Pacific Northwest Laboratory Annual Report for 1982 to the DOE Office of Energy Research

Part 2 Ecological Sciences February 1983

**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
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Part 2 Environmental Sciences

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

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Pacific Northwest Laboratory
Richland, Washington 99352

PREFACE

This 1982 annual report from Pacific Northwest Laboratory (PNL) to the Department of Energy (DOE) describes research in environment, health, and safety conducted during fiscal year 1982. 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 Office of the Assistant Secretary for Environmental Protection, Safety and Emergency Preparedness. Each part consists of project reports authored by scientists from several PNL research departments, reflecting the interdisciplinary nature of the research effort.

The parts of the 1982 Annual Report are:

Part 1: Biomedical Sciences		
Program Manager - H. Drucker	D. L. Felton, 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	J. M. Nielsen, Report Coordinator	J. E. Danko, E. M. Toomey, Editors
Part 5: Environmental and Occupational Protection, Assessment, and Engineering		
Program Managers - S. Marks W. A. Glass	W. J. Bair, Report Coordinator	R. W. Baalman, 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

FOREWORD

Our Annual Report for 1982 has been substantially reduced in size in order to highlight major developments in six prime research areas funded by the Ecological Sciences Division of the Office of Energy Research. In their entirety, these research areas contain 23 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:

- Terrestrial and Riverine Ecology
- Marine Sciences
- Radionuclide Fate and Effects
- Ecological Effects of Coal Conversion
- Solid Waste: Mobilization, Fate and Effects
- Statistical and Theoretical 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

*Vaughan, B. E., and C. M. Novich, Editors. 1982. *Environmental Sciences Department, Annual Report for 1982 on Interagency/Contract Research*. Battelle, Pacific Northwest Laboratories, Richland, Washington.

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Research Highlights

INTRODUCTION

Research carried out by Pacific Northwest Laboratory for the Office of Energy Research's Ecological Research Division (ERD) is designed to systematically evaluate the problems that energy development activities pose to the environment. Another prime purpose is to identify areas in which energy developments must ultimately meet the intent of the National Environmental Policy Act (NEPA) and various health statutes.

The only way to reliably assess environmental problems is to develop a basic understanding of the environmental and ecological processes that control energy-related pollutants. With this fundamental knowledge, our research becomes pertinent to a wide range of environmental concerns, not just the areas specifically studied. For example, the Laboratory's investigations of the soil microbial processes by which trace metals are converted to organic complexes of lesser (or sometimes greater) toxicity adds to our knowledge of how chemical by-products are formed, how radionuclides interact with the environment, and how retorted shale constituents affect regional hydrology. We believe that the dual utility and broad application of basic research is a positive force in the scientific world and one worth continued efforts.

In conducting the research programs indicated below, we point out applications to practical problems, especially where the data may be directly used by others. As practical applications are not always immediately apparent, technologists may be sometimes impatient with the approach taken. They should understand, in these instances, that current estimates of certain ecological processes may be quantitatively provisional, without a statistical basis, and sometimes barely suited for even order-of-magnitude purposes. In such instances, risk is likely to be overestimated, and it is in everyone's interest that a groundwork be laid for the acquisition of meaningful data. This is especially true where provisional data often are the only information available to agencies responsible for setting standards.

Mention should also be made of the distinguished achievements of Dr. Raymond E. Wildung who, at the time this report went to press, received an Ernest O. Lawrence Memorial award for his work on biological availability. In conferring this honor, DOE Secretary Don Hodel called attention to contributions to the field of ecology in defining metabolic relationships that control pollutant transfer through soils to plants to animals; to the cycling of carbon in soils; to nutrient and chemical processes in lake systems; and to the organic complexation of plutonium as it affects biological availability.

Terrestrial and Riverine Ecology

The goals of the Terrestrial and Riverine Ecology Program are to find ways of detecting changes in ecosystems while these changes are still relatively small and amenable to technology design or control options, and to provide and maintain field facilities, such as the National Environmental Research Park (NERP) for extramural scientists doing environmental research for the Department of Energy. Our research approach has been to develop a large data base for use in characterizing biotic communities, successional processes, transport pathways, and ecosystem responses to various perturbations, especially within the western U.S. Useful information about the ecological resiliency of semi-arid western areas, where the most intensive energy development is likely to occur, is sorely lacking and must be sought.

The data bases we have developed cover at least a 15-year period—in some cases 25 years. As such, they provide valuable documentation not available from other government agencies or academic institutions. The research record leads us to conclude that environmental impacts from Hanford site operations have been negligible. Another achievement has been our success in designing and applying methods for reclaiming land at mining and processing sites in the semi-arid West.

Marine Sciences

In our marine program, we are quantifying the most important factors controlling the availability of energy-generated materials to marine organisms. Studies focus on chemical processes occurring at major interfaces (for example, between atmosphere and ocean, water and organism, sediment and organism) and on physiological processes within the organism. We have approached the work through a combination of field measurement and laboratory experiments, with particular emphasis on the biologically active chemical forms of specific elements and compounds. At the organism level, we examined the physiological processes associated with sequestration and detoxification of metals and adaptation to low-level exposures to metals. Results from this program are needed for predicting, and subsequently minimizing, long-range environmental consequences of current and future energy production processes located in coastal regions.

Radionuclide Fate and Effects

PNL has been a pioneer in researching the soil and plant processes that enhance biological availability of metals and other compounds which may become dispersed in the environment. Our current objective is to develop a basic understanding of radionuclide chemical behavior in soil systems and determine biological availability and transport through ecological pathways. By defining basic mechanisms at the chemical, cellular, and organism level, we are able to incorporate the information into predictive models, which are subsequently validated in the field at reactor and waste management sites.

Through our investigations of critical geochemical, microbial, and plant phenomena, we hope to improve markedly the precision with which human dose assessments are made. (Originally, the dose assessments were based on occupational instead of environmental routes of exposure.) Basic principles have now been established to use the results for predicting the behavior of non-nuclear pollutants such as heavy metals, and to provide a basis for designing remedial measures (chemical treatments, biobarriers) to minimize hazards associated with extended waste storage.

Ecological Effects of Coal Conversion

In this program, a basic contribution of PNL has been to develop experimental systems for measuring the biological effects of complex organic mixtures. For complex mixtures represented by coal conversion products, suitable approaches were not generally available earlier because information was lacking on the complicated partitioning that occurs between solid, solution, oil, and gas phases. Synthetic fuel oils from coal conversion were found to be potentially much more toxic than, and different in composition from, petroleum products, which had been studied extensively in earlier years. Because these and other complex organic mixtures will be of continuing concern, several related goals are being pursued: 1) to examine their potential for formation of transformation products as a result of chemical and biological activity; 2) to determine effects on ecosystems and component organisms, and 3) to establish their potential for food chain transfer of the toxic organic constituents to man.

The long-term fate of synfuels in the environment cannot be simply expressed because mixtures of organic compounds change over time. Some components are readily taken up from soil by food plants or are otherwise conserved in ecological systems. For synfuels, a data base on environmental fate scenarios, effects on ecosystem components, and food chain uptake will provide the basis for assessing and minimizing environmental risks. The information will also significantly improve our understanding of the behavior of complex organic mixtures from processes other than coal conversion.

Solid Waste Mobilization and Fate

In 1982, some of the principles developed earlier in the Radionuclide Fate and Effects Program were applied to organic and inorganic constituents of other kinds of solid waste. Our goal is to establish a sound scientific basis for predicting water and solute movement and biological availability of solutes in solid wastes disposed of to the ground. We want to document the effects of weathering and residence time at sites where waste constituents are expected to become a permanent part of the landscape. Our research approach, which is both laboratory and field oriented, is designed to identify parameters and validate models.

Research to date has disclosed a series of interrelated chemical and microbiological processes that alter the overall chemistry of wastes and leachates over time. New methods and concepts are being developed to measure and explain the integrated effects of these processes. The research is also demonstrating that the simple chemical assays proposed under the Resource Conservation and Recovery Act for estimating the long-term effects of solid waste disposal are not adequate for this task.

Statistical and Theoretical Research

Our aim in this unique program is to put environmental sampling on a sound quantitative basis, rather than let it rest on its current, largely descriptive basis. Two approaches are being followed: first, we are modifying the theoretical basis for cost-effective sampling paradigms, and second, we are developing improved methods for statistically analyzing and presenting data. Existing statistical approaches do not adequately measure environmental contaminants, nor the wild populations that we suspect may carry these contaminants.

The program enables us to recommend the optimal size of study plots, sampling intensity, field replication, and program duration. Such design information, in association with cost data for field collections, is needed to determine the utility of ecological sampling programs objectively. One can confidently expect this information to streamline environmental monitoring requirements and provide sounder assessments at the same time.

Terrestrial and Riverine Ecology

The conifer forests, tundra, and shrubsteppe regions of the world are sensitive environments, where man's activities induce sometimes subtle, sometimes dramatic effects. The construction and operation of energy facilities in these regions could destroy wildlife habitat and displace or harm useful plants and animal populations unless appropriate steps are taken to minimize adverse impacts. Disturbances in the operation of energy facilities sometimes also result when terrestrial ecosystems are not appropriately managed.

In fiscal year 1982, PNL researchers continued to conduct basic research on how, and how quickly, selected ecosystems recover from natural and man-induced changes. Our terrestrial investigations verified the correlation between the presence of desert shrubs and large ant populations at radioactive waste sites, and gave us insights into the effects of wildfire on the recovery of vegetation. We also discovered that sagebrush leaves selectively take up some, but not all, toxic elements produced by coal combustion. A major freshwater study this year showed that changes in a river's water level that lead to dewatering are more harmful to salmon alevins than to eggs.

TERRESTRIAL ECOLOGY

Nuclear energy research and development facilities at Hanford are buffered by large, unpopulated land areas as a health and safety measure. To take advantage of these extensive and undisturbed land holdings, the Arid Lands Ecology Reserve (ALE) and National Environmental Research Park (NERP) were established to promote basic short- and long-term ecological research (Figure 1). The studies have produced information on how the development and operation of energy technologies affect arid and semi-arid regions, and how such impacts can be mitigated.

About 300 university and college personnel have used the NERP site since 1968, the year the Arid Lands Ecology Reserve (a predecessor of the NERP) was established. Sixty dissertations at the Masters and Doctoral level have been co-sponsored by PNL, and well over 100 college and university faculty members have participated in these PNL programs. In addition, about 40 industrial or other agency consultants have contributed to the programs under our cognizance.

Our previous studies of other plant and animal life are now well-documented [see, for example, Hanford National Environmental Research Park (NERP): A Description Summary... by B. E. Vaughan and W. H. Rickard, PNL-2299].

Also, a land use history was compiled during 1982 as a guide to understanding some changes now obvious on the Hanford site. A summary of the information was presented by N. R. Hinds (see Presentations list).

Response of Ant Colonies to Vegetation Changes

Because ants can burrow deeply into earthen covers overlying shallow burial trenches containing radioactive wastes, they can bring soil particles and bits of waste material to the ground surface. By revegetating burial trenches with herbaceous plants instead of

FIGURE 1. Map Showing the Geographic Extent of the Shrubsteppe Region in the Northwestern United States in Relation to the National Environmental Research Parks in Washington and Idaho.

shrubs, the attraction of trenches as an ant habitat can probably be reduced.

Ants are common throughout the shrubsteppe region of the western United States. But because they are not active above ground in daylight hours, and because their colony entrances are usually located at the base of perennial plants, especially sagebrush shrubs, the ants tend to be inconspicuous and "obscure" to ecological investigations (Figure 2).

We suspected that the presence of shrubs might enhance certain plant communities as an ant habitat. As such, we estimated colony populations of the ant, *Companotus semi-testaceus*, using 2 x 2 meter plots in two separate plant communities--one that supported sagebrush and one that did not. (A wildfire in 1973 had destroyed the sagebrush on some plots, but the herbaceous understory plants survived the burning with little

FIGURE 2. Ants Are Not Ordinarily Expected to Move Significant Quantities of Soil; because they are most active at night, their activity is often overlooked. (Amounts of soil moved are equivalent to the weight of an average man per year for typical ant colonies.)

damage. The results of the study show that the shrubs (sagebrush) did attract more ants. Whereas 880 to 480 ant colonies per hectare were counted on the sagebrush plots, only 280 ant colonies were counted on the sagebrush-bare plots.

There may be secondary complications to these observed differences. Ants are believed to be important in shrubsteppe communities because they provide food for lizards and birds. Insofar as ants collect plant seeds as food and store them below ground, a sufficiently large ant population can interfere

with broadcast seedings on shrubsteppe ranges, thereby reducing the establishment of seedlings that eventually produce the preferred wildlife or livestock forages. In plant communities, ants may also control the propagation of insects that feed on foliage and sap.

Response of Plant Communities to Wildfire

In the aftermath of the 1981 summer wildfire at Hanford, some observers theorized that the fire had probably destroyed cheatgrass seeds and would now expose the soil surface to accelerated wind and water erosion. This year, we returned to the burn site--a major portion of the Arid Lands Ecology Reserve--to test this hypothesis.

Our studies show that many seeds survived the burning and that, with the onset of autumn rains, seeds germinated and seedlings were promptly established. By the end of winter, ground covers of 53% and 66% in the two burned study fields were recorded. Most of the cover was provided by cheatgrass, *Bromus tectorum*, and by an annual forb, *Sisymbrium altissimum*. At the end of the growing season in May, the amount of dry matter produced was 270 and 527 g/m² on the two fields, respectively. These yields were significantly greater than the 10-year average yield of 230 and 270 g/m² for the same two fields (Figure 3).

Cheatgrass communities are susceptible to summer wildfires but are not permanently damaged. The response of the cheatgrass communities to burning was prompt and cheatgrass prevented accelerated soil erosion without requiring artificial reseeding or fertilization. The findings indicate that annual plants can play important roles in revegetation in semi-arid regions.

Response of Sagebrush to Chemicals Added to the Soil on the Hanford Site

The use of coal to produce electricity is steadily increasing in the shrubsteppe region. Coal combustion deposits biologically toxic elements on soils and vegetation surrounding coal-fired steam electric plants, making it necessary to determine whether chemical deposits cause significant long-term damage in plants. We simulated a long-term buildup of boron, copper, manganese, molybdenum, and zinc in a typical shrubsteppe soil by applying quantities of H₃BO₃, CuCl₂·2H₂O, MnCl₂·4H₂O, Mo(VI) oxide, and

FIGURE 3. Studies Show That Sites Burned by Wildfire Are More Productive After a Fire Than Before a Fire. Recovery can be severely impaired by vehicular and other disturbances of the ground surface.

ZnCl₂ to the ground surface beneath 100 sagebrush plants, *Artemisia tridentata*, growing on the ALE site. After two growing seasons, the new-growth sagebrush leaves were picked by hand and chemically analyzed.

The results show that only molybdenum was readily taken up by sagebrush leaves. Molybdenum oxide added to the soil at 20 grams per 0.5 m² increased the molybdenum content from 2 parts per million (controls) to 45 ppm. Molybdenum is of concern to livestock growers because elevated concentrations in forage and

drinking water can cause a disease (molybdenosis) in cattle. The effects of metal toxicity on shrubsteppe wildlife will receive more attention in the future.

THE RESPONSE OF COLUMBIA RIVER FISH TO MAN-INDUCED FLOW ALTERATIONS

Several effects of energy production on river ecosystems have been studied on the 40-mile stretch of the Columbia River bordering the Hanford site. This year we 1) quantified the effect of extended one-time dewatering on intergravel eggs and alevins of chinook salmon; 2) identified several ecological factors that influence the survival of salmonid eggs during dewatering; and 3) developed a generic model to predict flow elevation and temperature at downstream locations on the basis of discharges at an upriver dam. We also conducted initial analyses of the relationships between energy production and river ecology.

The dewatering tests used 30 artificial redds (nests), each filled with gravel and supplied with flowing water at 10°C. The results show that the tolerance of eggs depends on whether or not they have hatched by the time the dewatering occurs (Figure 4). The two egg phases survived continuous dewatering for 12 consecutive days. The two alevin phases, however, did not tolerate 24-hr dewatering.

FIGURE 4. As Fertilized Fish Eggs Develop, They Become Progressively More Sensitive to Dewatering. (Intermediate stages of embryonic development are shown from right to left.)

Mortality of embryos after extended dewatering coincided with premature hatching. Mortality of alevins coincided with development of functional gills. Basically, we found that any reduction of stream flow that exposes spawning beds could create substantial losses, if hatching has occurred.

Dewatering of artificial redds under controlled conditions does not necessarily simulate field conditions. Our initial observations indicate that the survival of salmonid eggs, when redds are dewatered, could be influenced by the extremes in environmental temperatures. Abruptly exposing chinook salmon eggs, developing at 10°C to a higher temperature of 24°C, for 4 to 8 hr will cause mortality. Eggs are resistant to abrupt cold shock at temperatures above freezing for the same periods, but cannot tolerate freezing. High moisture content (near 100% relative humidity) is essential to normal egg development. Lower humidity levels, which might occur if the gravel dehydrates, extract moisture from the eggs and cause implosion of the cell walls.

The ability to predict changing physical conditions at specific locations in streams (e.g., differing flow elevations and temperatures) as upstream discharges are altered is essential to predicting and mitigating deleterious impacts. A mathematical model suitable for unstable flow analysis was developed to portray conditions on the mid-Columbia River, and was calibrated and verified over a range of flows. The model was adopted from an existing computer code called DWOPER (Dynamic Wave Operational model), which was developed by the National Weather Service. The model proved to be reasonably accurate (± 0.08 m flow elevation and ± 20 minutes surface wave timing), and is applicable to other river systems that are affected by acute changes in flow.

In the future, we will study the effects of multi-technology energy developments on riverine ecosystems. An initial step will be to systematically assess potential impacts and identify important research needs. Material emissions will receive close scrutiny.

DETERMINATION OF LONG-TERM BIOLOGICAL RESPONSES

Since 1978, PNL has been developing measurement techniques capable of detecting slowly paced ecological changes. This year, PNL researchers determined what kinds of long-term measurements are cost-effective and appropriate for several typical western ecosystems. Now nearing completion are optimal

field measurement techniques to estimate forest vigor and performance and to estimate levels of toxic contaminants in avian food chains in aquatic habitats. The methods under development should be ready for technology transfer in fiscal year 1984. Agencies with local, regional, or national responsibilities will then be able to use these proven methodologies for useful long-term ecological monitoring programs.

To detect changes in specific ecological processes, one must repeatedly examine the same place or process. This necessity suggests that a practical field method should be passive, nondestructive, and specific to the taxonomic level(s) specified by the objective. The ultimate product should be an accurate estimate of the cost of attaining a specified level of precision and power for a given measurement. In forests, we have focused on needlefall and litterfall; for aquatic-based food chains, we have concentrated on Great Blue Heron nesting areas.

In terms of forest monitoring, we were able to determine an optimal litter collector design and to compare the cost of using total litterfall with that of using needlefall alone. To determine the optimum sampler size, we determined time, costs, and variability associated with using collectors ranging from 0.01 to 0.78 m². We found the small sizes (0.01 to 0.05 m²) to be ten times more cost-effective than the larger collectors. An analysis of three years of needlefall collections at four sites indicated that needlefall was probably as likely to provide cost-effective estimates as was litterfall. Since needlefall is more directly responsive to pollutant stress, it usually should be the measurement used (Figure 5).

FIGURE 5. The Level of Effort Required to Use the Small Sampling Collectors Recommended by PNL is One-Tenth That of More Traditional, Large Collectors. A new sampling concept, validated in 1982, establishes that measurement of needlefall from trees is an optimal indicator of pollutant stress.

Our studies on food chain contamination involved nine heron rookeries in four widely separated river drainages. These data will show whether nesting colonies within a single drainage exhibit comparable levels of food chain contamination. To relate food chain contamination to biological response, we estimate fledging success within nesting colonies. We found that, for a cost of \$1000 per year, it may be feasible to detect a 10% change in fledging success within a colony with 90% certainty.

The progress this year leads to concrete expectations for the future. First, the design requirements and associated costs of minimum cost/minimum variance observations in closed-canopy forests should be known by FY 1984 (published results in 1985). Second, definition of the appropriate independent experimental units (that is, colonies or river drainages) will be defined for heron food chains by fiscal year 1984. The design most appropriate for nation-wide use should be published about a year later.

ECOLOGICAL INVESTIGATIONS IN ALASKA

To determine how caribou are influenced by the environmental changes brought about by oil and mineral resource development in Alaska, we are completing the long-term study of migratory movements of arctic caribou herds. Particular phenomena of interest are the caribou's forage consumption and energy requirements. It is expected that this task will be completed and phased out during fiscal year 1983.

We observed migrating caribou herds in the spring of 1982 to document their resting, feeding, and walking periods. Measurements of snow depth and hardness were made to determine the "training" paths of caribou over different types of terrain in north-eastern Alaska. Fecal pellets were collected and microscopically examined for plant fragments, which were used to identify the species of plants that constitute the caribou's diet. The nutritional content of fecal pellets was determined by chemical analysis. Feeding trials were also conducted on captive caribou at the University of Alaska, Fairbanks, to check the reliability of fecal-pellet analyses as a measure of the caribou diet. Results of these studies should be available during fiscal year 1983.

Animal Behavior And Habitat Changes Are a Sensitive Indicator of Environmental Impact in Areas of Rapid Resource Development. Studies of the Arctic caribou herds were completed during this reporting period.

ACKNOWLEDGMENTS

This program consists of research projects 80618/000618, 80836/000836, 81261/001261, 83009/003009, and 83272/003272, which are described in Field Task Proposal/Agreements (Form 5120.2). Principal investigators for these projects are C. D. Becker, L. E. Eberhardt, W. T. Hinds, W. H. Rickard, and L. E. Rogers. Alaskan studies are being conducted by the University of Alaska under a subcontract with Pacific Northwest Laboratory. Co-principal investigators are S. Duquette, University of Alaska, and D. R. Klein, Alaska Cooperative Wildlife Research Unit.

PUBLICATIONS

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Marine Research

Coastal marine ecosystems will absorb increasing quantities of energy-related contaminants as energy facilities are developed and expanded. Some contaminants are released in controlled effluents, but others may be discharged accidentally. In either case, the environment must be protected from potentially adverse effects. Currently, we are researching the transformation processes and long-term fate of numerous pollutants and measuring physical, chemical, and biological recovery rates. Determining how biota are initially affected by exposure to metal and hydrocarbons is another task of PNL's marine research.

Information on the extent to which trace metals in the coastal marine environment are taken up, distributed, bound within animals, or eventually detoxified is needed to determine safe thresholds that must be respected for animals and humans to remain healthy over the long term. Also under investigation are the origins and concentrations of trace metals within the surface microlayer, the underlying water column (soluble and particulate), and bottom sediment. This information is needed to determine whether inputs from energy-related activities can, in fact, upset the balance of natural processes and lead to the degradation of coastal ecosystems.

Although the sea surface microlayer is only micrometers thick, it acts as a major sink and transfer point for metals (Figure 1). It is in this layer that most of the larval forms of sea life exist. Metals enrichment that we have observed could affect the survival, productivity, and tolerance of biological communities on a global scale, thus it is important to know the extent to which organisms tolerate or can adapt to metal accumulations. We expect that results of this program can be used to measure the environmental impacts of current and planned future energy-production activities near coastal regions.

ENTRANCE, BEHAVIOR, AND FATE OF TRACE METALS IN COASTAL WATERS

During the past five years, we have studied the major constituents of atmospheric particulate matter that enter into and interact with the marine environment. The processes by which lead, silver, and cadmium enter Puget Sound are now known, as are their residence times and the natural processes that contribute to their removal. This information can be used not only to ascertain their behavior and fate in the environment, but

also to determine the natural origins, distributions, and concentrations of these and other marine contaminants.

Trace Metal Budgets for Puget Sound

For two years we have studied the geochemical processes by which metals are removed from coastal waters. One aspect of this work has been to determine the sources and fates of trace metals in Puget Sound, a large estuary flanked by the urban industrial centers of Seattle, Tacoma, and Bremerton. This year, we developed budgets for lead, silver, and cadmium. Budgets are useful for estimating the relative importance of trace-metal sources and sinks and can become a basis for making decisions about acceptable discharge rates. Lead, silver, and cadmium were chosen for our mass balance exercise because they enter Puget Sound in a variety of ways, such as through river discharge (including shoreline erosion), oceanic input, atmospheric deposition, and sewage discharges. The major processes by which these metals are removed are sedimentation and seawater transport from the Sound to the Pacific Ocean.

Traces of lead enter Puget Sound in nearly equal proportions from all four sources (Figure 2). Lead appears in both particulate and dissolved forms and becomes rapidly adsorbed

FIGURE 1. The Sea Surface Microlayer Acts as a Major Sink for Deposits of Trace Metals from the Atmosphere and Spills at Sea. Wave action and diffusion are two of many processes that control the movement of metals through the microlayer.

FIGURE 2. Metal Budgets for Central Puget Sound Show Significant Urban Inputs for Lead and Silver. Sedimentation is the main process for removing these elements. The cadmium budget differs substantially from the lead and silver budgets (see text).

on suspended particles. The residence time for particulate lead is short, usually only several weeks, and most of it is removed by sedimentation. Sediments deposited during the last several decades contain eight times more lead than sediments deposited earlier, reflecting the increases in urban and industrial discharges to the area over the years.

The silver budget (Figure 2) is similar to lead in that silver is introduced through industrial and municipal sources and is removed by sedimentation. Unlike lead, however, little silver enters the Sound through atmospheric deposition. At 20:1, the sediment enrichment ratio for silver is the highest of the 16 metals we have examined. One reason for the high ratio is the naturally low abundance of silver in environmental materials.

The cadmium budget (Figure 2) differs substantially from the other budgets. Nearly all cadmium input to the sound comes from one source: the inflow of Pacific Ocean water, which is naturally rich in cadmium (0.070 µg Cd/L). Man's contributions of cadmium to the Sound are thus relatively insignificant, and

little of this metal seems to have accumulated in the estuary's sediments.

Measuring Trace Metals in Marine Samples

Purchase of the Perkin-Elmer Z-5000 atomic absorption unit early in 1982 greatly expanded our analytical capabilities for studying trace metals in seawater. Now we can determine concentrations of several metals in untreated seawater, combining the techniques of matrix modification and Zeeman background correction. The metals and their detection limits are manganese (0.05 parts per billion, ppb), chromium (0.07 ppb), zinc (0.1 ppb), aluminum (0.3 ppb), and arsenic (0.4 ppb). Other metals that can be detected by direct injection at levels higher than ambient include beryllium (0.05 ppb) and iron (1 ppb). With the aid of preconcentration methods, the detection limits for several metals in seawater are now on the order of 0.0001 ppb.

The Zeeman graphite furnace atomic absorption (ZGFAA) system, also purchased in 1982, allows detection limits on the order of 0.002 ppm for metals in animal and plant tissues and geological samples. The use of this analytical system enabled us to measure thallium (Tl) in geological and biological materials for the first time, and to make accurate and precise measurements of cadmium in those materials as well. We are currently developing analytical techniques for measuring silver in all media.

TRANSPORT AND BIOAVAILABILITY OF TRACE METALS IN COASTAL ECOSYSTEMS

The transport, fate, and subsequent availability of energy effluents to marine organisms are controlled by chemical, physical, and biological processes in the marine environment. These processes, which occur at important interfaces between the atmosphere and ocean, water and organism, and sediment and organism, can decrease or eliminate potentially negative ecological effects.

Atmospheric Metals and the Sea Surface Microlayer

The sea surface microlayer (upper 1 mm of ocean surface) controls the exchange of materials between the earth's atmosphere and the oceans and provides an important habitat for the eggs and young of many invertebrates and fish. Airborne particulate matter must traverse the microlayer—a boundary having unique physical, chemical, and biological properties—before it enters the subsurface water.

In a study of energy effluents contained in the microlayer, PNL scientists found that the microlayer contains metals such as zinc, lead, silver and copper in concentrations 10 to 1000 times higher than the concentrations in the water only 10 cm below the surface. The metals enrichment in the microlayer could result from the relatively long time the metals are present in the microlayer. These preliminary findings are based on microcosm studies using water collected from Puget Sound.

We modeled the fate, flux rates, and residence times of metals in the microlayer, to which particles from urban air had been added to simulate atmospheric deposition. At low mixing rates, 3% to 27% of the metals remained in the microlayer indefinitely. At equilibrium, concentrations in the microlayer were 100 to 92,000 times greater than those in the water column (Figure 3).

Most of the metal in the water column was in the dissolved form. In contrast, the microlayer contained a high percentage of metal as particulate matter and large quantities of dissolved and potentially toxic metals as well.

These laboratory enrichments are somewhat higher than those found in the field, possibly because wind-induced mixing was absent in the microcosm experiments. Studies are now in progress to determine if a laboratory model that includes mixing will adequately describe the microlayer metals enrichment observed in the field.

We are now examining the effects of the high metal concentrations on microlayer biota. Studies may be made of the effects of atmospheric metals on the photosynthetic activity of plankton and on the hatching and survival rates of fish eggs.

FIGURE 3. Metal Concentrations in the Microlayer Were Greater Than Those in the Subsurface Water, as Indicated by the Enrichment Factors in Microcosm Tanks at Equilibrium.

Bioavailability and Sublethal Effects of Metals in Marine Shellfish

Metals such as copper may concentrate in marine sediments inhabited by large populations of clams, which burrow into marine sediments to escape their predators. We observed the burrowing behavior of littleneck clams (*Protothaca staminea*) in natural marine sediment that had been contaminated with copper. The sediment was mixed with a copper-seawater solution, rinsed with fresh seawater from Sequim Bay, Washington, and placed in trays with uncontaminated flowing seawater. Clams were placed on the sediment surface, and the time for each to achieve complete burial was recorded.

Copper levels of up to 17 parts per million (ppm) dry weight sediment had no significant effect on the clam burrowing time. Above this threshold, however, burrowing time increased logarithmically with increasing copper concentration. An increase to 33 ppm dry sediment resulted in a 100-fold increase in the mean burrowing time. In contrast, clams exposed to sediment mixed with tightly-bound (chelated) copper exhibited no significant change in burrowing time.

Burrowing time proved to be a sensitive measure of sublethal biological effects of metals sorbed to sediments, and may be particularly useful for measuring effects in situations where the metals might have long-term influence on survival of the clam populations.

Feeding habits of shellfish may strongly determine the degree to which they accumulate trace metals. In a 1982 study comparing suspension-feeding clams (*Protothaca staminea*) with deposit-feeding clams (*Macoma inquinata*), we observed that the deposit-feeding clam accumulated cadmium more rapidly. Projections of the cadmium uptake curve, however, indicated that the suspension-feeder eventually accumulated about 3.5 times more metal than the deposit feeder.

Marine shellfish concentrate dissolved metals from seawater using their gills. Bioconcentration factors (i.e., metal/g tissue ÷ metal/ml water) range up to 1000. Recent studies by others have led to the hypothesis that both bioconcentration and toxicity are related to how metals bind to sulfhydryl groups of carriers, enzymes, or proteins within organisms.

We examined our own data on bioconcentration of metals in light of the sulfhydryl-binding theory. Excised gills of the clam *Protothaca staminea* were exposed to nickel, cadmium,

copper, or mercury. We found that the less soluble the metal sulfide, the greater the bioconcentration. By measuring the bioconcentration of one metal in a particular organism and environment, we are now confident that one can predict, with reasonable accuracy, the bioconcentration of several other metals.

BIOLOGICAL EFFECTS OF TRACE METALS ON MARINE ANIMALS

Marine animals can respond to elevated concentrations of potentially toxic trace metals by using storage and detoxification systems. By examining the storage and detoxification mechanisms, we hope to better understand the abilities of marine species to adapt to and tolerate conditions of metal enrichment.

Enhanced Tolerance to Metal Toxicity

In previous work, we determined that low concentrations of mercury (0.05 to 0.5 parts per billion) could enhance the tolerance of the mussel *Mytilus edulis* to toxic concentrations of mercury. This enhanced tolerance may be linked to the induction of low-molecular-weight, metal-binding proteins.

We also found that copper at 5 ppb could induce enhanced mercury tolerance, but that neither cadmium at 1 ppb nor zinc at 10 ppb could do so. Tissue analysis showed that the copper was taken up and accumulated, but cadmium and zinc were not. The copper accumulation was also accompanied by induction of the metal-binding proteins.

We suspected that the mussel's tolerance to mercury was unaffected by cadmium or zinc in the above study because the cadmium and zinc concentrations were not high enough to result in either metal accumulation or in the induction of metal-binding proteins. To determine if this was true, we conducted an additional experiment with higher exposure concentrations of the two metals (i.e., 10 and 50 ppb cadmium and 50 and 250 ppb zinc). The results showed that the two cadmium concentrations and zinc at 50 ppb were capable of inducing enhanced tolerance to mercury toxicity. At 250 ppb, however, zinc was found to reduce tolerance to mercury, because zinc itself was toxic at this concentration. Metal accumulation was observed in all four cases. Both metals are known to induce the synthesis of metal-binding proteins.

Exposure to mercury, copper, cadmium, and zinc was able to enhance tolerance to mercury toxicity if exposure concentrations were sufficiently high so that both accumulation into

tissues and the induction of metal-binding proteins occurred. It appears that all four metals interact with a common system, most likely with the low-molecular-weight, metal-binding proteins believed to function in metal detoxification.

Immunoassays and High Performance Liquid Chromatography of Metal-Binding Proteins

Antibodies against low-molecular-weight, metal-binding proteins were developed in earlier work. Antibodies had to be developed before immunoassays for these proteins could be developed. In the past year, however, we discovered that the original protein preparation against which the antibodies were induced may contain low levels of minor contaminant proteins. Once the metal-binding proteins are further characterized and purified, we will continue with the immunoassay development. Immunoassays will allow us to quantify and localize metal-binding proteins directly in cells and tissues of exposed individuals.

A major problem with analyzing low-molecular-weight, metal-binding proteins is the time required for routine isolation. Currently, two days are required to analyze a single sample by gel chromatography, and only four samples can be analyzed each week. The use of high-performance liquid chromatography (HPLC) techniques can greatly alleviate this problem. We have found that a TSK-3000 SW column (Toyo Soda) can best achieve separation of this protein. The mobile phase that appears to be most suitable for separation is 0.2 M tris-HCl pH 7.4, 0.1 M NaCl. We have been able to separate both mercury-binding proteins and copper-binding proteins. The next objective will be to interface the HPLC system with an atomic absorption spectrophotometer so that the latter can serve as an in-line detector for our system. Use of HPLC in addition to immunoassays will greatly increase our current analytical capabilities.

Deep-Sea Hydrothermal Vent Study

This past year, G. Roesijadi participated in the Oasis Expedition, an international biological program to study the deep-sea hydrothermal vents at 21°N on the East Pacific Rise. These deep-sea environments are of particular interest to us because they are characterized by large deposits of trace metals. The unique animals inhabiting this environment exist on an ore-grade, metal-rich substratum. Understanding the mechanisms that enable these animals to adapt to such an environment may provide valuable information

on processes by which animals in coastal regions can adapt to elevated metal concentrations resulting from human activities.

Samples of the vent clam *Calyptogena magnifica* were collected during the Oasis Expedition and prepared for later analysis at the laboratory. Initial findings showed that these clams contain extremely high concentrations of trace metals. Zinc, copper, lead, silver, chromium, and arsenic were present at concentrations up to 1.5 orders of magnitude higher than concentrations in *Mytilus edulis*, a nearshore mussel. Low-molecular weight, metal-binding proteins were also present. Electron-dense intracellular granules in kidney cells contained iron, zinc, lead, copper, nickel, and silver. We will continue to examine the multi-elemental composition of these clams and also investigate the metal sequestration systems of these clams.

Examination of Foot-Long Clams from Recently Discovered Deep Sea Hydrothermal Vents in the Pacific Ocean May Show How the Animals Have Adapted to Extremely High Concentrations of Metals in this Ocean Environment. (Photo—Scripps Institution of Oceanography)

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Ecological Effects of Coal Conversion

Synthetic fuels from coal are part of a mix of energy sources that will help meet national energy needs. Many of the new technologies, including synfuels, will generate substantial quantities of waste materials and result in fugitive releases of potentially toxic materials. Many of these materials are not found in petroleum fuels, nor have their fate in and effects on the environment been established. Our studies have focused on the fate and effects of materials that might enter the environment by spillage. Our current emphasis is changing to examine long-term effects of materials entering the environment as a result of waste disposal.

Unusually rapid progress was made in understanding the distributional kinetics, biological transformation, and ecological effects of complex organic materials after they are released to aquatic and terrestrial ecosystems. We are now examining potential for bioaccumulation and transfer through food chains leading to people. In these studies we used a 2.9:1 blend of middle to heavy distillates from a solvent refined coal (SRC-II) pilot plant. This blend represents types of mixtures expected to result from coal liquefaction. Results of our studies continue to indicate that coal liquids are more toxic than petroleum-based fuel oils.

The organic materials found in synthetic fuels and in other energy sources have a wide range of physical and chemical properties. We are continuing to develop special methods of handling the materials, presenting them to test systems, and analyzing their chemical composition. The methods include chromatographic separations and identification of organic constituents of coal liquids, flow-through aquatic bioassays, and hydroponic and lysimeter evaluation of plant exposures. Through bioassays, behavioral testing, and microcosm studies, we are able to identify and assess organism and ecosystem response to stress and to the effects of complex hydrocarbons.

TRANSPORT AND FATE

Organic compounds found in complex mixtures released to the environment are distributed and transformed by natural processes into many components (Figure 1). Some organic compounds are water soluble and have toxicities that decrease with time; some compounds are transformed biologically as the substituted compounds are broken down by organisms. Some of the components, undoubtedly, are concentrated in food chains, and this is the focus of the current year's research.

Because composition of materials after release is constantly changing, the chemical dynamics must be defined before ecological effects can be quantified. We are continuing our studies of partitioning events that occur in aquatic systems (water and sediments) and soil systems (soil sorption, transformation by microbes). For example, sediments mixed with coal liquid and "weathered" in artificial streams for several weeks were analyzed for organic residuals. Samples were taken from both the upper and lower end of the streams and in different sediment strata. We measured rates of degradation and solubilization of several phenols and hydrocarbons; these compounds are known to be toxic to organisms. Results indicated that the lower

molecular weight phenols were rapidly desorbed from sediment. Less soluble hydrocarbons were persistent and remained in the sediment for a longer period. The higher molecular weight phenolic compounds and less soluble hydrocarbons are likely to have persistent biological activity.

In other experiments, we investigated the behavior of coal liquids in soil. Laboratory soil columns were used to study compound transport under hydrologically dynamic conditions. We also investigated adsorption reactions of primary amines in soil, and how these reactions can be reversed. In these systems, anilines were sorbed to soil more strongly than were phenols. Thus, the anilines can be expected to be more available for uptake and degradation, while the phenols will be more likely to enter ground-water systems. The microbial/biochemical transformation of alkyl anilines was investigated with single compounds and in the presence of coal liquid. Unlike certain other systems, the presence of the whole coal liquid does not substantially change the degradation of alkyl anilines.

During the past year, we also constructed a model of aquatic pathways to simulate

FIGURE 1. Natural Processes Can Transform and Distribute Complex Organic Mixtures and Thus Alter Their Concentrations in Aquatic and Soil Systems.

transport and fate of synfuel compounds in compartments of aquatic systems (Figure 2). The Aquatic Pathways Model (APM) was based on a review of existing models and was designed and implemented using rate constants for interactions of phenolic compounds in aquatic environments. By designing the APM to require only a few parameters, we are able to model the distribution of many compounds. The model runs on a microcomputer and is portable.

FIGURE 2. The Aquatic Pathways Model, Developed by PNL, Can Be Used to Simulate the Transport and Distribution of Many Organic Compounds in Aquatic Systems.

EFFECTS ON AQUATIC AND TERRESTRIAL SYSTEMS

Organisms representing several different trophic levels and ecological niches are also being investigated with respect to the toxicity of complex organic mixtures. These include algae, zooplankton, benthic invertebrates, and fish. Effects on behavior and growth are determined in each case. Laboratory stream studies are continuing for the purpose of assessing effects at the community level.

Terrestrial plant studies have included germination of seeds in soil contaminated with a coal liquid. Growth, seed production, and water uptake of barley have been monitored. We found earlier that coal liquids were much more toxic than petroleum fuel oils and that toxicity varied with the exposure method used. Although the toxicity decreased with weathering, our current findings show that the coal liquids were more toxic than petroleum, and germination and growth were significantly reduced by the coal material after a year of weathering. Mixing the coal material into the upper 10 cm of the soil column resulted in higher toxicity than simply spilling the material on the surface and covering it with 10 cm of clean soil. The difference appears to be related to more rapid partitioning of toxic volatile compounds to the air in the case of surface

contaminations. Clearly, in both aquatic and terrestrial research, understanding the results obtained and drawing conclusions from them will depend on the test system used.

We have found that the water-soluble fraction of a coal liquid material governs most of the observable biological effects, and it is more toxic to aquatic species than are petroleum fuel extracts. In earlier studies we examined toxicity to daphnids, chironomids, and fishes. During the past year *Corbicula* (freshwater clams) were exposed to a water-soluble fraction of the coal liquid for up to 14 days and then held in river water to examine latent effects. In contrast to most other freshwater invertebrates, clams are relatively sessile, long-lived, and have sufficient tissue for analysis of accumulated synfuel residuals. Clams cannot move away from a fuel spill and could accumulate significant quantities of toxic compounds. Mortality was noted only after 14 days exposure to 50 ppm total phenols, or levels three times greater than those acutely toxic to fathead minnows. The clams were more tolerant than fish species. Clams exposed to acutely lethal doses for 7 to 14 days exhibited valve closure, thus avoiding exposure to the toxicant. Sublethal effects on growth for surviving clams were not detected at test concentrations of 0.5, 5.0, or 50.0 ppm total phenols.

The response of fish to the same water-soluble fractions was assessed in behavioral studies. Because fish are mobile, they can potentially avoid exposure to toxic levels of coal liquids if they detect toxic compounds. Exploratory avoidance tests conducted this past year with juvenile rainbow trout indicated that in contrast to fathead minnow tested earlier, trout did not avoid acutely toxic concentrations of the water-soluble fraction (2.4 to 6.3 ppm total phenols).

Microcosm studies of streams are being used to examine the effects of coal liquids on aquatic communities. Last year the test material was mixed directly with stream sediments, and stream colonization was subsequently monitored. Chemical analyses were made of stream sediment and water samples to define the dynamics of the material in the sediment/water system. Because results of the microcosm studies showed a high variance in the community response measurement, this information was used to design a second experiment.

This year, the material was added to streams that had been colonized by naturally occurring organisms (primary producers, herbivores, and benthic organisms). Preliminary

results after 4 days of exposure and 10 days of subsequent recovery indicated that these treatments were more toxic to benthic organisms and herbivores than they were to periphyton. In both studies, herbivores were apparently less tolerant than primary producers. At low doses of coal material, algal biomass increased due to reduced predation following mortality of the herbivores.

FOOD CHAIN TRANSFER

Some of the components of synfuels are potentially hazardous to human health in other than occupational exposure situations. One potential route of exposure is through accumulation of these compounds into living tissue and subsequent transfer to people through food chains. We are currently examining the potential for bioaccumulation of components of organic mixtures in different organisms from both aquatic and terrestrial food chains.

Both uptake and depuration of synfuel materials are influenced by the physical and chemical properties of the materials. They are also influenced by the ecological relationships among organisms. Our aquatic food chain studies are designed to provide an understanding of these factors. Short-term uptake and elimination experiments were initiated to determine the potential for synfuel materials to enter food chains. Water extracts of coal synfuels contain relatively high concentrations of phenols and anilines. We used kinetic model theory to estimate bioconcentration factors (BCFs) of phenol and aniline from uptake and elimination data in the green alga, *Scenedesmus quadricauda*, and in the freshwater zooplankton, *Daphnia magna*. These organisms are representative of the base of most aquatic food chains. Studies were also undertaken to develop experimental methods for monitoring fate of coal liquid residuals in fish tissue.

In studies with *S. quadricauda*, which we analyzed in 1982, we found that the measured BCF exaggerated the amount of aniline and phenol available for biological transfer. Results indicated that the phytoplankton rapidly concentrated ^{14}C -labeled aniline and phenol from the water column. Because the compounds were eliminated rapidly, we thought initially that the potential for bioaccumulation appeared relatively low, but this turned out to be an incorrect inference. High performance liquid chromatography of both the filtrate and the algae subsequently showed that although concentrations of phenol and aniline remained constant in water during the 24-hr exposure period, these compounds were

rapidly degraded or metabolized in the algae. After 24 hr, aniline accounted for only 65% and phenol for only 37% of the acetone extractable radioactivity. Our work suggests a need for understanding both the quantity and form of compounds measured in aquatic organisms.

In juvenile rainbow trout, *Salmo gairdneri*, we also determined uptake and depuration rates of representative compounds. The trout were exposed to both a single compound and to several complex compounds in the mixture. The presence of the water-soluble fraction of coal liquid influenced the bioaccumulation of phenol alone.

About 35% less radioactivity was accumulated by fish exposed to equal concentrations of ^{14}C -phenol when coal liquid water solubles were present than when they were not present. This indicates that perhaps other lipophilic components compete with phenol for adsorption into tissue. An important finding in this work is that BCFs determined from measurements with pure compounds in the laboratory may seriously differ from BCFs in actual environmental situations. The simultaneous presence of a complex organic mixture may also influence the nature of absorption processes. We found that a larger portion of absorbed ^{14}C remained bound to trout tissue following single compound exposure than when

coal liquid components were present. To fully understand the uptake process, new analytical methods need to be developed to identify the compounds present in fish tissue. We have initiated development of such methods.

To follow up on trends observed in rainbow trout exposure, we also conducted complementary studies on daphnids (Figure 3). Initial studies with daphnids also indicated that single compound experiments do not always predict uptake accurately. Moreover, any measure of the potential for bioaccumulation must consider all environmental variables. It is noteworthy, in the uptake and depuration experiments mentioned (Figure 3), that a significant proportion of the accumulated material remained in the tissue after 20 hr of depuration. This may be important for estimating long-term accumulation in food chains. Estimation will require a substantial level of effort in the future, and we are preparing for such studies now.

Rate of uptake and extent of bioconcentration of fossil fuel components in terrestrial plants are also under study. We have shown, for example, that quinoline, a synfuel component, becomes concentrated in tissues of legumes; metabolic products and conjugates form concurrently in this case, as well.

FIGURE 3. Daphnids Exposed to Phenol Accumulated Less Radioactivity When Coal Liquid Water-Solubles Were Present Than When the Solubles Were Not Present. Results of this experiment are being used to determine how interactions between compounds influence the uptake kinetics of single compounds from a complex mixture.

In studies of soybean uptake of quinoline, we found that the compound was transported to plant stems. Detailed chemical analysis was used to identify metabolic products of the material. Additional studies are underway to determine how quinoline is metabolized in soybeans.

MEETINGS AND PUBLICATION ACTIVITIES

We participated in meetings held at Airlie House in January 1982 to plan and coordinate the Synfuels research program among the National Laboratories. In addition, we participated in several scientific meetings and published several papers in the open literature. We participated in the Oak Ridge Fifth Life Sciences Symposium on "Synthetic Fossil Fuel Technologies: Results of Health and Environmental Studies"; the annual meeting of the Society of Environmental Toxicology and Chemistry; and the Western Division American Fisheries Society Annual Meeting.

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Solid Wastes: Mobilization, Fate and Effects

The goal of this program is to establish a sound scientific basis for predicting water and solute movement and the biological availability of solutes in solid wastes disposed of to the ground. In current work, we are establishing how weathering and residence time affect the mobility of inorganic and organic pollutants from permanent waste sites, and how these pollutants could affect aquatic and terrestrial environments. The information developed also helps establish more precise engineering criteria for solid waste disposal, and procedures for mitigating possible long-term effects.

Current emphasis in the program has been on oil shale. Like other energy technologies that produce solid wastes, a mature oil shale industry would generate wastes by different retorting technologies and would dispose of wastes in different geographic locations. Several common processes, however, influence the mobilization, transport, and biological effects of solutes in wastes disposed of to the ground. To elucidate the processes, a conceptual basis was developed that uses linked geochemical and hydrologic models as a framework. The model is being used to:

- 1) integrate the activities of a multidisciplinary research team (geochemistry, microbiology, inorganic and organic chemistry, hydrology, biology) necessary to examine different, but related, environmental processes;
- and 2) focus research on priority, rate-controlling processes and functional relationships necessary for generic applicability of the results.

In laboratory investigations, we determined basic mechanisms controlling solute form and behavior. Residence time in the environment and concomitant field studies were then used to establish parameters for laboratory studies and validate models. The integrated effects of mineralogical and microbiological processes were found to control chemical dissolution, solute distribution between the solid and aqueous phases, and solute form. The same processes also control leachates from raw shale, retorted shale, and co-disposed waters and sludges.

Because we found that hydrologic processes govern the rate and extent of solute movement in waste embankments, we are examining leachate attenuation by waste liners and substrata. Key geochemical and hydrologic processes are being modeled to estimate hydrologic transport of the inorganic and organic solutes. The model is being validated at above-ground disposal sites; at the same time, we are determining aquatic toxicity, mutagenicity and availability of solutes for plant uptake, and how organic and inorganic solutes are mobilized with time and weathering.

Research in this program has demonstrated that simple assays required by the Resource Conservation Recovery Act are not adequate for estimating the long-term effects of oil shale solid waste disposal on water quality. This is because a complex succession of interrelated chemical and microbiological processes alter the entire chemistry of retorted shale embankments and leachates with time. We developed new methods and concepts to identify, measure, and explain the integrated effects of these processes. Similarly,

Organic Constituents Are Sometimes Important in Determining the Behavior and Long-Term Fate of Solid Wastes. Gel permeation chromatography is used to separate organic compounds from complex mixtures that leach from a solid waste pile.

coupling hydrologic modeling with laboratory and field measurements is allowing us to identify controls on water movement. Controls, for example, include the quantity of water added to the waste at time of disposal and the effects of water use by plants used in surface stabilization. Currently, we expect to define precisely the geochemical and hydrologic controls on rate and extent of these changes with time. We are incorporating this information into chemical and hydrologic models to describe solute attenuation and movement.

Although earlier emphasis was to develop an understanding of the processes influencing the behavior of solid wastes, the studies already have markedly improved capabilities for site evaluation and waste management. Our results are currently being used by several state and federal agencies in the permitting and regulatory process, and also by industry for design and construction of disposal sites.

During 1982, the hydrologic model UNSAT1D was adapted to simulate water movement in a conceptual scenario involving an embankment that contains retorted shale of known physical and hydrologic properties (Figure 1). Simulations were used to identify key controls on water movement. With assumptions for climate, retorted shale properties, and embankment construction, we made estimates of potential evapotranspiration (PET) for the region relative to retorted shale infiltration. The estimates indicate that although PET is far greater than precipitation, the distribution of rainfall throughout the year results in some water infiltration. Between 60% and 90% of the rainfall was lost to evaporation. Plant transpiration would further reduce the amount of infiltration. In this simulation, precipitation alone would not result in drainage from the base of the embankment over a 30-yr period if the shale was initially at 15% (dry weight basis) water content. At 15% water content, significant storage capacity remained, limiting the possibility of drainage for a number of years.

At initial water levels near saturation (~30% water content), drainage would occur after the first year and continue for the simulation period. After approximately 20 yr, the pile would approach an equilibrium state where the amount of drainage would nearly equal the amount of precipitation infiltrating at the surface. Initial water content will therefore likely be an important factor controlling leaching of solutes from retorted shale embankments.

FIGURE 1. Surface Evaporation and Transpiration, and Lateral Intrusion of Ground or Spring Waters Will Control the Leaching of the Shale Pile Interior. These complex events require a conceptual model for predicting long-term storage and leaching out of the embankment.

In addition to studying the geohydrologic events, we will examine how plants may modify constituents in the soil, particularly organic compounds derived from buried wastes (Figure 2). Exploratory work has been conducted under the coal conversion program, and further work is being considered for inclusion in a solid waste program.

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Radionuclide Fate and Effects

Naturally occurring and man-made radionuclides can be released into the environment at several stages in the nuclear fuel cycle. The releases may be planned or accidental, and both types can contaminate the environment. One goal of ecological research at PNL is to develop a basic understanding of how radionuclides behave in the environment, and to use this knowledge to build a scientific basis for making decisions about the siting, operation, and decommissioning of nuclear facilities and the methods for waste disposal.

The studies reported here deal with the full range of contaminant behavior and fate, from the initial physicochemical factors that govern radionuclide availability in terrestrial and aquatic environments to studies of contaminant transport by biological means. By design, we focus more on the biologically and chemically mediated transport processes and food-chain pathways than on the purely physical forms of contaminant transport, such as transport by wind and water.

A primary objective of the work is to develop realistic dose assessment models and to provide accurate parameters for applying those models. We use integrated laboratory and microcosm studies to define basic mechanisms; we use field studies to verify predictive models and to identify important processes at the community and ecosystem levels.

ENVIRONMENTAL TRANSPORT PROCESSES

Technetium is one of several radionuclides that could be released to the environment, primarily through the air. Recent research has shown that technetium is mobile in soils and is readily taken up by plants. These properties, combined with the element's long physical half-life, mean that humans could receive doses of technetium through food-chain transfer. Technetium's mobility in soil, which regulates its entry into the food chain, is influenced by pH, the presence of hydrous oxides of iron and manganese, the soil's organic matter content, and microbial activity. Technetium uptake by plants and observed toxicity in soil at levels $>0.1 \mu\text{g/g}$ indicate that technetium behaves like a nutrient. Our prior laboratory studies with technetium and nutrient anions suggested that plants react to technetium much like they react to sulfur. To examine this hypothesis, we analyzed extracts from several plant species allowed to grow with their roots in contact with TcO_4^- . Our results showed that technetium is incorporated into higher molecular weight organic molecules, and that the degree of incorporation depends on the plant species and plant component.

The chromatographic behavior of the soluble technetium fraction after gel permeation, when extracted from the roots of soybean (*Glycine max*) and garlic (*Allium sativum*) plants, is shown in Figure 1. Most of the technetium present in soybean roots is soluble and still present in the TcO_4^- form. Garlic roots, however, contain only a minor fraction of technetium present as TcO_4^- ; most

of the technetium is incorporated into organic materials having higher molecular weight, which elutes faster from the gel column.

In all species of plants examined to date, the quantity of technetium transported to the portions of the plants exposed to the air (e.g., leaves and stems) was reduced whenever a significant fraction of technetium in non- TcO_4^- forms was incorporated in the roots. Also, animal feeding studies conducted this year show that technetium is principally concentrated in the yolk of Japanese quail eggs and in the hair of mammals. Yolks and hair are sulfur-bearing tissues, and the concentration of technetium in them supports our hypothesis that technetium is a sulfur analog.

FIGURE 1. Technetium Is Incorporated into Several Organic Fractions Extractable from Plant Roots by Chromatography (see peaks at center). Anionic technetium (peaks at right) is ten-fold higher in soybean roots (solid line) than it is in garlic roots (dotted line).

In addition to our technetium work, we are studying the role of transuranic elements in food-chain transfers. Previous studies with plutonium and neptunium show that the roots of soybean and alfalfa plants absorb these elements and store them in their metabolic compartments. Plutonium supplied as $\text{Pu}(\text{NO}_3)_4$ to hydroponically grown plants is poorly absorbed by roots; less than 0.001% is transferred to shoot tissues. Root absorption can be increased by three orders of magnitude by using Pu_3DTPA_3 to maintain solution solubility. This treatment also increases the plutonium fraction transferred to shoot tissues. Most of the plutonium metabolized by roots and leaves is found in the MW fraction of solubles $>10,000$. In our experimental soybean plants, only 0.00035% of the absorbed plutonium was in the seed. Most of this ($>80\%$) was unsolubilized in the seed coat and would be unavailable for absorption if ingested by animals.

Our experiments with neptunium show that it behaves differently than plutonium. In soybean, alfalfa, and more recently, bushbean plants, absorption is nearly total; shoot tissues contained ~30%, 6%, and 40%, respectively, of the root-absorbed neptunium. Unlike plutonium, metabolized neptunium was distributed among several high molecular weight fractions, e.g., $>30,000$, 30,000-5000, 5000-500 and <500 . This was found in leaves, stems, and roots. For all plants studied, less than 5% of the neptunium was found in stem tissues, suggesting the presence of efficient plant solubilization processes. Neptunium was also found in seeds at maturity in bushbean plants. A substantially higher fraction was observed than that found with plutonium. Most of the seed-incorporated neptunium is soluble and extractable (~75%).

The soil processes that keep transuranic elements soluble prior to root absorption appear to influence greatly plant uptake and, therefore, the movement of these elements into the ingestion pathway (Figure 2). Differences in uptake among plants and the fractional distribution of plutonium and neptunium among component plant parts (seed, leaf/stem, root) reflect the fact that some crops (cereal grains, roots or tubers, leafy vegetables or forage) contribute more of certain elements to the food chain than others.

In addition to our laboratory studies, we are also conducting field studies with plutonium, americium, cesium, neptunium, and uranium. The need here is to determine what differences from the laboratory data can be expected as plants are exposed for long per

FIGURE 2. Most of the Plutonium Entering the Soil Remains Bound to the Soil Fraction, and the Amount Entering Man Through Food Chains is Small. The biologically available fraction entering man, however, can vary over several orders of magnitude depending on microbial and other soil processes.

iods of time under realistic environmental conditions. The experimental method involves agricultural and range plant species in contaminated outdoor soil columns known as lysimeters. We expect the responses to vary with temperature and moisture fluctuations, cropping schedules (for legumes, cereal grains, and grasses), and the type of tillage operation used. Annual progress involves maintaining the lysimeter system, recording climatic variables, and tilling, seeding, irrigating, weeding, and harvesting the crops.

Currently, comparison of ^{239}Pu uptake for peas and barley shows that the uptake of nitrate ^{239}Pu was 7 to 20 times greater than uptake of the oxide form. However, the uptake of both chemical forms decreased by about an order of magnitude over three growing seasons. This event would not be readily ascertainable by laboratory approaches. We also expect to differentiate the more important environmental and culture variables that influence contaminant availability.

RADIONUCLIDE TRANSPORT PARAMETERS

Radionuclide transport pathways are not known with certainty for many elements. Our work on transport pathways has permitted us to identify areas where clarification is most urgently needed. We recently conducted laboratory experiments on Japanese quail to estimate the transfer from feed to eggs and meaty tissues (Figure 3). The magnitude of this transfer is considerably greater (up to three

FIGURE 3. Eggs Proved to be an Unexpected Point of Accumulation for Technetium, and As Such Could Provide a Source of Contamination for Man. Technetium is probably a sulfur analog (see text).

orders of magnitude) than previously believed. This accumulation may be explained by the fact that technetium behaves like sulfur in biochemical pathways. We expect that these data will result in upward revisions of commonly used technetium transfer coefficients for the feed-to-fowl ingestion pathway. Revisions to calculated ingestion doses for man will follow.

Our work this year also involved reviewing the parameters used in dose assessment models for calculating the dose that humans would receive if they consumed fish from contaminated waters. Both abiotic and biotic factors have been under examination. We are seeking a general relationship for use in predicting the radionuclide concentrations in fish in different contaminated environments. Factors reviewed for the relationship include physicochemical speciation, the influence of stable isotope dilution, the presence of chemical analogs, and the known biological role of the radionuclide. Approximately 30 radionuclides common to the nuclear fuel cycle are included in this study. We expect this work to result in a reference volume or handbook for use in dose assessment.

ECOLOGICAL INTERACTIONS INFLUENCING REMEDIAL ACTIONS AT WASTE SITES

Radionuclides disposed of in shallow-land burial sites may be moved by root intrusions and animal burrowing. The extent to which biotic transport occurs depends on the type of plants and animal communities at a site. To determine the differences, we examined the occurrence of pocket mice, a common burrowing animal, in areas primarily vegetated by

either cheatgrass or wheatgrass. These two grasses can be used to stabilize the soil surface of low-level waste burial grounds in western climates. Our results show that pocket mice were five times more abundant in wheatgrass-dominated communities than in cheatgrass areas. We then calculated the relative radionuclide concentrations at the soil surface in each community and found that concentrations were higher and increased for a period of 70 to 100 years in wheatgrass communities because of the burrowing activity of these animals (Figure 4).

FIGURE 4. Burrowing of Small Animals Makes Radioactivity More Available to Grasses Growing on the Soil Surface. When burrowing activity is projected through a 200-year interval (using models developed in this program), we found that certain grasses favor transport of radioactivity to the surface.

The contribution of these biotic transport processes to radionuclide movement has yet to be evaluated quantitatively. The test results do imply that the choice of one surface cover over another can influence both the long-term biotic transport potential of radionuclides and their resultant dose to populations.

Field work on how to clean up inactive uranium mills entered its third season this year. Our goals have been to identify and develop methods for stabilizing the surface of tailing piles, and to find a way of preventing plants and animals from penetrating surface covers, thus allowing radon gas to escape. Field data obtained from the vicinity of inactive uranium mill tailings sites were grouped using the clustering technique into six general plant community types: steppe, annual steppe, shrub steppe, woodland, forb-dominated steppe, and salt-shrubs. The grouping will now help us identify the most important environmental factors that affect protective covers.

We conducted model simulations and field lysimeter studies of rock and plant covers to evaluate the effect that surface cover type has on moisture dynamics within the overburden and underlying tailings. The results of the simulations and the preliminary findings of the lysimeter studies show that rock covers increase the moisture content of soil by reducing surface runoff and evaporation. If a rock cover is applied to tailings in such a way that plant growth is eliminated, water will accumulate in the tailings and eventually percolate into the ground water. Soluble contaminants in tailings could thus contaminate groundwater.

A major product of this year's work is a plan for designing covers that satisfies the needs of a particular site (Figure 5). A designed cover must control surface erosion adequately and not jeopardize the containment of the biologically toxic components of the tailings. Adequate erosion control is, logically, that which is required to keep the protective layer intact for the design life of the containment system. A surface cover, therefore, can be any combination of soil, rock, and vegetation that is effective and economical.

A slow-release herbicide in a polymeric carrier was developed and tested during the past year. Our goal was to create an effective system for controlling plant root penetrations through the earthen covers used to cap uranium mill tailings piles. A polymer system containing 58% polymer, 24% herbicide, and 18% carbon black was developed and

tested. Our studies show that the minimum effective concentration of the herbicide in soil for our delivery system was in the range of 0.3 to 6.4 ppm for the species tested.

Loose rock, asphalt, and multilayered earthen barriers were tested to see if they could withstand the burrowing of townsend ground squirrels (*Spermophilus townsendii*) and white-tailed prairie dogs (*Cynomys leucurus*)--information that simply has not been available. Each barrier (Figure 6) was placed in two of eight outdoor pens; the remaining pens served as controls. The ground squirrels and prairie dogs burrowed a maximum of 15 cm into the rock layer of the multilayer earthen barriers. The deepest penetrations in all rock layers occurred along the walls of the boxes. No evidence of penetration by prairie dogs was found with the asphalt barriers. We found that nearly half of the soil was displaced by burrowing, but there were few distinct tunnels. The 3-inch-thick asphalt barriers were effective in preventing intrusion by both rodent species tested. The larger of the two animals, the prairie dogs, were able to move the rock more easily, but were still excluded by the barriers. This finding implies that intrusions into buried waste (uranium mill tailings in this case) by plants and animals can be effectively deterred by an adequately designed barrier system.

TRANSURANIC CHEMICAL SPECIES IN GROUNDWATER

The migration of mobile forms of the transuranic radionuclides in groundwater is being studied by a resin bed sorption technique. Previous studies at this site showed that traces of plutonium are transported in the groundwater in a soluble, anionic chemical form in which the plutonium is predominantly (~90%) in the V or VI oxidation state. A hydroxy carbonate anionic complex of the plutonium may be this mobile form. During fiscal year 1982, we installed a series of three new sampling wells. Once the mobile radionuclide chemical species form at the crib/trench, they pass through the soil column relatively unattenuated in the groundwater and do not accumulate in downstream soils.

ANALOGS FOR TRANSURANIC CHEMISTRIES

The goal of this research has been to develop quantitative understanding of the environmental behavior of naturally occurring rare earth elements so that the data might be used to estimate the behavior of some of the transuranic elements. Attention has been focused on americium and curium--transuranic

FIGURE 5. When Applying Ecological Data to a Surface Stabilization Problem, Many Detailed Considerations Must Be Systematically Evaluated. A significant product of the current year's work was development of the model shown above, which is designed to protect a containment system from erosion and biotic intrusion.

elements that also exist environmentally in the +3 oxidation state.

Data on rare earth element behaviors are being investigated for their effectiveness in estimating the radiological dose that man would receive from environmentally weathered transuranic elements. An important consideration in estimating human dose regards the concentrations of the natural rare earth elements in food products. If the hypotheses are correct, these rare earth concentration data might be useful in estimating the potential dietary intake of transuranic elements which have weathered in the environment for

long time periods. Foodstuffs, representing a typical "market basket" for the American populace, were obtained, and sub-samples of the edible inner portions were analyzed by radiochemical neutron activation analysis for the entire suite of rare earth elements. In general the compiled data demonstrate that the rare earths are present in foodstuffs at extremely low levels, near the detection limits of our state-of-the-art analytical methods (parts per billion). Concentrations in foods represent depletions of approximately 10^{-5} from the total soil concentrations based on average crustal abundance.

FIGURE 6. Information Has Not Previously Been Available on Practical Techniques to Prevent Animal Intrusion at Waste Burial Sites. Studies to optimize several different types of a barrier system showed that a properly configured asphalt layer was highly effective. Animals could penetrate rock layers, but did not burrow through the asphalt layer.

ACKNOWLEDGMENTS

This program consists of research projects 80551/000551, 80623/000623, 80099/003378, 80834/000834, 81329/0001329, and 82004/002004 which are described in Field Task/Proposal Agreements (Form 5120.2). Principal Investigators for these projects are L. L. Cadwell, T. R. Garland, D. E. Robertson, R. G. Schreckhise, W. Weimer and R. E. Wildung.

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Statistical and Theoretical Research

Research in fiscal year 1982 has led to the development of improved statistical methods for quantifying and assessing environmental changes produced by energy developments. In light of these advancements, the descriptive studies of years past have become obsolete.

At PNL, scientists are studying the fate of contaminants in ecosystems. Using controlled studies, census techniques, and models of population dynamics, we have developed methods capable of detecting changes in the abundance of animals as a result of either contaminant exposure or landscape manipulations (e.g., construction activities, cooling ponds, buried wastes of non-nuclear or nuclear origin).

Significant accomplishments include the creation of field designs to detect population impacts, new census procedures for small mammals, and methods for designing studies to determine where and how much of a contaminant is extant over certain landscapes.

A book describing these statistical methods is currently being written and will apply to a variety of environmental contaminants, including radionuclides. PNL scientists also have devised an analytical method for predicting the success of field experiments on wild populations. Two highlights of current research are the discoveries that population of free-roaming horse herds can double in four years and that grizzly bear populations may be substantially smaller than once thought. As stray horses become a public nuisance at DOE and other large Federal sites, it is important to determine their numbers. Similar statistical theory can be readily applied to other situations where wild animals are a problem of concern to other governmental agencies. Another book, on statistical aspects of radionuclide studies, is written specifically for researchers in radioecology; the book will be of use to scientists in other areas of research as well.

EXAMINING THE FATE OF CONTAMINANTS FROM ENERGY DEVELOPMENTS

Efficient field sampling designs, backed by statistical analyses, enable scientists to assess public health and safety impacts from exposure to radionuclides more quantitatively. The focus of this effort is on data handling and development; that is, describing the pattern of distribution of radionuclides in the environment, estimating concentrations within ecosystems, and designing sampling schemes to assess the need for cleanup activities. Such analyses tell us whether contaminant levels comply with environmental guidelines and regulations.

Communications between statisticians and scientists doing radionuclide studies are being improved by publishing a variety of articles of common interest and by distributing TRANS-STAT, a publically available newsletter, to Department of Energy contractors. Publication of the newsletter fulfills PNL's objective of making information available to principal users. The upcoming publication of a book based, in part, on past issues of TRANS-STAT will make these methods widely available to the general scientific community. Several commercial publishers have offered to publish the 17-chapter manuscript, which includes information on how to

estimate transfer coefficients using ratios, design field surveys and studies, assess the uncertainty of dose-transport model predictions, and estimate the spatial distribution and inventory of contaminants.

Three new topics were developed and published in TRAN-STAT during fiscal year 1982. Issues 17 and 18 of the newsletter completed a series on extreme-value theory in radionuclide studies; Issue 19 described statistical design aspects of finding "hot spots" of contamination in the environment; and Issue 20, contributed by the Los Alamos National Laboratory, examined ways of statistically estimating parameters in nonlinear transport models. Issues for the coming year will include articles on using ratios to estimate transfer coefficients, doing probability plotting, designing field studies, and assessing uncertainty in dose-transport models.

In 1982, we again assisted the Nevada Applied Ecology Group with statistical design and analysis aspects of radionuclide studies on the Nevada Test Site. This work involves the continued development of Kriging methods for estimating the spatial distribution of radionuclides. In other related work, we provided the NRC with statistical designs for its field studies on stabilizing tailings

piles. We also analyzed both uranium bio-assay and in vivo measurements from participating NRC licensed uranium mills (NRC funded). In addition, the PNL staff helped prepare a guide book on decommissioning and decontaminating waste sites for DOE. Of particular interest was DOE's decision to hold a workshop on the ocean disposal of low-level radioactive waste in which PNL staff participated (Division of Remedial Action).

CENSUSING WILD AND STRAY POPULATIONS OF LARGE ANIMALS

The construction and production operations of energy facilities potentially affect the dynamic responses of animals in their natural habitat, particularly at sites where the land holdings are extensive. Because populations of either wild or stray animals cannot be studied in the laboratory, accurate field techniques are needed to determine how these populations respond to environmental pressures. To date, several new census methods and population dynamics models have been developed by PNL scientists that allow the accurate census of large animals such as

horses. The methods are widely applicable to other animals such as the grizzly bear, the bowhead whale, and the manatee. Studies of the latter species are important to other agencies because the animals are included on the endangered species list or are actively considered for inclusion on that list. Our ability to cooperate with other concerned agencies has permitted the use of a wider data base needed for sound development of the statistical theory for wildlife of concern to DOE.

The National Marine Fisheries Service, the National Park Service, and the Fish and Wildlife Service have primary responsibility for managing these populations of large animals. Also involved are the International Whaling Commission, the U.S. Marine Mammal Commission, the National Academy of Sciences, and the Bureau of Land Management.

In a study of horse herds last year, PNL discovered that the size of a free-roaming herd may double in only four years. Direct aerial counts at a site in Oregon show that the herd's population increase rate approached 20% per year, a rate that can be achieved

Horses Can Be a Public Nuisance at Large Federal Sites. In Oregon, aerial counts taken this year indicate that wild horse herds have higher survival and reproduction rates than domestic horses (see text).

only if survival and reproduction rates are higher than those of domestic horses. Adult survival was found to be high.

Nine papers covering research on this project were published in fiscal year 1982. The investigators also contributed to the efforts of two national committees and held continuing faculty appointments at two universities and at the Joint Center for Graduate Study in Richland, Washington. Through these studies and our other methodology research, we are developing management approaches that seek to balance the real risks to an endangered species against energy development needs.

The PNL staff has also created and published a census method for use in the controlled removal of animals and continued to do research on population regulation and dynamics. The latter work included publishing an assessment of the unusual rate of growth in feral horses. Computer models to determine the population dynamics of a number of species were also implemented. Finally, we are working on a book that summarizes this area of statistical research.

DESIGNING STUDIES TO DETECT EFFECTS ON MOBILE SPECIES

Small, wild animals that are naturally present on the site of industrial facilities often cause a variety of safety and other problems. Their reclusive nature makes an accurate population count nearly impossible, so investigators must usually base their population studies on mere estimates of animal abundance. Unfortunately, population estimates tend to be imprecise. This reliance on estimation rather than on direct measurement distinguishes these particular wildlife studies from other ecological sampling programs.

Through computer studies, we have found that the standard statistical approaches (as used in agriculture, sociology, and industrial quality control) are not appropriate for quantifying effects on mobile wildlife species. Statistical tests supposedly performed at a significance level of $\alpha = 0.5$ are actually at the 0.3 or 0.4 level, values which are very little different from chance (i.e., 50/50). Similarly, when a population changes, the standard analyses are less likely to detect that change compared to the special statistical tests recently developed at PNL.

Data transformations have proved to be an important aspect of the development of the new statistical methods. We have found that

Population Abundance Is One Method of Measuring Whether Or Not Particular Animals Are Affected by Pollutants.

the appropriate transformation of abundance data depends on the type of census technique used. When a capture-tag-recapture method is used, a logarithmic transformation of the data is required. However, when a removal census (catch-per-unit-effort) is used, an "inverse" transformation is necessary. Appropriate data transformation can increase the power of statistical tests of treatment effects by as much as 20% with no additional expense to the field study.

Another area of improvement has been to devise new statistical methods (i.e., "treatment effects") when attempting to compare the effects of different treatments on animal populations, as for example testing for effects of pollutants. The traditional reason for censusing wild populations has been to estimate animal numbers in order to manage the resource purposely. This leads to inappropriate methods for comparing treatment effects. By using the "catch" index present in all wildlife census techniques, we have developed statistical tests that are 2 to 20 times more efficient than comparable methods which use the absolute abundance data from

wildlife censuses. Again these improvements do not increase the cost of field studies and, furthermore, sometimes can actually decrease the cost of expensive wildlife studies.

In reviewing the biological and statistical literature, we found no useful guidelines for designing effective field studies to test for changes in populations. The practical consequence of this finding is that many apparently well-conceived field studies are virtually designed to fail. In current work, therefore, we have developed the first analytical calculations for computing the probable success of proposed field experiments. To date, these analytical techniques enable us to make comparisons of only two populations at one time. For more complex scenarios involving multiple populations subject to environmental impacts, Monte Carlo computer techniques were used to assess probable success rate. We are now developing simple hand calculations for these larger field studies.

Specific information about the populations being compared is necessary to design cost-effective studies and may have to be obtained in advance of determining treatment effects. Information, however, is needed in the following areas:

- expected size of populations
- sampling precision
- variability in population numbers across the landscape
- costs of establishing study plots versus costs of trapping.

Field data and cost functions can then be combined to yield optimal field study designs.

Optimization techniques will be an important tool of the future, because they can be used to determine the most useful sampling program and cost-effective approach for a wildlife study. If the optimal design is determined to be economically unfeasible, all alternative approaches will necessarily also be ineffectual, thus suggesting that dollars for environmental studies should be spent elsewhere.

For conducting long-term studies of population dynamics, we have developed an algorithm that can be used to determine the optimal size of a study plot and the appropriate trapping effort to use in censusing a population. This algorithm eliminates much of the

guess work in designing a population study and results in the greatest sampling precision for the monitoring dollars available. We are now conducting research to extend this capability to multi-plot population studies.

Multi-plot population studies must balance trapping costs and census precision with the costs of establishing study sites and the natural heterogeneity in animal numbers across the landscape. During the last year, two important findings have brought us closer to optimizing such field studies, which are inherently more complicated. First, we developed and field tested a model for predicting the effect that alternative levels of effort may have on sampling precision. Second, we identified a relationship which allows us to predict plot-to-plot variance knowing mean animal abundance. These relationships will be used to determine the effect that alternative levels of field replication (number of plots) and sampling (trapping effort) will have on the capacity of field studies to detect reasonable changes in animal numbers with both statistical assurance and significance.

ACKNOWLEDGMENTS

This program consists of research projects 80184/003386, 80244/003913, and 82011/002011, which are described in Field Task Proposal/Agreements (Form 5120.2). Principal investigators for these projects are L. L. Eberhardt, J. M. Thomas, R. O. Gilbert, and J. R. Skalski.

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Interagency 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 DOE work.

BONNEVILLE POWER ADMINISTRATION

Environmental Information Gathering and Evaluation Related to River Operations (Principal Investigator: D. H. Fickeisen)

Biological Studies of a 1200-kV Prototype Transmission System near Lyons, Oregon (Principal Investigator: L. E. Rogers)

ENVIRONMENTAL PROTECTION AGENCY

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

Ecological Effects of New Coal Conversion Technologies (Principal Investigator: D. H. Fickeisen)

Quantitative Methods for Detecting Environmental Pollution (Principal Investigator: J. M. Thomas)

Radiation Dose-Risk Estimates Associated with the Practice of Deep Sea Disposal of Low-Level Nuclear Waste (Principal Investigator: W. L. Templeton)

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

Biomonitoring Using Honeybees (Principal Investigator: J. M. Thomas)

Field Evaluation of Hazardous Waste Site Bioassays (Principal Investigator: J. M. Thomas)

Collection of Sediment from Commencement Bay, Washington (Principal Investigator: E. A. Crecelius)

KNOLLS ATOMIC POWER LABORATORY

Benthic Boundary Layer Program (Principal Investigator: J. S. Young)

NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants (Principal Investigator: R. E. Wildung)

NATIONAL OCEANOGRAPHIC AND ATMOSPHERIC ADMINISTRATION

Effect of Oil on the Behavior of Clams and Worms (Principal Investigator: W. H. Pearson)

Organic Pollutants in Waterways Adjacent to Commencement Bay (Principal Investigator: R. G. Riley)

Dosing of Oysters with Stable and Radioactive Cadmium (Principal Investigator: J. T. Hardy)

Significance of Metal-Binding Proteins and Lysosome-Like Vesicles in Mussels in a Metal-Contaminated Environment: An Experimental Field Study (Principal Investigator: G. Roesijadi)

Outer Continental Shelf Environmental Assessment Program (Principal Investigator: W. H. Pearson)

History of Contaminant Accumulation in Puget Sound and Commencement Bay, Washington (Principal Investigator: E. A. Crecelius)

Fate of Pollutants Discharged to Puget Sound: Accumulation in Sediments and Marine Birds (Principal Investigator: R. G. Riley)

NUCLEAR REGULATORY COMMISSION

Environmental Standard Review Plans (Principal Investigator: D. G. Watson)

Quantitative Assessment of Aquatic Impacts of Power Plants (Principal Investigator: D. H. McKenzie)

NUCLEAR REGULATORY COMMISSION (contd)

Biocide By-Products in Aquatic Environments
(Principal Investigator: R. M. Bean)

Relevance of Biotic Paths to the Regulation
of Nuclear Waste Disposal (Principal Investi-
gator: D. H. McKenzie)

Changes in Bioavailability of Low-Level
Radioactive Effluents After Passage Through
Soil (Principal Investigator: D. G. Watson)

Statistical Methods for Siting and Managing
Low-Level Radioactive Waste Sites (Principal
Investigator: T. L. Page)

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

Relevance of Biotic Paths to the Regulation
of Nuclear Waste Disposal (Principal Investi-
gator: D. H. McKenzie)

OAK RIDGE NATIONAL LABORATORY

Coordination and Planning of Acid Deposition
Research for the Interagency Task Force
(Principal Investigator: D. H. McKenzie)

U.S. FISH & WILDLIFE SERVICE

Analytical Support for Studies of Effects of
Oil Production on Aquatic Organisms (Princi-
pal Investigator: R. G. Riley)

U.S. GEOLOGICAL SURVEY

Development of Cost-Effective Biomonitoring
Techniques for Federal Oil Shale Lease Tracts
(Principal Investigator: J. R. Skalski)

Other Highlights

OTHER HIGHLIGHTS

FACILITIES

In October 1982, U.S. Senator Henry M. Jackson dedicated Battelle's new Marine Research Laboratory at Sequim, Washington. About 150 people attended the dedication. The new facility adds 23,400 sq. ft. to pre-existing laboratory, office, and conference space, and it houses modern chemistry laboratories along with 35 new offices and support facilities. Other improvements were made to the older beach facilities, including upgrading the sea water systems to 250 gal/min capacity with backup. On the beach, a variety of experimental systems can now 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 is also developing, with corporate resources, a Disease Diagnostic Center at the Sequim laboratory. Facilities for the center include a complete immunology laboratory, where animal colonies are maintained for producing immune sera used in fish and shellfish testing. Disease control has 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.

Also during October, a long-awaited facility was completed by DOE for research on aerosols. 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, several field investigations have identified unexpected contaminant sources at industrial sites believed to be operating under acceptable environmental conditions. The new facility greatly augments capabilities for evaluating aerosol contamination. Included are a wind tunnel and a system of instruments and equipment for particle generation and analysis.

The wind tunnel, a 50-foot-long test apparatus, 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, a hot-wire anemometer, and a laser doppler velocimeter for measuring velocity profiles, turbulent intensity, and roughness length. Wind speeds can be

Completion of the New Hilltop Addition, Shown Here, Makes the Marine Research Laboratory at Sequim, Washington, a Major West Coast Facility. Laboratory, office and conference space total 40,000 sq. ft., with fully equipped sea water facilities and holding tanks.

Expansion of the Marine Research Laboratory at Sequim was Completed in 1982. Attending the dedication were (left to right) Senator Henry Jackson, Sherwood Fawcett, and Burton and Artis Vaughan.

A 50-Foot-Long Wind Tunnel Is One of the Specially Designed Facilities Used to Study Airborne Contaminants.

achieved for 2 to 65 mph, 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 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 studies. These include an evaporative-condensation generator for 0.03 to 1.3 μm monodispersed particles, a vibrating orifice gen-

The Center for Marine Disease Control, A Component of MRL, Sequim, is Equipped for Diagnosis and Research on Aquatic Animal Diseases. Shown is typical apparatus for retinal diagnostic observation of fish.

erator 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 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 ; a remote laser particle spectrometer for measuring velocity and size in the range from 1 to 200 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.

STAFF

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

American Society for Testing and Materials (ASTM)

C. D. Becker: Task Group on Procedures for Acute Tests with Dispersants and Oil, member

T. M. Poston: Committee E-47. Toxicity Testing, member

Bureau of Land Management

J. W. Anderson: Scientific Advisory Committee for Research on the Studies Program for the Outer Continental Shelf, member

Department of Energy

Raymond E. Wildung: Executive Com., Oil Shale Task Force, member

Federal Marine Mammal Commission

L. Lee Eberhardt: Com. Scientific Advisors, member

International Atomic Energy Agency

W. L. Templeton: Consultant Group on Safety Series #5 (Disposal of Radioactivity into the Oceans), member

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