Pacific Northwest Laboratory Annual Report for 1984 to the DOE Office of Energy Research

**Part 2 Ecological Sciences
February 1985**

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

**Pacific Northwest Laboratory
Operated for the U.S. Department of Energy
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PACIFIC NORTHWEST LABORATORY
operated by
BATTELLE
for the
UNITED STATES DEPARTMENT OF ENERGY
under Contract DE-AC06-76RLO 1830

Printed in the United States of America
Available from
National Technical Information Service
United States Department of Commerce
5285 Port Royal Road
Springfield, Virginia 22161

NTIS Price Codes
Microfiche A01

Printed Copy

Pages	Price Codes
001-025	A02
026-050	A03
051-075	A04
076-100	A05
101-125	A06
126-150	A07
151-175	A08
176-200	A09
201-225	A010
226-250	A011
251-275	A012
276-300	A013

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B. E. Vaughan and Staff Members
of Pacific Northwest Laboratory

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Pacific Northwest Laboratory
Richland, Washington 99352

PREFACE

This 1984 annual report from Pacific Northwest Laboratory (PNL) to the Department of Energy (DOE) describes research in environment, health, and safety conducted during fiscal year 1984. The report again consists of five parts, each in a separate volume.

The five parts of the report are oriented to particular segments of our program. Parts 1 to 4 report on research performed for the DOE Office of Health and Environmental Research in the Office of Energy Research. Part 5 reports progress on all research performed for the Assistant Secretary for Policy, Safety, and Environment. In some instances, the volumes report on research funded by other DOE components or by other governmental entities under interagency agreements. Each part consists of project reports authored by scientists from several PNL research departments, reflecting the multidisciplinary nature of the research effort.

The parts of the 1984 Annual Report are:

Part 1: Biomedical Sciences Program Manager - J. F. Park	D. L. Felton, Report Coordinator and Editor
Part 2: Environmental Sciences Program Manager - B. E. Vaughan	B. E. Vaughan, Report Coordinator C. M. Novich, Editor
Part 3: Atmospheric Sciences Program Manager - C. E. Elderkin	N. S. Laulainen, Report Coordinator E. L. Owczarski, Editor
Part 4: Physical Sciences Program Manager - J. M. Nielsen	R. M. Garcia, Report Coordinator J. E. Danko, Editor
Part 5: Overview and Assessment Program Manager - W. A. Glass	R. W. Baalman, Report Coordinator and Editor

Activities of the scientists whose work is described in this annual report are broader in scope than the articles indicate. PNL staff have responded to numerous requests from DOE during the year for planning, for service on various task groups, and for special assistance.

Credit for this annual report goes to many scientists who performed the research and wrote the individual project reports, to the program managers who directed the

research and coordinated the technical progress reports, to the editors who edited the individual project reports and assembled the five parts, and to Ray Baalman, editor in chief, who directed the total effort.

W. J. Bair, Manager
S. Marks, Associate Manager
Environment, Health and Safety
Research Program

Previous reports in this series:

Annual Report for

1951	W-25021, HW-25709
1952	HW-27814, HW-28636
1953	HW-30437, HW-30464
1954	HW-30306, HW-33128, HW-35905, HW-35917
1955	HW-39558, HW-41315, HW-41500
1956	HW-47500
1957	HW-53500
1958	HW-59500
1959	HW-63824, HW-65500
1960	HW-69500, HW-70050
1961	HW-72500, HW-73337
1962	HW-76000, HW-77609
1963	HW-80500, HW-81746
1964	BNWL-122
1965	BNWL-280; BNWL-235, Vol. 1-4; BNWL-361
1966	BNWL-480, Vol. 1; BNWL-481, Vol. 2, Pt. 1-4
1967	BNWL-714, Vol. 1; BNWL-715, Vol. 2, Pt. 1-4
1968	BNWL-1050, Vol. 1, Pt. 1-2; BNWL-1051, Vol. 2, Pt. 1-3
1969	BNWL-1306, Vol. 1, Pt. 1-2; BNWL-1307, Vol. 2, Pt. 1-3
1970	BNWL-1550, Vol. 1, Pt. 1-2; BNWL-1551, Vol. 2, Pt. 1-2
1971	BNWL-1650, Vol. 1, Pt. 1-2; BNWL-1651, Vol. 2, Pt. 1-2
1972	BNWL-1750, Vol. 1, Pt. 1-2; BNWL-1751, Vol. 2, Pt. 1-2
1973	BNWL-1850, Pt. 1-4
1974	BNWL-1950, Pt. 1-4
1975	BNWL-2000, Pt. 1-4
1976	BNWL-2100, Pt. 1-5
1977	PNL-2500, Pt. 1-5
1978	PNL-2850, Pt. 1-5
1979	PNL-3300, Pt. 1-5
1980	PNL-3700, Pt. 1-5
1981	PNL-4100, Pt. 1-5
1982	PNL-4600, Pt. 1-5
1983	PNL-5000, Pt. 1-5

FOREWORD

Our Annual Report for 1984 has followed the previous report in highlighting major developments in five prime research areas funded by the Ecological Sciences Division of the Office of Energy Research. In their entirety, these research areas contain 19 individual projects (Field Task Proposal/Agreements, Form 5120.2), each covering specialized tasks. Rather than reproduce detailed information that can be found in Forms 5120.2 or in published reports, we have provided here a concise annual review of the overall program. We hope this will prove to be more useful to field directors, agency office heads, oversight committees, and others who will not have time for details principally of interest to research managers.

The following research areas are highlighted:

- Western Arid and Semi-Arid Ecosystems
- Marine Sciences
- Mobilization, Fate and Effects of Chemical Wastes
- Radionuclide Fate and Effects
- Statistical and Quantitative Research.

The research described in this report was funded through DOE's Environmental Research Program (HA-02-03) from three budget sources:

HA-02-03-01	Identify Transport & Conversions of Energy-Related Pollutants in the Environment
HA-02-03-02	Biological Responses
HA-02-03-03	Research Logistics, Operations and Support

For your convenience, a listing of interagency services agreements for PNL staff is provided at the end of this report. A separate annual report* should be consulted for highlights of Interagency/Contract research carried out by PNL staff.

Burton E. Vaughan
Subprogram Manager,
Ecological Sciences

*C. M. Novich and B. E. Vaughan, Editors. *Environmental Sciences Department, Annual Report for 1984 on Interagency and Other Research*. Battelle, Pacific Northwest Laboratories, Richland, Washington (in preparation).

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Research
Highlights

INTRODUCTION

Environmental research at PNL is designed to evaluate systematically the problems that energy development activities pose to the environment and to identify areas in which energy developments must ultimately meet the intent of the National Environmental Policy Act (NEPA) and various health statutes.

The research program is based on coordinated studies of two kinds: sophisticated laboratory experiments to investigate the intricate mechanisms of an ecosystem, and field studies involving freshwater, marine, and forest or semi-arid terrestrial environments. Special areas at the Hanford Site have been set aside and especially equipped for field studies, marine studies are done at Battelle's Marine Research Laboratory at Sequim, on the Olympic Peninsula. Work is also carried out in other parts of the United States to deal with existing pollutants at problem locations.

Because of great numbers of chemical and radiochemical contaminants that enter the environment, research effort is focused on investigating environmental processes that are generic in nature, i.e., that simultaneously affect several constituents. For example, the Laboratory is investigating the soil microbial processes by which trace metals are converted to organic complexes of lesser (or sometimes greater) toxicity. These studies help us understand the mechanisms by which numerous other chemical by-products are formed in soil systems, how radionuclides interact with the environment, and how co-disposed organic constituents may alter regional hydrology.

Research is currently being conducted in five areas: the terrestrial ecology of semi-arid sites; marine sciences; radionuclide fate and effects; waste mobilization, fate and effects; and theoretical (statistical) research on environmental sampling. The environmental laboratories are uniquely equipped for both indoor and outdoor studies at either the whole-organism level or the biochemical level.

The research knowledge and experience gained in these studies can be applied to work for other agencies of DOE and the Federal government. PNL has made considerable progress in evaluating the potential impacts of energy policy on the environment, including such national concerns as the acidification of western lakes and forests, and effects of toxic substances in food chains on human health. Some of these applications are listed in the Interagency Research section at the end of this report.

Western Arid and Semi-Arid Ecosystems

The purpose of the research conducted as part of the western Semi-Arid Ecosystems program is to understand how arid and semi-arid landscapes respond to a wide range of natural and human-related disturbances. The central theme of this research is to understand the basic functional processes and controlling mechanisms inherent in natural systems. We expect that changes associated with environmental stresses can be anticipated and the biological recovery process predicted once these basic relationships are understood. This knowledge will provide a strong theoretical basis for future decision-making policies, environmental assessments, and resource management decisions, not only at Hanford but also for habitats throughout the semi-arid region of the western United States.

The research base established through this program is also available to extramural scientists. Various university faculty and graduate students continue to participate by conducting research of interest to the Department of Energy as part of the National Environmental Research Park (NERP) Program. Most of this research has focused on establishing a data set to characterize the various biotic communities, successional processes, transfer pathways, and climatological patterns at Hanford.

NATIONAL ENVIRONMENTAL RESEARCH PARK

About one-third of the land area of the contiguous United States is grasslands. The arid and semi-arid grasslands of western North America are shown in Figure 1. The shrub-steppe is among the largest of the grassland regions in North America. It extends from western Wyoming into northern Utah and southern Idaho, through eastern Oregon, into eastern Washington, and south into northern California and Nevada.

Much of the current information concerning the structure and functioning of shrub-steppe ecosystems in the Pacific Northwest is the result of research conducted on the Arid Land Ecology (ALE) Reserve located on the Hanford NERP in south-central Washington. The 120-square-mile ALE Reserve was established in 1968 to provide protected areas for long-term ecological research. Since then, PNL has worked closely with university and college personnel. We have cosponsored graduate dissertations and have encouraged participation in NERP programs by faculty members and consultants from industry and state and Federal agencies.

The Hanford NERP Serves as a Focal Point for University and Government Research

The Hanford NERP serves as a point of contact between the various research components of PNL and the environmental surveillance programs conducted on the Hanford Site with regard to land, water and wildlife habitats. The NERP is also a point of contact between

FIGURE 1. One of the few places where the shrub-steppe region is being studied in detail is the Arid Land Ecology (ALE) Reserve in south-central Washington. The shrub-steppe is among the largest semi-arid grassland regions in North America and is the site of major energy developments.

state and federal agencies that have responsibilities for research and management of wildlife resources at the state and regional levels.

The Hanford NERP provides a mechanism for University researchers to gain access to the ALE Site to conduct research funded by sponsors other than DOE. In 1984, two university researchers had such access to the ALE Site as guest users. The studies are funded by Washington State University and the National Science Foundation and are concerned with the response of native plants to ash-fall from Mt. St. Helens and the response of soil-dwelling algae to wildfire.

Graduate student research on the Hanford NERP in 1983-84 centered upon Hanford Site wildlife resources, especially the resident populations of the American elk (Cervus elaphus), coyote (Canis latrans), the western Canada goose (Branta canadensis moffitti) and the great blue heron (Ardea herodias). Student research was also initiated on the nitrogen-fixing, leguminous plant Lupinus leucophyllous.

Three students completed their requirements for the Master of Science degree and graduated in 1984. At the present time six students are onsite, representing Washington State University, the University of Washington, Oregon State University, the University of Idaho and Western Washington University.

A brief synopsis of the on-going student research is summarized in the following text.

Radiotransmitters Facilitate Research on Movement and Abundance of Wildlife

Brood ecology studies of the western Canada goose were conducted in the spring and summer of 1983 and 1984. Radiotransmitters were attached to parent geese as a way to monitor their daily movements along a 40-kilometer section of the Columbia River. Data sets are in the analysis stage. Generally the data indicate that predation upon goslings is severe. Of 23 broods fitted with radiotransmitters in 1984, nine were entirely lost. Other broods had at least some mortality. Information on riverine habitat use and other habitat requirements of broods has also been obtained.

Detailed studies of the American elk on the ALE Site were conducted in 1983 and 1984. These show that the herd is increasing. Eleven young animals were added to the herd in 1983 and 13 in 1984. The total population is 55 animals. Studies (Figure 2) indicate that elk seldom leave the ALE Reserve. In the few instances when individual elk did leave, the travel direction was to the northwest. To date, all animals that have left have returned to the ALE site, where peak resident population levels coincide with the onset of the autumn rutting season.

FIGURE 2. This elk has been fitted with a radiotransmitter so that scientists can monitor its activities on the Arid Lands Ecology Reserve. Studies have indicated that elk rarely leave the Reserve.

Home ranges of Hanford elk have been determined using eight animals fitted with radio-transmitters. These data show much larger ranges than those of elk inhabiting forested regions of the United States. This is likely a behavioral response to the open, treeless habitat of the ALE site.

A massive wildfire in mid-August 1984 burned approximately 80% of the ALE Reserve. However, at the time of the fire the elk herd was utilizing the 20 percent of the reserve that did not burn. There were no elk mortalities due to the fire or to the intense fire suppression activities that accompanied it.

The resident, breeding population of elk on the ALE Site represents the only known occurrence of elk to inhabit the hot, dry, treeless shrub-steppe region of Washington in nearly 200 years of recorded history. Studies are in progress utilizing radiotransmitted heart rate pulses and body and subcutaneous temperature sensors on two males to determine their physiological responses to an environment characterized by intense summer drought, dessicated forages, and the virtual absence of a concealing vegetative cover. Data are also being collected to characterize the microclimates associated with draws and ravines which act as sites of thermal and concealing cover.

An ecological study of coyote populations on the ALE Reserve was initiated in 1984. To date, four coyotes have been captured, fitted with radiotransmitters and released. Tracer amounts of specific radionuclides were injected into the blood stream of each animal so that the feces could be collected, analyzed radiochemically and used as a way to obtain a quantitative estimate of the size of the coyote population on the ALE site.

The great blue heron is a large, fish-eating bird that nests on the Hanford Site. Graduate student research is centered on the use of heron colonies as biological indicators of organic and trace metal contaminants in the environment.

Botanical Studies in the Hanford NERP Provide Information on the Behavior of Nitrogen-Fixing Plants in Sagebrush-Bunchgrass Communities

A study was initiated to investigate chemical resource allocations in populations of blue-

flowering lupines. Study plots were established in the spring of 1984. Density counts were made, individual plants were permanently marked for future identification, and seeds of individual plants were collected for experimental purposes and chemical analyses. This study is expected to extend over a 2-year period and contribute to the re-directed OHER-sponsored terrestrial ecology programs aimed at obtaining a basic science understanding of plant, animals, geochemical, and hydrologic processes in arid and semi-arid regions.

DYNAMICS OF ARID LAND ECOSYSTEMS

A major new research effort at ALE, a well-protected part of the Hanford NERP, is designed to further scientific understanding of globally important ecosystems. The goal of this program, "Dynamics of Arid and Semi-Arid Ecosystems," is to quantify the responses of semi-arid ecosystems to both natural and man-related disturbances. Earlier studies at ALE have provided a substantial data base for characterizing major biotic communities, successional processes, transfer pathways, and the role of remnant shrub-steppe habitats as refuge for native biota. Some examples are provided below.

A Model Helps Researchers Study Water Balance

Soil water accretion and depletion patterns are central to ecosystem functioning, since the availability of free water largely governs the distribution of biota in time and space. We used a simulation model to evaluate changes in soil, plant, and microclimate on the water balance in a cheatgrass community. A simulation of soil water dynamics over a 13-year period is shown in Figure 3. The general trend of changes in soil water was adequately simulated for most years, but the detailed changes in soil water dynamics were not accurately portrayed for all years. Further research will address the discrepancies between measured and modeled water balance. It is likely that inadequate knowledge exists concerning the hydraulic properties of the soil, micro-meteorological patterns, or the influence of plant transpiration on water dynamics and water balance.

FIGURE 3. A model is being tested to help identify the environmental conditions that affect water balance in a semi-arid ecosystem. Further work is underway to determine variance between simulated and measured values.

Precipitation is Only One of Many Factors Affecting Plant Production

Primary production provides the chemical energy needed to run ecosystems. The dynamics of primary production can vary among plant community types and, hence, can affect ecosystem functioning (Figure 4). The herbaceous shoot production in a sagebrush-bunchgrass community in relatively undisturbed condition is compared to a cheatgrass community that has occupied an abandoned cultivated field since 1943 (Figure 5). Peak shoot production of the sagebrush-bunchgrass community averaged 70 g/m^2 , while the cheatgrass community averaged 225 g/m^2 . Yearly shoot production in the cheatgrass community was more variable than in the sagebrush-bunchgrass community. During 1972, shoot production peaked in April rather than May, and was the lowest of the four years, even though this was not the year of lowest precipitation. More shoot biomass was produced the following year, the year of lowest precipitation, than during the 1972 season. This suggests that plant growth responds to factors other than growing-season precipitation

alone. It is likely that temperature and the availability of essential mineral nutrients, nitrogen and phosphorus are influencing shoot production. Future research will focus on quantifying the abiotic and biotic factors controlling vegetative production in the shrub-steppe region.

Vegetative Structure Influences Animal Capture Studies

Vegetation species composition and structure are important factors in determining animal abundance for different portions of shrub-steppe landscapes. Results of a two-year study showed fewer small-mammal captures in cheatgrass communities as compared to sagebrush-bunchgrass communities (Figure 6). The vegetational structure of cheatgrass communities is a short-statured sward comprised of stems of cheatgrass that can be as dense as $10,000 \text{ per m}^2$. In contrast, sagebrush-bunchgrass communities were comprised of widely separated grass tussocks, $1-4 \text{ per m}^2$, interspersed between even more widely spaced sagebrush shrubs. Results from this study showed that the probabilities of animal

FIGURE 4. This figure contrasts the organic productivity of grasses in a semi-arid ecosystem. Researchers have observed that cheatgrass, an introduced plant, is much more productive than the native grasses.

FIGURE 5. Growth of cheatgrass and bunchgrasses decelerates in June as soil water decreases. However, factors other than precipitation also influence plant growth, and these are being studied.

FIGURE 6. Vegetative structure influences trap capture probabilities for small mammals. This has important implications for sampling techniques in environmental studies.

capture differed between the sagebrush-bunchgrass and cheatgrass communities, indicating that vegetative structure may be influencing trap captures of small mammals. Capture-recapture probabilities, then, may not only vary between plant communities, but they may also change from year to year depending on the density and amount of herbage produced, and on changes in species composition. Capture probabilities could also vary seasonally from the initiation of plant growth in the fall to maturation of seeds in the spring. This points out the need for additional information concerning the influence of vegetation on estimates of animal abundance between different plant communities.

ACKNOWLEDGMENTS

Principal Investigators for these respective projects are L. E. Rogers (FTPA 80618/000618) and W. H. Rickard (FTPA 81261/001261).

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Books, G. G. "Anion Interactions with Mid-Columbia River Water Level Fluctuations." Northwest Sci. (accepted).

Hinds, N. R. "Ecological Perspectives on Land Use History: The Arid Lands Ecology Reserve (ALE)." Northwest Sci. (submitted).

Hinds, W. T. "How to Create Good Ecological Monitoring Designs." Environ. Conser. (submitted).

McCorquodale, S. M. "Archeological Evidence of Elk in the Columbia Basin." Northwest Sci. (accepted).

McShane, M. C., and R. H. Sauer. "A Comparison of the Effects of Burning and Clipping on Bluebunch Wheatgrass." Northwest Sci. (accepted).

Rickard, W. H. "Shoot Production and Mineral Nutrient Assimilation in Cheatgrass Communities on the Arid Lands Ecology Site." Northwest Sci. (accepted).

Rickard, W. H. "Impact of Cattle Grazing on a Relatively Undisturbed Sagebrush-Bunchgrass Community." Northwest Sci. (accepted).

Rickard, W. H., and R. E. Fitzner. "Mineral Content of Canada Goose Eggs and Their Implications for Wildlife Surveillance." Northwest Sci. (accepted).

Rickard, W. H., and D. G. Watson. "Four Decades of Man-Induced Perturbations along the Mid-Columbia River: Impacts on Fish and Wildlife." Environ. Conser. (submitted).

THESES COMPLETED

Books, G. G. 1984. Anion Community Interactions with Columbia River Water Level Fluctuations. M.S. Thesis. Department of Environmental Sciences, Washington State University, Pullman (W. H. Rickard onsite advisor).

Jacques, I. D. 1984. Self-Revegetation of a Sagebrush-Bunchgrass Community After Surface Blading by Bulldozer. M.S. Thesis. Program in General Biology, Washington State University, Pullman (W. H. Rickard onsite advisor).

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Marine Sciences

The continued development of energy facilities has resulted in the addition of increasing amounts of contaminants to coastal waters. We are studying the entrance, behavior, and fate of various contaminants in order to determine their effects on coastal marine ecosystems. Through field and laboratory work, we have examined the ways in which trace metals and other contaminants enter the ecosystem and their effects on marine animals, including those in the microlayer at the sea surface and those in the benthic layer (bottom dwellers). One result of our studies is an increased ability to predict, and subsequently to minimize, the environmental consequences of current and future energy production activities in coastal areas.

TOXICITY OF SEA-SURFACE CONTAMINANTS

The objective of this program is to understand and predict the fate and biological effects of materials in the sea-surface microlayer. Biological processes in the sea surface are important because energy-generated contaminants concentrate at the sea surface and inhibit important biological processes such as photosynthesis and the development and productivity of marine organisms.

Biological Processes in the Sea Surface Are Important

The sea surface covers 71% of the Earth's surface and affects the global cycling of gases, particulate materials, and contaminants between the oceans and the atmosphere. Our research, and that of others, indicates that the sea-surface microlayer (the upper 50 micrometers of the ocean's surface) is typically a site of intense biological activity. The biological communities, or neuston, that inhabit the sea surface influence the chemistry of the interface and, hence, may influence interactions between air and sea. Research this year further confirmed and documented that biological activity in the microlayer affects sulfur dioxide production, bacterial recycling, and carbon dioxide reduction.

Recent evidence indicates that a natural source of sulfur dioxide to the atmosphere arises from dimethyl sulfide produced by marine phytoplankton. A particularly intense source appears to derive from phytoneuston (microalgae) living in the surface microlayer. We hope to examine this theory in future laboratory microcosm experiments.

The microlayer typically supports dense populations of bacteria, which help recycle and

biodegrade organic particles that float and collect at the sea surface. Our sampling program in Puget Sound indicates that bacterial densities in the top 20 micrometers of the Sound are 1000 to 100,000 times greater than in bulk seawater.

Research has demonstrated that of the approximately 5 billion metric tons of carbon dioxide released to the atmosphere each year from the combustion of fossil fuel, about 1 billion tons enter the oceans through the sea-surface microlayer. Our studies have shown, further, that the unique physical, chemical and biological characteristics of the microlayer could significantly alter or control the flux of atmospheric carbon dioxide into ocean surface waters. Evidence suggests that photosynthesis of phytoplankton is inhibited by high light energy or other forms of stress and may result in the increased release of extracellular carbon.

The Sea-Surface Microlayer Serves as a Habitat for an Abundant and Distinct Community of Organisms

Many commercially important fish and shellfish have neustonic eggs and larval stages that occur in high densities in the upper few centimeters of the water. For example, recent studies show that 90% to 99% of all blue crab larvae and 81% of all lobster larvae, as well as a substantial amount of Dungeness crab larvae at different sites, are found in the upper few centimeters of the water surface. In the Oregon shelf area, more than 40 species of larval ichthyoneuston were collected with a surface neuston net. High densities of larval Pacific sand lance (*Ammodytes hexapterus*) (an important prey species for salmon), English sole (*Parophrys vetulus*), and sand sole (*Psettichthys melanostictus*) were found in the surface layer.

Sea-surface microlayer contaminants could reduce the recruitment of several such fish, which depend upon the microlayer habitat during development from egg to embryo and from early larval to juvenile stages.

No studies in Puget Sound have specifically assessed the importance of the sea-surface microlayer to fisheries recruitment. In a NOAA study in the Strait of Juan de Fuca, however, samples were collected with a surface "pleuston" (actually neuston) net. The report emphasized the importance of the sea surface to fishery recruitment. In April, the total density of fish eggs was 1666 times greater in the microlayer than in the subsurface water. In fact, 38% of all the eggs in the upper 50 meters were restricted to the microlayer. The report* stated:

"It is evident from the large number of eggs captured by the pleuston sampler that many fish eggs aggregate in the extreme upper water layer.... Hexagrammos spp. (greenlings) were the most numerous larvae taken in the pleuston sampler. They were only rarely seen in any oblique net samples.... Fish eggs and larvae are particularly sensitive to oil pollution because many forms aggregate at the surface.... The recruitment of such species might be seriously disrupted by a single coincidental pollution episode...."

The Sea-Surface Microlayer is a Site of Intense Productivity (Carbon Reduction)

Microcosm tank samples of microlayer and bulk water were collected from Sequim Bay (Figure 1). Quadruplicate samples were collected from two field sites, an area of clean water and a section with a visible slick about 100 meters from the clean area. Samples from both slick and non-slick areas were exposed to full sunlight, reduced sunlight, and darkness.

Visual observations and use of a technique to measure layer thickness suggested major dif-

*Chester, A. J., D. M. Damkaer, D. B. Dey, G. A. Heron, J. D. Larrance. 1980. Plankton of the Strait of Juan de Fuca 1975 to 1977. Energy/Environment Program report, EPA-600/F-80-032, available from National Technical Information Service, Springfield, Virginia.

FIGURE 1. This apparatus is used to collect samples of the sea-surface microlayer.

ferences in the quantity of the organic materials active in the microlayer of the two field sites. The abundance of microalgal taxa differed greatly between the sea-surface microlayer and the bulk water. At both slick and non-slick sites, total densities of phytoneuston were 37 to 154 times greater than phytoplankton densities. Many species which were present in the microlayer were absent from the bulk water, and vice versa. The phytoneuston community was dominated by microflagellates and small pennate diatoms. These results indicate that the phytoneuston is indeed a distinct community and is qualitatively and quantitatively different from the phytoplankton community.

Under all conditions tested, reduction of inorganic carbon to particulate organic form (microalgal photosynthesis) per unit volume of surface water was greater in the microlayer than in the bulk seawater. Under natural summer solar flux, mean primary production rates (light minus dark bottles) for phytoplankton in the bulk seawater were about 5 to 9 milligrams of carbon per meter per hour (values typical for Puget Sound at this time of year). Ratios of photosynthesis in the microlayer to that in the bulk seawater were 17 in the slick area and 2 in the non-slick area. The higher productivity in the slick area may result from greater concentrations of dissolved inorganic or organic nutrients in such slicks.

Even though the photosynthetic rates were greater in the microlayer than in the bulk water, phytoneuston were, to some extent, photoinhibited by ambient solar flux. Reducing the ambient solar flux by 90% reduced phytoplankton productivity to about 50% of the rate in full sunlight, but increased phytoneuston productivity by 150% to 900%. Thus, under conditions of subdued light and in the absence of photoinhibition (typical winter conditions in the Pacific Northwest), carbon reduction is 30 to 50 times greater in the sea-surface microlayer than in the bulk seawater. This confirms our previous measurements made during winter conditions, which indicated productivities 20 to 150 times greater in the microlayer.

Extracellular Carbon Release is Higher in Phytoneuston than in Phytoplankton

The quantity of extracellular organic carbon released during photosynthesis was determined using techniques developed in earlier work. Microlayer and bulk water samples were collected from laboratory microcosms and preserved in Lugol's iodine and examined microscopically in the laboratory. The densities of individual species were enumerated in replicate subsamples.

The purging method was effective in removing virtually all (99.9%) of the NaH_2CO_3 from the filtrate samples, leaving only dissolved organic carbon. As with particulate carbon, dissolved organic carbon production was much greater in neuston than in plankton (N/P = 20 to 85). Under conditions of either high or low light, approximately 3% to 8% of the

inorganic carbon reduced to particulate organic carbon was released in the form of extracellular dissolved organic carbon. In both neuston and plankton, extracellular carbon release was greater in the dark, when it represented 16% to 65% of the reduced particulate carbon.

In general, neuston productivity (the rate of reduction of inorganic to organic carbon) was considerably higher than plankton productivity, and the release of extracellular dissolved organic carbon increased logarithmically with increasing particulate carbon reduction.

Contaminants Concentrate at the Sea Surface

We have found that in some parts of Puget Sound, microlayers are highly contaminated with a variety of toxic chemicals. We are investigating the significance of this contamination to the reproductive success of valued resource species.

Our previous work has shown that the sea-surface microlayer of Puget Sound contains concentrations of several potentially toxic metals that are 100 to 1000 times greater than those in the bulk water. Further work using capillary gas chromatography has now revealed high concentrations of aromatic hydrocarbons and as-yet-unidentified chlorinated organics in the microlayer of Hylebos Waterway, in Tacoma, Washington. Total aromatic concentrations were more than 2400 times greater in the microlayer than in the bulk seawater (Table 1).

Sea-Surface Contaminants Can Inhibit Reproduction of Fish and Shellfish

Eggs of English sole are less than 1 millimeter in diameter and float at or near the surface. Since microlayer contaminants are generally concentrated in the upper 50 micrometers, at least the upper surface of the eggs would be exposed continuously to such contaminants. Our sampling indicates that high densities of sole eggs float on the surface of Puget Sound throughout the early spring months.

Our studies show that microlayer material is extremely toxic to neustonic fish eggs--much more so than bulk seawater from the same location. In one experiment, microlayer water was collected with a glass plate sampler from

TABLE 1. Concentrations (ng/liter) of aromatic hydrocarbons from Hylebos Waterway were greater in the microlayer than in the subsurface water. The concentration of contaminants in the microlayer can affect the physiology of numerous marine organisms.

Microlayer Dissolved Component	Ratio Microlayer ($<0.5\mu\text{m}$)	Microlayer Particulate	Bulk Water Total	Microlayer/ Total	Bulk Water
Naphthalene	0	0	0	0	∞
Benzothiophene	0	275	275	0	∞
2-Methylnaphthalene	0	1,186	1,186	0	∞
1-Methylnaphthalene	0	1,117	1,117	0	∞
Biphenyl	0	100	100	0	∞
2,6-Dimethylnaphthalene	0	132	132	0	∞
2,3,6-Trimethylnaphthalene	0	0	0	0	0
Fluorene	0	94	94	0	∞
Dibenzothiophene	0	2,040	2,040	0	∞
Phenanthrene	97	5,410	5,507	0	∞
Anthracene	0	372	372	0	∞
1-Methylphenanthrene	143	2,755	2,898	51	57
Fluoranthene	308	32,604	32,912	27	1,219
Pyrene	81	19,720	19,801	0	∞
Chrysene	0	46,593	46,593	0	∞
B(E)pyrene	0	28,285	28,285	0	∞
B(A)pyrene	0	39,619	39,619	0	∞
Perylene	0	11,152	11,152	0	∞
Totals	629	191,454	192,083	78	$>2,461$

Hylebos Waterway in Commencement Bay, an area where the sediments are contaminated with aromatic hydrocarbons, synthetic organics, and metals. Bulk seawater was collected from a depth of 20 centimeters at the same location.

The Hylebos bulk seawater was clear, while the microlayer appeared as dirty brown water full of particles and detritus. Neustonic fish eggs (sand sole) were collected from a clean area (Sequim Bay) and exposed to Sequim Bay seawater, Sequim Bay microlayer, Hylebos seawater, and Hylebos microlayer. Exposures included controls adjusted for salinity differences between the Sequim and Hylebos microlayers.

During 96-hour exposures, larval survival decreased logarithmically with increasing concentrations of the Hylebos microlayer material (Figure 2). In fact, the first examination after 24 hours revealed that all eggs exposed to 100% Hylebos microlayer were dead (i.e., turned white, sank, and did not hatch). Even at dilutions of 1:100 of the Hylebos microlayer with clean seawater, hatching success was reduced and larvae displayed a high incidence of morphological

FIGURE 2. Fish eggs were exposed to water from Hylebos waterway. As the concentration of water from the microlayer increased, so did the mortality rate of the eggs. This is indicative of the extreme toxicity of materials in the microlayer.

abnormalities such as bent spines. Differences in pH and salinity did not account for the observed effects.

METABOLISM OF TRACE METALS IN MARINE ANIMALS

Marine organisms have mechanisms that allow them to store and detoxify metals such as

mercury, cadmium, copper, and zinc. Knowledge of the nature and operation of these mechanisms can be used for assessing the effects of such substances on marine organisms and for developing predictions on the effects of future energy-producing activities.

In previous research, we demonstrated or confirmed the existence of low-molecular-weight, metal-binding proteins in several marine invertebrate species. Work with the marine mussel (*Mytilus edulis*) showed that induction of such proteins was correlated with enhanced tolerance to the toxicity of mercury. Low-level exposures to mercury, cadmium, copper, and zinc resulted in both the apparent induction of these proteins and enhanced mercury tolerance. Efforts during the past year have concentrated on the isolation and purification of the low-molecular-weight, mercury-binding proteins of the marine mussel and the development of an antibody against such substances. Additional work was also conducted on the status of metals in the hydrothermal vent clam (*Calyptogena magnifica*).

Mercury-Binding Proteins of the Marine Mussel Are Complex and Challenging

The low-molecular-weight, metal-binding proteins of the marine mussel represent a novel protein system whose identity has not been definitely established. Several studies from this and other laboratories, however, have been published on physiological correlates or biochemical characterization. Additionally, no specific assay for these proteins currently exists. For these reasons, efforts were undertaken to isolate and purify the mercury-binding proteins of *M. edulis*. Purified protein preparations can be used to characterize and identify the proteins and to develop antibodies for use in highly specific immunoassays.

Progress to date has shown that the low-molecular-weight, mercury-binding proteins exist as two molecular-weight variants: 20,000 to 25,000 and 10,000 to 12,000. The former, which are the primary mercury-binding species, occur as dimers, which can be divided into sub-unit components by treatment with 1% 2-mercaptoethanol, a reducing agent that can cleave disulfide bonds.

Further separation of the dimer form by DEAE anion exchange chromatography resulted in the isolation of three separate charge variants (Figure 3). Amino acid analysis of two of these showed similar compositions of 26% half-cystine and very low levels of phenylalanine, tryosine, histidine, and leucine. These compositions are very similar to those of the metallothioneins, which are believed to be involved in metal detoxification. One of the peaks isolated above by DEAE anion exchange chromatography is currently being used as an antigen in the development of antibodies against the mercury-binding proteins.

Separation of the dimer form by anion exchange, high-performance liquid chromatography indicated greater complexity with the resolution of six mercury-binding protein peaks (Figure 4). The identities of these six peaks are under investigation.

Although the results described above are preliminary, they do indicate considerable complexity of the mercury-binding proteins of *Mytilus edulis*. The proteins occur in both dimer and monomer form; the dimer can be separated into at least three, and possibly six, different charge variants.

Hydrothermal Vent Clams Take in Large Quantities of Trace Metals

Specimens of the hydrothermal vent clam were collected at a depth of 2600 meters from 21°N on the East Pacific Rise in the spring of 1982. The elemental composition was determined for the total soft tissues, individual

FIGURE 3. Mercury-binding proteins were analyzed by DEAE ion exchange chromatography to determine the fraction at which they separated from the protein. One of the several peaks is being studied as a possible antigen.

FIGURE 4. Mercury-binding proteins analyzed by high performance liquid chromatography provided resolution of up to six mercury-binding protein peaks.

organs, and the shell. The soft parts contained high concentrations of a number of trace metals. Iron, copper, and zinc at 760, 148, and 2152 $\mu\text{g/g}$ dry weight, respectively, were present at the highest concentrations. Silver and antimony showed the greatest enrichments when concentrations in *C. magnifica* were compared with those of the mussel, *Mytilus edulis*, from a relatively pristine environment. Gills, kidney, pericardium, and mantle were involved in the concentration of various metals; the degree of concentration depended on the particular metal in question. The shell was not a major site for metal accumulation or deposition.

Detailed examination of the kidney revealed the presence of numerous metal-rich, intracellular inclusions, ranging from small granules to large concretions. These inclusions apparently serve as sites for intracellular deposition and sequestration of metals. The metal composition of these granules and concretions varied, but they were generally rich in iron, zinc, lead, copper, silver, and nickel. A large percentage of the kidney iron was soluble and bound to a protein which was tentatively identified as hemoglobin, a protein present in high concentrations in the clam's tissues. Cadmium was also found in the soluble fraction of tissues. Sephadex and DEAE chromatography of the soluble fraction showed that cadmium was associated with low-molecular-weight proteins with chromatographic properties similar to metallothionein found in other organisms.

UPTAKE AND DEPURATION OF SILVER IN MUSSELS

Previous studies conducted at PNL and other laboratories have shown that mussels rapidly accumulate heavy metals when exposed to seawater containing elevated concentrations. Silver is one of the heavy metals that is frequently elevated in the soft tissue of mussels collected from urban embayments. The concentration of silver in soft tissue of mussels from Sequim Bay, an uncontaminated site, is in the range of 0.06 to 0.08 $\mu\text{g/g}$ dry weight. In contrast, mussels collected from urban areas of Puget Sound, such as Seattle and Tacoma, contain 0.1 to 0.4 $\mu\text{g/g}$ Ag.

We have completed a series of experiments that were designed to determine whether mussels and clams regulate their body burden of silver when exposed to concentrations within a factor of 10 of the silver concentration in uncontaminated seawater. The concentration of silver in Sequim Bay water is usually in the range of 0.5 to 1.0 ng/L.

In the experiment, 16 mussels were exposed in flowing, unfiltered seawater that was dosed with radiolabeled $^{110\text{m}}\text{Ag}$ at 3.1 nci/L and stable silver of 0.8 ng/L. The exposure contained approximately twice the silver concentration as the seawater in Sequim Bay where the mussels were collected. The mussels were exposed for 3 weeks. The $^{110\text{m}}\text{Ag}$ activity was determined approximately twice a week by counting the live animals on a gamma detector, then returning the mussel to the tank. After 3 weeks, the accumulation of $^{110\text{m}}\text{Ag}$ appeared to stop, and six mussels were sacrificed for analysis of stable silver in soft tissue (Figure 5). The remaining mussels were allowed to depurate in the same exposure system, but no silver was added during the depuration period. The depuration of $^{110\text{m}}\text{Ag}$ was almost complete within 5 weeks after silver dosing ceased (Figure 5).

The concentration of stable silver in the mussels that were not depurated was not significantly different from unexposed mussels. The concentration of silver in exposed mussels was $0.072 \pm 0.043 \mu\text{g/g}$, compared to $0.079 \pm 0.062 \mu\text{g/g}$ in controls. This indicated that the turnover of silver is rapid in

FIGURE 5. Mussels appear to regulate the body burden of silver. Peak body burdens of ^{110}Ag occurred within three weeks after the mussels were exposed to a high concentration of the metal. Tissue concentration of labeled Ag was rapidly exchanged with non-isotopic silver after this source was removed.

mussels exposed to low concentrations. Using the specific activity of the dosing solution, it was possible to calculate that 50% of the silver body burden was turned over during the three-week uptake period, but the body burden did not change significantly. Because of the relatively large variation in uptake rates between individual mussels, it is difficult to determine changes in groups of individuals.

These results indicate that the body burden of silver in mussels collected from the field will reflect silver concentration in seawater during the previous several weeks. We are now analyzing the tissue of mussels and clams exposed to various concentrations of silver to determine the relationship between seawater concentration and body burden.

PARTITIONING COEFFICIENTS (K_p) FOR METALS IN SEDIMENTS AND WATER COLUMN

In order to predict the effects of contaminant releases on coastal marine ecosystems, we must first understand the processes by which such contaminants are made available to marine organisms. The biological availability and toxicity of metals in sediments to

bottom-dwelling organisms are influenced by both the geochemical interactions of the metals with the sediment particles and the chemical forms of the soluble metals in porewater. Metals are sorbed or desorbed by various coatings on sediment particles, thus controlling the concentrations of soluble metals in sediment porewater. Since the biological accumulation of soluble metals from sediment porewater is related to sediment toxicity, development of methods that can determine or predict the concentrations and chemical speciation of soluble metals in porewater may lead to better understanding of the relationship between sediment bulk chemistry and toxicity.

During the past year we have measured the concentrations of metals in porewater extracted from Puget Sound sediments. We have also measured the concentrations of metals in the solid phases of these sediments. These data enable us to calculate the ratio of solid metal to soluble metal in sediment, referred to as a partitioning coefficient (K_p). Variations in the K_p for a specific metal may reflect changes in either the chemical form of the metal or the number and nature of the adsorption sites on the particles.

We have calculated the partitioning coefficients for several metals in Puget Sound sediment; Puget Sound water column; western Washington rivers; and Hyco Reservoir, a lake in North Carolina that receives coal fly ash. Next, we plan to determine the association of metals with different coatings or solid phases in sediments. Three sediment coatings (iron oxides, manganese oxides, and organic matter) have been identified as the major chemical phases that control the porewater concentrations of most heavy metals. The partitioning of metal between porewater and these coatings varies greatly with metal and coating.

Eventually we will be able to calculate the concentration of soluble metal in interstitial water by knowing the amount of phases present and the total metal in the sediment. Assuming that certain species of metals in porewater are biologically available, we hope to establish the relationship between those metals and sediment chemistry.

ACKNOWLEDGMENTS

Principal Investigators for these respective projects are J. T. Hardy (FTP 81332/001332), G. Roesijadi (FTP 80644/000644), and E. A. Crecelius (FTP 80641/000641).

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Mobilization, Fate and Effects of Energy-Derived Chemicals

A number of energy technologies produce liquid and solid effluents. The compositions of these effluents differ between and even within technologies, and both inorganic and organic chemicals are of concern. Environmental pathways for transport, transformation and accumulation of these compounds often result in the potential for human exposure to toxic materials. In recent years, we have made progress in delineating the major environmental routes for such exposure. However, the current state of the art does not permit an adequate evaluation of the relative importance of many detailed processes known to affect transport and transformation of solutes, or the biological availability, uptake, and metabolism of particular chemical forms of these contaminants.

We are studying the hydrochemical processes governing transport and chemical form of solutes resulting from energy development and also the uptake and metabolism of these chemicals by aquatic organisms (fish and shellfish) that may be present in receiving waters. The program focuses particularly on commonalities in abiotic and biotic processes controlling chemical transport and effects.

HYDROCHEMICAL PHENOMENA CONTROLLING SOLUTE BEHAVIOR

The composition of solutes mobilized from wastes and transported in subsurface environments will differ with the type of waste, its residence time in the environment, and the land surface and subsurface characteristics of the disposal site. Several fundamental processes, however, control solute concentration and form at any point in time and space. When water moves through wastes and substrata, the integrated effects of mineralogical and microbiological processes control chemical dissolution, solute distribution between the solid and aqueous phases, and solute form. Hydrologic processes govern the rate and extent of solute movement.

A conceptual basis was developed for research that uses linked geochemical and hydrologic models as a framework for examination of these processes. The models serve to integrate the activities of a multidisciplinary research team (geochemistry, microbiology, inorganic and organic chemistry, and hydrology) and to focus research on priority, rate-controlling processes and functional relationships necessary for generic applicability of the results. Laboratory studies are used to establish basic mechanisms and functional relationships. Studies in controlled field facilities establish the range for parameters influencing these relationships and allow validation of models.

Currently, investigations of the behavior of inorganic solutes are being completed. Research emphasis is on defining the behavior

of a well-rationalized set of organic compounds and organometallic complexes originating from energy-related activities.

Inorganic Solubility Controls

Research on long-term solubility phenomena in wastes have focused on alkaline, high-ionic-strength conditions characteristic of many energy-derived wastes disposed to the ground. Investigations of recarbonization reactions which occur in alkaline wastes with environmental exposure have recently been completed. Ninety-day equilibrations of solid waste materials in deionized water with argon, N_2/CO_2 , and N_2/O_2 atmospheres demonstrated the important role of $CO_2(g)$ absorption on leachate chemistry through precipitation of anhydrous and hydrated carbonate minerals. There were, however, soluble constituents in many leachates, perhaps organic acids, which reduced the rate of heterogeneous nucleation of calcite in spite of highly supersaturated conditions. A comparison between the time-dependent solubility behavior under argon with the N_2/O_2 system suggested the importance of nonbiological inorganic sulfur oxidation on the continued release of soluble constituents from the waste matrix.

We found that carbonate precipitation scavenged soluble metals from the leachate. Studies were therefore initiated to investigate the carbonate phases that precipitated during recarbonization and their capacity to remove soluble constituents from the leachates by coprecipitation, solid solution formation, or occlusion. Calcite seed crystals

were placed in leachate extracts from solid wastes and equilibrated with time for two weeks under two N_2/CO_2 atmospheres. During this period of initial recarbonization, carbonate overgrowths formed on the seed crystals. Two polymorphs of $CaCO_3$ were identified in the overgrowths: calcite and aragonite. The overgrowths contained boron, strontium, and barium in addition to calcium carbonate ($CaCO_3$), verifying the importance of coprecipitation in minor element solubility in waste leachates.

Modeling Inorganic Solubility Controls

Solubility calculations provide insight into the reactions controlling solute concentration and form. The MINTEQ geochemical model was applied to calculation of the behavior of the major ions calcium (Ca) and sulfate (SO_4) in solutions from batch leaching equilibration of water with an alkaline waste. The quantity of readily soluble calcium and sulfate was sufficient to cause immediate saturation of the water with anhydrite ($CaSO_4$). Under these conditions, the anhydrite begins to precipitate. As calcium salts, oxides, and hydroxides continue to dissolve, the aqueous phase becomes saturated with portlandite [$Ca(OH)_2$], a mineral that forms during the setting of cement. At reaction times longer than 100 hours, calcium concentrations begin to decrease. This coincides with fluorite (CaF_2) moving from a state of considerable oversaturation to one of equilibrium with the dissolved calcium and fluoride. It appears that, as is commonly observed, time is required for sufficient fluorite crystals to nucleate and allow significant calcium to precipitate via this mineral. As fluorite precipitation proceeds, depleting the dissolved calcium, dissolved sulfate increases. This indicates redissolution of some of the anhydrite that had precipitated earlier. The effectiveness of these two solids in limiting calcium and sulfate solubility is indicated by the fact that, without exception, all samples fall within the error band about the calculated equilibrium lines (Figure 1).

Solubility controls have been tentatively identified for molybdenum, manganese, barium, and strontium.

FIGURE 1. Fluorite and anhydrite are effective in limiting the solubility of calcium and sulfate in waste leachates. All samples fall within the calculated error band (dashed lines) around the equilibrium lines.

Linking Inorganic Chemical and Hydrologic Transport Models

Major progress was achieved in interfacing geochemical models with hydrologic transport models. A simple water flow model, SATCOL, was coupled with MININR (a compact version of the geochemical code MINTEQ developed earlier in this program) and used to predict effluent chemistry from a column of the alkaline waste described above. The model calculated equilibrium precipitation/dissolution of solid phases and aqueous speciation and gas phase equilibrium. Based on assumed solid phases present in the column, the coupled code correctly predicted the transport of sulfate, potassium, sodium, calcium, and pH but did not predict magnesium and carbonate concentrations satisfactorily. Limitations of the model include uncertainties in the solid-phase estimates required to initialize the geochemical code and numerical dispersion produced by the simplified flow calculation. Solutions to these problems, based on new concepts and approaches, are under development.

Effects of Environmental Factors on Water Available for Transport

In addition to vertical transport, an unsaturated flow code was also used to describe water balance in ground-disposed waste. Initial moisture, content of the waste, precipitation and potential evapotranspiration were found to have the most significant influence on water balance. The sensitivity analysis also demonstrated that the flow model is sensitive to parameters describing the sink term (plant uptake); hence, plant rooting depth and density were found to have significant effect on the flow. The model was also applied to an arid site to evaluate its performance using long-term (13-year) water storage data. The transpiration and water loss mechanisms were modeled with reasonable success for some years but not for others, indicating a need to develop a more coherent picture of actual evapotranspiration under arid water conditions.

Subsurface Behavior of Organic Compounds and Mixtures

Subsurface sorption reactions and microbiologic degradation of important energy-derived organic compounds are being investigated to provide a fundamental understanding of the factors influencing subsurface attenuation and persistence of organic chemicals. The program utilizes a series of model compounds previously selected for use in the DOE Subsurface Transport Program, subsoils of diverse properties from energy development regions, and pure-phase mineral materials to probe important surface and biochemical phenomena controlling both subsurface behavior of organic compounds and the chemical interactions that occur under conditions of multiple chemical exposure. Experimental research over the past year has focused on 1) the subsurface sorption of two-ring nitrogen heterocycles and the influence of co-contaminants, and 2) a survey of the relative importance of aerobic and anaerobic degradation in controlling the subsurface persistence of two-ring heteroatomic organic compounds.

Sorption studies have demonstrated that major differences exist in the subsurface behavior of basic (e.g., quinoline, $pK_a = 4.90$) and neutral (e.g., indole) N-heterocycles. Quinoline is strongly sorbed in acid subsoils and

weakly in basic subsoils, suggesting the importance of a cation exchange mechanism. Supporting this proposed mechanism, quinoline adsorption was shown to be a function of the activity of the cationic quinoline species and the surface area and cation exchange capacity of the subsoil. Acid and basic subsoils with markedly different adsorption properties at their natural pH were shown to adsorb equal quantities of quinoline per unit of surface area at the same pH. Thus, a single mathematical expression can be derived to describe pH-dependent adsorption in subsoil. In contrast, indole, a nonionizable neutral N-heterocycle, is weakly adsorbed in both acid and basic subsoils through bonding to hydrophobic mineral surfaces. The sorption of indole closely parallels that of quinoline at pH levels where quinoline exists as the neutral compound (Figure 2). This similarity in behavior suggests a comparable adsorption mechanism for the two neutral compounds.

The adsorption of quinoline in presence of co-contaminants was investigated in acid and basic subsoils to elucidate chemical interactions which may occur in presence of complex organic mixtures. The presences of pyridine and acridine in dilute concentrations reduce quinoline adsorption in acid subsoil, but

FIGURE 2. Subsoil properties are as important in the sorption of organic compounds as the concentration and form of the compound. In acid subsoils, quinoline is much more strongly adsorbed than indole because quinoline becomes protonated. When quinoline is neutral, as in a basic subsoil, its sorption behavior is identical to indole.

have no effect on the weakly adsorbing basic material. The influence of acridine is particularly noteworthy as this compound reduced sorption of low levels of quinoline by 60% (Figure 3). These findings are significant because they suggest that the competition between charged/polar sorbing organics, even at low environmental concentrations (e.g., $<10^{-6}$ M), will reduce subsurface compound sorption under conditions of multiple chemical exposure. When all sorbing compounds are neutral, however, competitive effects may be minimal.

Key factors controlling subsurface microbial degradation of two-ring, heteroatomic aromatic compounds have been identified. Availability of oxygen, magnitude of sorption, and compound molecular properties were found to govern the microbial degradation of indole, quinoline, α -naphthol, and α -aminonaphthalene in subsoils differing in important chemical and mineralogical properties. Aerobic and anaerobic degradation rates differed markedly. To illustrate, anaerobic degradation of α -aminonaphthalene was not observed for 15 to 20 weeks, whereas aerobic degradation began within one week. Sorbed compounds were apparently unavailable for the microorganisms, as exemplified by quinoline, which was readily degraded in basic subsoils, but only slowly degraded in acidic subsurface materials where it is strongly sorbed. The effect of pH on degradation rate remains to be determined, however. Degradation varied markedly between each compound class. For example, in basic subsoils, anaerobic degradation occurred within 3 to 4 weeks for the N-heterocycles (indole and quinoline), but 20 to 30 weeks were required before degradation of the ring-substituted compounds (α -naphthol and α -aminonaphthalene) was detected. The lengthy adaptation periods for α -naphthol and α -aminonaphthalene suggest that under conditions of high groundwater velocities and low sorption, a significant portion of these contaminants would be immune to degradation because they would migrate faster than the microbial community could respond. These new findings point to the importance of microorganisms in detoxifying potentially mutagenic compounds in the subsurface and highlight the need for incorporating microbial activity in hydrologic transport models to allow quantitative prediction of subsurface persistence and movement of these compounds.

FIGURE 3. The presence of acridine decreases the adsorption of quinoline in acid soil. The presence of co-contaminants is one of many factors that influence the behavior of wastes in the soil.

CONTAMINANT TRANSFER THROUGH AQUATIC ORGANISMS

Aquatic environments serve as sinks for environmental contaminants transported through surface and subsurface wastes. Aquatic biota can therefore serve as important links in the transformation of contaminants from water systems to people. The objective of our current research is to generate information needed to understand biological processes that control the fate of organic contaminants in freshwater environments.

A major task accomplished within the framework of these objectives is the preparation of a comprehensive report synthesizing the information derived from our extensive laboratory research on the behavior of complex mixtures in aquatic environments. This document, edited by D. H. Fickeisen and B. E. Vaughan, together with a current critical review by B. E. Vaughan (see publications), describes the current state of aquatic research with organic mixtures, and it sets the stage for our present research.

The objectives of our current program are to increase our understanding of the biological processes controlling uptake, elimination, and storage of organic materials in organisms involved in pathways leading to man. Meeting these research objectives requires specialized analytical tools and laboratory techniques, because many organic molecules are dramatically altered in their physical and chemical properties when absorbed and metabolized by living organisms.

In current experiments, we are studying the fate of organic chemicals in trout after bioabsorption from the water column, and after absorption from the gut through feeding. The tissue distribution and metabolic pathways resulting from the different exposures are being examined, with particular emphasis on form and potential for storage in edible tissues. Initial experiments with direct absorption of 1 ppm radiolabeled quinoline from water through skin and gill membrane demonstrated that most of the absorbed chemical was present in the fish in the form of conjugated hydroxylated metabolites. Seven different metabolic forms were isolated, and were found to be concentrated primarily in the gall bladder. Concentrations of quinoline and metabolites in other tissues after 48 hours' exposure and 24 hours' depuration did not exceed the initial water concentration. Not all the absorbed quinoline could be accounted for as quinoline or hydroxylated conjugates; another metabolic form, as yet unidentified, was found to predominate in the edible flesh of the exposed trout.

In our most recent experiments, special feeding procedures were developed to ensure that test trout would ingest a specified quantity of radiolabeled quinoline through natural feeding. These procedures were developed so

that anaesthetization and gavage of test fish (a common experimental procedure in gut absorption studies) could be obviated, and to avoid changes in biological function because of trauma. The fish were fed daily for 10 days, then fed pellets containing no quinoline for an additional 7 days. The responses of gall bladder, gut, and muscle tissue with time are presented in Figure 4. Tissue distribution found at the end of the exposure and end of elimination period are shown in Figure 5. Although concentrations of quinoline and metabolites in gall and gut were found to be orders of magnitude higher than in muscle, muscle tissue contained almost one third of the total quinoline radiolabel after the 7-day elimination period. This material is not eliminated rapidly, in contrast to that from the gall bladder.

Analysis of tissues from the feeding experiment revealed that little of the hydroxylated quinoline metabolite was present, even in the gall bladder, which contained the highest concentration of quinoline-derived material. The bulk of the altered quinoline material exhibits extraction behavior similar to the unidentified material from the water-column absorption experiments.

Biochemical experiments with trout liver tissue fractions have shown that the liver

FIGURE 4. Trout were fed pellets containing quinoline for 10 days. Over time, the distribution of quinoline varied in different parts of the body.

FIGURE 5. Seven days after the end of the ingestion period, almost one-third of the quinoline and its metabolites was found in muscle tissue, the part of the fish usually consumed by humans.

microsomes contain the quinoline hydroxylating enzymes. Although more work is required for assay optimization, microsomal hydroxylation can be readily accomplished *in vitro*. On the other hand, conditions have not been found that result in the formation of the unidentified material occurring from absorption through the gut.

These experiments suggest that route of absorption can have a profound influence on the fate of an organic material in trout, and therefore may also substantially influence its subsequent transfer through the environment. Experiments are being conducted with other examples of organic contaminants, such as naphthol and indole, and with other aquatic species, such as crayfish, in order to determine whether these initial results with trout have general applications.

ACKNOWLEDGMENTS

Principal Investigators for these respective projects are R. M. Bean (FTPA 83237/003237), R. E. Wildung (FTPA 81264/004285), and J. M. Zachara (FTPA 80505/004445).

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Radionuclide Transport Research

PNL research on the mechanisms of radionuclide transport in the terrestrial environment began in 1974 as an outgrowth of the recognition that many factors contribute to the accessibility of radionuclides for human contact. While these factors may be more significant than occupational exposure, they are also more difficult to understand and monitor. Living forms are extremely influential in radionuclide behavior and transport, and this group of studies focuses on the nature and interrelationships of processes occurring in soil, plants, and animals. The work summarized below explores the behavior of radionuclides in soils and plants and the ways in which they are changed by metabolic processes. A second emphasis is on biological transport of radionuclides by mammals, insects, and fish.

CHARACTERIZATION OF PLANT-PRODUCED LIGANDS

Research by PNL over the years has demonstrated that some elements that are chemically insoluble in soil are often made more soluble by plants for transport through the food chain. Plants modify radionuclides by root excretion and also by metabolism after uptake. Those modified forms, in turn, affect gastrointestinal absorption by animals that ingest the plants, further altering the possibilities for human exposure.

The primary objective of this program is to identify and quantify those physiochemical and biological processes that influence the long-term transport of radionuclides through the environment and to man. A complex interaction exists among soil chemical and microbial processes, and plant and animal processes associated with the food chain leading to man. The common denominator in each of these processes is chemistry or chemical form. Plants represent a very important link between man and radionuclides in soils. Terrestrial plants, because of changing environmental stresses, must be able to adapt metabolically to a range of chemical insults. To meet this need, plants have evolved relatively efficient processes to solubilize and adsorb soil nutrients (including trace elements, which encompass the radionuclides), to allow for their transport within the plant, and, should a trace element concentration exceed physiological limits, to employ processes to compartmentalize or chemically complex potentially toxic elements. Each of these processes, to some degree, involves chemical complexation by way of plant-produced organic ligands.

In the current study of radionuclide complexes and plant ligands, focus is on three aspects of the problem: determination of the composition of root exudates and decomposi-

tion products, and their interactions with radionuclides in soil; the chemical forms of radionuclides freely soluble and mobile in the plant (xylem exudates), and the chemical forms of elements once metabolized or stored in plant tissues. This approach, although seemingly complex, is simplified by several factors: there are a limited number of complexing ligands, these fall into a limited number of relatively low molecular weight chemical classes, and many of these classes are ubiquitous in the plant kingdom. In fact, many of these compounds are products both of soil microbial processes and biomass decomposition. To date, studies at PNL and elsewhere have shown that compounds of low molecular weight, including organic acids, amino acids, small peptides, catechols and small mixed ligands, can effectively complex and chemically stabilize trace elements. The resulting species may be mobile both in soil and in the food chain.

Efforts to date have concentrated on soil microbial ligands, plant transport ligands, and storage forms of radionuclides. These efforts are currently being expanded to include root secretions and their soil metabolic products, the latter resulting from microbial growth and metabolism. The experimental approach used includes procedures to extract and fractionate solubles using a combination of size exclusion column methods and coupled cation-anion column methods to separate compound class fractions, and subsequently to characterize in detail both molecular weight and class fractions by means of high-resolution liquid chromatography and GC-mass spectrometry. With this methodology, we can also identify trace elements associated with each molecular weight fraction, and "back react" both molecular weight and compound class fractions with specific radionuclides for evaluation of stability constants.

The usefulness of this approach and methodology is demonstrated in Figure 1. Shown are the results of a series of experiments in which radionuclide complexation in whole xylem exudates of soybean was compared to class fractions of exudates and inorganic species. It should be noted that these xylem exudates represent the transport fluid that carries the majority of inorganic elements from root to shoot tissues. In addition, all cations transported in plants, with the possible exception of K^+ , are chemically stabilized by organic ligands to prevent hydrolysis and exchange with cell wall material while in transport. In the example shown, inorganic forms of Pu^{4+} , Fe^{3+} , Ni^{2+} , and Cd^{2+} were amended to either whole xylem exudates (reaction mixture 1) or individual compound classes isolated from exudates (reaction mixtures 2, 3, 4 and 5), subjected to thin layer electrophoresis, and the resulting complexation patterns compared in the reaction mixtures and in the organic forms of the spikes (reaction mixture 6).

A comparison of the complexation patterns observed demonstrates both the range of organic complexation potential in biological systems and the usefulness of the techniques. In Plate A of Figure 1, plutonium amended to whole xylem exudates is principally anionic and, based on complexation patterns for com-

pound classes, is chemically stabilized by organic acids. Similarly, most iron amended to exudates is complexed by components isolated in the organic acid fraction, with one of the major transport forms composed of an amino acid/peptide (Plate B). Two of the iron complexes are cationic, not found in exudate fractions, and may represent mixed organic acid/amino acid ligands. Whole exudates amended with nickel (Plate C) show the presence of two major anionic complexes and one cationic complex. These are formed in the amino acid/peptide and organic acid reaction mixtures. It should be noted that the complexation patterns obtained for plutonium, iron, and nickel with the laboratory-prepared (in-vitro) exudates are similar to those obtained in vivo from plants absorbing these elements through their roots. The complexation behavior of cadmium varies from that of the other elements studied; a higher percentage of cadmium remains at the origin (neutral or hydrolyzed) in vitro than in the in vivo treatments noted above. This would suggest that elements are chemically complexed soon after root absorption. Even with this limitation, the two cadmium-containing ligands formed in whole exudates are shown to be formed as a result of complexation with organic acid and amino acid/peptide constituents of transport fluids.

These types of studies serve to demonstrate the importance of biological complexation processes in chemically stabilizing and solubilizing trace elements, particularly the polyvalent elements (plutonium and iron) resolved in exudates, compared to their inorganic controls. In addition, it should be noted that the complexes formed in xylem exudates are not biologically unique and, that they undoubtedly form the basis for complexation during root and plant litter decomposition and, possibly, microbial decomposition processes.

FIGURE 1. Chromatography patterns were used to compare the metabolites formed when plutonium, iron, nickel, and cadmium enter the plant vascular system. Tracer concentrations of the elements were amended to whole xylem exudates (1), amino acid/peptide fractions (2), organic acid fractions (3), neutral fractions (4), polyacidic fractions (5) and a non-organic matrix (6). Complexation patterns were subsequently determined using electrophoresis techniques.

THE METABOLIC BEHAVIOR OF TECHNETIUM IN PLANTS

Technetium is perhaps the most mobile of the radionuclides transported in the terrestrial food chain. The behavior of technetium in the environment is a subject of great concern in Europe. PNL's research is of particular interest to the Europeans because PNL is the only U.S. laboratory that has continuously

been involved in technetium research since 1975, and one of only two still engaged in this line of research. One goal of the current research has been to understand what happens to technetium when it is absorbed by plants.

These studies have demonstrated that the physiological basis for the relatively high technetium soil/plant concentration ratios, observed in both field and laboratory studies, is the chemical similarity between the pertechnetate ion and several nutrient and nonnutrient ions. It is this chemical similarity that enables the pertechnetate ion to compete for, and use, transport sites and plant membrane transport processes normally functioning in the absorption of sulfate, phosphate, and selenate. It is currently assumed that the chemistry of pertechnetate and its nutrient analogs, particularly sulfate and selenate, forms the basis of its observed phytotoxicity in plants. This characteristic could permit us, eventually, to understand the processes involved in technetium metabolism and its ultimate chemical fate in plants, and allow us to assess the importance of these chemical transformations on transfer processes in the food chain.

The most recent PNL studies on the chemical fate of technetium in plants have proceeded along two lines. The first involves the screening of a wide variety of plant species, including a range of species known to metabolize sulfur in different ways, to determine both the range of absorption and transfer within the plant and the molecular weight distributions for soluble components. The second approach involves a comparative evaluation of the metabolic fate of technetium, sulfur, and selenium in a limited number of plant species. This approach is based on the assumption that the fate of technetium in plants is related to its chemical similarities to these elements.

In the examination of a number of plant species, our objectives have been to compare plant uptake rates, tissue distributions, and the partitioning of technetium within tissues, and to determine the relative molecular weight distribution of technetium in the soluble fractions of tissues. Of the 14 plant species screened, the five *Allium* species (representing onion, garlic and leek) exhibited the lowest root absorption rates (0.16

to 1.1 $\mu\text{g Tc/g dry wt root/hr}$) and lowest leaf technetium concentration (4 to 13 $\mu\text{g/g dry wt}$). The remaining agronomic species exhibited absorption rates from 1 to 4.4 $\mu\text{g Tc/g dry wt root/hr}$, and leaf accumulations of 12 to 130 $\mu\text{g Tc/g dry wt tissue}$.

If we assume that technetium is an analog for sulfur with respect to root absorption, then the comparatively low absorption rates for the *Allium* species may be related to their high sulfur fertility requirements during cultivation, i.e., inefficient uptake of sulfur. When the distributions of technetium in various plant tissues were compared, a range in behavior was observed. In the *Allium* species, the storage organ had less than 7% of the accumulated technetium, while the roots contained 28% to 60% of the remaining activity. Interestingly, bulb onion is the only *Allium* species where major accumulation is in the leaves (64%). In the other species studied, radish and soybean represent the extremes; we observed root accumulations of 6% and 26%, respectively.

The chemical fate of technetium in tissues of these plant species was determined by fractionation and ultrafiltration. The vast majority (>90%) of the technetium associated with these tissues is soluble and extractable. The exceptions occurred in radish root and soybean leaves, which had 23% and 32%, respectively, of the accumulated technetium associated with the insoluble residue. Ultrafiltration of the solubles to partition the technetium associated with high (>20,000), intermediate (20,000 to 500), and low (<500) molecular weight (MW) components showed an interesting trend. The *Allium* species generally showed technetium to be associated with intermediate to low MW compounds, while the other plant species (radish, soybean and mustard) showed the majority of the technetium accumulated in leaves and roots to be associated with high to intermediate MW components. The generally high solubility of technetium in plant tissues is unusual, not only for radionuclides, but even for many nutrient ions.

Recent efforts have been directed towards understanding the mechanisms controlling the metabolic fate of technetium in plants. In these studies, we are evaluating the comparative chemical behavior of pertechnetate, sulfate, and selenate in soybean and onion. In

general, technetium exhibits substantially more solubility, in both species, than either sulfate or selenate. Unlike technetium, sulfur and selenium are found to be associated with the organelle fractions of root and leaves of both soybean and onion. While the behavior of sulfur and selenium is as expected, the metabolic fate of technetium remains uncertain.

Chromatographic separations of the soluble fractions of soybean leaves supplied with either technetium, sulfur, or selenium exhibited some interesting trends with respect to protein molecular weight distributions and tracer distributions (Figure 2). For example, radiolabeled sulfur is associated with a broad range of molecular weight components, with 58% of the activity found in the high MW (>200,000) proteins (fraction 6)--a normal pattern, considering that plants need sulfur amino acids for the synthesis of proteins. The protein distributions observed for soybean leaf solubles in the presence of radiolabeled selenium (unlabeled sulfur present) is similar to that in the absence of selenium (radiolabeled sulfur). However, the distribution of tracer selenium differs from that of tracer sulfur, in that only 10% is associated with proteins of high molecular weight, and >30% is associated with components of intermediate molecular weight (fractions 8 and 9; note that fraction 7 contains primarily selenate). In comparison, the presence of technetium results in a noticeable shift in protein distribution, namely a 50% increase in the high molecular weight fraction (6). In addition, >70% of the technetium present in the soluble proteins is associated with components of intermediate molecular weight (fractions 7 and 9), while only 15% of the technetium is found in the high-molecular-weight fraction (6).

Based on the protein distributions and the distribution of technetium, it is possible that technetium is partially substituting for sulfur in proteins of low to intermediate molecular weight, and to some extent in those of high molecular weight. If it is assumed that these are biologically non-functional, due to the inability of technetium to form structural bonding patterns analogous to disulfide linkages, then the increase in fraction 6 protein could represent a response

FIGURE 2. Proteins containing selenium, technetium, and sulfur exhibit different trends with respect to protein molecular weight distribution in soybean leaves. Such comparative studies help us determine the metabolic fate of technetium.

of the plant to the need for specific proteins (i.e., functional proteins).

While these studies are far from definitive, efforts are continuing. We are focusing on the isolation and characterization of specific proteins containing sulfur, selenium, and technetium in an effort to determine the sites and patterns of accumulation for each of the elements. By starting at the amino acid level, where sulfur and selenium reduction and incorporation is known to occur, progressing through characteristic peptides, and, subsequently, through the structured proteins, it should be possible to resolve the metabolic fate of technetium.

RADIONUCLIDES TRANSPORTED BY INSECTS AND FISH

Recent field studies in radioecology have emphasized plant and animal processes as they relate to biological transport of environmental radiocontaminants. Our objectives have been to identify basic transport processes and quantify them so that we can observe and predict long-term contaminant behavior in the environment. PNL's work with honeybees and other insects, and also with fish, has helped to clarify the processes of radionuclide transport.

Observations on the occurrence of feral honeybee colonies in the environs of waste management sites prompted investigation of the potential role of honeybees in waste transport. Although several studies conducted elsewhere have focused on the possible accumulation of contaminants in honey, we theorized that pollen, because of its relatively high natural concentrations of potassium (of which cesium is an analog), might be more likely to contain selected plant-derived contaminants. Therefore, we sampled both pollen and honey from hives that were located within 1.6 kilometers of six different low-level radioactive waste management areas on the Hanford Site. A control location on the Arid Lands Ecology Reserve was also sampled. Pollen samples were collected in pollen traps at the entrances to hives as bees returned from foraging (Figure 3). Honey was collected at the end of the season by removing honey and comb from frames.

While honeybees forage, thousands of individual pollen grains attach to a bee's hair-covered exterior. The bee combs the pollen grains from its body surface and packs them onto spines on its hind legs for transport to the hives. Each individual pellet carried on one hind leg is referred to as a pollen load.

Pollen samples collected in the early summer (June and July) and again in the fall (September and October) were analyzed for gamma-emitting radionuclides. Five of six waste site locations yielded pollen with contaminant concentrations greater than that observed at the control site. Maximum concentrations of cesium-137 in whole pollen samples ranged up to 391 pCi/g dry weight.

Honey samples were commonly below detection limits for cesium-137. When cesium was detected in honey, it was at concentrations one

FIGURE 3. In this photo, a PNL scientist removes pollen from a collection box attached to a honeybee hive. The pollen was analyzed for evidence of radionuclides.

to two orders of magnitude less than in pollen.

Iterative subsampling was used to examine the homogeneity of cesium-137 in contaminated pollen samples. We found that the cesium was non-uniformly distributed. For example, one contaminated pollen sample contained measurable radioactivity in only 6 of 211 individual pollen loads. Concentrations of cesium-137 in individual pollen pellets, however, ranged up to 121 nCi/g.

These observations, together with knowledge concerning foraging patterns of honeybees, suggest that contaminants are not widely distributed in the areas where the bees foraged. We propose two hypotheses to explain the transport pathway leading from the contaminant source to the pollen. One hypothesis maintains that few individual plants (among those used by the bees) are rooted in contaminated soils and that the plant roots absorb the radioactive materials, which are then translocated to the pollen. This hypothesis suggests that the bees collect contaminated pollen grains. A second hypothesis maintains that the bees occasionally collect pollen-sized radioactive particles that become incorporated into the pollen load and are returned to the hive with the pollen. Our microscopic examination of individual pollen loads does reveal small amounts of debris among the pollen grains.

Work is underway to determine how the contaminants are distributed within pollen loads and thereby to identify the environmental pathway by which contaminants accumulate in bee-collected pollen. Selected pollen samples are currently being analyzed for strontium and plutonium.

Since bees collect thousands of pollen samples from a relatively large area during a short time, they have the potential to provide useful and economical samples for contaminant monitoring. Plants and animals are also involved in the vertical redistribution of contaminants in buried waste. Previously, we reported on observed surface contamination in an experimental facility that resulted from soil cast to the surface by the burrowing bee, *Agapostemon texanus*. Our calculations showed that the small burrowing invertebrates were capable of moving relatively large quantities of some contaminants in comparison to the quantities that could be brought to the surface by crop plants rooted in the same contaminated soil.

In addition to the burrowing bee, we identified a wasp, *Tachytes distinctus*, that was transporting actinide elements from a below-ground contaminated layer to the soil surface. Liquid latex was poured into a number of burrow systems, allowed to solidify and later excavated to determine the depth, pattern, and volume of burrows (Figure 4). For the wasp, mean burrow lengths and diameters were 490 and 12.6 millimeters, respectively. The longest burrow measured was 1240 milli-

meters. Burrowing occurred most frequently in irrigated test plots and along roadsides where surface water tended to accumulate. The burrow entrances penetrated vertically 12 to 32 centimeters into the soil, and then usually curved to parallel the soil surface for the remainder of their length. Average burrow volumes were approximately 60 cubic centimeters.

A recently developed biological transport (BIOPORT) model can be used to predict the long-term behavior of buried contaminants subsequent to intrusion by plant roots and burrowing animals. Translocation by plants and movement of soil by burrowing animals results in a redistribution of radioactive materials into the previously uncontaminated soil profile above buried waste and onto the soil surface. Study has shown that these biological transport processes, which may be viewed as nuisance problems in the short term, have potential for significant accumulated contaminant transport when occurring over a number of years.

An extensive review of concentration ratios of 26 radionuclides in fish has provided a basis for revising and updating parameter values used in assessing dose to humans from ingestion of fish. Attempts were made to evaluate biotic and abiotic environmental factors that influence concentration ratios. For example, feeding characteristics of the fish (omnivorous, planktivorous, or predaceous) appear to influence the magnitude of radionuclide uptake in their tissues, all

FIGURE 4. Excavated latex casts reveal the depth and extent of wasp burrowing systems. Invertebrates, such as the wasp, redistribute buried contaminants during burrowing.

other factors being equal. The PNL review includes a discussion of environmental variables that influence radionuclide concentrations in fish tissues. We believe that individuals conducting dose assessments can use these relationships, together with some relatively simple environmental variables describing the sites, to choose concentration ratios that are both appropriate and meaningful for their situations, thereby improving the quality of dose assessments.

In the future, research will be extended to examination of the effects of plant root depth on waste distribution. Radionuclide uptake by plants having only a fraction of their root mass in contact with the waste (those with deep, penetrating roots) is inadequately represented by available data. Sensitivity analyses indicate that knowledge of root depth is important in predicting radionuclide transport through the soil profile by plants. We are currently reviewing methods to assess more effectively the functional root depth of semi-arid plant species relative to waste burial sites.

A great deal remains to be learned about the character and activities of radionuclides in the soil and the functions of living forms in altering the nature of radionuclides and effecting radionuclide transport. The PNL research represents a continuing effort to comprehend the nature and activities of radionuclides and their impact on the environment and humans.

ACKNOWLEDGMENTS

Principal Investigators for these respective research projects are L. L. Cadwell (FTPA 80834/000834), T. R. Garland, D. A. Cataldo (FTPA 80623/000623), and R. E. Wildung (FTPA 80551/000551).

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Statistical and Quantitative Research on Wild Populations

The objectives of these PNL projects are to evaluate the population dynamics of plants and animals and to conduct research on quantitative methods suitable for evaluating the responses of natural populations to energy-related impacts.

POPULATION STUDIES OF LARGE ANIMALS

Our work in recent years has demonstrated that classical models for population dynamics, being based largely on laboratory data for small organisms, cannot be extrapolated accurately to large, wild mammals. The research is needed; for example, populations of large, free-roaming animals can pose problems with regard to safety and industrial operations at DOE sites. Consequently, we have been seeking data on free-living populations of large animals from various habitats.

Useful population data on animals with long life-spans take decades to accumulate and require extensive manpower and other resources. We have been obtaining data through long-term collaborative efforts with scientists from other agencies, helping them with quantitative analyses in exchange for opportunities to work with the data thus collected.

The species studied include such diverse forms as bowhead whales, elk, fur seals, grizzly bears, sea otters, Antarctic seals, and feral horses. Two research projects on the Yellowstone grizzly bear have recently been completed, one summarizing and analyzing recent population data, and the second projecting possible future trends for the small population that exists now.

Survival of Grizzly Bears May Be Linked to Their Spatial Patterns

Spatial patterns in habitat are a poorly understood facet of population dynamics in virtually any wild species. Such an understanding is a key factor, for example, in efforts to prevent extinction of the grizzly species in the contiguous United States. Work is underway to assess the spatial patterns of grizzly bears in their natural habitats.

The grizzly bear population is small and threatened. The proximate cause of recent grizzly declines has been identified as excess mortality of mature adult females. The survival of an entire grizzly population is

delicate; it may hinge on the survival or loss of one or two adult females per year. Therefore, the importance of minimizing negative impacts on the bear population becomes crucial. Most of the grizzly population decline is due, directly and indirectly, to human activities. The major need, then, is to alter interactions between bears and humans in ways that will ensure long-term survival of the grizzly. Inasmuch as there are perhaps 30 to 40 mature adult females in the Yellowstone population under study, assessing these issues is both challenging and difficult.

A Model May Help Predict Survival and Reproduction of Large Mammals

In another aspect of our research on population dynamics, we have developed a new, generalized model for studying large animals. The "net maternity function" model is based on the product of a survivorship function and a reproduction function. The model is expected to be useful for comparative purposes and as a way to develop an approximate model when, as usually is the case, field data are limited. Data on about a dozen species were used in formulating the model. A number of other factors were considered in the study, including maximal growth rates for some large-mammal populations, and a bias due to senescence that apparently has a significant influence on estimates of survival based on age structure or tag returns.

QUANTIFYING ENERGY-RELATED EFFECTS ON MOBILE SPECIES

The economic constraints of conducting wildlife investigations often force a compromise between the scope of a field study and the precision of population estimates or the statistical power when comparing different populations. In practice, this compromise is often based on personal experience or intuition rather than quantitative judgement and fact. The effect is that many wildlife investigations do not achieve the potential

they are capable of attaining. In a survey of field experiments where the objective was to test effects on wild populations, half of the studies did not have the field replication needed for valid statistical hypotheses tests. The remaining half had insufficient replication so that all but the most obvious changes in population measures would be undetected.

Current practices in the design of wildlife studies rarely reflect these concerns about the validity and efficiency of field studies. Within economic constraints, a range of alternative design options usually exists. The spectrum of alternative designs for wildlife experiments ranges from a few "intensively" sampled plots to "extensive" designs with numerous populations located across the landscape which are only superficially sampled. Because the ability to quantitatively test for effects on wild populations drastically differs among these design options, not all selections are equally acceptable. The purpose of our research conducted during FY 1984 has been to devise objective, quantifiable criteria for identifying the most effective and efficient field designs.

The identification of the most efficient study approach is the process of design optimization. An optimal design allocates field efforts where the sources of experimental error are the greatest and where field efforts will contribute most to the power of statistical tests. Some of our most interesting results of optimization occur in the analysis of multi-plot, mark-recapture studies to estimate animal abundance. An investigator has the options of sampling a number of populations or concentrating on estimating the demographic parameters of a few populations. Research on optimality indicates (Figure 1) that intensive trapping effort should be sacrificed for greater replication of the test conditions. Furthermore, the optimal level of trapping effort quickly stabilizes, at a level far below the amount required to completely enumerate population levels. Unfortunately, this finding is unappreciated by most wildlife investigators--often to the detriment of the research. Cost effectiveness and reliability of results should improve significantly as design optimization is utilized in wildlife studies.

FIGURE 1. When designing a sampling program, it is usually more efficient to use fewer traps per plot (samples) for greater replication of experimental conditions. The power curves indicate the optimal numbers of study plots and trapping effort needed in a wildlife experiment. The optimal trapping effort (dotted line) is seen to stabilize at about 100 traps per plot.

An important aspect of the research on design optimization has been to make the statistical methodology understandable and tractable to a broad spectrum of skill levels. A graphical technique has been developed (Figure 1) that can easily identify optimal design configurations without sophisticated mathematics, with improved insight, and in situations which otherwise defy analytic techniques. The approach is the same whether we are designing a simple single-plot study to estimate animal abundance or a multi-plot, multi-year impact study. By plotting separately the solutions to a cost function and to the non-central test distribution, the optimal design is identified by the single point where the cost and power (or precision) contours share a common tangent. In Figure 1, several solutions for different program budgets and different levels for the power of the test (e.g., 0.70, 0.90) of hypotheses have been identified. Identification of several solutions enables the behavior of opti-

increase. This allows us to understand general strategies of experimental design.

The design of wildlife investigations should be based upon the best information and/or beliefs about the possible states of nature. The optimization procedures described serve to formulate these beliefs in a manner conducive to objective design making. The results of the research during FY 1984 may help assure that population studies are designed consistent with this information. Nothing less should be expected of field studies whose results may influence resource management decisions.

ACKNOWLEDGMENTS

Principal Investigators for these respective projects are L. L. Eberhardt (80184/003386), and J. R. Skalski (80244/003913).

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Eberhardt, L. L. "Assessing the Dynamics of Wild Populations." J. Wildl. Manage. (submitted).

Skalski, J. R. "Construction of Cost Functions for Wildlife Research." Wildl. Soc. Bull. (submitted).

Skalski, J. R. "A Variable Effort Technique for Estimating the Areal Mean Abundance of Animals." J. Wildl. Manage. (submitted).

Other Research

Environmental and energy assessment, to be effective, requires the cooperation of many governmental agencies and sponsors. The research topics identified in this section are complementary to work discussed in other sections of this report, but do not duplicate our work for DOE's Office of Energy Research.

U.S. ARMY

Acute Toxicity of Smoke Screen Materials to Aquatic Organisms (Principal Investigator: P. Van Voris)

Evaluate and Characterize Mechanism Controlling Transport Fate and Effects of Army Smokes in the Aerosol Wind Tunnel (Principal Investigator: P. Van Voris)

ARMY CORPS OF ENGINEERS

Analyze Sediments, Tissues and Water for Polyaromatic Hydrocarbons and Metals (Principal Investigator: J. W. Anderson)

Data Collection and Analyses for Everett Harbor Sediments (Principal Investigator: J. W. Anderson)

Conduct Oceanographic Cruises for Selection and Characterization of Ocean Disposal Sites off Grays Harbor, Washington (Principal Investigator: W. H. Pearson)

Characterization of Sediments from the Duwamish Waterway and from Neah and Clallam Bays (Principal Investigator: J. W. Anderson)

BONNEVILLE POWER ADMINISTRATION

White Sturgeon Workshop to Identify and Prioritize Research Needs (Principal Investigator: D. H. Fickeisen)

DEPARTMENT OF ENERGY/RICHLAND OPERATIONS

Wildlife Census (Principal Investigator: W. H. Rickard)

PCB Study (Principal Investigator: R. G. Riley)

DEPARTMENT OF ENERGY

BWIP Sorption Studies (Principal Investigator: L. L. Ames)

BWIP Solubility Studies (Principal Investigator: L. L. Ames)

Coal Liquefaction Environmental Studies--DOE Fossil Energy Program (Principal Investigator: D. D. Dauble)

Cover Technology Evaluation for Uranium Mill Tailings--UMTRA (Principal Investigator: P. A. Beedlow)

Commercial Nuclear Waste--International Support (Principal Investigator: W. L. Templeton)

ENVIRONMENTAL PROTECTION AGENCY

Fate and Effects of Toxic Substances on Ecosystem Processes (Principal Investigator: J. W. Anderson)

Acute Effects of New Coal Conversion Technologies on Aquatic Systems (Principal Investigator: D. H. Fickeisen)

Preparation of Superfund Technical Resources Documents (Principal Investigator: D. A. Neitzel)

Statistical and Biomathematical Assistance on Network Biomonitoring Using Honey Bees (Principal Investigator: J. M. Thomas)

Field Assessment of Indigenous Biota in a Semi-Arid High Plains Ecoregion Impacted by Hazardous Materials (Principal Investigator: J. M. Thomas)

Cold Climate Research and Development Program (Principal Investigator: J. B. States)

Prepare a User's Guide for Applying Chemical Spill Agents (Principal Investigator: J. W. Anderson)

Technical Support Related to Sea Disposal of Radioactive Waste (Principal Investigator: W. L. Templeton)

Technical and Management Analyses to Support the Puget Sound Water Quality Management Program (Principal Investigator: J. A. Strand)

Preparation of Superfund Technical Resources Documents (Principal Investigator: D. A. Neitzel)

Coordination and Staff Support for Acid Deposition Research Staff (Principal Investigator: W. E. Fallon)

Screening Model Development for Radiological Assessment (Principal Investigator: W. L. Templeton)

KAPL

Food-Chain Transfer of Corrosion Products from Radioactive Stainless Steel in One-Step Marine Food Chain (Principal Investigator: J. S. Young)

Support Effort to Environmental Impact Statement on Disposal of Decommissioned Naval Nuclear Reactor Plants (Principal Investigator: W. L. Templeton)

NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION

Characterize and Quantify Organic Constituents of Marine Waters and Pore Waters Affected by Leachates of Log Bark at Log Transfer Facilities (Principal Investigator: R. G. Riley)

Provide Assistance to National Marine Pollution Issues Workshop (Principal Investigator: D. H. Fickeisen)

Perform Pathology and Immunology of Salmon Disease (Principal Investigator: R. A. Elston)

Investigation of Chemical and Physical Properties of Marine Sediments (Principal Investigator: E. A. Crecelius)

NUCLEAR REGULATORY COMMISSION

Identification and Physiochemical Characteristics of Mobile Radionuclides in Groundwater at Several Low-Level Waste Disposal Sites at Chalk River, Canada (Principal Investigator: D. E. Robertson, Radiological Sciences Department)

Relevance of Biotic Pathways to the Regulation of Nuclear Waste Disposal (Principal Investigator: D. H. McKenzie)

Effects on Bioavailability of Low-Level Wastes Resulting from Chemical Changes in Wastes During Transport through Soil (Principal Investigator: W. H. Rickard)

Application of Statistics in Siting and Managing Commercial Low-Level Radioactive Waste Disposal Sites (Principal Investigator: J. M. Thomas)

Safety Implications of Auxiliary Systems Biofouling at Nuclear Power Plants (Principal Investigator: T. L. Page)

Soil, Biotic, and Hydrologic Processes Governing Mobility and Biological Availability in Low-Level Waste Disposal Sites (Principal Investigator: R. E. Wildung)

ROCKWELL HANFORD OPERATIONS

Sampling and Analysis of Gable Mt. Pond, U Pond, and S Pond Sediments for PCB's (Principal Investigator: R. G. Riley)

UNC NUCLEAR INDUSTRIES

Thermal Plume Characterization Studies at N Reactor (Principal Investigator: T. L. Page)

100 Area Biotic Transport Studies (Principal Investigator: T. L. Page)

Facilities and
Staff

FACILITIES AND STAFF

FACILITIES

Battelle's Marine Research Laboratory at Sequim, Washington, houses 40,000 sq. ft. of modern chemistry laboratories, offices, conference space, and support facilities. On the beach, a variety of experimental systems can be maintained, such as isolated rooms for behavioral studies and high-capacity flow-through tanks, in appropriate arrays to do statistical verification studies. Exposure systems can support selected marine organism communities for long periods of time.

Since identifying and preventing disease in marine species is a major concern in the Northwest, Battelle has also developed, with corporate resources, a Disease Diagnostic Center at the Sequim laboratory. Facilities for the center include a complete immunochemistry laboratory, where animal colonies are maintained for producing immune sera used in fish and shellfish testing. Disease control has an important bearing on all sponsored studies at the laboratory. Apart from DOE work at Sequim (described in an earlier section of this report), PNL continues projects for several agencies on coastal ecosystems research, bioassay chemistry, fate of oil and dispersant chemicals, and sediment biogeochemistry.

Battelle's toxic/hazardous aerosol exposure facility enables scientists to generate and evaluate a wide range of aerosols under simulated field conditions. In recent years,

scientists have come to realize that airborne contaminants, which enter foodstuffs directly through deposition and retention on edible plants, may sometimes present a more serious problem than has been previously acknowledged. Also, field investigations have identified unexpected contaminant sources at industrial sites believed to be operating under acceptable environmental conditions.

The facility greatly augments capabilities for evaluating aerosol contamination and allows for controlled environmental modeling of aerosols and gases, singly and in combination. Included are a wind tunnel and a system of instruments and equipment for particle generation and analysis. The wind tunnel is used to simulate aerosol transport and deposition at variable conditions of wind velocity, turbulence, temperature, pressure, and humidity. It is equipped with NBS standard pitot tubes and hot-wire and hot-film anemometers for measuring mean velocities, velocity, profiles, and turbulence characteristics. Wind speeds of 1 to 65 mph can be achieved in the 20-ft-long, 80-ft³, test section, with assured laminar flow from 0 to 25 mph. Turbulent flow can be produced at all speeds. The apparatus has a closed-loop design, HEPA filters, and a test section with eight 2 x 2-ft ports. It provides full containment and internal washdown for hazardous materials applications.

A wide array of standard particle generation systems are readily available for aerosol

The Marine Research Laboratory at Sequim, Washington is situated on a hilltop overlooking Sequim Bay; this major West Coast facility has over 40,000 sq. ft. of laboratory, office and conference space, including fully equipped sea water facilities and holding tanks.

A 50-foot-long wind tunnel is one of the specially designed facilities used to study airborne contaminants.

studies. These include an evaporative-condensation generator for 0.03 to 1.3 μm monodispersed particles, a vibrating orifice generator for 0.5 to 30 μm monodispersed particles, and nebulization generators for labelled monodispersed and polydispersed particles. In addition, special particle generation systems or accessory devices can be developed as needed.

Characterization equipment includes a remote laser particle spectral analyzer for measuring aerosol size distribution, concentration, and velocity in ranges from 1 to 200 μm and 1 to 50 m/s; a 16-channel, high-resolution optical particle counter that counts and sizes particles from 0.3 to 20 μm (concentration limit of 2×10^6 particles/ ft^3); an electrical analyzer for measuring size distribution from 0.0032 to 1.0 μm ; and a mass monitor. Infrared laser spectrometry or gas chromatographic-mass spectrometry are used to analyze gaseous and organic pollutants and to characterize their molecular form in real time. Supporting the facility is a major aerosol physics laboratory where basic research related to sampling and generation of experimental aerosols is carried out. Scanning electron microscopy and energy dispersive x-ray analysis with automated particle sizing are available in the laboratory.

The center for marine disease control, a component of MRL, Sequim, is equipped for diagnosis and research on aquatic animal diseases. Shown is a typical apparatus for retinal diagnostic observation of fish.

STAFF

Several PNL staff members serve on national committees or commissions or hold editorships on important journals. Staff activities at the national and international level include the following:

American Fisheries Society

C. D. Becker: Coordinator, Fisheries Lab Series

American Institute of Biological Sciences

L. E. Rogers: Consultant, Evaluated literature on biological effects of extremely low frequency non-ionizing radiation; wrote resource paper "Ecological Effects of Extremely Low Frequency Electromagnetic Fields"

Argonne National Laboratories

D. H. McKenzie: Committee Member, National Acid Precipitation Assessment Program, Acid Rain Watershed Working Group

R. E. Wildung: Chairman, Environmental Research Division Review Committee

Department of Natural Resources

R. E. Fitzner: Member, Natural Heritage Advisory Council (appointed by Head of Washington State Department of Game)

Environmental Protection Agency

P. Van Voris: Member, National Panel to review Environmental Biotechnology Regulations and Implications

Illinois Natural History Survey

C. E. Cushing: 1985 Chairman, External Advisory Committee, Long-Term Ecological Research, Large River Project

International Atomic Energy Agency

W. L. Templeton: Chairman, Program Review Committee of International Laboratory of Marine Radioactivity; Member, Technical Committee to Revise Definition and Recommendations for Ocean Dumping for the London Dumping Convention

National Council on Radiation Protection and Measurement

W. L. Templeton: Member, Scientific Committee #64, Radionuclides in the Environment; Co-chairman, Scientific Committee #64, Task Group #6

R. E. Wildung: Committee Member, Scientific Committee #64, Task Group #7

Non-Game Advisory Council

R. E. Fitzner: (Appointed by Commissioner of Public Lands, Washington State)

North American Benthological Society

C. E. Cushing: Member of Executive Committee

Northwest Scientific Association

W. H. Rickard: Board Member and Trustee for a two-year term beginning in 1985

Organization for Economic Cooperation and Development/Nuclear Energy Agency

W. L. Templeton: Member, Task Group on Co-ordinated Research and Environmental Surveillance Program; Chairman, Executive Group, Committee on Co-ordinated Research and Environmental Surveillance Program; Member, Ad Hoc Review Committee on Site Suitability of North East Atlantic Dump Site

U.S. Army Corps of Engineers

J. W. Anderson: Consultant, Waterways Experiment Station

U.S. Department of Interior

W. H. Rickard: Consultant, Evaluation of natural areas in the Pacific Northwest

Wildlife Society

R. E. Fitzner: President, Washington Chapter

Editorial Positions

C. D. Becker: Associate Editor, North American Journal of Fisheries Management

C. E. Cushing: Editor, Bulletin of the Ecological Society of America; Editor, Bulletin of the North American Benthological Society

G. Roesijadi: U.S. Editor, Marine Environmental Research

J. A. Strand: Correspondent, Coastal Ocean Assessment News - Pacific Region

P. Van Voris: Editor, Environmental Management Journal, Ecotox Special Issue Editor

PRESENTATIONS

Apts, C. W., J. T. Hardy, E. A. Crecelius, and N. S. Bloom. 1984. "A Comparison of Metal Concentrations in the Sea-Surface Microlayer and Subsurface Bulk Water at Two Bays in Puget Sound." Presented in 47th Annual Meeting of the American Society of Limnology and Oceanography, June 11-14, 1984, Vancouver, British Columbia, Canada.

Becker, C. D., and D. A. Neitzel. 1983. "Salmonid Redd Dewatering: What Do We Know?" Presented at Salmonid Reproduction, an International Symposium, October 31-November 3, 1983, Bellevue, Washington.

Beedlow, P. A., and N. R. Hinds. 1984. "The Ceres Device for Detecting Stress Effects in Plants." Poster session presented at the 16th Air Pollution Workshop, April 30-May 4, 1984, Banff, Alberta, Canada.

Beedlow, P. A. 1984. "Patterns of Disturbance and Recovery in Arid and Semi-Arid Ecosystems." Presented at the Annual Ecological Research Division Terrestrial Ecology Meeting, June 5-6, 1984, Gaithersburg, Maryland.

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Crecelius, E. A. 1984. "Temporal Trends of Contamination of Puget Sound." Presented at Ocean Disposal Symposium, September 10, 1984, Corvallis, Oregon.

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Cushing, C. E. 1984. "Lotic Research in Arid and Semi-Arid Ecosystems." Presented at the Department of Energy's Energy Research Division Annual Meeting, June 5-6, 1984, Washington, D.C.

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Dauble, D. D., R. M. Bean, R. W. Hanf, Jr., and B. L. Thomas. 1984. "How Fish Respond to Organic Contaminants in the Environment: Uptake and Fate of Materials from Water and the Diet." Presented at the Annual Meeting of the Western Division of American Fisheries Society and Western Association Fish and Wildlife, July 15-20, 1984, Victoria, British Columbia, Canada.

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Eberhardt, L. E. 1983. "Brood Ecology of Canada Geese on the Hanford Reach." Presented at the Annual Meeting of Washington State Wildlife Society, December 9, 1983, Richland, Washington.

Felice, L. J., J. M. Zachara, and R. G. Riley. 1983. "The Selection of Organic Compounds for Subsurface Transport Studies." Position paper requested by Dr. Frank Wobber, Department of Energy, November 30, 1983, Washington, D.C.

Felice, L. J., J. M. Zachara, and R. L. Schmidt. 1984. "Quinoline Partitioning in Subsurface Materials: Adsorption, Desorption, and Solute Competition." Presented at the International Conference on Ground Water Quality, March 26, 1984, Tulsa, Oklahoma.

Fitzner, R. E. 1984. "Animal Populations of the Shrub-Steppe: Response to Disturbances." Presented at the Department of Energy meeting on Research in Arid and Semi-Arid Lands, June 5-6, 1984, Washington, D.C.

Fitzner, R. E. 1984. "Bird Assemblages of Pristine and Old Field Communities in South-central Washington." Presented at the Pacific Northwest Bird and Mammal Society Meeting, May 5, 1984, Seattle, Washington.

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Hardy, J. T., and C. W. Apts. 1984. "Phytoneuston Productivity and Effects of Atmospheric Particulate Matter." Presented at the 47th Annual Meeting of the American Society of Limnology and Oceanography, June 11-14, 1984, Vancouver, British Columbia, Canada.

Hardy, J. T. 1984. "Toxicity of Sea Surface Contaminants." Presented at the Pacific Northwest Association of Toxicologists Meeting, September 29, 1984, Sequim, Washington.

Hardy, J. T., and C. W. Apts. 1983. "Sea Surface Microlayer Metals Enrichments in Two Puget Sound Bays." Presented at Pacific Estuarine Research Society Meeting, September 30 - October 1, 1983. Bellingham, Washington.

Hardy, J. T. 1984. "Phytoneuston: Plants of the Sea Surface." Presented at the American Association for the Advancement of Science Symposium Annual Meeting, May 24-29, 1984, New York, New York.

Hinds, W. T. 1984. "Landscape Heterogeneity and Plot Replication on the ALE Reserve." Presented at the Department of Energy Meeting on Research in Arid and Semi-arid Lands, June 5-6, 1984, Washington, D.C.

Jenne, E. A., K. M. Krupka, and T. R. Garland. 1983. "Further Validation of Equilibrium Thermodynamic Speciation of Uranium." Presented at the Sixth Workshop on Environmental Research for Actinide Elements, November 7-11, 1983, Hilton Head Island, South Carolina.

Jenne, E. A. 1983. "Speciation in Natural and Polluted Waters." Presented at the International School on Metal Complexes in Solution, October 3-8, 1983, Palermo, Italy.

Jenne, E. A., and K. M. Krupka. 1984. "Validation of Geochemical Models." Presented at Workshop on Fundamental Geochemistry Needs for Nuclear Waste Isolation, June 20-21, 1984, Los Alamos, New Mexico.

McCorquodale, S. M. 1983. "The Elk of the Arid Lands Ecology Reserve: A Preliminary Status Report." Presented at the Annual Meeting of Washington State Wildlife Society, December 9, 1983, Richland, Washington.

Rickard, W. H. 1984. "Shoot Production and Mineral Nutrient Assimilation in Cheatgrass Communities on the Arid Lands Ecology Site." Presented at Department of Energy meeting on Research in Arid and Semi-Arid Lands, June 4-6, 1984, Washington, D.C.

Rickard, W. H. 1984. "Mineral Nutrients and Production in Cheatgrass Communities in Southcentral Washington." Presented at the Northwest Scientific Association and Montana Academy of Sciences Conference, March 21-24, 1984, Missoula, Montana.

Roesijadi, G. 1984. "Studies on the Mercury-Binding Proteins from the Marine Mussel, *Mytilus edulis*." Presented at NIEHS International Conference Workshop on Bio-

chemistry of High Affinity Metal-Binding Proteins in Non-Mammalian Species: Implications for Human Health, September 21, 1984, Triangle Park, North Carolina.

Roesijadi, G. 1984. "Enhanced Mercury Tolerance in *Mytilus edulis*: Influence of Low-Level Prior Exposure to Hg, Cu, Cd and Zn." Presented at the Pollution Tolerance Symposium AAAS Annual Meeting, May 29, 1984, New York, New York.

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