrier particles may be significantly different from that observed in laboratory studies involving pure PAH deposited on filters and other substrates. In this study, a matrix of laboratory exposures has been conducted with three representative PAH, benzo[a]pyrene (BaP), perylene, and the nitro-PAH derivative 1-nitropyrene. BaP was included as a "reference compound" since it has often been considered as a surrogate for other PAH in a number of environmental chemistry and health studies. Perylene, an isomer of BaP, was selected to study possible differences in reactivity among isomeric PAH. Nitropyrene was selected as a representative of the nitro-PAH compounds, and has recently received much attention as a potent mutagen (8) and as a major contributor to the overall mutagenic activity of particulate diesel exhaust (9).

Known amounts of the three compounds were deposited on glass fiber and Teflon filters as well as on coal fly ash, diesel exhaust, and ambient particles and were exposed to pure air, to pure air containing ~100 ppb of the atmospheric pollutants ozone, nitrogen dioxide, and sulfur dioxide and to particle-free ambient air. All exposures were carried out for 3 h in the dark at sampling face velocities equivalent to those typical of high-volume sampling.

Experimental Section

Only a summary of the experimental protocol is given here. A more detailed account of experimental procedures can be found elsewhere (10).

Filter Exposure Protocol. Benzo[a]pyrene (Aldrich Chemical Co.; Purity ≥98%), perylene (Aldrich; >99%), and 1-nitropyrene (Pfaltz & Bauer) were employed without further purification. Fluorescence analysis of 1-nitropyrene revealed the presence of pyrene as a minor impurity (0.3%). Exposure studies were conducted with mixtures of the three PAH spiked on 25-mm diameter Teflon (Zefluor PTFE membrane, 1 μm pore size, Ghia Corp.) and glass fiber filters (Gelman Sciences Inc. type A/E) loaded with coal fly ash, diesel exhaust, or ambient particulate matter. Fly ash (postelectrostatic precipitator, collected at a coal-fired power plant during normal operation) was deposited on the filters by using a fluidized bed apparatus. The fly ash particles had a mass median diameter of 4–7 μm and a specific surface area of ~2 m^2/g. Diesel exhaust samples were obtained from a Mercedes 240D car running idle, with the raw exhaust collected in a 4-m^3 FEP Teflon container and deposited on the filters by sampling from the container for ~15 s at a flow rate of 10 L min^{-1}. Ambient particulate matter samples were collected in the Los Angeles area. Carbon analyses (11) were performed on filters selected at random prior to spiking. Total, organic, and elemental carbon concentrations were 42.3, 11.9, and 30.4 μg cm^{-2} for fly ash, 3.3, 1.3, and 2.0 μg cm^{-2} for fly ash, and 35.9, 28.2, and 7.7 μg cm^{-2} for ambient samples, respectively. Total carbon accounted for ~3%, ~10%, and ~60% of the mass for fly ash, ambient particles, and diesel exhaust particles, respectively.

Spiked amounts (200 μL of CH$_2$Cl$_2$ solution) were 0.19 ± 0.01, 0.97 ± 0.05, and 15.5 ± 0.08 μg/filter for benzo[a]pyrene, perylene, and 1-nitropyrene, respectively. In order to minimize PAH photodecomposition, filter spiking and all subsequent sample handling steps were carried out in a glove box located in a room equipped with inactinic light.

Exposures to pollutant gases were carried out by using 4-m^3 FEP Teflon containers covered with black plastic

Table I. Summary of Experimental Conditions

run no.	exposure type	concn of added pollutant, ppb[a]	T, °C	RH, %	matrix air pollutants concn, ppb				
					NO	NO₂	SO₂	O₃	PAN
1	pure air		20	50	<5	<3	0	0	0
2	pure air		22	50	<5	<3	0	0	0
3	NO₂[b]	100 (90–120)	22	57	10		2.5	~5	0
4	NO₂[b]	95 (90–103)	26.5	46	8		1	~5	0
5	ozone	100 (94–102)	19	55	5	5	0		0
6	ozone	100 (90–103)	18	54	2	2	0		0
7	SO₂	105 (100–130)	21	53	2	0		2	0
8	SO₂	103 (95–107)	22	48	2	3		2	0
9	ambient air		25	44	4	17[c]	0	20	<2
10	ambient air		31	54	4	18[c]	0	40	<2

[a] Average; range in parentheses. [b] With nitric acid impurity removed by using nylon filters. [c] May include some nitric acid; see text.

film, with eight filters mounted on open-face 25-mm diameter polycarbonate filter holders inside the container and exposed simultaneously to the gaseous pollutant(s) of interest at a flow rate of 10.4 ± 0.2 L min⁻¹. This flow rate was selected so as to carry out the exposures at the same face velocity as that of a high-volume sample collected at a typical flow rate of 40 cfm (1.1×10^3 L min⁻¹).

All sets of filters exposed simultaneously included one sample of each type, i.e., PAH on two filter media, Teflon and glass, and four substrates, blank filter, fly ash, diesel exhaust, and ambient particulate matter. All exposures were carried out in duplicate.

Purified, humid air [~50% relative humidity (RH)] was delivered by an Aadco Model 737-14 air purification apparatus. Ozone was produced by using a Welsbach Model T-408 ozone generator. Nitrogen dioxide and sulfur dioxide were from compressed gas cylinders (100 ppm in N₂). Nylon filters (Ghia Corp., M8PL) were inserted upstream of the Teflon container to remove any nitric acid (12) present as impurity in the NO₂ cylinder. The absence of nitric acid in the matrix during NO₂ exposure runs was also verified by ion chromatography analysis of samples collected on nylon filters. The filter-deposited PAH were also exposed to particle-free polluted ambient Camarillo, CA, air, with ambient particles removed by using a glass fiber filter.

Pollutant concentrations were measured throughout the exposure runs by using instruments calibrated and operated according to U.S. Environmental Protection Agency procedures. NO₂ was monitored with a Thermo Electron 14 B/E chemiluminescent analyzer, ozone with a Dasibi 1003 AH ultraviolet photometer, and SO₂ with a Monitor Labs ML 8850 pulsed fluorescent analyzer.

Particle-free, purified air contained only traces of nitric oxide, nitrogen dioxide, ozone, and sulfur dioxide. Soot-catalyzed oxidation of SO₂ to sulfuric acid may take place on filters in humid air in the dark (13). Thus, trace levels of sulfuric acid, which was not measured, were probably present in some of our SO₂ exposure experiments. The glass filter employed to remove particles from ambient air may remove some of the nitric acid (14).

Analytical Protocol. Exposed filters were extracted by sonication with 2 mL of methylene chloride for 3 h. Benzo[ghi]perylene (0.43 µg/sample) was added as an internal standard. A 400-µL aliquot of the CH₂Cl₂ extract was diluted with equal volume of acetonitrile, and 5-µL aliquots of the mixture were analyzed by high-pressure liquid chromatography (Altex Model 332 liquid chromatograph) by using a 150 × 4.6 mm C₁₈ ultrasphere ODS reversed-phase column and isocratic elution with a mixture of 75% by volume acetonitrile and 25% water saturated with methylene chloride, at a flow rate of 1.5 mL min⁻¹.

Benzo[a]pyrene and perylene were quantitated by fluorescence (Schoeffel Model FS 970 fluorescent detector; λ excitation = 280 nm, λ cutoff = 370 nm). Nitropyrene was quantitated on the basis of its absorption at 254 nm by using an Altex Model 155-30 ultraviolet detector. Calibration curves relating concentrations to the compound/internal standard peak area ratios were constructed for each PAH. Accuracy was verified by comparison with the National Bureau of Standards reference material no. 1647, which contains benzo[a]pyrene and other PAH but not perylene and 1-nitropyrene. Reproducibility on six sets of replicate analyses was 3.1 ± 2.7% for 1-nitropyrene, 6.3 ± 3.1% for perylene, and 7.8 ± 2.5% for BaP at nominal concentrations of 15.5, 0.97, and 0.19 µg/filter, respectively. Studies conducted with the three PAH spiked on Teflon and glass filters and on glass filters loaded with ambient particulate matter indicated recoveries for the entire analytical protocol of 93 ± 5% (1σ, n = 6) for BaP (concentration range 22–520 ng/filter), 116 ± 9% for perylene (130–955 ng/filter), and 101 ± 6% for 1-nitropyrene (3–78 µg/filter).

Results and Discussion

Ten sets of exposures were carried out in the dark with PAH exposed for 3 h to purified air (control experiments), to 100 ppb of nitric acid free NO₂, 100 ppb of ozone, 100 ppb of SO₂, and particle-free ambient air. Pollutant concentrations and other parameters for all exposures in the dark are listed in Table I. Perylene, benzo[a]pyrene, and 1-nitropyrene concentrations for each type of exposure are listed in Tables II–VI and are summarized in Table VII. Within the experimental precision, no losses of 1-nitropyrene, benzo[a]pyrene, or perylene were observed. In addition, no new peaks indicative of reaction products were recorded in either ultraviolet or fluorescence chromatograms. At the detection limits afforded by our analytical protocol, any reaction product equivalent to ~0.2% of the initial amount of 1-nitropyrene (assuming similar absorption at 254 nm) would have been detected in the ultraviolet chromatogram, while even lower fractional amounts would have been seen in the fluorescence chromatogram.

The results listed in Tables II–VII show *no evidence for physical or chemical removal of BaP, perylene, and 1-nitropyrene under our experimental conditions, irrespective of filter type, carrier particles, and pollutant gases.* Thus, our results are at odds with the commonly accepted concept of substantial chemical transformations, be they filter promoted or not, of PAH by interactions with atmospheric pollutant gases. Particularly, the nitro-PAH 1-nitropyrene, a potent, direct-acting mutagen, was found to be resistant to oxidation, further nitration, or other

filter type	substrate	benzo[a]pyrene		perylene		1-nitropyrene	
		run 1[a]	run 2	run 1	run 2	run 1	run 2
Teflon	blank	0.180	0.170	1.02	1.16	15.7	15.3
	ambient	0.140	0.187	1.00	1.23	13.8	17.1
	fly ash	0.156	0.184	1.17	1.44	15.4	15.0
	diesel	0.152	0.192	1.05	1.18	15.5	16.4
glass	blank	0.185	0.190	1.32	1.24	15.6	15.4
	ambient	0.205		1.10		17.2	
	fly ash	0.182	0.180	1.09	1.11	14.2	17.1
	diesel	0.158	0.172, 0.182[b]	0.95	1.36, 1.43[b]	15.5	17.1, 18.9[b]
spiked amount, μg/filter		0.19 ± 0.01[c]		0.97 ± 0.05		15.5 ± 0.8	

[a] See Table I for details. [b] Replicate analyses. [c] Relative standard deviation of 5% computed from gravimetric and volumetric uncertainties in preparing and spiking PAH solutions on filters.

filter type	substrate	benzo[a]pyrene		perylene		1-nitropyrene	
		run 7[a]	run 8	run 7	run 8	run 7	run 8
Teflon	blank	0.190	0.244, 0.275[b]	1.15	1.42, 1.68[b]	14.3	17.8, 16.6[b]
	ambient	0.205, 0.174[b]	0.244	1.32, 1.30[b]	1.42	17.1, 16.9[b]	18.1
	fly ash	0.208	0.208	1.37	1.18	17.1	16.3
	diesel	0.232	0.240, 0.214[b]	1.32	1.35, 1.21[b]	17.6	17.1, 17.2
glass	blank	0.227	0.222	1.16	1.05	16.9	16.5
	ambient	0.204	0.196	1.27	1.15	14.4	17.6
	fly ash	0.207	0.223	1.13	1.11	16.0	16.3
	diesel	0.221	0.195	1.19	1.21	16.5	13.9
spiked amount, μg/filter		0.19 ± 0.01		0.97 ± 0.05		15.5 ± 0.8	

[a] See Table I for details. [b] Replicate analyses.

filter type	substrate	benzo[a]pyrene		perylene		1-nitropyrene	
		run 5[a]	run 6	run 5	run 6	run 5	run 6
Teflon	blank	0.206	0.178	1.07	0.907	15.3	16.6
	ambient	0.206	0.197	1.17	1.20	16.7	15.5
	fly ash	0.203	0.212, 0.190[b]	1.10	1.26, 1.11[b]	17.3	17.1, 16.1[b]
	diesel	0.185	0.174	1.00	0.76	17.2	17.6
glass	blank	0.183	0.192	0.99	1.03	15.0	15.4
	ambient	0.194	0.250	1.24	1.36	16.3	16.6
	fly ash	0.185	0.178	1.06	0.99	16.9	16.3
	diesel	0.163	0.195	0.92	1.08	15.8	15.9
spiked amount, μg/filter		0.19 ± 0.01		0.97 ± 0.05		15.5 ± 0.8	

[a] See Table I for details. [b] Replicate analyses.

chemical reactions under the conditions of our study.

Because of the implications of these results with respect to atmospheric sampling and atmospheric transformations of PAH, we have compared in the following paragraphs our results with existing literature data for each type of pollutant studied. Since heterogeneous transformations of PAH are expected to be a complex function of PAH state (pure of adsorbed), PAH concentration, substrate, and pollutant gas concentration (6, 15, 16), the discussion presented below emphasizes differences in experimental conditions and their relevance to conditions prevailing when polluted atmospheres are sampled.

Exposure to Purified Air. In the dark, PAH exposed to pure air may be lost by physical processes (evaporation, "blowoff") as well as by chemical reactions such as oxidation and/or substrate-dependent decomposition reactions. Neither type of loss process appears to be significant

for the three PAH studied under our conditions irrespective of substrate (Table II). Korfmacher et al. (15) observed the decomposition, in the dark, of PAH adsorbed on coal fly ash. While substantial decomposition was observed for several PAH, only very slow decomposition was reported for BaP. Perylene and 1-nitropyrene were not investigated. In our study, there is not evidence for fly ash induced decomposition of these three PAH in the dark.

In agreement with our results, Butler and Crossley (17) found no evidence for oxidation or decomposition for 10 PAH (including BaP) adsorbed on burner soot and exposed to air for up to ~4 months. Amounts of PAH employed ranged from ~50 μg to ~5 mg. The amount of BaP exposed was ~90 μg, i.e., ~400 times higher than that employed in our study. Pitts et al. (4) reported small yields of BaP quinones when 6–10 mg of BaP deposited on a glass filter was exposed to purified air for 24 h. The amounts

Table V. PAH Exposure to 100 ppb of Nitric Acid Free Nitrogen Dioxide in the Dark

| | | concn after exposure, µg/filter | | | | | |
| | | benzo-[a]pyrene | | perylene | | 1-nitro-pyrene | |
filter type	substrate	run 3	run 4	run 3	run 4	run 3	run 4
Teflon	blank	0.208	0.196	1.28	1.00	15.1	16.1
	ambient	0.171	0.199	1.22	1.14	14.0	15.4
	fly ash	0.197	0.205	1.14	1.20	15.7	14.9
	diesel	0.222	0.193	1.30	1.08	16.8	15.7
glass	blank	0.188	0.210	1.13	1.18	14.3	15.9
	ambient	0.181	0.199	1.11	1.07	14.4	15.8
	fly ash	0.178	0.205	0.92	1.10	13.7	15.2
	diesel	0.180	0.194	0.98	1.12	14.4	17.6
spiked amount, µg/filter		0.19 ± 0.01		0.97 ± 0.05		15.5 ± 0.8	

[a] See Table I for details.

of BaP deposited per filter area unit and the volume of air employed were ~10^3 and 25 times higher, respectively, than those employed in this work.

Exposure to Sulfur Dioxide. In the bulk liquid phase, PAH react readily with sulfuric acid at room temperature to form polycyclic aromatic sulfonic acids (18). These sulfonic acids, along with other sulfur-containing products, were obtained by Jäger and Rakovic (19) upon exposure of BaP and pyrene adsorbed on fly ash and alumina to 10% SO_2 in air. At lower, but still high, SO_2 levels of ~100 ppm, the conflicting results of Tebbens et al. (20) and Hughes et al. (7) for soot and fly ash, respectively, may be indicative of substrate-specific heterogeneous effects. Tebbens et al. (20) observed significant loss of BaP adsorbed on propane burner soot and exposed to 50–80 ppm of SO_2 for 4 h in the dark. In contrast, Hughes et al. (7)

reported no reaction for BaP and pyrene adsorbed on coal fly ash (50–150 µg of PAH/g of ash), silica gel, and alumina and exposed to 100 ppm of SO_2 in the dark.

At a lower SO_2 concentration more relevant to atmospheric and plume conditions, PAH do not appear to react with SO_2. Butler and Crossley (17) report no reaction between 10 PAH adsorbed on burner soot (phenanthrene, coronene, fluorant΄ ιe, chrysene, BaP, pyrene, benzo[a]anthracene, benzo[e]pyrene, benzo[ghi]perylene, and anthanthrene, 0.1–8 mg/g of soot) and exposed to 5 ppm of SO_2 in the dark for up to 100 days. In our experiments conducted at even lower concentrations, no reaction was observed in the dark between 100 ppb of SO_2 and BaP, perylene, and 1-nitropyrene deposited on glass and Teflon filters loaded with fly ash, diesel exhaust particles, and ambient particulate matter (Table III).

Exposure to Ozone. Kotin et al. (1) first reported substantial reactions between BaP deposited on a filter and ozone, NO_2, and mixtures of O_3 and NO_2. Lane and Katz (21) and Katz et al. (22) studied the reactions of nine PAH including BaP deposited on cellulose TLC plates impregnated with solutions containing ~100 µg/mL of each PAH and exposed to 0.2–2.3 ppm of ozone. BaP half-lives ranged from 0.1 to 0.6 h and decreased with increasing ozone levels. Pitts et al. (4) exposed 6–10 mg of BaP deposited on glass filters to 11 ppm of ozone for 24 h and identified the BaP diones and other oxidation products. In our study conducted using much lower amounts of PAH, lower ozone concentrations typical of ambient levels (100 ppb), and a shorter exposure time, BaP, perylene, and 1-nitropyrene were found to be resistant to ozonation in the dark (Table IV).

Exposure to Nitrogen Dioxide. Following the early work of Kotin et al. (1), who reported 60% loss of BaP deposited on filters and exposed to NO_2, a number of studies have documented the loss of PAH and/or the

Table VI. Exposures to Particle-Free, Ambient Camarillo Air in the Dark

| | | concn after exposure, µg/filter | | | | | |
| | | benzo[a]pyrene | | perylene | | 1-nitropyrene | |
filter type	substrate	run 9[a]	run 10	run 9	run 10	run 9	run 10
Teflon	blank	0.192	0.180	1.00	1.29	14.6	14.2
	ambient	0.210	0.242	1.21	1.20	17.3	18.0
	fly ash	0.198	0.216	0.94	1.13	16.3	16.9
	diesel	0.230	0.222	1.21	1.15	17.6	16.8
glass	blank	0.175	0.185	0.84	0.94	13.5	14.4
	ambient	0.217, 0.196[b]	0.198	1.30, 1.17[b]	1.12	16.7, 16.4[b]	16.1
	fly ash	0.172	0.166	0.82	0.87	14.6	13.9
	diesel	0.170	0.187	0.71	0.96	15.0	15.7
spiked amounts, µg/filter		0.19 ± 0.01		0.97 ± 0.05		15.5 ± 0.8	

[a] See Table I for details. [b] Replicate analyses.

Table VII. Summary of PAH Exposures to Single Pollutants in the Dark

| | | PAH concentration, µg/filter[a] | | |
filter type	substrate	BaP	perylene	1-nitropyrene
Teflon	blank	0.196 ± 0.025 (12.7)	1.14 ± 0.18 (15.8)	15.4 ± 1.0 (6.5)
	ambient	0.198 ± 0.031 (15.6)	1.21 ± 0.11 (9.0)	16.3 ± 1.5 (9.2)
	fly ash	0.198 ± 0.017 (8.6)	1.18 ± 0.14 (11.7)	16.1 ± 0.9 (5.6)
	diesel	0.203 ± 0.027 (13.3)	1.13 ± 0.17 (15.0)	16.8 ± 0.7 (4.2)
glass	blank	0.196 ± 0.018 (9.2)	1.09 ± 0.14 (12.8)	15.3 ± 1.0 (6.5)
	ambient	0.204 ± 0.019 (9.3)	1.18 ± 0.10 (8.5)	16.1 ± 1.1 (6.8)
	fly ash	0.188 ± 0.018 (9.6)	1.02 ± 0.11 (10.8)	15.4 ± 1.3 (8.4)
	diesel	0.184 ± 0.019 (10.3)	1.05 ± 0.19 (18.1)	15.8 ± 1.3 (8.2)
spiked amount, µg/filter		0.19 ± 0.01	0.97 ± 0.05	15.5 ± 0.8

[a] Mean ± 1σ of 10 exposure runs listed in Tables II–VI; relative standard deviation (RSD, %) in parentheses.

Table VIII. Comparison of This Work with Recent Literature Data for PAH Exposures to NO_2 in the Dark

	this work	Tokiwa et al. (8)	Pitts et al. (4)
PAH studied	BaP, perylene, and 1-nitropyrene	pyrene	BaP and perylene
filter type	Teflon (Ghia Zefluor) and glass (Gelman A/E)	paper (Toyo no. 2)	glass (Gelman A/E)
amount of PAH on filter, μg	0.19 (BaP), 0.97 (perylene), 15.5 (nitropyrene)	10×10^3	$(6-10) \times 10^3$
filter sampling area, cm^2	3.8	23.7	406
amount of PAH per filter area unit ($\mu g/cm^2$)	0.046 (BaP)	422	14.8-24.6
relative to this work	(1.0)	9.2×10^3	322-535
NO_2 concentration, ppm	0.1	1, 10	0.25-1.3
exposure time, h	3	24	8-24
flow rate, L/min	10	2	28.3
NO_2 dose, ppm m^3	0.24	2.9-28.8	3.4-53
relative to this work	(1.0)	12-120	14-220
product of NO_2 dose and PAH amount per filter area unit, relative to this work	(1.0)	1.1×10^5-1.1×10^6	4.5×10^3-1.2×10^5
nitric acid impurity in matrix air			
ppb	0 (removed)	not measured	~3-15
% of NO_2	0		~1.1%
nitro PAH yield			
BaP	0		18% (8 h, NO_2 = 0.25 ppm) 40% (8 h, NO_2 = 1 ppm)
perylene	0		not reported
pyrene		0.02% (NO_2 = 1 ppm), 2.85% (NO_2 = 1 ppm + added nitric acid ~20 ppb)	
1-nitropyrene	0		

formation of nitro-PAH compounds upon exposure to NO_2 in the dark (3, 4, 6, 7, 17, 23, 24). In contrast, we find no measurable reaction in the dark between nitrogen dioxide and the three PAH, benzo[a]pyrene, perylene, and 1-nitropyrene, under our experimental conditions (Tables V).

The apparent discrepancy between our results and those of earlier studies may be rationalized in terms of PAH concentrations and physical state, NO_2 levels and exposure dose, presence of nitric acid in the matrix air, and substrate-specific effects. A comparison of our work with selected recent literature data concerning interactions of PAH with NO_2 is given in Table VIII according to PAH studied, substrate, exposure time, gaseous pollutant concentration, and other pertinent parameters. The importance of these parameters is discussed further below.

PAH Concentration. Owing to the complexity of heterogeneous reactions between substrate-adsorbed or filter-deposited PAH and pollutant gases, no simple relationship is expected between nitro-PAH yields and reactant concentrations (i.e., PAH and NO_2). However, for a given set of substrate and experimental conditions, the extent of nitration is expected to vary with PAH and/or NO_2 levels. For example, the study of Pitts et al. (4) involved ~10 mg of pure BaP deposited on a high-volume glass filter. This is equivalent to a BaP concentration of ~25 μg cm^{-2}, i.e., ~500 times higher than that of 46 ng cm^{-2} employed in our study. For comparison, a 24-h high-volume sample from an urban atmosphere containing a typical BaP concentration of 1×10^{-3} μg m^{-3} would yield a BaP loading of only ~5 ng cm^{-2}, i.e., ~10 times *less* than that we employed.

NO_2 Dose. Tokiwa et al. (8) prepared the nitro derivatives of chrysene, fluoranthene, fluorene, pyrene, and phenanthrene by exposure to 10 ppm of NO_2 for 24 h at

a flow rate of 2 L min^{-1}. Pitts et al. (4) exposed BaP to 1.3 ppm of NO_2 for 24 h at a flow rate of ~30 L min^{-1}. The PAH were thus exposed to NO_2 doses of ~29 and 56 ppm m^3, respectively (or ~55 and 106 mg of NO_2). Both investigators noted a substantial decrease in nitro-PAH yields when lower NO_2 concentrations were used, e.g., yields of only a few percent at NO_2 levels of 1 ppm in the study of Tokiwa and co-workers (8). For comparison, total exposure to NO_2 in our study was only 0.24 ppm m^3 (450 μg), i.e., ~100 times lower than in the studies discussed above.

Substrate-Specific Effects. Recent studies have shown that nitration of PAH is highly substrate dependent (7). Jäger and Hanus (6) studied BaP and pyrene adsorbed on silica gel, alumina, fly ash, and carbon (soot). Of these substrates, carbon was the least conductive to nitration, with 6-nitro-BaP and 1-nitropyrene yields of only 0.4%. Butler and Crossley (17) exposed burner soot to 100 ppm of oxides of nitrogen (NO plus NO_2) for up to 51 days and reported half-lives for 10 PAH ranging from ~4 to 30 days. The half-life of BaP was 7 days, and perylene and 1-nitropyrene were not studied. These studies indicate that even at high PAH and/or NO_2 levels, the reactivity of PAH towards nitration may be drastically reduced for PAH adsorbed on soot particles.

Presence of Nitric Acid. In the bulk liquid phase, nitro-PAH are prepared by reaction of the parent PAH with nitric acid (25, 26). Thus, direct nitration of particulate PAH by nitric acid may take place if $HONO_2$ is present as an impurity in the matrix air and thus interferes with studies of PAH exposure to NO_2. Indeed, Tokiwa et al. (23) obtained a 150-fold increase in 1-nitropyrene yield when adding ~20 ppb of nitric acid to pure air containing 10 ppm of NO_2. Nitric acid was a reported impurity (~1% or 10 ppb) in the NO_2 exposure study of Pitts et al. (4).

Table IX. Exposure of Benzo[a]pyrene to Nitrogen Dioxide in the Dark at Higher Reactant Concentrations[a]

filter	substrate	BaP, μg/filter
Teflon	blank	190
	ambient	197
	fly ash	210
	diesel	204
	unexposed ambient	199, 201
glass	blank	189
	ambient	192
	fly ash	234
	diesel	215
	unexposed ambient	186

[a] Exposure conditions: BaP = 200 ± 10 μg/filter, NO_2 = 260 ppb (NO = 17 ppb, O_3 = 0), exposure time = 8 h, flow rate = 10 L min^{-1}, T = 17 °C, RH = 54%, and nitric acid ⩽ 0.09 ppb.

Table X. Passive Exposure of Filter-Deposited BaP to Nitric Acid Vapor

			reaction products	
			yield, % of BaP[a]	
retention time, min	ultraviolet chromatogram	fluorescent chromatogram	Teflon blank	Teflon + diesel exhaust[b]
2.68	X	no fluorescence	7.3	10.3
2.79	no absorption	X		
3.27	X	no fluorescence	3.6	6.6
5.24	X	X	26.1	33.9
6.23	X	no fluorescence	37.7	36.6

[a] Assuming same absorption as BaP at 254 nm. [b] Differences in yields may be indicative of substrate-specific effects.

Thus, the apparent nitration of PAH by NO_2 reported in earlier work may in fact be due to low levels of nitric acid, which in our study was removed from the matrix air by using nylon filters.

In order to investigate further the relative importance of NO_2 and nitric acid in the nitration of filter-deposited PAH, two additional exposure experiments were carried out. The first experiment involved higher amounts of BaP (200 ± 10 instead of 0.19 ± 0.01 μg/filter), higher levels of NO_2 (260 instead of 100 ppb), and a longer exposure time (8 instead of 3 h). The exposure flow rate was 10 L min^{-1} as before. Nitric acid, measured in the matrix air with two nylon filters in series, was below our detection limit of 0.09 ppb. The results, listed in Table IX, show that no measurable loss of BaP was observed under these conditions. No new peaks indicative of reaction products were detected in either ultraviolet or fluorescence chromatograms. Thus, even when the BaP concentration was increased by a factor of ~1050 and the NO_2 dose (concentration × exposure time) by a factor of ~7, there was no evidence for nitration of BaP by NO_2 in nitric acid free air, irrespective of substrate.

In the second experiment, BaP deposited on a Teflon filter and on a Teflon filter loaded with diesel exhaust particles as described before was subjected to passive exposures to nitric acid vapor in pure air. The BaP concentration was 200 μg/filter as in the preceding run. Nitric acid vapor was allowed to reach its equilibrium concentration at 20 °C and 1 atm in a closed container.

After passive exposure for 20 h, the two filter samples were extracted and analyzed as described before. No detectable amount of BaP was left on either filter, and our detection limit of ~0.02 μg indicates a >99.99% conversion to reaction products. Four new peaks appeared in the ultraviolet chromatogram, only one of which was recorded on the fluorescence chromatogram. The four peaks most likely correspond to three nitro-BaP isomers, i.e., the 6-nitro, 3-nitro, and 1-nitro based on PAH ring site reactivity considerations (25), as well as possibly a dinitropyrene isomer (the 1,6- or 3,6-dinitro-BaP). As is indicated in Table X, yields estimated assuming that the products absorb like BaP at 254 nm were 75% for BaP on the Teflon filter and 87% for BaP on diesel exhaust particles. Since nitro-BaP isomers in fact absorb less than BaP at 254 nm (4), our estimated yields are conservative lower limits for the actual yields.

A second peak, not detected in the ultraviolet chromatogram, was present in the fluorescence chromatogram. Keeping in mind that nitric acid is an oxidant as well as a nitrating agent, we speculate that this peak may correspond to a BaP quinone, formed either directly from BaP

or by oxidation of one of the nitro-BaP isomers.

The two experiments described above strongly suggest that nitric acid, rather than NO_2, should be considered in studies of PAH nitration. Studies conducted with much lower nitric acid levels relevant to the atmospheric situation are in progress in our laboratory.

Exposure to Particle-Free Ambient Air Pollutant Mixtures in the Dark. Exposures to particle-free ambient Camarillo, CA, air yielded no evidence of PAH loss (Table VI). These exposures were carried out on days of reasonably good air quality, with NO_x ≤ 25 ppb, O_3 ≤ 40 ppb, and PAN ≤ 2 ppb. Only trace levels of nitric acid, which was not measured, are expected to coexist with these low levels of ambient pollutants. In addition, some of the ambient nitric acid, if any, may have been removed on the glass filter employed to remove particles from the ambient air prior to PAH exposure.

Few literature data are available for comparison with our results. Fox and Olive (27) exposed anthracene adsorbed onto airborne particulate matter to suburban Austin, TX, air and found only traces of anthraquinone, the major expected oxidation product, after 4 days of exposure in the dark. In apparent contrast, Peters and Seifert (28) noted substantial losses for ^{14}C-labeled BaP deposited on high-volume filters and exposed to ambient air in Berlin, Germany. BaP losses were correlated with ambient ozone levels, a behavior consistent with the involvement of oxidative processes or other chemical reactions. Differences in nature of the PAH studied, experimental conditions, and pollutant composition of the ambient air make it difficult to compare our results with those of the above studies, and additional work with PAH exposed to ambient atmospheres is needed.

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Organic Free Radicals Associated with Idiopathic Liver Lesions of English Sole (*Parophrys vetulus*) from Polluted Marine Environments

Donald C. Malins,* Mark S. Myers, and William T. Roubal

Northwest and Alaska Fisheries Center, Environmental Conservation Division, Seattle, Washington 98112

■ Organic free radicals in freeze-dried liver microsomes from English sole containing idiopathic liver lesions (e.g., hepatocellular carcinomas), as well as from animals without liver lesions, were measured by electron paramagnetic resonance (EPR) spectroscopy. The concentration of free radicals was significantly higher in microsomes from livers with lesions compared to microsomes from livers without lesions. The EPR spectra of free radicals from the livers of the lesion-containing fish had characteristics similar to the spectrum from the "aromatic fraction" of sediment extracts incubated with liver microsomes isolated from healthy fish. The free radicals appeared to be of the XP· type in which the X· moiety is derived from a polynuclear aromatic compound linked to a macromolecule, e.g., a protein. However, the exact relationship between the free radicals and the development of lesions is not presently understood.

Introduction

Recently it was shown that the prevalence of liver neoplasms in English sole was positively correlated with the concentration of aromatic hydrocarbons in bottom sediments (*1–3*). Likewise, certain other liver lesions were positively correlated with aromatic hydrocarbons present in the sediment (*2, 3*).

Among metabolites arising from the biotransformation of organic xenobiotics such as aromatic hydrocarbons are compounds derived from one-electron oxidations, i.e., compounds containing one or more unpaired electrons (free radicals). The involvement of free radicals in biological damage is well documented (*4–8*). Mason (*9*) discussed the formation of free radical intermediates in the metabolism of paraquat, 5-methylphenazinium methyl sulfate, chloropromazine drugs, and thiyl, azo, and nitro aromatic compounds. Trush et al. (*10*) also discussed the activation of pharmacologic agents (e.g., adriamycin, nitrofurantoin, estradiol, ellipticine, and benzo[a]pyrene) to free radical intermediates.

Several aromatic hydrocarbons and their substituted derivatives are converted enzymatically to free radicals which have received attention in studies of mutagenicity and/or carcinogenicity. For example, *N*-hydroxy-2-(acetylamino)fluorene is transformed enzymatically to a free radical–lipid adduct which is mutagenic (*8*). Benzo[a]pyrene is converted to 6-oxybenzo[a]pyrene, which will bind to DNA (*11*). Anthanthrene undergoes conversion to a semiquinone free radical while 10-azabenzo[a]pyrene is converted to 6-oxy-10-azabenzo[a]pyrene; both bind covalently to poly(G) (*12*).

Although the formation of organic free radicals in biological systems is well-known, little appears to have been reported on the direct identification of free radicals derived from xenobiotics in fish. Many potential sources of free radicals (e.g., polynuclear aromatic hydrocarbons and scores of halogenated compounds) are present in sediments of Puget Sound from which the English sole were captured (*1, 3*); total aromatic hydrocarbons range as high as 63 ppm in certain sediments (*3*). Because free radicals arising from xenobiotics may contribute to liver abnormalities in fish, we undertook a study of the free radical burden in the livers of English sole obtained from waterways adjacent to urban and nonurban areas of Puget Sound (*1, 3, 13*). We measured the total free radical burden in freeze-dried
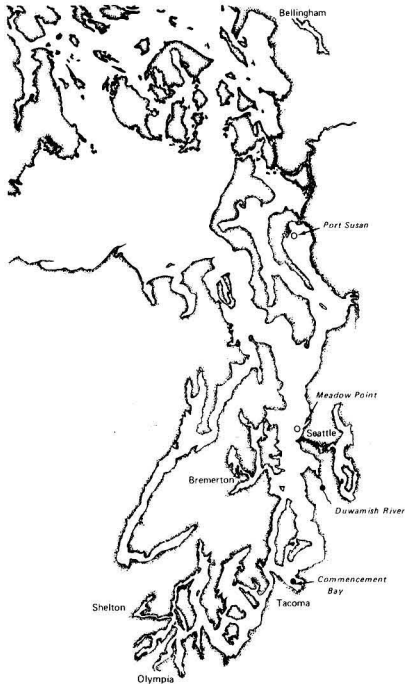
Figure 1. Sampling locations in Puget Sound, WA. Open circles (O) indicate nonindustrialized embayments. Closed circles (●) indicate urban embayments adjacent to industrial activity.
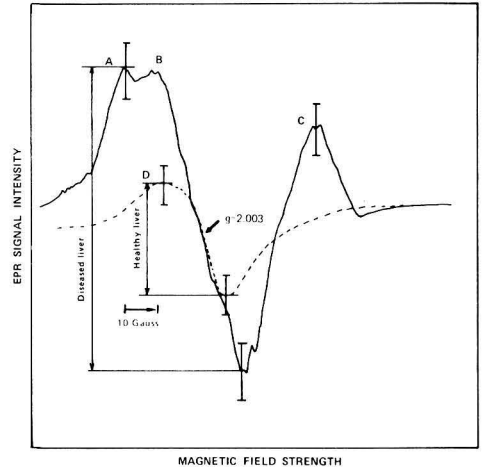

Figure 2. Typical EPR spectra for freeze-dried liver microsomes from English sole. Solid line (major signals A, B, and C) is the spectrum (mean signal intensity ± SD) for free radicals in 42 separate samples containing idiopathic lesions from the Duwamish River fish. The single resonance (D; dashed line) is the spectrum (mean signal intensity ± SD) for free radicals in seven separate liver samples devoid of lesions (Meadow Point fish). The spectra were recorded at room temperature.

liver microsomes using EPR spectroscopy in an attempt to correlate elevated levels of free radicals with liver lesions.

Materials and Methods

Adult English sole (*Parophrys vetulus*), 24–38 cm in length, were captured by otter trawl from the Duwamish River and Commencement Bay (urban embayments adjacent to industrial activity; Figure 1) and from the nonindustrialized embayments of Meadow Point and Port Susan (3) (Figure 1). The fish were weighed and the lengths recorded; then the fish were necropsied. Any gross liver lesions were described and recorded. A portion of each liver was preserved in Dietrich's fixative (1); the remainder of the liver was placed in screwcap vials, wrapped in aluminum foil, and submerged in liquid nitrogen for transfer to the laboratory. Isolation of liver microsomes, followed immediately by free radical determinations, were begun within $2^1/_2$–3 h from the time of catching the fish.

Liver microsomes were isolated as described by Schnell et al. (14), except that 1.15% KCl was used instead of sucrose. The microsomes were frozen at −60 °C, lyophilized in the dark, and then transferred under oxygen-free nitrogen to a glove box purged with nitrogen.

The dry preparations were placed in tared, nitrogen-purged 3-mm i.d. thin-walled quartz EPR sample tubes. The tubes were then capped with tightly fitting plastic plugs and immediately scanned over a 200-G range on a Varian E-4 EPR spectrometer with 4 mW of radio-frequency power, a gain setting of 8.0×10^4, a 1- or 2-s time constant, and a 16-min scan time. Optimum sensitivity was obtained by using 4 G of field modulation (a value which did not result in line broadening). In order to compare individual samples, the vertical distance from the top of peak A to the bottom of the peak (diseased tissue; Figure 2) and the vertical distance D (for healthy tissues;

Figure 2) were all normalized to 15 mg of sample. Peak A was used as a basis of comparison for diseased tissue because it corresponded more nearly to the location in the magnetic scan for free radicals reported to be specifically related to carcinogenesis (15). All spectra were recorded at 22 °C.

The lyophilization of microsomes was checked for possible artifact formation [discussed by Ts'o et al. (7) as possibly resulting from improper sample handling and reflected in the appearance of EPR signals in the $g = 2.005$ region, coincident with lipid peroxidation] as follows: microscomes free of detectable free radicals (from lesion-free fish) were extracted with chloroform–methanol in order to remove lipids, then suspended in 1.15% KCl solution (10 mL), and stirred under nitrogen with a polyunsaturated lipid mixture (salmon oil; 75:25 w/w) shown previously to undergo rapid autoxidation and formation of free radicals when exposed to air in a protein matrix (16). The mixture was lyophilized and checked by EPR for free radicals derived from products of lipid oxidation.

Liver tissue samples placed in Dietrich's fixative were processed by standard histologic procedures, embedded in paraffin and sectioned at 5 m, and routinely stained with Mayer's hematoxylin and eosin–phloxine stains (17). Additional special stains were Perl's Prussian Blue for hemosiderin (18), periodic acid–Schiff stain (17), and Masson's trichrome stain (17). All liver sections were examined by light microscopy. The person performing the histopathological diagnoses had no knowledge of the area of capture of the fish or results of EPR analyses. After all liver sections had been examined, the EPR data were made available, and statistical comparisons were made. These included comparing EPR values from fish from different sampling areas and from fish with and without significant idiopathic hepatic lesions. The lengths of the fish within these groups were also compared. The statistical analysis used in these comparisons was one-way analysis of variance (19) and the Student–Newman–Keuls multiple range test (20, 21).

Samples (~1 kg) of bottom sediments from the Duwamish River and Meadow Point were extracted by using

the procedure described by Malins et al. (1). For the analysis of organic compounds, extracts were fractionated by using both silica gel and Sephadex LH-20 column chromatography according to procedures of Brown et al. (22), Ramos and Prohaska (23), and Bruya and MacLeod (24). The aromatic fraction (Duwamish River sediment) contained about 85% aromatic hydrocarbons, 5% halogenated polynuclear aromatic hydrocarbons, 4% nitrogen-containing heterocyclic compounds, and 6% PCBs as determined by capillary gas–liquid chromatography using flame ionization and electron capture detectors (22–24). About 61% of the hydrocarbon fraction consisted of chrysene, benzo[a]pyrene, benzofluoranthenes, and benzanthracenes. The amount of polynuclear aromatic hydrocarbons in Meadow Point sediment was about $1/150$ of that in the Duwamish River samples.

By use of the method of Lesko et al. (6), the fractions were incubated for 12 min, in separate tests, with liver homogenates prepared from lesion-free English sole from Meadow Point. Reactions were stopped by the addition of 0.5 mL of 10% sodium dodecyl sulfate, and the mixtures were extracted under subdued light employing homogenization with 200 mL of cold, deoxygenated benzene. The resulting benzene solutions were passed through filter paper, reduced in the dark almost to dryness in a rotary evaporator at 30 °C, transferred to EPR sample tubes, and frozen at −15 °C. The frozen solutions were degassed by quickly thawing under vacuum. The freeze–thaw sequence was repeated 3 times. The degassed solutions were then analyzed by EPR spectroscopy.

Results

Microscopic examination of liver tissues from individual fish revealed a broad spectrum of idiopathic lesions (Table I). These lesions were within the general categories of degenerative, regenerative, "preneoplastic", neoplastic, and nonneoplastic/proliferative diseases and intrahepatocellular storage disorders. The degenerative lesions were megalocytic hepatosis (MH) and hepatocellular necrosis/degeneration (HN/D); the single regenerative lesion was hepatocellular regeneration (HR); the suspected preneoplastic lesion was nodular eosinophilic hepatocellular hypertrophy (NEH); neoplastic conditions were minimum deviation basophilic nodule (MDN), liver cell adenoma (LCA), hepatocellular carcinoma (HC), cholangiocellular carcinoma (CC), and mixed hepatocellular/cholangiocellular carcinoma (MC); the nonneoplastic/proliferative disorder was cholangiofibrosis (CF); intrahepatocellular storage disorders included hepatocellular fatty change (FC) and hemosiderosis (HS). These lesions in English sole have been described in detail by McCain et al. (13).

Most liver samples with idiopathic lesions contained multiple lesion types. The majority of sole with such lesions (51 of 54 fish) were from the highly polluted Duwamish River and Commencement Bay sites. The only lesions detected in Port Susan sole were FC and HS (intrahepatocellular storage disorders) and a single case of MH. Idiopathic liver lesions were not detected in fish from Meadow Point; however, all fish examined from Commencement Bay had such lesions.

Table I also gives the EPR signal intensity data for English sole. Independent data sets of EPR signal intensity values (sets 1–6) were formed based on sampling sites and the presence or absence of histologically detected idiopathic liver lesions. The sets of English sole for which these data were calculated were (1) Duwamish River sole with idiopathic liver lesions, (2) Duwamish River sole without idiopathic liver lesions (normal livers), (3) Commencement Bay sole with idiopathic liver lesions (all sole

Table I. Histopathological Diagnoses and Electron Paramagnetic Resonance (EPR) Signal Intensities for Liver from English Sole from Puget Sound, Washington

set	mean EPR signal intensity X̄	±SD	N	range	95% CI for mean	idiopathic liver lesions,[a] frequency (prevalence, %) MH	HN/D	FC	HS	HR	NEH	MDN	LCA	HC	CC	CF	MC	NSL	mean length, mm, ±SD
(1) Duwamish River English sole (idiopathic liver lesions)	5.89	3.43	42	1.7–17.1	±1.07	11 (26)	17 (40)	17 (40)	11 (26)	13 (31)	16 (38)	7 (17)	18 (43)	13 (31)	9 (21)	3 (7)	1 (2)	0 (0)	312 ± 40
(2) Duwamish River English sole (normal livers)	1.85	1.16	15	0.0–4.0	±0.64	–	–	–	–	–	–	–	–	–	–	–	–	15 (100)	292 ± 29
(3) Commencement Bay English sole (idiopathic liver lesions)	5.62	1.83	9	3.3–7.8	±1.41	3 (33)	7 (78)	5 (56)	2 (22)	4 (44)	4 (44)	1 (11)	4 (44)	3 (33)	0 (0)	1 (11)	0 (0)	0 (0)	301 ± 15
(4) Meadow Point English sole (all normal livers)	2.11	1.07	7	0.0–3.0	±0.96	1 (33)	0 (0)	1 (33)	3 (100)	4 (44)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (11)	0 (0)	7 (100)	301 ± 35
(5) Port Susan English sole (idiopathic liver lesions)	5.07	2.45	3	3.6–7.9	±6.10	1 (33)	0 (0)	1 (33)	3 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	321 ± 13
(6) Port Susan English sole (normal livers)	1.87	1.10	18	0.0–4.1	±0.55	–	–	–	–	–	–	–	–	–	–	–	–	18 (100)	308 ± 40
normal English sole with normal livers from all areas sampled	1.91	1.09	40	0.0–4.1	±0.35	–	–	–	–	–	–	–	–	–	–	–	–	40 (100)	301 ± 36
English sole with idiopathic liver lesions from all areas sampled	5.80	3.14	54	1.7–17.1	±0.86	15 (28)	24 (44)	23 (43)	16 (30)	17 (31)	20 (37)	8 (15)	22 (41)	16 (30)	9 (17)	4 (7)	1 (2)	0 (0)	311 ± 35

[a] Explanation of idiopathic liver lesions and symbols: MH, megalocytic hepatosis; HN/D, hepatocellular necrosis, degeneration (including hyaline degeneration); FC, fatty change/fatty degeneration; HS, hemosiderosis; HR, hepatocellular regeneration; NEH, nodular eosinophilic hypertrophy; MDN, minimum deviation basophilic nodule (early hepatocellular neoplasm); LCA, liver cell adenoma (benign neoplasm); HC, hepatocellular carcinoma (malignant neoplasm); CC, cholangiocellular carcinoma (malignant intrahepatic biliary neoplasm); CF, cholangiofibrosis/adenofibrosis (nonneoplastic); MC, mixed carcinoma (malignant neoplasm containing biliary and hepatocellular elements); NSL, no significant liver lesions; (–), not applicable.

Table II. Analysis of Variance of EPR Signal Intensities for English Sole Livers

source of variation	sum of squares	deg of freedom	mean square	F value	level of significance (p)
all samples	16.6706	5	3.3341	16.4	<0.001
normal sole vs. sole with lesions	16.5956	1	16.5956	81.9	<0.001
Duwamish River sole with lesions vs. Commencement Bay sole with lesions	0.0064	1	0.0064	<1	NS[a]
Duwamish River sole with lesions and Commencement Bay sole with lesions vs. Port Susan sole with lesions	0.0171	1	0.0171	<1	NS
Meadow Point normal sole vs. Port Susan normal sole	0.0421	1	0.0421	<1	NS
Meadow Point normal sole and Port Susan normal sole vs. Duwamish River normal sole	0.0094	1	0.0094	<1	NS
error	17.8288	88	0.2026		

[a] NS, not significant.

Table III. Relationship between Idiopathic Liver Lesion Type and Magnitude of EPR Signal

	MH	HN/D	FC	HS	HR	NEH	MDN	LCA	HC	CC	CF
				(A) Specific Lesion Types							
mean EPR signal intensity in fish with specific condition ±SD	5.10 ± 1.82	5.43 ± 2.00	5.13 ± 2.48	7.52 ± 3.68	5.07 ± 2.56	6.03 ± 3.29	3.70 ± 1.62	6.46 ± 3.19	6.82 ± 3.80	6.20 ± 4.13	8.80 ± 1.90
no. of cases	15	24	23	16	17	20	8	22	16	9	4

analysis of variance

	sum of squares	deg of freedom	mean square	F value
treatment	176.01	10	17.60	2.07 ($p < 0.1$)
error	1385.23	163	8.50	
total	1561.24	173		

(B) Broad Lesion Categories

	degenerative/ necrotic lesions	regenerative lesions	preneoplastic lesions	neoplastic lesions	proliferative lesions	storage disorders
mean EPR signal intensity ±SD	5.48 ± 2.26	5.07 ± 2.56	6.03 ± 3.29	6.09 ± 2.98	8.80 ± 1.90	5.91 ± 3.30
no. of cases	29	17	20	36	4	32

analysis of variance

	sum of squares	deg of freedom	mean square	F value
treatment	52.01	5	10.4	1.23 (NS)
error	1114.01	132	8.44	
total	1166.02	137		

had at least one of these lesions), (4) all Meadow Point sole (none contained liver lesions), (5) Port Susan sole with idiopathic liver lesions, and (6) Port Susan sole with normal livers. Included in Table I, as a summary, are the EPR signal intensity data for sole with normal livers from all sampling sites and sole with idiopathic liver lesions from all sampling sites.

The six independent samples (1–6, Table I) were compared by using one-way analysis of variance. Because the statistical deviation appeared to increase with the mean EPR signal intensity for a particular sample (coefficients of variation for all normal sole and sole with liver lesions were similar—0.57 and 0.54, respectively), the data were first transformed to the log scale (19). The resulting analysis of variance data, shown in Table II, demonstrates significant differences among all samples ($F = 16.4$; $F_{0.01}(5, 80) = 4.99$; $p < 0.001$).

Comparisons of interest were made by partitioning the sum of squares into five independent tests (corresponding to the 5 degrees of freedom for all samples) (25). The variance between normal sole and sole with idiopathic liver lesions accounted for the bulk of the variation among samples ($F = 81.9$; $p < 0.001$). The remaining variation between areas within the normal groups and within the groups with liver lesions was very small in comparison and was not statistically significant.

An effort was made to determine if EPR signal intensity was related to the presence of individual specific lesion types (MH, HN/D, NEH, HS, etc.) or to the broader lesion categories (degenerative, regenerative, preneoplastic, neoplastic, proliferative, and intrahepatocellular storage disorders), that is, to determine if lesion types could be distinguished from one another on the basis of EPR signal intensity. One-way analysis of variance was performed on the EPR data grouped by specific lesion types (Table IIIA) and grouped into the broader lesion categories (Table IIIB).

Analysis of variance of the EPR data grouped by specific lesion types failed to detect significant variations between the groups tested (Table IIIA). In addition, analysis of variance of the EPR data grouped by general lesion categories failed to detect significant variations between the groups tested (Table IIIB).

Comparison of total length measurements (an estimation of age) of sole from the six independent samples by one-way analysis of variance failed to show any significant differences among the groups. Mean lengths and standard deviations for the six independent samples are shown in Table I.

Lyophilization of microsomes did not appear to give rise to artifacts [appearance of free radicals due to lipid oxidation (7)] as evidenced by the lack of detectable free
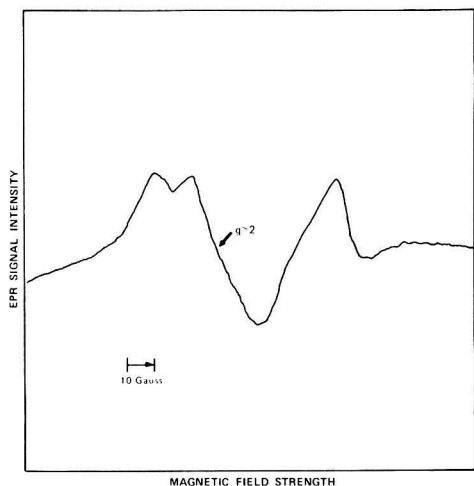
Figure 3. EPR spectrum of free radicals in the solid state at room temperature. The spectrum was obtained by adding a benzene solution of xenobiotic-derived free radicals (formed when the aromatic hydrocarbon fraction of bottom sediment from the Duwamish River was incubated with lesion-free liver homogenate) to freeze-dried liver microsomes from healthy fish. The benzene was then evaporated from the matrix in the dark under a stream of nitrogen.

radicals in microsomal reaction mixtures containing added polyunsaturated lipids shown previously to undergo rapid autoxidation in air (16).

Typical EPR spectra for microsomes from lesion-containing and lesion-free livers are shown in Figure 2. Spectra for lesion-free livers consisted of a single resonance (D) with a g value of 2.003; freeze-dried tissue from a number of sources, e.g., seeds, leaves, and various animal tissues including healthy liver, often shows such a signal ($g = 2.003$–2.005), a resonance which has been attributed to semiquinone type free radicals, the source of which is not known with certainty (26, 27). Microsomes from livers with lesions showed three overlapping EPR signals (peaks A, B, and C, Figure 2). Because of overlap, it was not possible to accurately measure line widths nor was it possible to measure the g value of peak A. Peak B was about 22 G wide, whereas peak A appeared to be about 34 G wide. The line width of peak C was not measured because of uncertainty in assigning boundaries to the absorption comprising this portion of the spectrum.

Free radicals were formed when liver homogenates from lesion-free Meadow Point fish were incubated with the aromatic hydrocarbon fraction isolated from bottom sediment of the Duwamish River. However, free radicals were not detected when the alkane, PCB, and polar fractions from Duwamish River sediment were incubated with the liver preparations; none of the organic fractions from Meadow Point sediment gave rise to detectable levels of free radicals.

When a benzene solution of the free radicals formed by incubating the aromatic fraction with liver homogenates was quickly mixed under nitrogen in the dark with freeze-dried liver microsomes prepared from lesion-free Meadow Point English sole and the benzene was quickly removed under a stream of nitrogen, the spectrum shown in Figure 3 was obtained. When the experiment was performed in this manner, the time between radical formation and the EPR analysis was short (compared to the time required for lyophilization of the incubation mixture), resulting in optimum sensitivity. Line widths for the two

major peaks comprising the left-hand portion of the spectrum were approximately 22 and 35 G. The g value measurements were not made. Free radicals were not detected in the nonincubated aromatic fraction, nor was there any difference in the EPR spectrum of the lyophilized microscomes when the microsomes were mixed with the nonincubated aromatic fraction.

Discussion

Microsomes from English sole livers with idiopathic lesions contained a statistically greater concentration of organic free radicals than did the microsomes from livers without detected lesions. Moreover, the spectrum for diseased livers (three major EPR signals; Figure 2) indicates that the free radicals in lesion-containing tissues differ from those in lesion-free tissues (a single EPR absorption; Figure 2).

Increased levels of certain free radicals appear to be associated with chemical carcinogenesis. For example, Kotrikadze et al. (28) reported a gradual increase over a 30-day period in the free radical content of the hepatic microsomal fraction from rats injected with the carcinogen benzo[a]pyrene; however, no information was given on the identity of the free radicals. Gutierrez et al. (29) reported that free radical accumulations increased in livers of rats with tumors induced by a single dose of 7,12-dimethylbenzanthracene.

In other studies, Vithayathil et al. (15) reported that developing liver tumors in rats fed 2-(aminoacetyl)-fluoranthene gave an EPR spectrum with signals at $g = 2.005$ and $g = 2.035$. Vithayanthil et al. viewed the EPR signal with $g = 2.035$ to be specifically related to carcinogenesis and claimed that this signal was an indication that the tissues would later become tumorous. EPR spectra for English sole liver microsomes with hepatic lesions in the present study are similar to those reported by Vithayathil et al. to the extent that the g value of the second major EPR signal (peak A; Figure 2) is greater than 2.003 and is located to the left of the central resonance as was the EPR signal with $g = 2.035$ [Vithayathil et al. (15)]. Line widths for EPR signals in the two studies were also approximately the same.

Of the four fractions (alkane, aromatic, PCB, and polar) isolated from Duwamish River bottom sediment, only the aromatic fraction gave rise to detectable levels of organic free radicals when incubated with healthy English sole liver homogenates. Many carcinogenic polynuclear aromatic compounds have low ionization potentials and form free radicals under conditions which are mild, in comparison, to those required for generation of free radicals from other classes of compounds such as polychlorinated biphenyls (PCBs) or compounds comprising one or two benzenoid rings (e.g., benzene and naphthalene). A high percentage of the aromatic fraction is composed of polynuclear aromatic compounds, several of which yield free radicals with lifetimes which allow EPR determinations to be made at room temperature (6–8, 11, 12). Thus, polynuclear aromatic compounds are a likely source of the free radicals.

Three types of organic free radicals may contribute to the EPR spectrum obtained with microsomes from English sole with lesions (Figure 2). These are X·, XP·, and P·, where X· is a xenobiotic free radical (e.g., obtained from a polynuclear aromatic compound), XP· is X· covalently linked to a protein or other macromolecule, and P· is a free radical of a macromolecule (e.g., a macromolecule containing an oxygen, nitrogen, carbon, or sulfur free radical) which may have been produced either by the reaction of X· with P or through tissue breakdown.
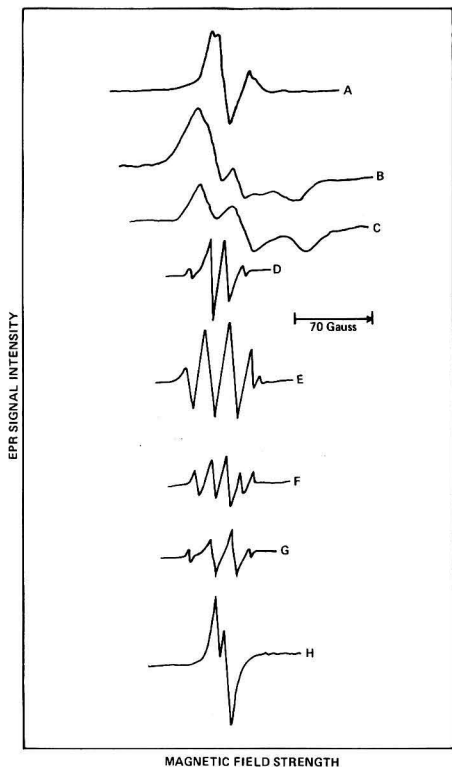
Figure 4. EPR spectrum of freeze-dried liver microsomes from English sole with idiopathic liver lesions together with EPR spectra for irradiated dry biological materials. Spectra for irradiated materials (B–H), reported in the literature, were redrawn to the same scale used for spectrum A (the EPR spectrum for liver microsomes from English sole with idiopathic liver lesions) in order to facilitate the comparisons. (B) Irradiated feathers, adapted from Gordy et al. (30). (C) Irradiated hair, adapted from Gordy et al. (30). (D) Irradiated collagen, adapted from Gordy and Shields (31). (E) Irradiated alanine, adapted from Gordy et al. (30). (F) Irradiated keratin, adapted from Gordy and Miyagawa (32). (G) Irradiated zein, adapted from Gordy and Miyagawa (32). (H) Irradiated fish scale, adapted from Gordy et al. (30).

To determine whether P· type free radicals contributed significantly to the spectrum for diseased liver (Figure 2), we compared a number of EPR spectra (30–32) for irradiated dry proteins, powdered amino acids, and peptides (examples of P· type free radicals) with spectra for the liver microsomes from English sole with liver lesions; representative spectra are shown in Figure 4. In some cases, the spread (in gauss) for irradiated materials was much greater than that for the spectra of microsomes from diseased livers (~140 G vs. ~70 G; Figure 4B,C). In other cases, in which the spread was similar to that for liver microsomes from lesion-containing fish (Figure 4D–H), the irradiated materials exhibited multiple lines which were well resolved, with line widths of 4–10 G. These line widths were less than half of those recorded for spectra of liver microsomes from lesion-containing English sole. Therefore, based solely on comparisons of spectral characteristics, P· type free radicals do not appear to contribute in a major way to the EPR spectra obtained with microsomes from lesion-containing English sole liver.

The presence of X· was not indicated because treatment of the diseased liver microsomes with organic solvents did not result in the liberation of detectable amounts of soluble free radicals. Moreover, the line widths for microsomes

from diseased livers (>20 G; Figure 2) appear to be considerably greater than the line widths for X· type free radicals in the solid state. For example, a solid matrix of 6-oxybenzo[a]pyrene on an inert support exhibits a line width of about 5 G (33). However, when the aromatic fraction was incubated with liver homogenates from the healthy fish, appreciable amounts of soluble free radical X· were produced as evidenced by its extraction in organic solvents. This can be explained, in part, if the conversion of X· to XP· in this system is a relatively slow process. Also, in this system, the ratio of X· to protein was substantially greater than in diseased tissue.

When 6-oxybenzo[a]pyrene was added to cytochrome c, salmon sperm DNA, bovine serum albumin, and egg albumin, in separate tests (33), the spectra for these preparations were similar to those for the left-hand portion of the spectrum (peaks A and B, Figure 2) for lesion-containing livers. Spectra with line shapes approximating this left-hand portion have been reported for dry complexes prepared by reacting free radicals derived from benz[c]-acridines with bovine serum albumin (34).

On the basis of the evidence discussed, we favor XP· (where X = a polynuclear aromatic structure) as a contributor to the EPR spectrum for liver microsomes from English sole with liver lesions. However, the possible presence of free radicals derived from xenobiotics other than polynuclear aromatic molecules cannot be ruled out. For example, the spectrum for liver microsomes (Figure 2) of English sole with liver lesions may indicate more than one radical species (as evidenced by the three-line pattern) or g anisotropies for a single dominant species (or series of similar species). Stier et al. (35) reported that nitroxides were formed when carcinogenic aromatic amines were incubated with rabbit liver microsomes; in solution, the nitroxides typically showed three, symmetrical hyperfine lines by EPR. However, when covalently bound to endogenous constituents of microsomal membranes, the nitroxides exhibited an EPR pattern of three broadened and unsymmetrical lines. Clearly, more work on free radical formation and characterization in English sole liver remains to be done before an in-depth understanding is obtained about the role played by free radicals in the observed liver lesions.

The concern that lyophilization of microsomes might give rise to artifacts was allayed when it was determined that free radicals could not be detected in a matrix of microsomes containing added oxidizable lipids and then lyophilized. In this regard, Nakayama et al. (36), studying naphthylamine metabolism, lyophilized microsomes with no apparent formation of artifacts.

The question arose why an EPR spectrum (Figure 3), which was similar to that of the diseased tissue (Figure 2), was obtained by mixing free radicals generated from the aromatic fraction of Duwamish River bottom sediment with healthy liver microsomes, particularly when lesion-free fish from the Duwamish River did not exhibit a similar spectrum. One reason may be that defense mechanisms, by which free radicals are scavenged (e.g., antioxidants), are operative in healthy fish, and these defense systems may not be fully effective in animals exposed to high burdens of xenobiotics. In this case, the ability of fish to eliminate free radicals may be gradually impaired, and the free radicals may slowly accumulate and overwhelm the defense mechanisms, thus reaching the limit of detection by EPR.

Most fish with liver lesions contained multiple lesion types as indicated, yet it was not possible to form strictly independent samples and subsequently relate EPR signal

intensity to specific lesions or groups of lesions. However, the statistically significant elevation in mean EPR signal intensities obtained with English sole livers with lesions compared to livers without lesions strongly suggests that the formation of the free radical species is associated with one or more of these lesions.

The differences between mean EPR signal intensities in sole with and without hepatic lesions are not apparently attributable to increasing age of fish because no significant length differences were detected among the six independent samples by analysis of variance. The association of elevated free radical levels in English sole livers with lesions is strengthened by the fact that English sole with normal livers from separate geographic areas were not significantly different with respect to EPR signal intensity, nor were English sole with liver lesions from separate sampling sites in Puget Sound.

In conclusion, free radicals of the XP· type (in which X· appears to be derived from a xenobiotic—possibly a polynuclear aromatic compound) were detected in English sole liver microsomes isolated from fish with idiopathic liver lesions. However, the question of the structure(s) of the free radicals and how the free radicals—believed to be pollution related—are associated with lesion formation remains to be determined.

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Adsorption and Surface Precipitation of Metals on Amorphous Iron Oxyhydroxide

Mark M. Benjamin

Environmental Engineering and Science, Department of Civil Engineering, University of Washington, Seattle, Washington 98195

■ The effects of Cd, Cu, Co, and Zn on sorption of CrO_4 and SeO_4 onto $Fe_2O_3 \cdot H_2O(am)$ have been investigated. Sorption of the anions is unaffected by the presence of the cations unless the cations form a surface precipitate, presumably $M(OH)_2(s)$. Generally when such a precipitate forms, sorption of the anion increases. If a surface precipitate does not form, competition between the metal and the anion for surface sites and electrostatic effects of the cations on anion adsorption are both negligible, even at very high apparent adsorption densities of the metals (1 mol of M/mol of Fe). Adsorption of metals increases their tendency to precipitate in some systems and decreases it in others. The results suggest a technique which may be useful in detecting the formation of surface precipitates.

Introduction

It is now well-accepted that adsorption phenomena play an important role in transport, chemical reactions, biotransformation, and the ultimate fate of trace constituents in many aquatic systems. Concomitant with increased awareness of the importance of sorption phenomena has been an increase in the number and complexity of adsorption experiments reported in the literature and of adsorption models proposed to explain the experimental results. There has recently been a convergence in the conceptual framework of most models, so that many of the underlying principles of adsorption reactions are now agreed upon. Though there is still some disagreement as to which model represents the structure of the electrical double layer most realistically, Westall and co-workers (1, 2) have shown that several models can reproduce experimental results equally well, at least for the relatively simple systems they analyzed. They point out, however, that a clear distinction among the models may emerge from the study of more complex systems.

Extending experimental and theoretical investigations to systems containing more than one potential adsorbate can provide insight unattainable from single-adsorbate systems and may provide a technique for choosing among the available models. At the least, such studies will help define the conditions under which results of simple laboratory experiments can be extrapolated to more complex chemical systems. Interactions between adsorbed ions may be either direct (e.g., chemical reactions between two ions at the surface) or indirect, through mutual effects on the adsorbent. This study was undertaken to determine which of the numerous possible adsorbate–adsorbate interactions in systems with several adsorbates is actually significant, to determine the conditions under which surface precipitation of an adsorbate occurs, and to study the effect of surface precipitation on the behavior of other ions in the system.

The adsorbates studied were four cationic transition metals (Cd, Cu, Co, and Zn) and two oxyanions (CrO_4 and SeO_4), and the adsorbent was amorphous iron oxyhydroxide ($Fe_2O_3 \cdot H_2O(am)$). These systems were chosen because the surface properties of the solid have been characterized extensively (3, 4) and adsorption of each of

the ions on $Fe_2O_3 \cdot H_2O(am)$ has been studied in systems containing only one strong adsorbate (3–6). Although $Fe_2O_3 \cdot H_2O(am)$ does not have a well-defined crystal structure, some aspects of its morphology during precipitation and aging have been described by Murphy et al. (7–9). Its surface site density (9.8 sites/nm²), specific surface area (600 m²/g of $Fe_2O_3 \cdot H_2O$), point of zero charge (pH 7.9), intrinsic acidity constants, and surface charge–surface potential relationship have also been characterized (3–11). In addition, its sorption properties have been studied in systems with Ca, Cu, Zn, Pb, Cd, Ag, Hg, CrO_4, SeO_4, SeO_3, SO_4, AsO_3, AsO_4, and several other ions over a wide range of conditions (4–6, 10–14). The sorption behavior of these ions in one-adsorbate systems is very similar whether the adsorbent is amorphous or well crystallized (10, 12, 15–19). It seems likely that the patterns of multiple-adsorbate interactions observed on the amorphous oxide would apply on well-crystallized solids as well. The effects of high concentrations of oxyanions on sorption of trace cationic metals were reported in a previous publication (6). In this paper the complementary data for the effects of high concentrations of strongly binding cations on sorption of trace metal oxyanions are reported.

Materials and Methods

Amorphous iron oxyhydroxide was prepared by dropwise addition of CO_3-free 1 M NaOH to an acidified (pH 2) solution containing the desired concentration of $Fe(NO_3)_3$ and enough $NaNO_3$ to give an ionic strength of 0.1 M after addition of all reagents. The neutralized suspension was kept at pH 7 for 1 h, then adjusted to the desired pH for the adsorption experiment, and equilibrated for one additional hour. The suspension was continuously purged with CO_2-free N_2 and was kept at 20 °C.

Iron oxyhydroxide prepared in this way is a reproducible adsorbent with well-established properties. At room temperature the solid attains a pseudoequilibrium condition within a few minutes which is stable with respect to surface properties and adsorption characteristics for at least several weeks (20, 21).

The adsorbate ions were added as the sodium (for CrO_4 and SeO_4) or nitrate (for Cd, Cu, Co, and Zn) salts from stock solutions of analytical grade reagents. In some cases radioactive tracers were also added at this time. All adsorbates were added to the suspension within 5 min of one another. The pH of the suspension was adjusted to a value 0.5–1.0 unit lower than that where cationic metal sorption was expected; i.e., the pH was adjusted so that there was zero metal sorption in the initial sample.

Sequential 20-mL aliquots of the suspension were transferred to N_2-purged glass centrifuge tubes, and the pH of the bulk suspension was increased between aliquots by addition of appropriate volumes (50–1000 µL) of 0.1 or 1.0 M NaOH. The tubes were capped and placed on an end-over-end roller for 2 h at room temperature (20 ± 3 °C).

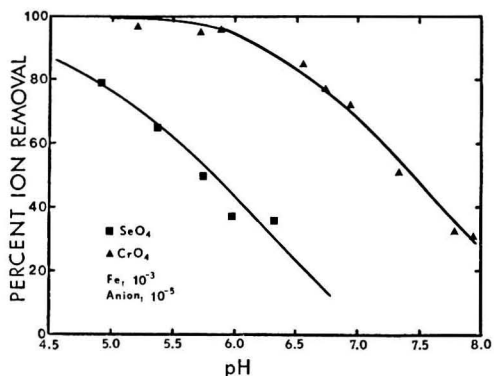The pH in each tube was then measured, and the suspensions were centrifuged. The fractional removal from

Figure 1. Adsorption of CrO_4 and SeO_4 onto $Fe_2O_3 \cdot H_2O$(am) in systems containing only one adsorbate.

solution was computed by comparing the concentration of metal in the supernatant with that in a sample of the bulk slurry. Concentrations were determined by the activity of radioactive tracer for Cd, Zn, Co, and SeO_4 and by atomic adsorption spectrophotometry for Cu and CrO_4.

The kinetics of adsorption onto $Fe_2O_3 \cdot H_2O$(am) and many other oxides involve a rapid initial step which typically takes 5–30 min and which is sometimes followed by a slower step during which the adsorption density may increase at the rate of a few percent per day (10, 22, 23). The experiments reported here had equilibration periods long enough for the first step to be complete while minimizing the effect of the second step.

Results and Discussion

Adsorption and Precipitation in Single Metal Systems. The extent of adsorption of metal or metalloid ions onto oxide surfaces in systems containing only one strongly sorbing noncomplexed solute and one adsorbent depends primarily on the identity and the total concentrations of adsorbate and adsorbent and on the solution pH. Typically a cationic metal shifts from being mostly dissolved at low pH to mostly sorbed at higher pH, as pH increases through a critical range 1–2 pH units wide (4, 10–12, 17–19). Metal oxyanions similarly exhibit a critical pH range, except their partitioning shifts in the opposite direction as pH increases, i.e., from mostly sorbed to mostly dissolved (3, 13, 15, 16). Although the location of the critical pH range can be adjusted by changing the system composition, the general shape of the pH-adsorption edge is similar in almost all systems.

Adsorption of all the anionic and cationic adsorbates studied onto $Fe_2O_3 \cdot H_2O$(am) from single adsorbate solutions is shown in Figures 1 and 2, respectively. The strong dependence of sorption on pH noted above was observed in all these systems. The high concentrations of cations used reflect the fact that in the multiple adsorbate systems to be discussed later, only very large cation concentrations affected anion adsorption significantly.

Metal removal from solution is compared in systems with and without $Fe_2O_3 \cdot H_2O$(am) in Figure 2. Figure 2A also presents results of replicate tests conducted about a year apart. When iron is present, the removal "edge" is quite reproducible. In the absence of iron, a relatively steep edge was observed each time the experiment was run, and the edge was at a higher pH than when iron was
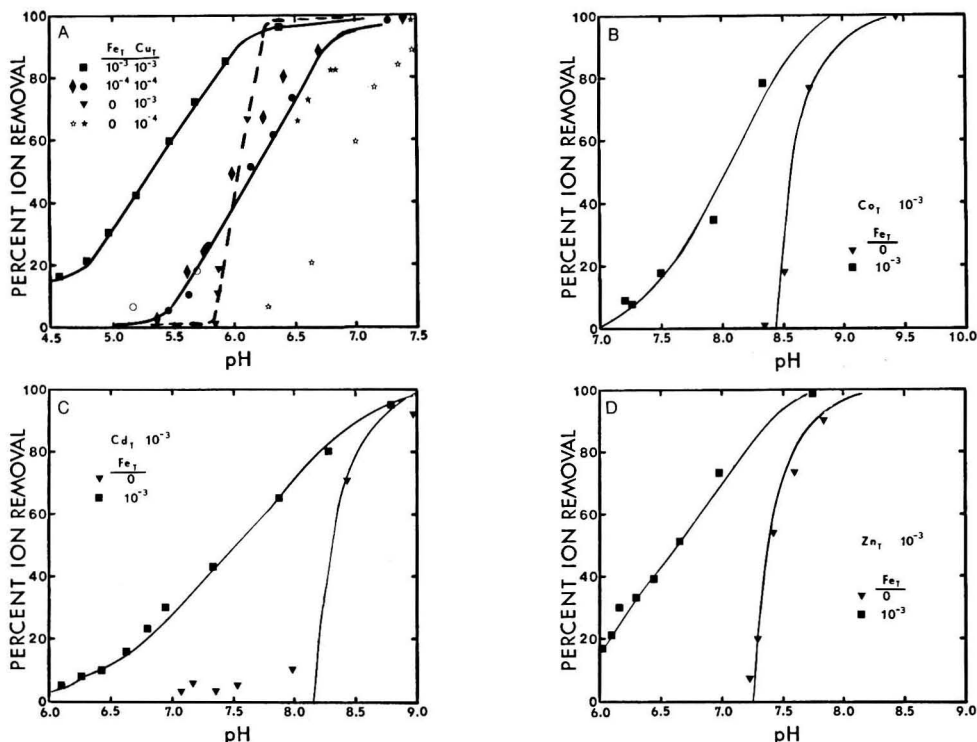
Figure 2. Comparison of the fractional removal of metal ions from solutions in the presence and absence of $Fe_2O_3 \cdot H_2O$(am): (A) Cu; (B) Co; (C) Cd; (D) Zn. Data of Swallow et al. (5) are presented for comparison (O). The lines for the Fe-free systems are drawn consistent with the estimates of K_{so} in Table I. For the systems with 10^{-4} M Cu_T, data are presented from replicate tests conducted about a year apart.

Table I. Stability Constants for Complexes and Precipitates Possible in the Systems Studied[a]

	hydrolysis[b]		precipitation of M(OH)$_2$(s),[b]	complexation,[c]
	p$K*_1$	p$K*_2$	pK_{so}[d]	log K_{1,SeO_4}
Cu	>8.0	>9.3	19.9	2.2[e]
Co	9.7	9.1	14.5	2.7
Cd	10.1	10.2	15.1	2.3
Zn	9.0	7.9	16.9	2.2

chromate precipitation: p$K_{so,CuCrO_4}$ = 5.4[c]
chromate acidity: p$K_{a,HCrO_4}$ = 6.5[c]

[a] Stability constants for species not shown, such as MCrO$_4$ complexes, were not available in ref 25 and are unlikely to be important in the systems studied. [b] From ref 24. [c] From ref 25. [d] Estimates based on the data in Figure 2. Activity corrections were made by using the Davies equation. The crystallinity of the solid phase formed was not investigated. [e] Estimate based on the stability of the CuSO$_4$(aq) complex.

present. However, the pH of the edge varied over a range of about 0.5 pH unit. Subsequent experiments did not narrow this range, and the reason for the variation is not clear. However, since the results from any one experiment were self-consistent and since metal removal was measured directly in each experiment where it was an important factor, this variation in the exact pH of the removal edge is not critical to the conclusions.

Apparent solubility products for the metal oxides or hydroxides, computed based on the data from the iron-free systems, are summarized in Table I, along with other pertinent equilibrium constants. For all four metals studied the metal is removed from solution at a significantly lower pH in a system containing Fe$_2$O$_3$·H$_2$O than when it is absent. In addition, the removal efficiency is less strongly dependent on pH when the iron is present. Since the total concentration of metal (M$_T$) was very large in these systems and was equal to the concentration of Fe comprising the adsorbent, it is arguable whether the reaction responsible for metal removal was sorption or surface precipitation. Regardless of which removal mechanism was operating, Figure 2 shows that the presence of Fe$_2$O$_3$·H$_2$O(am) is important in the metal removal process in these systems. Furthermore, the role of Fe$_2$O$_3$·H$_2$O(am) must be more significant than simply providing crystal nucleation sites for the precipitating metal. If Fe$_2$O$_3$·H$_2$O(am) were acting strictly as a nucleating agent for the metal oxides and thus minimizing supersaturation in solution, then as soon as MO(s) or M(OH)$_2$(s) crystals formed in the iron-free systems they would grow until the same equilibrium condition was reached as in the systems containing iron. In experiments in which 20–80% of the metal was removed from solution in the iron-free systems, MO(s) or M(OH)$_2$(s) crystals must have formed. Nevertheless, they reached an equilibrium condition with more metal in solution than systems at the same pH containing iron. Since crystal nuclei were present in both systems at these pHs and they had different equilibrium compositions, the presence of the iron must have altered the equilibrium state and not just the nucleation kinetics. This conclusion is further supported by the slope of the metal removal curves in the presence of Fe$_2$O$_3$·H$_2$O(am), which are inconsistent with simple precipitation of MO(s) or M(OH)$_2$(s). The results do not rule out the possibility that a surface precipitate formed in the systems with iron. However, if surface precipitation was the mechanism for metal removal in these systems, the activity of the surface precipitate phase must have been

Table II. Effects of Various Parameters on Sorption in Systems with Multiple Adsorbates

parameter	effect of increasing parameter on metal (M) sorption
pH	typically a dramatic increase (decrease) in sorption of cations (anions) as pH increases through a critical pH range
any other adsorbate (Ad)	competition for sorption sites; M sorption decreases
any ionized adsorbate (Ad^{m+} or Ad^{p-})	alteration of potential in electrical double layer; effect depends on overall stoichiometry of sorption reactions; normally adsorption of cations makes surface more attractive to anions and less attractive to other cations
sparingly soluble adsorbate	new surface phase may form; surface phase may consist of adsorbate plus M^{n+}, OH$^-$, constituent ion of adsorbent, or some combination of these; effect on M depends on adsorbent properties of new phase
adsorbate which can form a soluble complex with the metal	depends on relative adsorptive bond strength of complexes compared to uncomplexed ion
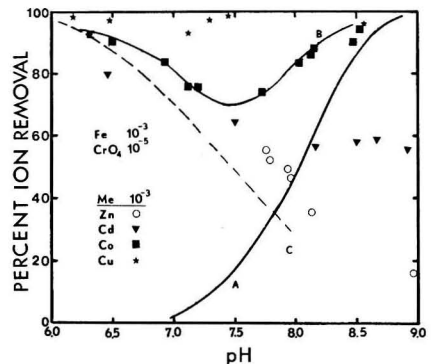
Figure 3. Effect of 10^{-3} M Cu, Co, Cd, or Zn on CrO$_4$ sorption onto Fe$_2$O$_3$·H$_2$O(am). The data points all represent fractional removal of CrO$_4$ from solution. The solid lines represent fractional removal of Co (A) and CrO$_4$ (B) in the system with 10^{-3} M Co$_T$. The dashed line represents CrO$_4$ removal in a metal-free system. The data for line A are in Figure 2B, and those for line C are in Figure 1.

less than that of the freshly precipitated metal oxide or hydroxide formed in bulk solution. Such a situation may arise due to formation of a solid solution rather than a pure solid (26), formation of a more stable crystalline phase on the surface than forms in bulk, or any factor that causes the activity coefficient for the surface precipitate to be less than 1.0.

Adsorption in Multiadsorbate Systems. The effect of metal ions on the sorption behavior of CrO$_4$ and SeO$_4$ was investigated next. Possible interactions between these species are summarized in Table II. Available stability constants indicate that complexation and/or precipitation reactions between the metals and oxyanions were not significant in any of the systems studied. Preliminary experiments indicated that cationic metal concentrations of 10^{-4} M or less have no effect on sorption of CrO$_4$ or SeO$_4$ onto Fe$_2$O$_3$·H$_2$O(am). However, when the metal concen-
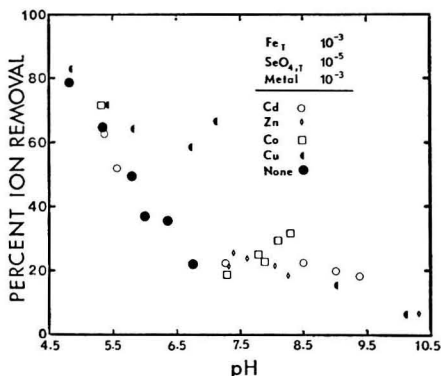
Figure 4. Effect of 10^{-3} M Cu, Co, Cd, or Zn on SeO$_4$ sorption onto Fe$_2$O$_3$·H$_2$O(am).

tration is increased to 10^{-3} M, the effect on anion sorption is generally large (Figures 3 and 4). Anion removal from solution is enhanced, and the magnitude of the change increases in the order Zn < Cd < Co < Cu. As would be expected, the effect of the metals on anion sorption is slight in the pH region where metal sorption is slight and increases dramatically in the pH region where most of the metal begins to sorb. This point is demonstrated clearly in Figure 3 for a solution containing 10^{-3} M Co$_T$. In the figure, the broken line shows the sorption of CrO$_4$ on Fe$_2$O$_3$·H$_2$O(am) in the absence of any added metal. The solid lines labeled A and B represent removal of Co and CrO$_4$, respectively, when 10^{-3} M Co is added to the system. As soon as removal of Co becomes significant (pH ~7.3), there is a dramatic increase in CrO$_4$ removal.

The metals alter sorption of SeO$_4$ in much the same way as, but to a lesser extent than, sorption of CrO$_4$ (Figure 4). As is the case for CrO$_4$, metal concentrations of 10^{-4} M or less have little effect, and a concentration of 10^{-3} M M$_T$ increases sorption of the anion, with the magnitude of the effect increasing in the order Zn < Cd < Co < Cu. Adsorption behavior of CrO$_4$ and SeO$_4$ is quite similar to that of other oxyanions (e.g., AsO$_3$, SeO$_3$, and PO$_4$) in systems with no metal added (11, 13, 16, 27, 28). Therefore, it seems likely that the trends observed for CrO$_4$ and SeO$_4$ when metals are present would apply for other oxyanions and other oxide sorbents as well. Since the qualitative effects of the metals were identical for the two anions studied, and since they were quantitatively more dramatic for CrO$_4$, further experimental work was conducted only in systems with CrO$_4$ as a model anionic adsorbate.

Two plausible explanations for the enhanced anion removal in systems containing 10^{-3} M Cu$_T$, Cd$_T$, or Co$_T$ are that (1) the metals sorb, making the surface potential more positive, and therefore more favorable for anion sorption, or (2) the metals form surface precipitates onto which anions sorb more strongly than they do onto Fe$_2$O$_3$·H$_2$O-(am). The first possibility was tested by measuring CrO$_4$ removal from solutions containing an order of magnitude less of each reagent (10^{-4} M Fe$_T$, 10^{-4} M Cu$_T$ or Co$_T$, and 10^{-6} M CrO$_4$) except the background electrolyte (10^{-1} M NaNO$_3$). For a given pH under conditions where almost all the Cu or Co is bound to the surface, the adsorption density (1.0 mol of M/mol of Fe) and the surface electrical potential should be approximately the same in these systems as in the more concentrated systems. Thus, the electrostatic interactions between the surface and CrO$_4$ should be approximately the same in the two systems. Nevertheless, the high adsorption density of metal had no
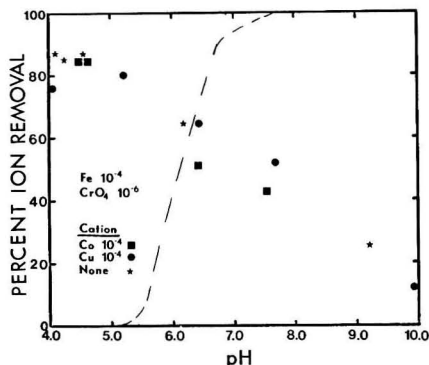
Figure 5. Effect of 10^{-4} M Cu or Co on CrO$_4$ sorption onto Fe$_2$O$_3$·H$_2$O(am) in systems one-tenth as concentrated as those in Figure 3. The dashed lined shows fractional removal of Cu in this system and is taken from the data in Figure 2A.
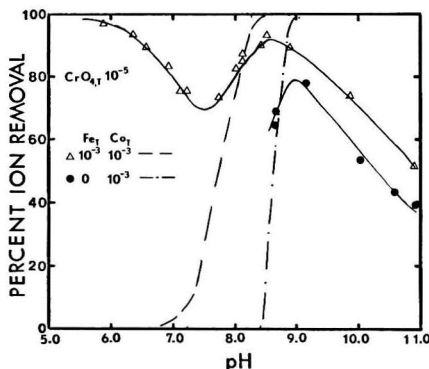
Figure 6. Sorption of CrO$_4$ onto freshly precipitated Co(OH)$_2$(s) (10^{-3} M Co$_T$) compared with sorption in a system with 10^{-3} M Co$_T$ plus 10^{-3} M Fe$_T$. The data points all represent CrO$_4$ sorption, and the broken lines represent removal of Co from solution.

effect on CrO$_4$ sorption in the less concentrated systems (Figure 5). This effectively rules out the possibility that metals enhance CrO$_4$ removal primarily by electrostatic interactions.

The possibility that in the systems containing 10^{-3} M metal the CrO$_4$ is adsorbing onto surface precipitates of the metal oxides is supported by the results of experiments in which the sorption of CrO$_4$ onto pure metal oxides was studied. For instance, Figure 6 shows a comparison of CrO$_4$ sorption in two systems, each containing 10^{-3} M Co and differing only in that one system also contains 10^{-3} M Fe. As soon as Co(OH)$_2$(s) starts precipitating in the system without iron, a significant fraction of the CrO$_4$ is removed from solution. The pH dependence of CrO$_4$ sorption is almost identical in the two systems and is very different from that on Fe$_2$O$_3$·H$_2$O(am) in the absence of cobalt. In each case CrO$_4$ removal increases markedly as Co is removed from solution up to a pH between 8 and 9, and then CrO$_4$ is released back to solution as the pH is raised further.

A similar argument can be made for CrO$_4$ sorption onto pure Cu(OH)$_2$(s). Figure 7 indicates that Cu(OH)$_2$(s) is a significantly stronger sorbent for CrO$_4$ than Fe$_2$O$_3$·H$_2$O-(am). On the basis of this result, one would expect CrO$_4$ sorption to be enhanced if a Cu(OH)$_2$(s) surface precipitate forms on Fe$_2$O$_3$·H$_2$O(am). As noted earlier, such an enhancement is observed when 10^{-3} Cu is added to a system containing 10^{-3} M Fe and 10^{-5} M CrO$_4$ (Figure 3). Interest-
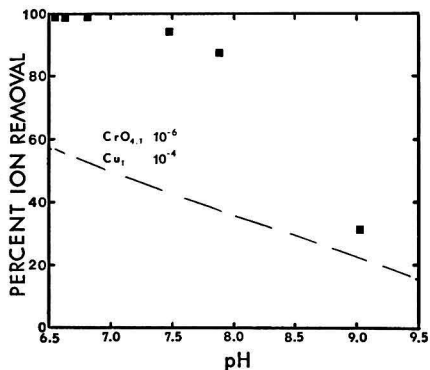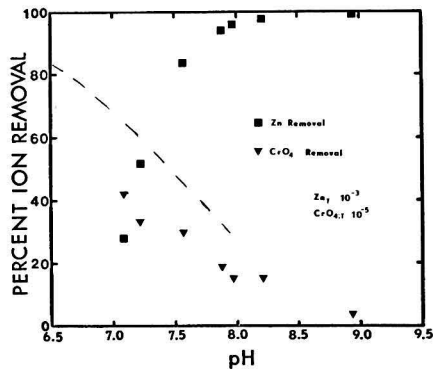
Figure 7. Sorption of 10^{-6} M CrO_4 onto freshly precipitated $Cu(OH)_2(s)$ (10^{-4} M Cu_T). The dashed line represents sorption of CrO_4 in a system containing 0.5×10^{-4} M $Fe_2O_3 \cdot H_2O(am)$ with or without 10^{-4} M Cu_T added (from Figure 5).

Figure 8. Sorption of 10^{-5} M CrO_4 onto freshly precipitated $Zn(OH)_2(s)$ (10^{-3} M Zn_T). The dashed line represents sorption of 10^{-5} M CrO_4 onto $Fe_2O_3 \cdot H_2O(am)$ (10^{-3} M Fe_T) and is taken from the data in Figure 1.

ingly, neither Cu nor Co had any effect on CrO_4 sorption in systems one-tenth as concentrated as those discussed above (Figure 5). This suggests that in those systems the metal is removed from solution without forming a new surface phase. Although sorption of CrO_4 onto pure Cd-$(OH)_2(s)$ was not investigated, the increase in CrO_4 removal from solution concomitant with removal of Cd is consistent with the hypothesis that the CrO_4 is binding to a new surface phase in this system also.

Zinc had a much smaller effect on CrO_4 sorption than any of the other metals investigated (Figure 3). The reason for this may be either that even at high adsorption density Zn binds to $Fe_2O_3 \cdot H_2O$ without forming a surface precipitate or that CrO_4 binds less strongly to a surface precipitate of Zn than to one of Cu, Co, or Cd. Sorption of CrO_4 onto 10^{-3} M freshly precipitated $Zn(OH)_2(s)$ is approximately as strong as or perhaps slightly weaker than that on 0.5×10^{-3} M $Fe_2O_3 \cdot H_2O(am)$ (Figure 8). Thus, even if Zn did form a surface precipitate on $Fe_2O_3 \cdot H_2O(am)$, the effect on CrO_4 sorption would be small; i.e., sorption of CrO_4 is not a sensitive indicator of $Zn(OH)_2(s)$ surface precipitation. On the basis of the evidence for precipitation of Cu, Cd, and Co under similar conditions, surface precipitation of $Zn(OH)_2(s)$ in the 10^{-3} M Zn_T system seems likely. However, it cannot be asserted with certainty at this time.

In summary the different effects of metals on CrO_4 sorption in these systems can be explained if the adsorbed metals form CrO_4-sorbing surface precipitates in the more concentrated systems but adsorb without precipitating in less concentrated systems. The case for surface precipitation in systems containing 10^{-3} M metal is based on the dramatic change in CrO_4 sorption behavior and the similarity of that behavior to CrO_4 sorption on the pure metal oxides. The presumption that a surface precipitate rather than a bulk precipitate of the metal oxide forms stems from the significant effect of Fe in enhancing metal removal in these systems. Finally, the case against metals affecting CrO_4 sorption primarily through electrostatics rests on two arguments: the rather striking similarity already alluded to of CrO_4 sorption onto $Co(OH)_2(s)$ and onto $Fe_2O_3 \cdot H_2O(am)$ plus Co, and the very different behavior of CrO_4 in two systems in which the electrostatics would be the same if no surface precipitate formed. While the surface precipitate has not been observed directly and hence its existence not proven unequivocally, there does not appear to be any other hypothesis that satisfactorily explains the results in all the systems studied.

Surface Precipitation in Other Systems. The conversion of adsorbed metal ions into a surface precipitate of metal oxide or hydroxide has been suggested or inferred by several investigators (29–32). For instance, James and Healy (29) inferred that $Co(OH)_2(s)$ was precipitating on the surface of several oxides based on similarities between the electrophoretic mobility of $SiO_2(s)$ particles with bound Co and that of $Co(OH)_2(s)$ particles. These investigators did not report how precipitation of surface oxides affected sorption of other ions in solution. Obviously this effect depends on the extent of coverage by the secondary phase and its porosity to adsorbate ions, as well as its own properties as a sorbent. The fact that sorption of CrO_4 onto a bulk precipitate of $Co(OH)_2$ is similar to, but slightly weaker than, that onto a surface precipitate (Figure 6) indicates that the surface precipitate may be slightly more "active" or may have more available sorption sites.

The conditions under which adsorbed ions start forming a surface precipitate are extremely difficult to characterize in single-adsorbate systems. Veith and Sposito (33) and Corey (26) have cautioned that results from experiments in which a surface precipitate has formed can easily be misinterpreted as adsorption phenomena. The results presented here suggest that changes in sorption of another adsorbate may be a sensitive indicator of the surface precipitation reaction.

Adsorbate Interactions in Systems without Surface Precipitation. In many ways, the systems in which addition of metals had no effect on the removal of anions from solution are more striking than those where a surface precipitate formed. It was noted earlier that metal concentrations of 10^{-4} M had no effect on CrO_4 or SeO_4 sorption onto $Fe_2O_3 \cdot H_2O(am)$. Even in the absence of surface precipitation of a metal hydroxide phase, one might expect the sorbed metal to compete with the anions for surface sites or to alter the surface electrical potential enough that anion sorption would be noticeably affected. The lack of either competitive or electrostatic interactions between adsorbed metals and anionic metalloid ions is demonstrated most dramatically in Figure 5 for a system containing 10^{-4} M Fe_T, 10^{-4} M Cu_T, and 10^{-6} M $CrO_{4,T}$. Even at pH 7.0, when the adsorption density of Cu is 1 mol of Cu adsorbed/mol of Fe, the sorption of CrO_4 is identical with that in a system with no Cu. Davis and Leckie (11) have estimated an adsorption site density of 0.87 mol of sites/mol of Fe for $Fe_2O_3 \cdot H_2O(am)$. Obviously in the experimental system described here most if not all of the available sorption sites should be occupied by Cu at pH 7.

The case against precipitation of $Cu(OH)_2(s)$ in this system is illustrated by comparing the results in Figure 7 and Figure 5. While 100% of the CrO_4 in solution adsorbs to 10^{-4} M $Cu(OH)_2$ at pH 7.0 (Figure 7), the same amount of Cu has no effect on CrO_4 sorption if the Cu is bound to $Fe_2O_3 \cdot H_2O(am)$ (Figure 5). Since it was shown earlier that surface precipitates behave quite similarly to bulk precipitates with respect to CrO_4 sorption, the only reasonable conclusion is that no surface precipitate is forming in the less concentrated system.

The term "adsorption density" must be used cautiously in systems such as described in Figure 7, since conceptually adsorption is obviously precluded under conditions where more metal is removed from solution than the number of adsorption sites available. However, if one defines adsorption in a macroscopic sense as removal of a chemical species from solution in response to the presence of a surface and in the absence of other chemical reactions such as precipitation, the results in systems with 10^{-4} M Cu and 10^{-4} M Fe appear to qualify as adsorption phenomena. Apparently CrO_4 and Cu bind to entirely different groups of sites on $Fe_2O_3 \cdot H_2O(am)$, and any changes in the average surface potential caused by Cu sorption affect CrO_4 sorption minimally.

Failure of competitive adsorption data to conform to the expected results based on a single-site Langmuir adsorption model has been reported often and has often been used as evidence for the presence of more than one type of surface site (4, 12, 19, 27). However, these reports have involved competition between pairs of cations or pairs of anions and usually indicate that there is some competition for surface sites at high adsorption density. The remarkable aspect of the results from this study is that even when the adsorption density of metal is very large, competitive interactions are totally absent. Similarly, electrostatic interactions among adsorbed ions are also absent. Anderson and Malotky (16) have shown that the electrophoretic mobility of oxides is altered when arsenate adsorbs and that the change in surface charge decreases the driving force for adsorption of additional arsenate ions. Similar inferences have been made by Davis and Leckie (11) for sorption of cations. However, the present study indicates that high adsorption densities of metal do not significantly alter the driving force for anion adsorption. Apparently, metal sorption does not significantly alter the interfacial potential at the locus where the anions sorb.

The results with respect to both competition and electrostatics are consistent with those of a previous study (6) in which high adsorption densities of several anions (AsO_4, AsO_3, SeO_4, SeO_3, and CrO_4) did not affect sorption of trace amounts of cations (Cd, Zn, Cu, and Co) at all unless a surface precipitate formed. Taken together, these studies suggest that while there may be several types of cation-binding sites on $Fe_2O_3 \cdot H_2O(am)$ and several types of anion-binding sites, the differences between the two groups are large and are much greater than the differences within each.

This study also points out an interesting relationship between adsorption and surface precipitation which has not been appreciated previously. It has already been noted that adsorption of high concentrations of metals promotes surface precipitation of oxides at pH values where precipitation does not occur in bulk solution (10^{-3} M M_T, 10^{-3} M Fe_T). It has also been argued that in a system containing 10^{-4} M Cu_T and 10^{-4} M Fe_T, no surface precipitate forms even when the Cu is completely removed from solution (pH 7–8). However, Figure 2A shows that $Cu(OH)_2(s)$ will precipitate at pH ~6.5 from a solution con-

taining 10^{-4} M Cu_T if $Fe_2O_3 \cdot H_2O(am)$ is absent. Thus, in this case, the adsorption reaction prevents $Cu(OH)_2(s)$ precipitation. In other words, adsorption can either increase or decrease the effective solubility product of a metal oxide, depending on the total concentration of metal and adsorbent in the system.

Summary and Conclusions

In summary, many systems in which adsorption is significant contain several potential adsorbates. Previous studies have elucidated the sorption behavior of individual ions from single-sorbate solutions, but few have addressed the question of how adsorbate ions interact with one another and with the surface in systems containing more than one strongly binding ion. Some of the possible interactions in these systems include competition for surface sites, alteration of the surface electrical potential resulting in a change in the Coulombic interactions between ions and the surface, or precipitation of a new surface phase. This work has shown that the effect of Cd, Co, Cu, or Zn on sorption of CrO_4 or SeO_4 onto $Fe_2O_3 \cdot H_2O(am)$ is small unless a surface precipitate of the metal oxide or hydroxide forms. If such a precipitate does form, sorption of the anion is increased, except in the case of Zn where anion sorption is about the same in the presence or absence of the surface precipitate. Whereas surface precipitation is extremely difficult to detect by direct observation or analysis of the precipitating ion, in many cases it is relatively easy to detect by its effect on sorption of another ion.

The presence of the iron adsorbent increases the tendency for a new phase to precipitate from concentrated solutions (10^{-3} M M_T) but interferes with precipitation from less concentrated systems (10^{-4} M M_T).

When a surface precipitate does not form, extremely large apparent adsorption densities of metal (1 mol of M/mol of Fe) have no effect on CrO_4 or SeO_4 sorption, indicating that the metals and anions bind to different groups of sites. The lack of any significant effect on anion sorption in some systems with large metal adsorption densities indicates that at the sites where anions are binding, the surface potential is not much different than in a metal-free system.

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Kinetics of the Short-Term Consumption of Chlorine by Fulvic Acid

Robert G. Qualls and J. Donald Johnson*

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

■ Concern over the environmental effects of chlorination has prompted efforts to minimize the amount of chlorine necessary to prevent fouling of power plant condensers. In this study we developed a kinetic model of the short-term consumption of chlorine by fulvic acid in natural fresh waters. Fulvic acids isolated from several sources were used. The reactions were adequately described by the sum of two second-order reactions. Concentrations of fulvic acid reaction sites were related to fulvic acid carbon concentrations. Rates of chlorine consumption by fulvic acids isolated from three diverse sources were quite similar, suggesting that the kinetic expressions may be generally applicable. The fulvic acid expressions were integrated in a larger kinetic model describing reactions of chlorine with ammonia, a model dipeptide, and fulvic acid to simulate chlorine consumption in a river water sample. The simulation matched the data measured in the river water quite well. When conditions typical of "once-through" cooling water chlorination were used, the most important reactions consuming free residual chlorine were formation of NH_2Cl and consumption by humic substances in the sample. Most reduction in total residual chlorine was caused by humic substances.

Introduction

Large quantities of water are required for the cooling systems of both nuclear and coal-fired power plants. This water is generally chlorinated to prevent fouling of the condensers. Since chlorine consumption by natural waters is difficult to predict, the common practice has been to add much more than is necessary to ensure that excess chlorine

remained throughout the system. Recently, however, there have been efforts to minimize the amounts of chlorine residuals discharged with cooling water because of the toxicity of chlorine residuals to aquatic organisms and concern for mutagenic substances formed in chlorinated water (*1–4*). Moss et al. (*5*) have provided experimental data on the minimum free residual chlorine (FRC) concentrations necessary to maintain condenser performance at several Tennessee Valley Authority power plants.

There have been several efforts to model the kinetics of chlorine consumption in natural waters so that sufficient, but not excessive, amounts of chlorine can be added. The primary basis for natural water chlorination models has been the Morris and Wei studies (*6, 7*) of reactions of NH_3 and chlorine in pure water. Their kinetic model was tested and modified by Saunier and Selleck (*8*). In addition to ammonia, natural waters contain other components that consume chlorine. These substances are primarily organic N compounds and dissolved humic substances. Efforts to model the chlorination of cooling waters have been carried out by Nelson (*9*), Lietzke (*10*), Haag and Lietzke (*11*), and Zielke et al. (*12*). The first simply involved a mass balance approach whereas the others incorporated reaction kinetics involving chlorine and NH_3. None of the models have been successful in simulating the free residual chlorine (FRC) concentrations because of the lack of data on the kinetics of chlorine consumption by organic compounds in natural water.

The specific chemical composition of the dissolved organic matter in natural water remains largely unknown. At best, only broad classifications by molecular size and

solubility have been made. The major single solubility class of the dissolved organic carbon (DOC) is the humic substances. They typically comprise 30–80% of the DOC in natural water (13, 14). The major component of the aquatic humics is fulvic acid (13).

It has been recognized that there is frequently a correlation between the color of water and its chlorine demand (15). In many natural waters humic substances may constitute the major non-nitrogenous chlorine demand. Recently, the chlorination of humic substances has received a great deal of attention. Most of this work has centered on chloroform production (e.g., see ref 15). Kinetic work has been concerned with long-term reactions over hours or days (16, 17).

The general objective of this study was to develop kinetic expressions for the consumption of oxidizing chlorine by organic substances in natural fresh water. Although our main concern was with predicting the consumption of free residual chlorine and the production of chloramines in power plant cooling waters, the results may be equally applicable to other uses of chlorination such as for municipal waters, especially where the short-term reactions are of concern. More specifically, our objectives were (1) to develop kinetic equations for chlorine consumption by isolated fulvic acids for a 5-min period, (2) to test the generality of our equations by comparing consumption by humic substances from different sources, and (3) to simulate the short-term chlorine consumption in a natural water sample using an extended model incorporating these equations.

Materials and Methods

Reagents and Analyses. Chlorine demand free water (18) was used to dilute all solutions. The source of chlorine for all experiments was Fisher reagent grade NaOCl. Concentration of the stock was determined by amperometric titration (18). Fisher phenylarsine oxide solution was used as the titrant in amperometric titration. It was standardized against G. Frederick Smith primary standard grade $KH(IO_3)_2$.

For solutions buffered between pH 6.5 and pH 8.0 a phosphate buffer was used. Buffers (Fisher reagent grade) were filtered, chlorinated to destroy trace amounts of reducing agents, and then UV irradiated to remove residual chlorine. Solutions were adjusted to a desired pH by adding 0.2 M K_2HPO_4 and 0.2 M KH_2PO_4 in a ratio to yield the desired pH approximately and then were adjusted precisely (± 0.05 pH unit) with 0.5 N NaOH or 0.5 N HCl by using an Orion 91-15 combination pH probe.

For experiments requiring rapid quenching of the reactions the DPD–ferrous ammonium sulfate (DPD–FAS) procedure was used (18). Reactions were initiated by injecting 0.1- or 0.2-mL aliquots of chlorine stock from an automatic pipet into a rapidly stirred 25- or 50-mL sample. Observations of dye indicated that mixing was complete in less than 0.25 s. For the slower reactions, the beaker was sealed with Parafilm and incubated. The reaction was stopped by rapidly pouring the sample into another beaker containing DPD reagent and buffer. A technique was developed such that reactions could be stopped after 2 s with quite reproducible results. Titration for FRC was followed by titration for combined residual chlorine (CRC). Reactions of the CRC would not be stopped by the initial DPD reagent, but only trace amounts of CRC were formed, and they did not change significantly with time.

Experiments with river water at longer times were done with amperometric titrations (apparatus described in Johnson and Inman (19)). Titrations were done rapidly by using an automatic pipet to bring the titration near the

end point instantly. The titration was completed rapidly while recording current response on a chart recorder. In this way, the titration was completed in less than 15 s. Amperometric titrations and DPD–FAS titrations were compared during every experiment.

Ammonia was determined by the colorimetric phenol–hypochlorite nitroprusside procedure (20). Organic N was determined by Kjeldahl digestion (21). Digests were neutralized and ammonia was measured as above. Dissolved organic carbon (DOC) was determined with a Dormann/Envirotech DC-54 carbon analyzer.

Kinetics of Chlorine Consumption by Fulvic Acids. Rates of chlorine consumption of fulvic acids isolated from three sources were measured at varying initial chlorine and carbon concentrations. The fulvic acids from one source (University Lake, NC) were investigated intensively to developed rate expressions. Fulvic acids from the other sources were compared under a more limited set of initial concentrations.

Fulvic acids from University Lake and Black Lake (NC) were isolated and purified in large quantities as part of another project (22) by the methods of Thurman and Malcolm (13). Briefly, water was acidified, and the humic substances were adsorbed to XAD-8 resin by liquid chromatography, eluted in basic solution, acidified, and centrifuged to remove humic acids. Fulvic acids were desalted and then dried by lyophilization. Humic materials from the Cape Fear River were not completely purified, and humic acids were not separated from the acidified water. The humic acid fraction was a minor component of the Cape Fear sample since no visible floc formed in the acidified sample. Other isolation steps were followed as cited above, but the eluted fulvic humic solution was not desalted or dried. Because the Cape Fear humic concentrate was not desalted and dried, the highest carbon concentration used in the experiments contained about 1 mM Na_2SO_4 and 3.4 μM NH_3. Other fulvic acids solutions did not contain detectable (0.2 μM) ammonia.

Stock fulvic acid solutions of 46 mg/L C concentration were made from the dried (or concentrated Cape Fear) material and neutralized with NaOH. Carbon concentrations used in experiments were 1.84, 3.68, 5.52, and 9.20 mg/L C (153, 307, 460, and 767 μM C, respectively). Initial NaOCl concentrations were 5, 10, 20, 30, and 50 μM Cl. Solutions were buffered with 5 mM phosphate. To determine if the buffer had an effect on the reaction rates, phosphate concentrations of 1, 2, and 5 mM were compared in one experiment for one set of initial conditions. A 5 mM carbonate buffer was also used and showed similar results although pH was less stable. For one set of reaction conditions (307 μM C and 20 μM Cl) reaction rates at pH 6.5, 7.0, 7.5, and 8.0 were compared with phosphate buffer. All experiments were performed at 25.0 °C (± 0.02).

Natural Water Samples. A water sample from the Cape Fear River was filtered through a 0.45-μm Millipore filter. The pH of the sample was 6.9, and the concentrations of NH_3-N, DON, and DOC were 0.28, 0.42, and 10 mg/L (20, 20, and 8.33 μM), respectively.

Samples were chlorinated as described in the previous section but analyzed by amperometric titration. "Blanks", chlorine demand free water treated and incubated in the same manner as samples, were included in each set of experiments. They indicated no significant chlorine loss during the experimental procedures.

Simulation. Simulation of chlorination reactions was performed with the Continuous Systems Modelling Program, a widely available Fortran-based program for solving a set of simultaneous differential equations by numerical
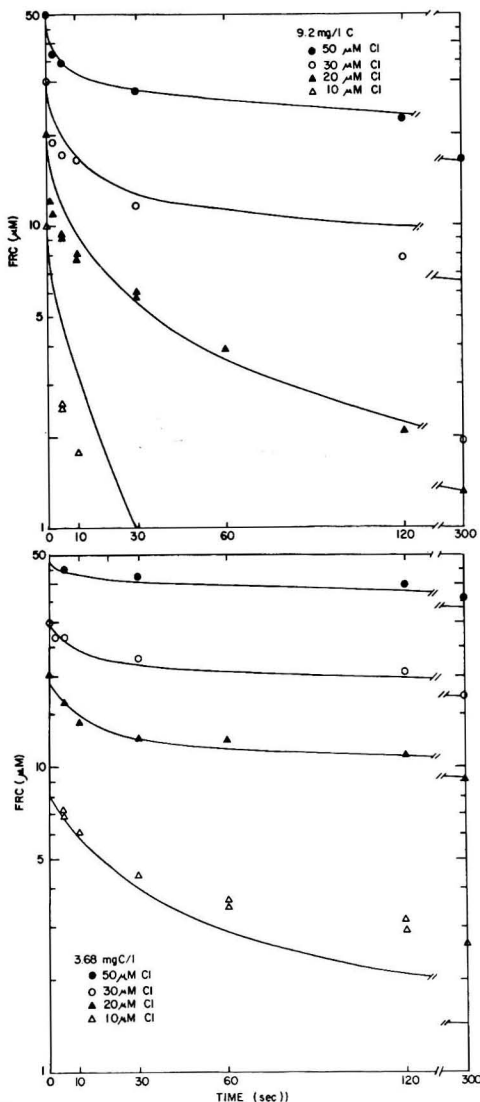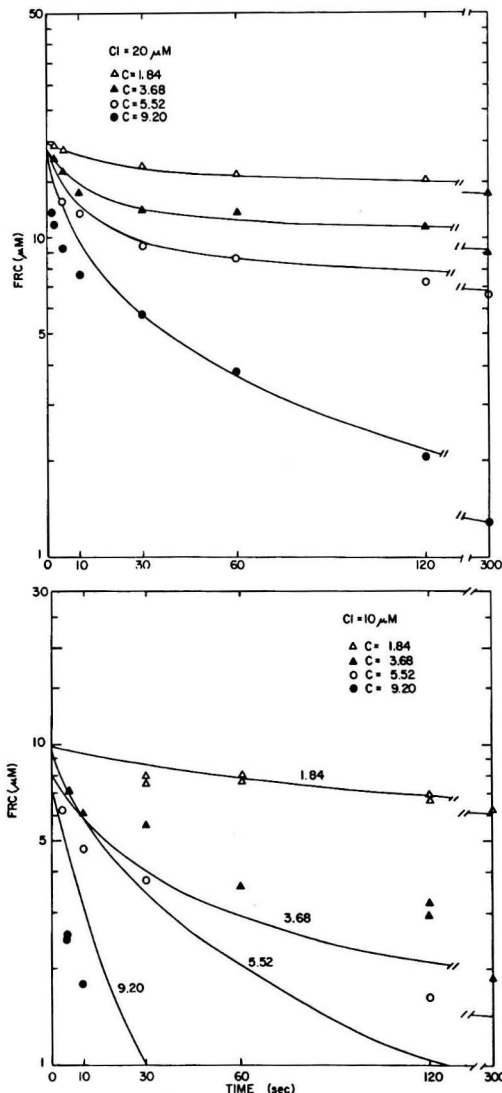
Figure 1. Rate of free residual chlorine (FRC) consumption by fulvic acid (3.68 and 9.20 mg/L carbon) as a function of initial chlorine concentrations. Data points are experimental values. Solid lines represent model predictions.

Figure 2. Rate of free residual chlorine (FRC) consumption by fulvic acid as a function of fulvic acid concentration (in mg/L as carbon). Data points are experimental values. Solid lines represent model predictions.

techniques (IBM, CSMP III reference manual). Initial concentrations of H^+, NH_3, chlorine, chloramine-forming organic N, and fulvic acid reactive sites were specified. Rates of each reaction were expressed as the rate law for the reaction. The rate of change in concentration of each species (e.g., NH_3) was expressed as the sum of rates due to each reaction. The system of differential equations was solved by a special algorithm for "stiff" differential equations.

Results and Discussion

The decline in FRC in University Lake fulvic acid solutions is shown in Figures 1 and 2. The data are shown on a semilog plot to reveal any first-order behavior of chlorine. Figure 1 compares reactions over a range of initial chlorine concentrations for two fulvic acid concen-

trations. Figure 2 compares a range of initial fulvic acid concentrations for the same initial chlorine concentrations. Note that the log scale has the effect of magnifying differences at lower concentrations where analytical variability was greater.

We found the following trends in the fulvic acid experiments: (1) It appeared possible to divide the reaction into two processes, one with a fast rate, occurring in less than about 30 s in most cases, and another slower process continuing throughout the experiment. (2) The amount of chlorine consumed in the initial fast process varied relatively little among different initial chlorine concentrations for a particular carbon concentration. (3) Except for cases where the amount of Cl added initially was low and almost exhausted by the initial phase, the data were nearly linear from 30 to 300 s, and they were nearly parallel

for different initial concentrations. This suggests that the second phase may be nearly pseudo first order with respect to Cl concentration.

These observations led us to the following simplified model for the reactions. We assumed that there were two reactions consuming FRC, a fast reaction, which is essentially complete in less than 1 min, and a slower reaction involving a moderate excess of fulvic acid reaction sites. The idea that the first reaction proceeds to completion quickly explains the observation that similar amounts of chlorine are consumed in the "fast" part of the curve as initial chlorine concentrations are varied, at a particular carbon concentration. On the basis of this assumption, one might express number of reaction sites for this fast reaction as a function of the amount of chlorine consumed initially. The assumption that the slower reaction proceeds in a moderate excess of fulvic acid sites is consistent with the pseudo-first-order behavior of some of the reactions between 1 and 5 min.

We represented these reactions with the following empirical rate expression:

$$dCl/dt = k_1[Cl][F_1] + k_2[Cl][F_2] \quad (1)$$

where dCl/dt is the rate of FRC disappearance, [Cl] is the FRC concentration, k_1 and k_2 are rate constants for the fast and slow reactions, respectively, and $[F_1]$ and $[F_2]$ are the concentrations of sites on the fulvic acids for the fast and slow reactions, respectively.

Kinetic analysis of this reaction scheme presents special problems. Unlike most situations where the initial molar concentrations of the reactants are known, we have no a priori way of estimating the concentrations of reacting sites of the fulvic acid. The total concentrations of reactive sites were simply related to the total molar carbon concentration by the following equations:

$$[F_1] = X_1[C] \quad (2)$$

$$[F_2] = X_2[C] \quad (3)$$

where X_1 and X_2 are the moles of reaction sites per mole of carbon. The parameters X_1 and X_2 were initially estimated indirectly from the amount of chlorine consumed during various parts of the reaction as a function of carbon concentration.

We estimated the parameter X_1 by extrapolating the trends from 1 to 5 min back to time zero, continuing the slight curvilinear trends as closely as possible. The difference between the initial FRC concentration and the extrapolated value was considered a rough initial estimate of F_1 or the amount of FRC consumed by the completed fast reaction. These values were plotted as a function of [C] to give estimates of X_1. For example, the estimate of F_1 varied from 4.9 to 5.7 μM for 3.68 mg/L C.

The parameter X_2 was estimated by considering the amount of chlorine consumed in 5 min minus that consumed by the first reaction. The molarity of sites for the second reaction we considered to be at least in 5-fold excess, as a first approximation, to yield the pseudo-first-order behavior observed bewteen 1 and 5 min.

The rate constant k_1 was estimated by noting the time necessary for a consumption of FRC corresponding to $^1/_2$ F_1, i.e., the half-life with respect to F_1. The half-life with respect to one component may be derived from the second-order integrated equation (23) and

$$k_1 = \frac{1}{b_0 - a_0} \ln \left[\frac{a_0}{b_0} \left(\frac{(2b_0) - 1}{a_0} \right) \right] \frac{1}{t_{1/2}} \quad (4)$$

where $t_{1/2}$ is the time when half of $[F_1]$ remains, b_0 is the initial FRC concentration, and a_0 is the initial $[F_1]$.

Table I. Combined Residual Chlorine Present in Chlorinated University Lake Fulvic Acid Solutions at 30 s with 20 μM Cl Added Initially

mg of C/L	CRC, μM
1.84	1.3
3.68	1.7
5.52	2.2
9.20	3.9

The rate constant k_2 was estimated by drawing tangents to the extrapolated lines for the long-term reaction at time zero and thus estimating the initial rate. Then

$$dCl'/dt = k_2[Cl]_0[F_2]_0 \quad (5)$$

where dCl'/dt is the initial rate of the slow reaction and the other concentrations are the initial concentrations.

From the above initial estimates, the best values for k_1, k_2, X_1, and X_2 were found by iterative simulation. Various combinations of values were tried until simulations yielded good fits for all sets of initial conditions. The following values were found: $k_1 = 6.3 \times 10^{-3}$ M^{-1} s^{-1}; $X_1 = 0.020$ M sites/M C; $k_2 = 4.2 \times 10^{-5}$ M^{-1} s^{-1}; $X_2 = 0.078$ M sites/M C.

The values for X_1 and X_2 meant that, for example, the concentrations $[F_1]$ and $[F_2]$ for the 9.2 mg of C/L experiments were 15 and 60 μM, respectively.

The curves generated by the above model are shown as solid lines in Figures 1 and 2. In most cases, the model fit the data reasonably well across the range of initial chlorine and carbon concentrations. The fit of data for 10 μM initial chlorine (Figure 2) does not appear as good as other data, but the log scale tends to magnify those differences, and analytical variability was higher at concentrations below 5 μM.

Small amounts of combined residual chlorine (CRC) were formed in all the fulvic acid solutions (Table I). The CRC appeared to have been formed before the first analysis (2 s) and usually remained approximately constant throughout the 5-min periods. The amounts formed were greater in higher concentrations of fulvic acid but was not always in exact proportion to the concentrations of fulvic acids. No CRC was formed in solutions containing only buffer and no fulvic acid. The University Lake and Black Lake fulvic stocks contained no ammonia, so we concluded that the CRC was formed from the nitrogen compounds in the purified fulvic acids. If the moles of CRC formed are taken as an estimate of the moles of N which reacted, then only about 13–20% of the N reacted with the chlorine in the Black Lake isolated fulvic acid samples to produce chloramines.

The humics from the Cape Fear River were isolated but not as purified as were the other fulvic acid samples, so they contained traces of ammonia in addition to organic N. The 9.2 mg of C/L solutions contained 3.8 μM NH$_3$, for example, which resulted in formation of predictable amounts of CRC.

In modeling the chlorine consumption, the small amount of CRC formed was treated as occurring before the other reactions began. Thus, the initial FRC concentrations were calculated as the FRC added minus the CRC present at the first analyses. We suspect that that assumption was an oversimplification but served our purposes for an empirical model.
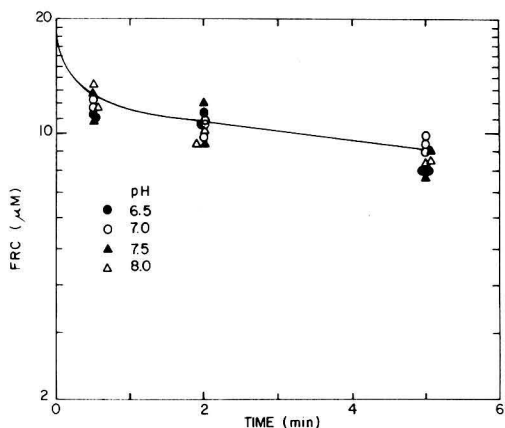
The rates of chlorine consumption by humic substances from three diverse sources were quite similar (Figure 3). Comparison of the three sources was made on the basis of chlorine consumed (FRC added, minus TRC) because of small differences in the amount of CRC initially formed between the different sources. The comparisons were
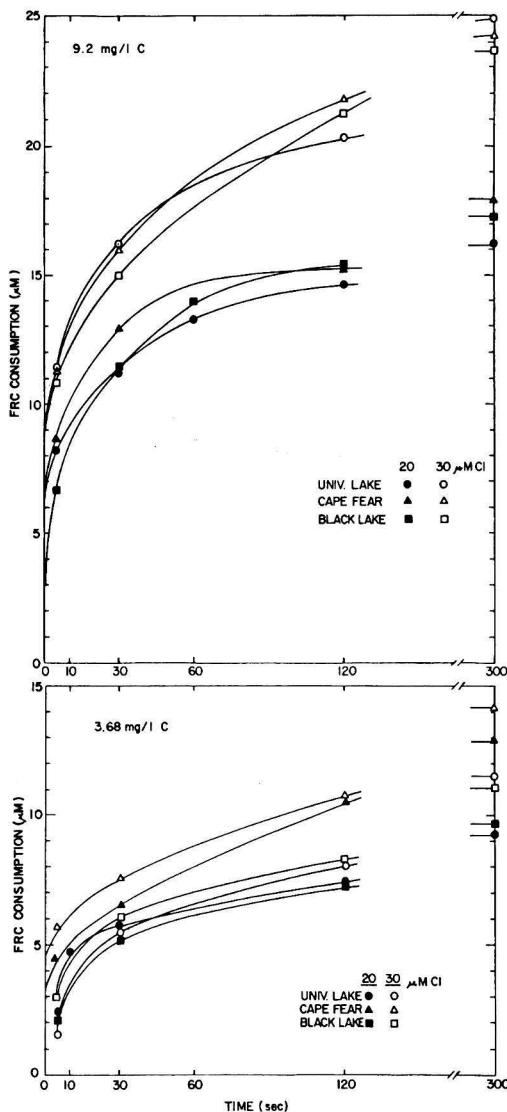
Figure 3. Comparison of rates of chlorine (FRC) consumption by fulvic acid from different sources. The solid and open points represent two levels of chlorine added initially. The lines were drawn through the data by eye.

Figure 4. Rate of chlorine (FRC) consumption by 3.68 mg of C/L of fulvic acid as a function of pH; 20 μM chlorine was added initially. The solid line represents the model prediction for pH 7.

made at two initial humic substance concentrations and at two initial FRC concentrations. At the 3.68 mg of C/L concentration, the Cape Fear chlorine consumption differed somewhat from that of the other samples; however, the differences were not great. The similarity of chlorine consumption rates of samples from different locations supports the general applicability of the model used here.

The similarities in the chlorine consumption rates are surprising considering the very different ecosystems from which the substances were isolated. Fulvic acids are frequently regarded as a heterogeneous group of complex structures. However, Liao et al. (22) found that the oxidation breakdown products of fulvic and humic acids from Black Lake and Lake Drummond were qualitatively similar. The similarity of the initial chlorine consumption rates observed in this study may reflect some basic structural properties common to fulvic acids.

The model using two competitive second-order reactions to describe the short-term rates of FRC consumption by fulvic acids was intended as an empirical model to predict FRC concentrations. Because of the complexity of the proposed structures of fulvic acids, it is more likely that there are a number of types of sites with different reaction rates. However, the fit of the model over a range of concentrations of both reactants may indicate some degree of reality to the model. It seems clear that there are at least two discrete groups of reactions; the fast and slow reactions. A portion of the sites of the fast reactions could be much faster without being detected in the data; however, the exhaustion of that group of sites seems obvious. In reaction carried to longer times by using higher concentrations of chlorine added, the curves plotted on a semilog graph continue to decline in slope. This trend is represented well by the model for the slow reaction, but we suspect that it may be due to a number of reactions with gradually declining rate constants.

The long-term reactions resulting in ring cleavage and trihalomethane production would undoubtedly be subject to quite different kinetics. The number of sites for the fast reactions, as represented in the model, corresponded to one site per 51 carbon atoms. These hypothetical sites were relatively rare. It is possible that some of the N in the fulvic acids could react with Cl and not appear as CRC in the analyses. The number of sites for the slow reaction was about one site per 12 or 13 carbon atoms. The interpretation of the parameters used in the model, though, must only be speculation.

A set of experiments were done to determine the FRC consumption rates as a function of pH (Figure 4). There was little consistent difference in the rates in the pH range 6.5–8.0. The absence of large differences in rate as a function of pH is surprising since the proportion of $HOCl/OCl^-$ varies greatly in that range. The HOCl species is likely to be the reactive form (24). The decrease in the proportion of the chlorine in the HOCl form at higher pH may be offset by an increase in the reactivity of the fulvic acid sites.

Simulations. To test the application of the kinetic equations for fulvic acid chlorine consumption in a natural water situation, we integrated the equations into a larger model containing kinetic equations for chlorination of NH_3 and organic N. The rates of chlorine consumption were
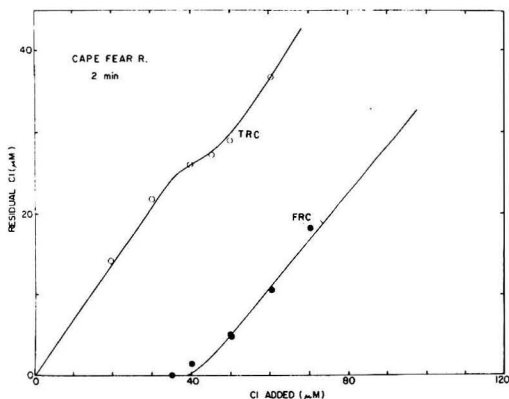
Figure 5. Simulated (lines) vs. measured (data points) residual Cl after 2 min as a function of chlorine added initially for the Cape Fear River water sample (pH 6.9, 25 °C).

measured in a sample of Cape Fear River water and were compared to a simulation by using the kinetic model. The kinetic model is discussed thoroughly elsewhere (25). It incorporated the Morris–Wei model of chlorine–ammonia reactions (7) as modified by Saunier and Selleck (8). It used the reactions of glycylglycine as a model for the reactions of the chloramine-forming organic N compounds. We used rate constants for the formation of (N-chloroglycyl)glycine and (N,N-dichloroglycyl)glycine from Margerum et al. (26), and for the decay of (N,N-dichloroglycyl)glycine from Johnson and Qualls (25). In experiments described elsewhere (25) we found that the organic N which formed chloramines was about 10% of the DON in this sample. An equation for the consumption of NH_2Cl by fulvic acid was also included (25).

We assumed that all of the DOC in the river water sample was fulvic acid. That almost all of the DOC were humic substances was supported by the UV absorbance at 254 nm of the sample and the isolated humic substances (at the same pH and ionic strength). The temperature was 25 °C for the experiment and simulation.

The experiment and simulation showed the concentrations of FRC and TRC at 2 min as a function of FRC added. The 2-min period was considered representative of the time between chlorination and contact with the condensers in a once-through cooling system (5). This sort of curve is used to choose an initial chlorine dose sufficient to yield about 3 μM FRC at the condensers, which was about 50 μM Cl added in this case.

The simulation matched the observed data reasonably well (Figure 5). For chlorine doses less than the stoichiometric amount necessary to satisfy the NH_3 demand (20 μM) the amount of CRC (TRC – FRC) formed was dependent largely on the competition between NH_3 and the fulvic acid "fast" reaction sites for the added FRC. Hence, the simulation was quite sensitive in that area to the relative value of the rate constants for those fast reactions. If the experiment with 50 μM chlorine added (which would yield a few micromolar FRC at 2 min) is considered under these model conditions, the relative rate of major FRC consuming reactions in the Cape Fear River water was (from fastest to slowest) (1) organic chloramine formation, (2) NH_2Cl formation, (3) consumption by the fast fulvic reaction, (4) $NHCl_2$ formation, and (5) consumption by the "slower" fulvic acid reaction. However, for the same initial conditions, at 2 min, the total FRC consumption was due mainly to (largest to smallest): (1)

NH_2Cl formation, (2) the fast consumption by fulvic acid (only slightly less than (1)), (3) organic chloramine formation, and (4) the slower consumption by fulvic acid. The reduction in TRC was due mostly to consumption by fulvic acid. While these categories represent the simplified assumptions of the model, the role of the fulvic acid reactions are obvious. The magnitude of these various reactions will vary with the concentration of the chlorine-consuming substances found in other waters. However, we preformed similar experiments and simulations of samples from the relatively unpolluted Tennessee River and the moderately polluted Holston River (TN). Both the order of the relative rates and total FRC consumption by the simulated reactions were the same as for the Cape Fear experiments (25).

The application of the model presented for chlorination of fulvic acids requires data on the concentration of fulvic acid in terms of carbon content. While it may be true in many waters that most of the DOC is humic substances, it would be more accurate to have some surrogate measure to estimate the proportion of the DOC that is humic substances. Either UV absorbance or color might provide an approximate measurement.

Because we have been primarily concerned with chlorination of cooling waters, we have restricted ourselves to the reactions occurring during the first 5 min after chlorine addition. For applications in which the longer term reactions are of concern, such as municipal water chlorination, more research is needed. While the two additive terms of our model may suffice to predict the longer term reactions, a third term could easily be calculated when the other terms are known. Nevertheless, the critical competition with NH_3 and organic N largely occurs within the first 5 min. Other research which requires attention is the similarity of the reactions of chlorine with humic acid to those with fulvic acid, the reaction of NH_2Cl and fulvic acid, and determination of the temperature coefficients for these reactions.

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