**Pacific Northwest Laboratory
Annual Report for 1979
to the DOE Assistant Secretary
for Environment**

Part 2 Ecological Sciences February 1980

**Prepared for the U.S. Department of Energy
under Contract EY-76-C-06-1830**

**Pacific Northwest Laboratory
Operated for the U.S. Department of Energy
by Battelle Memorial Institute**

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PACIFIC NORTHWEST LABORATORY
operated by
BATTELLE
for the
UNITED STATES DEPARTMENT OF ENERGY
Under Contract EY-76-C-06-1830

Printed in the United States of America
Available from
National Technical Information Service
United States Department of Commerce
5285 Port Royal Road
Springfield, Virginia 22151
Price: Printed Copy \$____*; Microfiche \$3.00

*Pages	NTIS Selling Price
001-025	\$4.00
026-050	\$4.50
051-075	\$5.25
076-100	\$6.00
101-125	\$6.50
126-150	\$7.25
151-175	\$8.00
176-200	\$9.00
201-225	\$9.25
226-250	\$9.50
251-275	\$10.75
276-300	\$11.00

3 3679 00054 5568

PNL-3300 PT2
UC-11

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

February 1980

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Pacific Northwest Laboratory
Richland, Washington 99352

PREFACE

Pacific Northwest Laboratory's (PNL) 1979 Annual Report to the Department of Energy (DOE) Assistant Secretary for Environment describes research in environment, health, and safety conducted during fiscal year 1979. 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 1-4 report on research performed for the DOE Office of Health and Environmental Research. Part 5 reports progress on all other research performed for the Assistant Secretary for Environment, including the Office of Technology Impacts and the Office of Environmental Compliance and Overview. Each part consists of project reports authored by scientists from several PNL research departments, reflecting the interdisciplinary nature of the research effort. Parts 1-4 are organized primarily by energy technology.

The parts of the 1979 Annual Report are:

Part 1: Biomedical Sciences

Program Manager - H. Drucker

D. L. Felton, Editor

Part 2: Ecological Sciences

Program Manager - B. E. Vaughan

B. E. Vaughan, Report Coordinator

C. H. Connally, Editor

Part 3: Atmospheric Sciences

Program Manager - C. E. Elderkin

R. L. Drake, Report Coordinator

G. B. Long, Editor

Part 4: Physical Sciences

Program Manager - J. M. Nielsen

J. M. Nielsen, Report Coordinator

J. L. Hooper, Editor

Part 5: Environmental Assessment, Control,
Health and Safety.

Program Managers - D. L. Hessel

S. Marks

W. J. Bair, Report Coordinator

C. M. Unruh

R. W. Baalman, C. W. Dotson, Editors

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 Dr. 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

FOREWORD

This volume of the Annual Report describes all of PNL's work in the ecological sciences funded by the Office of Health and Environmental Research (OHER). This covers ecological research in the following budget activity categories:

G - Environment

GK - Multi-Resource

GK-01-02-03-1 Identification Transport and Conversion
of Energy-Related Pollutants in the Environment

GK-01-02-03-2 Environmental Effects of Energy-Related
Processes and Pollutants in the Environment

GK-01-02-03-3 Energy-Related Supporting Research

GK-01-02-03-4 Environmental Operations and DOE Support Activities

For convenience, organization charts for the several participating departments and sections of PNL can be found at the back of the report.

Wherever possible, we have grouped the individual research projects (Field Task Proposals) according to the energy technology they primarily support. With the abstracts that follow, principal investigators are listed who may be contacted for further information about individual projects. This format should make the report useful not only to OHER but to managers of technology programs as well. The reader can turn to an appropriate section of this report set off by a blue divider page to find those ecological investigations by PNL that are pertinent to his concerns. The reader should note that for several large programs, research on biomedical effects, atmospheric dispersal and chemical characterization is reported separately in other volumes of the Annual Report (see Preface). This involves principally the Synfuels (Section 4), Nuclear Fission (Section 5), Nuclear Fusion (Section 8) and Electric Field (Section 11) research programs.

PNL's direct experience with the oil, synfuels and shale industries, and with other agencies concerned with energy installations, has contributed in a particularly pertinent way to the understanding of the major environmental problems that the technologies will need assistance in solving. Not all such work is reported herein. Nevertheless, all work for other agencies and industry can be located in the bibliography named below^(a). Projects funded specifically on the basis of interagency agreement with DOE are briefly described in Section 12 of this report.

In our ongoing discussions with technology representatives, a misunderstanding frequently occurs concerning the purposes of DOE work in the ecological sciences and concerning the usefulness of the quantitative data developed in these programs. Basically, the ecological programs are designed to assist the technologies in meeting the intent of the National Environmental Policy Act. In meeting this objective, part of the work is designed to anticipate problems "around-the-corner," so that the developing technology may be suitably modified before major capital investment is committed. Theoretical studies, particularly those described in Sections 7 and 10 of this report, are concerned with improving the basis for measuring environmental effects. In many situations, current measurements of ecological processes are highly provisional, not having adequate statistical basis, and better suited as order-of-magnitude estimates only. In these areas, it is in the interest of the technologies that a groundwork be laid for reliable data, acquisition of which is likely to be required by the agencies responsible for setting standards.

Several large segments of programs were transferred to the Environmental Protection Agency (EPA) in October 1979. These projects are reported here because the work was substantively conducted under DOE sponsorship. The projects transferred include Methodology for Collecting and Interpreting Ecological Data (Section 7), petroleum effluent effects studies in Marine Research (Section 6) and a portion of the bioassay work in Synfuels (Section 4). At the close of FY 1980, these efforts will be reported in a separate annual report to EPA.

In December 1979, the Battelle trustees approved construction of a new laboratory to expand PNL's Marine Research Laboratory at Sequim, Washington. The six-million-dollar expansion will approximately double the size of present office, laboratory, and wet laboratory facilities used by PNL's Marine Sciences Section. With construction of the Northern Tier Pipeline now imminent and with other new energy developments in the Pacific Northwest, the decision to expand the Marine Research Laboratory reflects Battelle's confidence in regional research development and the need for it.

Significant staff involvement in scientific activities at a national level continued during 1979. Dr. Jack Anderson was appointed to the National Oceanographic and Atmospheric Administration/Environmental Research Laboratory Amoco-Cadiz Research Assessment Committee. Mr. William Templeton was appointed to Scientific Committee #64 of the National Council on Radiation Protection and Measurements (NCRP). Dr. Lee Eberhardt continued on the Federal Marine Mammal Commission and on the Committee of Scientific Advisors on Marine Mammals, and was also appointed to the National Academy of Science Committee on Free-Roaming Wild Horses and Burrows. Dr. Ray Wildung continued as a member of the NAS Committee on Accessory (Trace) Elements (Chairman of the Oil Shale Panel) and as a member of the NAS Board of Mineral Resources Committee on Soil as a Mineral Resource. Dr. Richard Foster also was appointed to Scientific Committee #64 of the NCRP. In addition, several staff members served as associate editors of various learned journals (W. Ted Hinds, Ecology; C. Dale Becker, Trans. Amer. Fish. Soc.; and Jack Anderson, J. Marine Environ. Res.).

Burton E. Vaughan
Subprogram Manager
Ecological Sciences

- (a) A Bibliography of Environmental Research
PNL-SA-4655, Rev. #5
Ecological Sciences Department
Pacific Northwest Laboratory
Richland, WA 99352

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1 National Environmental Research Park and Land Use

NATIONAL ENVIRONMENTAL RESEARCH PARK AND LAND USE

- **Hanford National Environmental Research Park**
- **Terrestrial Ecology (ALE Administration)**
- **Restoration of Surface-Mined Lands**
- **Long-Term Ecological Monitoring**

The U.S. Department of Energy has large land holdings in the arid and semi-arid regions of the western United States. One of the most strategically situated of these is the U.S. DOE Hanford Site in the state of Washington. The Hanford Site currently contains the obsolete facilities developed for wartime plutonium production and stores large amounts of radioactive waste materials. As an additional health and safety measure, these facilities were buffered by a large land area unoccupied by people. Today this buffer zone provides some of the least disturbed and therefore most desirable land for long- and short-term terrestrial and aquatic ecological research in the United States. In recognition of this research potential, the Arid Lands Ecology (ALE) Reserve and the Hanford National Environmental Research Park (NERP) were established to promote the use of the Hanford Site for ecological research, especially studies related to energy technologies and their potential for ecological impacts in arid regions.

The terrestrial ecology studies, most of which are conducted on the Hanford Site, are concerned with short- and long-term energy technology impacts, construction and operational impacts, and natural perturbations in shrub-steppe ecosystems. These projects provide the kind of information needed by the National Environmental Policy Act (NEPA), as well as advancing the state of ecological knowledge in the scientific community at large. Land restoration studies, located in Washington and in Arizona, are aimed at testing alternative ways to successfully revegetate surface-mined lands in most arid parts of the western United States.

Some effluents of energy technology are not confined to the land holdings of any particular state, federal or private ownership and are dispersed widely over regional landscapes. Air pollution is one of these. The ecological monitoring of long-term effects investigates the widespread subtle but potentially damaging effects of air pollution in the Pacific Northwest, one of the most ecologically diverse regions in North America.

Throughout Annual Report:

- Bullets denote WPAS titles.
- ® Use of trade name does not imply Battelle endorsement.

• National Environmental Research Park

Principal Investigators: W. H. Rickard and M. L. Warner

Other Investigator: J. L. Warren

The Hanford National Environmental Research Park (NERP) is an outdoor laboratory dedicated to education and research leading to a better understanding of ecological systems and the impact of energy technology development upon them. The Hanford NERP is one of four federally designated research parks in the United States. It supports ecosystems representative of western shrub-steppe, some of which are believed to be in near-pristine condition. These ecosystems serve as baseline cases suitable for objectively judging the magnitude and direction of ecosystem-level changes induced by time and man's manipulation of the environment.

Educational Use

The Hanford NERP is used for study by graduate students and faculty from various universities. Presently, two Northwest Colleges and Universities for Science (NORCUS) students, both from Washington State University, are using the facilities and land for research. Mr. Bradley DeWaard is investigating the ecological behavior of Canada goose (*Branta canadensis*) broods along the Hanford reach of the Columbia River. Mr. Peter Bookman is using radio-isotopes to determine plant root distribution and biomass. Dr. Richard Newsome, a faculty member of Beloit University, is investigating the taxonomy of a native perennial grass, bottlebrush squirrel-tail (*Sitanion hystrix*), in relation to new concepts of numerical taxonomy. Dr. Newsome is a NORCUS Faculty Appointee.

Other students are also using the Hanford NERP for research. Ms. M. C. McShane is investigating the effect of fire on bluebunch wheatgrass (*Agropyron spicatum*), and Ms. M. A. Parkhurst is investigating the ecological distribution of pink balsamroot (*Balsamorhiza rosea*), a threatened species. Both of these students are enrolled at the Joint Center for Graduate Study, Richland, Washington.

Research Use by University Staff

Two National Science Foundation projects continue to use the Hanford NERP as field study sites. Dr. James Kenagy of the University of Washington is investigating

the ecological physiology of desert rodents, and Dr. Richard Mack, Washington State University, is studying the environmental factors affecting seedling survival of native and introduced grasses in arid and semi-arid climates.

Cooperative Studies with State and Federal Wildlife Agencies

The Washington State Game Department has expressed concern about the existence of a self-established herd of elk (*Cervus canadensis*) on the Hanford NERP because of potential damage claims by neighboring farmers and ranchers. The elk herd consists of about 10 animals. At least two calves were born in 1979. The herd is manageable at this size and provides unusual opportunities for ecological studies of large ungulates in a harsh environment.

The Washington State Game Department has provided personnel to suppress the coyote (*Canis latrans*) population responsible for the decline of the nesting Canada goose (*Branta canadensis*) population on Columbia River islands. This is the second season of coyote suppression. It is too early yet to tell if it has benefited the goose population.

Historically, migrating waterfowl have used the Columbia River as a resting stop. In recent years the number of ducks, including mallards (*Anas platyrhynchos*), and geese using the Columbia River has declined. PNL staff members are using radiotelemetry techniques to determine the local movements of mallards.

Other NERP Projects and Concerns

Endangered and Threatened Species

An uncommon species found on the Hanford NERP is the American bald eagle (*Haliaeetus leucocephalus*). It is considered threatened in the State of Washington and endangered in most other states. Historically, small numbers of eagles have used the Hanford reach of the Columbia River as a feeding area. The eagles feed primarily on carcasses of spawned-out salmon. In recent years the number of eagles wintering along the Hanford reach has increased slightly.

Fire

A lightning-ignited fire burned about 2500 ha of sagebrush/bunchgrass vegetation on the Arid Lands Ecology (ALE) Reserve in July 1979. New roads and vehicle tracks were left by fire suppression activities, and one study plot was lost in the same manner. No damage to facilities was reported. Human disturbances of the landscape will be visible long after the effects of the fire have disappeared.

Stray Livestock

No stray livestock wandered onto the ALE Reserve in 1979. However, about 11 stray horses inhabit the Hanford Site. Two foals were born in 1979. If the stray horse population increases, management practices will need to be implemented to prevent depletion of and damage to range forage.

NERP-Related Research

NERP research in 1979 centered on investigating the use of desert shrubs as ecological monitors of air pollution, especially by trace metals. Special litterfall collectors have been tested as a nondestructive and systematic way to obtain leaf material for chemical analyses. Samples of desert shrub leaves have been obtained through cooperative studies with the NERP at the Idaho National Engineering Laboratory and the NERP at the Los Alamos Scientific Laboratory. The initial results of chemical analyses of fallen leaves of desert shrubs collected in 1978 is shown in Table 1, compared with National Bureau of Standards orchard leaf samples.

TABLE 1. Trace Metals Detected in Litterfall from Three National Environmental Research Parks Compared with Trace Metals in National Bureau of Standards Orchard Leaf Samples(a)

Site	Species	Metals in Litterfall, $\mu\text{g/g}$					
		% Ash	B	Cu	Mn	Mo	Zn
Hanford Site	Sagebrush(b)	8.63	73	15	180	2.3	27
	Sagebrush(c)	7.85	74	11	90	2.0	14
	Bitterbrush	5.02	77	10	93	2.4	14
	Greasewood	27.37	59	8.3	202	1.9	30
	Hopsage	11.44	40	5.9	147	6.0	14
Idaho National Engineering Laboratory	Sagebrush	9.23	42	24	106	2.3	47
	Bitterbrush	6.52	47	8.5	53	1.9	15
	Greasewood	25.37	26	11	146	3.1	30
	Hopsage	18.69	54	4.5	118	2.7	23
Los Alamos Scientific Laboratory	Sagebrush	7.69	57	12	102	2.7	50
National Bureau of Standards	Orchard Leaves	—	30	13	87	<0.5	25
	Certified Value	—	33	12	91	0.3	25

(a) Because the western NERP's are located in regions remote from industrial air pollution, these values represent indigenous or baseline levels. Any increase in these levels can be attributed to anthropocentric induced changes in trace elements.

(b) Low-elevation stand

(c) High-elevation stand

• Terrestrial Ecology

Principal Investigators: L. E. Rogers and W. H. Rickard

Other Investigators: R. E. Fitzner, K. A. Gano, M. L. Warner, J. L. Warren and R. H. Sauer

Technical Assistants: M. A. Combs, C. A. Lee and L. F. Nelson

The Terrestrial Ecology program, conducted on the Arid Lands Ecology (ALE) Reserve, supports long-term basic ecological studies needed for understanding how ecosystems function under both natural and man-induced stressing agents. Major current and recent study topics include the impact of plowing, cattle grazing and wildfire; revegetation of steep slopes; leaf fall monitoring of desert shrubs for ecological trends; endangered species; biotic transport (food webs) analysis; plant phenology (climatic associations); population studies of shrub-steppe biota (birds, mammals, insects) and wildlife utilization of seral plant communities.

Two of the tasks under the Terrestrial Ecology program are designed to protect the ALE Reserve from unauthorized entry and to collect microclimatic data throughout the Reserve, which spans approximately 120 square miles. Maintenance of fence boundaries and periodic aerial surveillance insure the Reserve's protection. Twenty-six meteorological stations at different locations on the Reserve provide climatic information.

Wildlife utilization of disturbed plant communities is presented in detail for this year's Annual Report. This section of the Annual Report also summarizes a 10-year comparison of aboveground primary production of plant communities in near-pristine condition with those of self-established plant communities on plowed ground and the results of a study of shrub productivity, as measured by litterfall collection, before, during and after a drought year.

Wildlife Utilization of Disturbed Plant Communities

Disturbance of native habitats in the semi-arid western regions resulting from siting of energy-related facilities will almost surely create seral annual grass communities. Past research here has shown that these communities are able to maintain themselves longer than 30 years. Our study of wildlife utilization is designed to evaluate relevant sampling techniques and to provide a data base to ascertain what these changes mean to resident wildlife. The study compares the vegetation, ground-dwelling beetles, small mammals, birds and large mammals on two each adjacent replicated plots of native bunchgrass and cheatgrass community types. The cheatgrass community plots were old agricultural fields that, because of plowing, had been invaded by alien cheatgrass to a greater extent than pristine communities are invaded.

Vegetation

Descriptions of the vegetation on each plot were obtained using phytomass and canopy coverage estimates. Phytomass samples were randomly selected, then clipped, sorted by species and dried. Twelve 0.032-m² samples from the invaded plots and ten 0.5-m² samples from the pristine plots were collected twice during the 1979 growing season.

Both the invaded and pristine plots yielded similar amounts of green vegetation early in the season, but the cheatgrass community surpassed the bunchgrass community in vegetative production as the season progressed. Phytomass values were three to four times larger than that from the pristine fields on the second harvest period (Table 2). This is attributable to the success of cheatgrass both in density and seed production on the invaded plots. Cheatgrass seeds resulted in 21% and 48% of the dry weight on the two

TABLE 2. Phytomass (g/m² + S.E.) and Canopy Cover on Pristine Plots and Plots Invaded by Alien Vegetation

Study Area		Phytomass				Total Cover	
		Total Live	Litter	Total Live	Seed	Percent-age	Number of Species
		April(a)		June(b)			
Invaded Plots	#1	38.1 ± 5.5	846.1 ± 88.4	195.3 ± 43.2	40.4 ± 5.9	42.7	5
	#3	23.4 ± 8.6	726.9 ± 167.0	166.8 ± 18.0	80.0 ± 14.5	38.6	5
Pristine Plots	#2	41.4 ± 6.0	163.4 ± 26.8	51.4 ± 9.8	0.6 ± 0.2	74.9	19
	#4	29.8 ± 4.0	106.8 ± 12.4	40.4 ± 9.4	0.8 ± 0.3	60.6	14

(a)Harvest day 1

(b)Harvest day 2

invaded plots while bluebunch wheatgrass (*Agropyron spicatum*) seeds only contributed 1% and 2% of dry weight on the pristine plots.

Large amounts of litter also characterize the two invaded plots. Almost continuous mats of fallen litter cover the floor of both plots. However, much of the litter taken from the pristine fields was standing litter composed of bluebunch wheatgrass and Sandberg bluegrass. Here, areas between vegetation were largely bare.

Canopy cover was estimated. The pristine fields had a larger percentage of total cover and a greater variety of species than did the invaded fields. The pristine plots were dominated by bluebunch wheatgrass and had a large diversity of growth forms, including shrubs and many species of annual and perennial forbs. The invaded fields contained a very low diversity of plant species, with cheatgrass and introduced weeds predominating. However, some native forbs, particularly lupine, are becoming reestablished on the invaded plots.

In addition to phytomass and cover data, phenological descriptive data were taken throughout the 1979 growing season. These data established the duration of foliar growth and seed production important in identifying potential food source supplies for wildlife throughout the year.

Ground-Dwelling Beetles

Ground-dwelling beetles are known to be important components in the diet of many wildlife species. The abundance of this insect group may influence the attractiveness of a habitat to a particular wildlife species. The abundance of ground-dwelling beetles on the invaded and native grassland study areas is shown in Table 3. Some beetles, such as *Eleodes novoverrucula*, *Amara* sp. and *Harpalus* sp. clearly appear to prefer the cheatgrass habitat. Others, such as *Eleodes granulata*, are only common in the native area.

Small Mammals

The relative abundance and species composition of small mammal populations on the two plots were compared. Four trapping sessions were conducted during the year for five consecutive nights each; live traps were used on the first two nights and snap traps on the following three nights. Live-trapping provided mark-capture information, while use of snap traps provided an estimate of relative abundance.

A total of seven small mammal species were captured during the four sessions (Table 4). They were, in order of abundance, the Great Basin pocket mouse (*Perognathus parvus*), deer mouse (*Peromyscus maniculatus*), western harvest mouse (*Reithrodontomys megalotis*),

TABLE 3. Ground-Dwelling Beetles on Pristine Plots and Plots Invaded by Alien Vegetation^(a)

Taxa	October 1978		March 1979		July 1979		September 1979	
	Invaded Plot	Pristine Plot	Invaded Plot	Pristine Plot	Invaded Plot	Pristine Plot	Invaded Plot	Pristine Plot
<i>Tenebrionidae</i>								
<i>Stenomorpha puncticollis</i>	5	4					7	12
<i>Philolithus densicollis</i>							1	12
<i>Eleodes novoverrucula</i>	2	1	1	0	40	23	67	17
<i>Eleodes hispilabris</i>					14	12	10	31
<i>Eleodes obscura</i>					0	4	3	1
<i>Eleodes nigrina</i>					9	32	2	7
<i>Eleodes granulata</i>					1	18	0	11
<i>Coniontis setosa</i>					15	3	4	48
<i>Coniontis ovalis</i>							15	1
<i>Coniontis lanata</i>					29	0	2	0
<i>Eleodes humeralis</i>	0	1						
Totals	7	6	1	0	108	92	111	140
<i>Carabidae</i>								
<i>Amara</i> sp.	4	5	0	2	16	1	5	1
<i>Harpalus</i> sp.	5	0	11	0	31	0	37	0
<i>Calosoma luxatum</i>			0	9				
<i>Agonum jejunum</i>			0	7				
<i>Cymindris</i> sp.					0	3		
Totals	9	5	11	18	47	4	42	1
GRAND TOTALS	16	11	12	18	155	96	153	142

^(a) Each study site is comprised of two replicates. The totals shown are for seven-day collection periods.

TABLE 4. Small Mammal Captures in Pristine Plots and In Plots Invaded by Alien Vegetation^(a)

Species Trapped	November 1978		March 1979		June 1979		August 1979 ^(b)	
	Invaded Plots	Pristine Plots	Invaded Plots	Pristine Plots	Invaded Plots	Pristine Plots	Invaded Plots	Pristine Plots
Pocket Mouse	9	62	1	24	14	40	7	28
Deer Mouse	1	6		8	0	1		
Harvest Mouse		1		9			0	2
Montane Vole			2	1	1	0		
House Mouse	1							
Pocket Gopher	1	1						
Merriam Shrew	1	1	1	1				
Total	13	71	4	43	15	41	7	30

^(a) Each study site is comprised of two replicates. The totals shown are for seven-day collection periods.

^(b) Trapping session was terminated after four days because grasshoppers had sprung more than half of the snap traps, causing unequal trapability.

montane vole (*Microtus montanus*), house mouse (*Mus musculus*), pocket gopher (*Thomomys talpoides*), and the merriam shrew (*Sorex merriami*). The pocket mouse was the most frequently captured species and was most common in the bunchgrass community. Deer mice were also more frequently caught in the bunchgrass than in the cheatgrass community, mostly during November and March. Most harvest mice

were also caught during March. Other species were only infrequently captured.

Birds

Seven species of birds occurred in the invaded fields, while 19 species inhabited the pristine areas (Table 5). The sage sparrows (*Amphispiza belli*), Brewer's sparrows

TABLE 5. Average Number of Birds Sighted per Census on Pristine Plots and Plots Invaded by Alien Vegetation

Species	Invaded Plots											
	O	N	D	J	F	M	A	M	J	J	A	S
Horned lark	7			2.3		31	21	23.5	9.7	25	5	11
Meadow lark	0.7					7	9	10.5	10.7	2	17	8
Vesper sparrow												
Savannah sparrow								0.5				
Sage sparrow												
Brewer's sparrow												
Lark sparrow												
White-crowned sparrow												
Hungarian partridge												
Chuckar				15.3								
Goldfinch												
Robin												
Barn swallow												1
Mourning dove								0.5				3
Magpie												
Loggerhead shrike						0.3						
Raven												
Marsh hawk												
Golden eagle												

Species	Pristine Plots											
	O	N	D	J	F	M	A	M	J	J	A	S
Horned lark	7			6.3		24	18.5	16.3	13	6	4	3
Meadow lark	4					21.7	24	26.7	21	32	35	26
Vesper sparrow							9.5	11	15	1		2
Savannah sparrow							1.5	1				
Sage sparrow						1.3		0.7	1	1	2	
Brewer's sparrow								5.7	1.7			
Lark sparrow												3
White-crowned sparrow								37				
Hungarian partridge	1.7					0.7	1		3.3	2		
Chuckar				3.3		12.3	11	1.3	1.3			
Goldfinch	0.3											
Robin						0.7						
Barn swallow											1	
Mourning dove								1				
Magpie	0.3			1.3		21						
Loggerhead shrike	0.7			0.3								
Raven				1.3		1						
Marsh hawk						0.3						
Golden eagle				0.3								

(*Spizella breweri*) and vesper sparrows (*Poocetes gramineus*) were observed nesting in the pristine fields but never in the cheatgrass fields. Horned larks (*Erimophila alpestris*) and meadowlarks (*Sturnella neglecta*) occurred commonly in both areas, but meadowlarks tended to nest in the pristine areas. Predatory and semipredatory birds, which include loggerhead shrikes (*Lanius ludovicianus*), magpies (*Pica pica*), ravens (*Corvus corax*), marsh hawks (*Circus cyaneus*) and golden eagles (*Aquila chrysaetos*) occurred almost exclusively in the native fields.

Large Mammals

All observations of medium-sized and large mammals in the cheatgrass plots and native plots were recorded whenever bird surveys were conducted. During the three consecutive days of monthly bird surveys, deer and elk were observed regularly. Undoubtedly, many of the same animals were noted more than once. In order to arrive at a "relative use" index for the areas, the highest daily count figures for each of the two habitat types were used.

The medium-sized and large mammals noted during this study included coyotes, mule deer, elk and porcupines (Table 6). Mule deer were most abundant in native areas while elk were most often observed in the cheatgrass habitats. Coyote and porcupine observations were too few to allow association with any particular habitat type.

Primary Productivity of Dryland Grass Communities: A 10-Year Summary

Measurements of aboveground primary productivity, expressed in grams per square meter per year ($\text{g}^{-2} \text{yr}^{-1}$), were made in three grass communities on the ALE Reserve. One site, at an elevation of 400 m, supports a plant community dominated by native perennials, chiefly bluebunch wheatgrass (*Agropyron spicatum*). This site is in nearly pristine condition, as evidenced by the virtual absence of alien plants. The other sites are located near, but not adjacent to, the native grassland site, one at 305 m and the other at 520 m elevation. These two sites are dominated by cheatgrass (*Bromus tectorum*), an alien annual grass of European origin. Cheatgrass has dominated these sites with little reinvasion by native species since cultivation of the fields ceased over 36 years ago. None of the study sites have been grazed by cattle or sheep since 1943 when the land was initially incorporated into the boundaries of the Hanford Site.

Over a 10-year period, self-established cheatgrass communities were more productive, on the average, than the native grass community. Mean productivity averaged $219 \text{ g}^{-2} \text{yr}^{-1}$ for the cheatgrass community at the higher elevation and $270 \text{ g}^{-2} \text{yr}^{-1}$ at the lower elevation (Table 7). The native grass community had a mean productivity of only $64 \text{ gm}^{-2} \text{yr}^{-1}$ over an eight-year period. The lower-elevation cheatgrass community was more productive than any of the 10 native grassland sites

TABLE 6. Large and Medium-Sized Mammals Recorded in Pristine Plots and Plots Invaded by Alien Vegetation

Month Recorded	Species							
	Mule Deer		Elk		Coyote		Porcupine	
	Invaded Plot	Pristine Plot	Invaded Plot	Pristine Plot	Invaded Plot	Pristine Plot	Invaded Plot	Pristine Plot
October		9					1	
January		10	2		2	2		
March								
April	3							
May	2	6	5					
June		7	4					
July	2		9	9				
August	3				2			
September	0							
Total	10	32	20	9	4	2	1	

TABLE 7. Aboveground Primary Production and Growing Season Precipitation for Cheatgrass and Bunchgrass Communities

Year	Production (g/m ² /yr)				Precipitation (cm)		
	Cheatgrass		Bunchgrass		Cheatgrass		Bunchgrass
	520 m	305 m	400 m (Site 1)	400 m (Site 2)	520 m	305 m	400 m (Site 1)
1969	226	327			25.1	21.7	24.5
1970	208	145			21.6	19.3	25.0
1971	263	216	72		20.9	17.3	21.4
1972	107	128	73		18.8	16.4	17.4
1973	266	233	62		14.3	11.5	12.9
1974	282	397	92 ^(a)	78	36.4	31.4	36.2
1975	237	309	115	70	25.3	23.0	24.8
1976	97	134	74	58	18.9	17.0	20.5
1977	82	13	10	11	8.4	6.1	6.0
1978	425	797	89	87	26.2	20.2	22.8
Mean	219	270		64 ^(c)	21.6	18.4	21.2
Coefficient of Variation	0.47	0.80		0.36			
Efficiency ^(b)	10.1	14.7		3.0			

(a) A fire had occurred on the site during August 1973. Figures below here are for the burned portion of the site. Mean is based on the unburned portion plus production for 1971 through 1973.

(b) Efficiency = production/precipitation (cm).

(c) This mean is derived from the first three figures in the Site 1 column and all the Site 2 figures. Because Site 1 burned, its mean was not calculated.

studied in the International Biological Program's (IBP) Grassland Biome network, although it is located in one of the most arid portions of North American grasslands. Mean productivity at the IBP sites ranged from a low of 21 g⁻² yr⁻¹ at a desert site in New Mexico to a high of 241 g⁻² yr⁻¹ at a mountain meadow in Montana. All of these grasslands fall at the low end of the range of 100 to 1500 g⁻² yr⁻¹ for temperate grasslands worldwide.

The two cheatgrass communities were more productive than the native grass community, and also more variable. Annual productivity at the lower cheatgrass site ranged from 13 to 797 g⁻² with a coefficient of variation of 0.80. For the cheatgrass site at the higher elevation, the range was 82 to 425 g⁻² with a coefficient of variation of 0.47. The native grass community's productivity ranged

from 11 to 86 g⁻² with a coefficient of variation of 0.36. All three sites showed very low production in 1977, a drought year (Table 7). Some of the aboveground production in the native community is in tissues unaccounted for here, such as wood and leaves of sagebrush (*Artemisia tridentata*). Nevertheless, production of sagebrush is not nearly enough to account for the wide differences observed between the native and alien communities. A double sampling method of estimating sagebrush phytomass in this same community indicates that aboveground production is only about 10 g⁻² yr⁻¹. Sagebrush, although conspicuous, is not an important contributor to primary production in this community.

A fire burned through a portion of the native community in August 1973, killing sagebrush but leaving the herbaceous vegetation relatively unaffected. The burning

stimulated herbage production in 1974, 1975 and 1976, but the burned and unburned portions of the site yielded similar amounts of dry matter hereafter in the absence of live sagebrush in the burned area (Table 7).

In the dry climate of the Hanford Site, available soil water is important in sustaining plant growth. The cheatgrass communities were much more efficient at converting annual precipitation to dry matter than was the native community. The cheatgrass sites produced 14.7 and 10.1 g dry matter per centimeter of precipitation; the native community produced only 3.0 (Table 7).

Although growing season (October through May) precipitation is important in determining annual productivity, it alone was found to be a poor predictor. Linear regression of annual production based upon growing season precipitation produced r^2 values of only 0.33 for the upper cheatgrass community, 0.27 for the lower cheatgrass site and 0.37 for the native grass community.

The cheatgrass communities are more productive than the native communities, most probably because cheatgrass is more effective in exploiting shallow soil water. It has a very fibrous root system and tends to form a closed rather than an open canopy. Yet why do the cheatgrass communities persist? Native grass communities as typified by those studied here have the advantage of a long evolution of adapting to the harsh desert environment. Perennial grasses are long-lived and good competitors for space because individual plants are relatively large. Seeds need not ripen often to maintain the community. Undisturbed native grass communities can successfully resist invasion by alien annuals, even after burning. However, once the soil is drastically disturbed, as by plowing, invading native grass seedlings must compete with alien seedlings, such as cheatgrass. Numerous studies have shown that autumn-germinating cheatgrass seedlings preempt the available soil moisture. Spring-germinating native grass seedlings are then unable to obtain sufficient moisture in the presence of the more developed cheatgrass root systems. The early vigor of cheatgrass seedlings and the abundance of viable seeds with the ability to lie dormant during an especially unfavorable year allow cheatgrass to maintain site dominance for decades. In eastern Washington, cheatgrass communities have successfully resisted reinvasion by native species for 50 years.

Cheatgrass is generally an unwelcome invader in dry western U.S. grasslands. Its establishment signals the loss of perennial

grass and forb species valued as livestock forage. Although cheatgrass is very nutritious when green and palatable to all classes of livestock, it is regarded as of little economic value. Extreme year-to-year and site-to-site variation in yield, as demonstrated by this study, and a very short grazing season make conventional livestock management in cheatgrass communities difficult to practice.

Cheatgrass-dominated communities also have less aesthetic appeal than native ones. The native bunchgrass communities are diverse in plant species composition and life forms. During the spring, showy flowered forbs such as balsamroot (Balsamorhiza careyana), phlox (Phlox longifolia), lupine (Lupinus laxiflorus), sego lily (Calochortus macrocarpus), cluster lily (Brodiaea douglasii) and thread-leaf fleabane (Erigeron filifolius) lend color to an otherwise drab landscape. Sagebrush, too, has a pleasant camphor-turpentine aroma. Cheatgrass-dominated communities, in contrast, are monotonously uniform. They typically support only a small number of annual forb species, most of which are aliens. The mustards, Descurainia spp. and tumble mustard (Sisymbrium altissimum), are the most conspicuous.

Despite these negative features, cheatgrass has value as a prompt soil stabilizer. Its vigor and persistence are valuable assets in revegetation of land not to be grazed by livestock, such as shallow burial sites for radioactive wastes. Perhaps greater use of cheatgrass communities for livestock grazing can be devised through new and innovative research. Cheatgrass grazing potential could be more fully utilized if predictions of yield, based on environmental factors, were available. This study shows a poor relationship between growing season precipitation and productivity, suggesting that simplistic single-factor predictors are not likely to be very useful. Research conducted over a long period of time is needed to improve our understanding of the important environmental relationships that must be incorporated into a useful predictive model. We know of no other studies in grasslands of this type lasting longer than the eight-to-ten-years' effort reported here. The IBP study sites, for example, were examined intensively for only two to three years.

Regardless of cheatgrass' present lack of economic value, it is clear that native perennial bunchgrass communities in the shrub-steppe region in Washington and Oregon are an ever-diminishing and perhaps irreplaceable living resource. Dryland wheat and irrigated agriculture have usurped thousands

of hectares. Since European man first occupied the regions 170 years ago, overgrazing by livestock has modified many more hectares. Even such "temporary" intrusions as off-road vehicular travel for recreation or fire suppression can permanently alter the landscape; they disturb the soil and destroy root systems of native plants, laying the ground open to invasion by alien species. Responsible land management requires that the long-term implications of any site disturbance on native grasslands be carefully considered. Clearly, grassland sites in native condition are scarce in the Pacific Northwest and the few remaining sites, of which the ALE Reserve is an outstanding example, will require considerable and thoughtful protection to conserve their integrity as baseline study sites.

Response of Shrubs to Severe Drought, Measured by Litterfall Collection

The annual drought on the Hanford Site was especially prolonged during the 1976-1977 plant growing season. Shrub litterfall on

the Hanford Site was collected monthly in the growing seasons before, during and after the 1977 drought to measure its effects on shrub productivity. This study was also conceived as a nondestructive way to monitor changes in chemical composition of shrub leaves from year to year.

Shrubs used in the investigation were located on four sites of different elevations. The four species of shrubs selected for study were greasewood (*Sarcobatus vermiculatus*), spiny hopsage (*Atriplex spinosa*), bitterbrush (*Purshia tridentata*) and sagebrush (*Artemisia tridentata*). Short lengths of polyvinyl chloride (PVC) irrigation pipe were used to collect litterfall from individual shrubs. One collector was placed under the canopy spread of each shrub and emptied of its contents monthly. Rain gauges, read monthly, were maintained at three sites.

The results of litterfall collections are summarized in Figure 1. Hopsage averaged

FIGURE 1. Summary of Monthly Litterfall Collections. Bars represent average values; lines represent extreme values.

2.12 g of dry litter during 1976. No measureable litter was collected in 1977 because hopsage failed to produce leaves that year. However, in 1978, hopsage averaged 3.65 g litter per collecting device. The 1978 litterfall collections were marked by an abundance of winged fruits. Litterfall collection peaked in July in both 1976 and 1978.

Bitterbrush produced, on the average, 2.95 g litter in 1976, 0.77 g in 1977 and 3.70 g in 1978. August was the peak month of litterfall collection. The duration of litterfall in 1978 extended later into the autumn in 1978 than it did in 1976 and 1977 (Figure 1).

Greasewood produced more litterfall than did the other shrub species, partly because greasewood plants are larger. Litterfall averaged 5.33 g in 1976 and only 1.29 g in 1977. Greasewood litterfall was clearly affected by the 1977 drought, even though greasewood has deep root systems and is regarded as a phreatophyte. The peak month of litterfall in 1976 and 1977 was November (Figure 1). In 1978, litterfall averaged 8.87 g and peaked in September (Figure 1).

Sagebrush, by far the most abundant shrub species on the ALE Reserve, was studied on two sites, one at low elevation and the other at high elevation. Sagebrush at the low-elevation site in 1976 yielded 2.69 g of litterfall in 1976, but only 1.46 g in 1977. Like the other shrub species, sagebrush was deleteriously affected by the 1977 drought. In 1978, sagebrush recovered from the drought and produced 3.99 g litterfall. There was no definite peak litterfall month in 1977, but July was the peak month for litterfall in 1976 and 1978 (Figure 1).

Sagebrush at the high-elevation site produced 2.14 g litter in 1976 and 2.88 g in 1977. Apparently the drought was not severe

there. In 1978, litterfall amounted to 5.09 g. It peaked during July in all three years, but litterfall was also heavy in August 1978 (Figure 1). In 1977, only 5 cm of precipitation was recorded at the low-elevation sites and only 7.3 cm at the high-elevation sagebrush site. The 1978 growing season was much moister; 25.4 cm was recorded at the high elevation site, 18.8 cm at the low-elevation sagebrush site and 15.3 cm at the greasewood-hopsage site.

Growth of shrubs, as measured by the amount of collected litterfall, was very limited in the drought year of 1977 at all sites except the high-elevation sagebrush site. Hopsage failed to produce leaves in 1977. However, growth for all species was restored in 1978 when precipitation was more normal. The failure of the high-elevation sagebrush site to reflect the 1977 drought is unexpected; the site may be more moist than the precipitation gauge indicates. Snow accumulates in drifts at this site and the soil water regime is probably more conducive to plant growth than it appears to be by the rain gauge indication. General observations also indicate that the site is more mesic than the others.

Other factors than drought can influence litterfall production. Insects, such as grasshoppers and the moth *Aroga websterii*, can defoliate shrubs. Mule deer (*Odocoileus hemionus*) browse upon bitterbrush, although there was no evidence that bitterbrush in this study was being eaten by deer. Jack-rabbits (*Lepus californicus*) found throughout the shrub-steppe vegetation, also eat shrub leaves. There was no indication that insects or vertebrate animal foraging significantly affected litterfall production during the period of this study. Nor was it likely that the plants were affected by herbicides drifting in from agricultural fields. Variations in litterfall production seem most directly linked with precipitation.

● Restoration of Surface-Mined Lands

Principal Investigator: R. H. Sauer

Other Investigator: M. L. Warner

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The total disruption of vegetative production resulting from surface mining in arid regions can be mitigated by using rainfall runoff from the spoil bank slopes to irrigate crops placed between rows of spoil banks. The Restoration of Surface-Mined Lands program at PNL focuses on demonstrating the feasibility of using mine spoil to collect and direct rainfall to a strip of best available soil placed between the spoil banks. The amount of runoff can be increased by applying water-repellent or pore-filling materials to the spoil slopes. In this way, the soil receives much more water. This improves the quality and quantity of biologic production without outside irrigation water. The advantages of this innovative approach are 1) reduced costs of handling spoil, 2) elimination of costs for irrigation equipment, supplies and energy, 3) reduced demand on scant supplies of good soil and 4) crops of increased economic value. This demonstration program is operational at two locations: the Hanford Site and an operating surface coal mine near Kayenta, Arizona.

This program is successfully demonstrating that crops can be grown in arid regions without irrigation by using the runoff from slopes of mine spoil. We will continue developing this approach by exploring new slope treatments, land shapes and crops to improve the net economic efficiency of this means of restoration in arid regions.

Program Demonstration on the Hanford Site

The Hanford Site is in one of the hottest and most arid areas in the western U.S. The scant annual rainfall (less than 15 cm) makes restoration difficult without irrigation water. For the demonstration, artificial spoil banks have been constructed. The slopes of these simulated spoils have been treated with butyl rubber sheeting, paraffin or rubberized asphalt. Some have been left untreated as controls. Grapes and wheat are planted in the topsoil between the slopes. Use of these treatments is intended to identify a long-lasting and relatively inexpensive means of collecting rainfall runoff.

From the time of the first wheat crop three years ago, the results have been very encouraging. Although the 1977 season was one of the driest on record in the region, a wheat crop was produced in the area between the slopes covered with rubber sheeting. No wheat was produced in flat areas without adjacent spoil banks. Rainfall in 1978 was normal, and wheat yield in the treated areas was four to five times that in the untreated

areas. In 1979, wheat growth in the treated valleys was vigorous in the spring until it was completely consumed by a regional infestation of migratory grasshoppers. This growth occurred in spite of a drought the previous fall that delayed germination, and record winter cold that killed some wheat plants. In this same period of time, the grapes have been maturing and would have produced fruit this year (a normal schedule for commercial vineyards) if locusts had not defoliated the vines. Barring extreme weather and insect populations, next and subsequent years should be successful.

Demonstration at Kayenta, Arizona

The site at Kayenta, Arizona was selected to demonstrate this innovative approach at an operational coal strip mine. The mine is located in the arid northeast corner of Arizona on the Navajo Indian Reservation and is operated by the Peabody Coal Company. Demonstration efforts at this site are being coordinated by Dr. John Thames of Arizona State University. During this second year of work at this site, design and construction of the

demonstration site were completed. The 150-m-long valleys with good soil and adjacent smoothed spoil banks were constructed and seed of four pasture grass/forb mixes broadcast onto the good soil. Some of the soil has been salted; some, compacted; and some has been left untreated. Despite an absence of significant rain after seeding, the test crops did germinate, and differences between controls and treatments were clearly visible. In the next and subsequent years, vegetation quality and yield are expected to increase with the occurrence of normal rainfall patterns and normal soil improvement with use.

System Analysis

To synthesize the data that are being collected from these sites, a mathematical

modeling effort was begun. The first version of a model was coded and is operational. This model was manipulated using data from the Hanford Site in an attempt to identify variables that may merit experimental manipulation at other sites because of their impact on net economic efficiencies. The model was found to be particularly sensitive to four variables: 1) cost of slope treatment, 2) ratio of cultivated area to total area, 3) quantity of spoil moved and 4) crop value. The model suggests that, of the four slope treatments at Hanford, paraffin will produce the highest net economic returns. The model will be refined and incorporated into program management as more data are applied to the model.

• Long-Term Ecological Monitoring

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Ecosystems of the Pacific Northwest, historically, have not been subjected to environmental degradation such as can be found in the Northeast. This opportunity to collect baseline data for both species and ecosystems requires application of ecological sampling which is appropriate for a wide spectrum of pollutant source types yet also appropriate for long-term studies. The Long-Term Ecological Monitoring program, begun in FY 1978, will provide the foundation for monitoring activities in the Northwest which will be responsible to DOE's specific needs and interest.

This research effort concentrates on the forests and shrub-steppes of Washington, Oregon and Idaho. Four components are currently underway: repeatable, nondestructive species-specific vegetation sampling; statistical designs in noncontrollable observational situations; animal species monitoring using selected points in species-specific behavior; and interagency coordination.

Vegetation Monitoring

FY 1979 was the first full year of monthly litterfall collection at the forest sites selected for ecological monitoring (Figure 2). Collections are made at Quinault Research Natural Area and Neskowin Crest Research Natural Area, which are representative of the Sitka spruce-western hemlock coastal forests, and at H. J. Andrews Experimental Forest and Wind River Research Natural Area, which are low-elevation Cascade forests dominated by Douglas fir and western hemlock. In the summer of 1979 a new collection site was established at the U.S. Navy Bombing Range, Boardman, Oregon. This site typifies the sagebrush-cheatgrass community of arid eastern Washington. Monitoring at this site is particularly important since it is located downwind from the Portland General Electric coal-fired plant scheduled for completion in 1980.

At each site, some initial vegetative characterization was done. For the forest sites, this included measuring tree diameters at breast height and rating dominance for all trees with a diameter greater than 5 cm growing in the one-hectare sampling

FIGURE 2. Distribution of Established Federal Research Natural Areas in Oregon and Washington

area. A species list was made of the shrubs and understory vegetation. At the shrub-steppe site, canopy cover estimates were taken for all shrub species.

Cooperative agreements were established with the Forest Service and Oregon State University for routine monthly sample collection and maintenance at the forested sites. In the laboratory, the samples are oven-dried and the litter is sorted into reproductive parts, epiphytes, needles and miscellaneous. These are then identified by species.

Statistical Design

Sampling methods were investigated to evaluate the field techniques implemented in FY 1979 to estimate the litterfall in coniferous forests. The purpose of the evaluation was to determine the degree of precision and reliability that existing field methods attained in estimating the biomass and composition of litterfall. We were attempting to estimate the effort required to measure litterfall with a precision to within 10% of the true mean value 90% of the time.

Analysis of six months of litterfall data indicated a general inverse relationship between the precision of a litterfall estimate and the volume of the litter sample collected. Because litterfall occurs on an annual cycle in coniferous forests, months with low volumes of litterfall were also characterized by litterfall estimates with reduced precision. Analysis revealed that needlefall in the coniferous forests could be more precisely estimated than total litterfall. Current sampling efforts were found to be generally less precise in estimating the relative needlefall composition by species than in estimating the mass of the needlefall. Only for predominant species could relative contribution to total needlefall be estimated with the prescribed precision.

Our initial designs for estimating litterfall were to use circular plots and subsampling along randomly selected line transects (radii or diameters of the circular plots). This method was found to possess possible biases. Study areas were reconstructed using simple random sampling to minimize apparent biases and improve precision of litterfall estimates.

Methods for estimating the mean litterfall or leaffall in sagebrush communities were investigated. A sampling method using double sampling for stratification was implemented on the Boardman site.

Animal Monitoring

In FY 1979, the major goal of the animal monitoring task was to select the species to be used for long-term monitoring. In selecting animals for monitoring, we concentrated on nonmigratory and easily observable species. Selection of species from more than one trophic level was also necessary for examining ecological systems. A primary consideration was to select the same or similar species at all of the monitoring sites.

To date, we have chosen to study long-eared owls (Asio otus) and screech owls (Otus asio) and small mammals, primarily deer mice (Peromyscus maniculatus) and pocket mice (Perognathus parvus). Owls occur at all of our monitoring sites and they probably prey at least partly upon deer mice and pocket mice. To study owls, we will collect castings and identify the prey items in them. The data obtained from casting analysis will enable us to examine food habits and diet variability of owl species among study sites. Castings can also be analyzed later for chemical residues. Any added eggs will be saved for chemical analysis and productivity data.

Small mammals will be trapped at each study site and a few individuals of key species (deer mice and pocket mice) will be saved for future chemical analysis. The trapping of small mammals will also enable us to quantitatively relate prey availability in the environment to owl prey selection and will provide data useful in assessing trophic dynamics from prey to predator.

As part of the owl studies, we have placed 100 nest boxes at four of the study sites (H. J. Andrews, Neskowin Crest, Quinalt and Wind River). We hope that owls will nest in the boxes, enabling us to study these birds in forested areas more easily. In dryland areas, it is easier to locate owls, since nest sites are few and owls are thus limited in their selection.

Interagency Coordination

During FY 1979 an interagency coordination task was organized. Interagency awareness is important because DOE is only one of several government agencies with responsibilities and interests in ecological monitoring. The dual purpose of the interagency coordination task is to define monitoring goals and methods and to promote communication with specific institutions and agencies which are interested in monitoring. The defining of monitoring has just begun. The long-term effort of interaction with sister agencies emphasizes field applications of

monitoring principles. Several federal agencies face increasing responsibilities for monitoring; the National Science Foundation, the Environmental Protection Agency and the Department of Energy are three of the most obvious. Others with related responsibilities include the National Park Service and the State Department (through international efforts such as the Man and the Biosphere program). Some cooperative efforts between DOE/PNL and these groups have already been formed. For example, the National Park Service has recently begun baseline examinations of environmental contamination in Biosphere Reserves (designated via Man and the Biosphere) on their land. The events leading to this effort and PNL's involvement appear in Figure 3. Because we think that a purely environmental approach to monitoring will lead to erroneous conclusions about biota, we are emphasizing the need for ecological measurements. Likewise, dialogue with the National Science Foundation has begun regarding their recommendations for long-term studies. This kind of interagency communication and other cooperative efforts will be a continuing portion of the PNL Ecological Monitoring Program.

FIGURE 3. Development of the 1979 Interagency Cooperative Venture in Olympia National Park

2 Alaskan Resource Research

ALASKAN RESOURCE RESEARCH

- **Ecological Investigation of Alaskan Resource Development**

Ecological studies of the consequences of resource development in northern Alaska began during 1974. They were an extension of radiation ecology investigations initiated in 1959 at Project Chariot and of environmental programs conducted at Prudhoe Bay from 1971 to 1973. Emphasis was initially placed on collecting baseline data on ecosystems that are or soon will be impacted by petroleum resource extraction and transportation. Quantitative information is currently being obtained on small mammal, bird and arctic fox populations in disturbed and control areas to assess the true environmental cost of resource development in a previously unimpacted region of great national interest.

● Ecological Investigation of Alaskan Resource Development

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The objective of this research is to provide an integrated program for the definition of ecological consequences of resource developments in northern Alaska. The qualitative and quantitative results obtained describe the environmental costs incurred by petroleum resource extraction and transportation, including interaction of wildlife populations with industrial activities. This section of the Annual Report presents information on impacted populations of arctic foxes, small mammals, and tundra-nesting birds in the Prudhoe Bay oil field and along the Trans-Alaska Pipeline and haul road; findings from similar studies from the Colville River Delta and other unimpacted habitats; field experiments to determine the sensitivity of lichen communities of the Brooks Range to sulfur dioxide concentrations likely to be encountered near pipeline pumping stations; and amounts of worldwide-fallout radionuclides in the lichen-caribou-Eskimo food chain.

Arctic Fox and Red Fox Studies

Arctic fox (*Alopex lagopus*) and red fox (*Vulpes vulpes*) studies were continued. Emphasis was on arctic foxes because they are the major mammalian predator of the region currently being developed for petroleum resources and because they are an important source of income to resident trappers throughout the circumpolar region. This year, tag returns from marked foxes captured by trappers indicated again that arctic foxes make extensive movements (up to 2,000 km) in relatively short periods of time and may extend the impact of apparently localized resource development (Figure 1).

Because of a lack of information on the Prudhoe Bay arctic fox population prior to petroleum developments, comparative observations were made of denning activities at Prudhoe Bay (disturbed habitat) through a University of Alaska subcontract and on the Colville River delta (undisturbed habitat) through a Pennsylvania State University subcontract (Figure 2). Results showed that arctic foxes are very adaptable and that reproduction was apparently not adversely affected by current petroleum development activities. The percentage of arctic fox dens examined since 1976 which have contained juveniles has been consistently greater at Prudhoe Bay than at the Colville River

delta site (Table 1), and the Prudhoe Bay region supports about three times as many fox families per unit area as does the Colville River delta. This higher production of Prudhoe Bay foxes may be related to artificial food sources (garbage and handouts from construction workers); however, variations in natural fox habitat quality between the two areas may be at least partially responsible. Food habits of the two populations are currently being examined to determine the importance of artificial foods.

The 1979 arctic fox populations, as measured by reproductive success on the two study areas, remained relatively high as compared to the 1977 low, although the population density of lemmings, a major food of arctic foxes, has not recovered at Prudhoe Bay as it has at the Colville River delta site (Table 2). It is generally assumed that the cyclic nature of arctic fox populations is closely related to that of lemmings; therefore, the recovery of fox reproduction at Prudhoe Bay without a concomitant increase in lemmings may be related to utilization of alternate food sources, either natural or artificial. In addition, it was noted that juvenile fox mortality appeared to be considerably higher at Prudhoe Bay this year than in any previous year, including 1977.

FIGURE 1. Map of Arctic North America Showing Long-Range Movements of Tagged Arctic Foxes

FIGURE 2. Map of Northern Alaska Showing Locations of Study Sites

A second and final year of a behavior study of the interactions of penned arctic and red foxes was conducted by a Pennsylvania State University subcontract at the U.S. Fish and Wildlife Service Northern Prairie Wildlife Research Center in Jamestown, North Dakota. The purpose of this investigation was to determine the relationships within and between two closely related species whose ranges overlap in the region currently being impacted by northern Alaska petroleum developments. Results from this year confirmed the preliminary data obtained in 1978 and showed that red foxes consistently displaced arctic foxes from preferred habitats. As a

result, it is believed that petroleum development activities, which appear to disturb arctic foxes less than red foxes, probably will have little impact on the population balance between these two species in jointly occupied areas.

During the late fall, winter and spring months, arctic foxes are reported to spend considerable time on the Beaufort Sea ice pack in areas that are currently proposed for petroleum development. Results from our investigation conducted during mid-March to mid-May of this year indicated that foxes are present up to at least 40 km offshore

TABLE 1. Comparison of Arctic Fox Reproduction as Measured by Percentage of Dens with Juveniles on the Prudhoe Bay (disturbed) and Colville River Delta (undisturbed) Study Sites

Year	Prudhoe Bay Site		Colville River Delta Site	
	Number of Dens Examined	Percentage with Juveniles	Number of Dens Examined	Percentage with Juveniles
1975	17	71	—	—
1976	20	85	30	37 ^(a)
1977	19	21	39	5
1978	10	50	46	44
1979	20	55	26	35

(a) Minimum percentage based on aerial survey only with no ground check.

TABLE 2. Arctic Fox and Lemming Population Indices at the Prudhoe Bay and Colville River Delta Study Sites

Year	Prudhoe Bay		Colville River Delta	
	Lemming Index(a)	Arctic Fox Numbers	Lemming Index(a)	Arctic Fox Numbers
1975	9.1	High	—	—
1976	8.7	High	—	Medium
1977	1.8	Low	2.7	Low
1978	0	Medium	8.7	Medium
1979	1.1	Medium	6.0	Medium

(a) Numbers captured per 1000 trap nights using trap lines described by F. A. Pitelka (1973), "Cyclic Patterns in Lemming Populations near Barrow, Alaska," in *Alaskan Arctic Tundra*, ed. M. E. Britton, pp. 199-215, Arctic Institute of North America Paper No. 25, AINA, Washington, D.C.

through mid-May, at which time arctic foxes on terrestrial habitats begin to whelp. It was also found that foxes do not congregate in large numbers or for long periods of time at food sources during spring months as they do during fall and winter. This provides critical information for designing future studies and for evaluating fox behavior in the context of petroleum developments.

Small Mammal Studies

A fourth summer of investigation at the Franklin Bluffs (buried pipeline) site (Figure 2) showed a continued reduction of

animal populations due to blocked drainage of runoff waters by the Trans-Alaska Pipeline haul road and a substantial decline in the small mammal population density from peak numbers recorded last year (Table 3). This year, the small mammal trapping grids were enlarged from 30.5 by 38.1 m (~0.116 ha) to 75 by 75 m (~0.562 ha). This was done to increase spacing of traps to 15 m, thereby decreasing trapping mortality and enabling more effective study of small-mammal home ranges. Five species were caught during 4802 trap-nights. The tundra vole (*Microtus oeconomus*) was most frequently caught (Table 4). The population density of

TABLE 3. Small Mammal Population Density at the Franklin Bluffs Study Site During the Summers of 1976 Through 1979

Year	Number of Animals			Area (ha)	Population(a)	Population Density (Number/ha)
	Marked	Captured	Recaptured			
1976	9	15	2	0.932	43 ± 468	52
1977	4	8	1	0.932	18 ± 84	19
1978	54	54	19	0.932	148 ± 668	159
1979	23	18	4	2.81	87 ± 938	31

(a) Modified Petersen (1896) Index, according to notation of Bailey (1951):

$$P_B = \frac{M(C+1)}{R+1} ; sp_B = \frac{M^2(C+1)(C-R)}{(R+1)^2(R+2)}$$

Bailey, N. T. J. 1951. "On Estimating the Size of Mobile Populations from Recapture Data." *Biometrika* 38(pts. 3-4): 293-306.

Petersen, C. G. J. 1896. *The Yearly Migration of Young Plaice Into Limfjord from the German Sea*. Rept. Danish Biol. Sta. for 1895, 6:1-77. Cited by Bailey (1951).

TABLE 4. Numbers of Small Mammals Captured at Franklin Bluffs and Trans-Alaska Pipeline Mile 12 Sites from July 21 to August 9, 1979

Site	Tundra Vole(a)		Singing Vole(b)		Red-Backed Vole(c)		Varying Lemming(d)		Brown Lemming(e)	
	Adult	Subadult	Adult	Subadult	Adult	Subadult	Adult	Subadult	Adult	Subadult
Franklin Bluffs	15	17	6	3	2	0	0	1	0	1
Trans-Alaska Pipeline Mile 12	3	2	0	0	0	0	1	2	0	2

(a) *Microtus oeconomus*

(b) *Microtus miurus*

(c) *Clethrionomys rutilus*

(d) *Dicrostonyx groenlandicus*

(e) *Lemmus trimucronatus*

both varying lemmings (*Dicrostonyx groenlandicus*) and brown lemmings (*Lemmus trimucronatus*) remained low.

Nineteen small-mammal trapping grids were located in suitable habitats within the new Trans-Alaska Pipeline mile 12 study site (Figure 2), located 50 km north of the Franklin Bluffs site. Fewer animals and fewer species were captured at that site during 7308 trap-nights. The population density of small mammals was estimated to be three animals per hectare.

Tundra-Nesting Bird Studies

The new 100-ha study site established at Trans-Alaska Pipeline mile 12 to compare the environmental impacts of buried versus above-ground pipeline construction modes contained 120 nests of 10 species of tundra-nesting birds. A total of 214 adult and nestling birds were banded and one previously banded bird was captured. Approximately 4 ha of habitat were lost to areas occupied by the pipeline, pipe pad and access road. Early in the breeding season another 14 ha of

habitat were unavailable to nesting birds because of the accumulation of snow and meltwater caused by the "snow fence" effect of the elevated pipeline. Late in the season these wet areas adjacent to the pipeline pad become important feeding areas for adults and young birds, and marked family groups were observed to move up to 1 km to utilize these areas. We estimate that habitats that would have supported about 11 nesting pairs of birds of six species were lost during the 1979 summer season as a result of the presence of the pipeline within the study site.

Tundra-nesting birds within the 100-ha Franklin Bluffs site continued to avoid the wet areas created by blocked drainage of runoff waters from the Trans-Alaska Pipeline haul road. Nesting densities were low within the haul road dust shadow, and the birds resumed nesting next to the buried pipeline pad. Phenologies of 65 nests of 12 species were documented at the Franklin Bluffs site. Eighty adult and nestling birds were banded for migration studies and four previously banded birds returned to the site.

Lichen Studies

Arizona State University subcontractors conducted additional studies on the systematic variation of gas exchange in arctic lichens. This work extended the observations made last year in *Cetraria cucullata* to three other species and documented the physiological basis for the observed differences in photosynthetic activity. A clear gradient was seen in $^{14}\text{CO}_2$ incorporation from the top to base and from proximal to distal portions of *Cladonia stellaris* (= *C. alpestris*) and *C. rangiferina* and from thalli margins inward for *Parmelia separata*. This gradient was most pronounced in photosynthesis, during which essentially all of the activity was restricted to the top half (0 to 4 cm) of the lichen mat. The gradient was positively correlated with differences in chlorophyll a and b concentrations. These data show that lichens are most active physiologically in a relatively small portion of their biomasses and that there is a steady progression of senescence in their older (lower) portions, reflected by a progressive decrease in both photosynthetic and respiratory activity.

Four 24-hr observations were made of photosynthetic rates and patterns in different depths of *C. stellaris* and *C. rangiferina* lichen communities. Biomass in 0.5-cm vertical segments of the lichen mats was determined to further define the eco-physiological aspects of these northern

Alaskan lichen communities that are vital winter range components for caribou.

Natural *Cladonia-Cetraria* lichen communities that were exposed to field fumigation by sulfur dioxide during summer periods of 1977 and 1978 were re-examined and found not recovering from exposures of 0.62 ± 0.31 ppm.

Radiation Ecology Studies

Amounts of worldwide-fallout ^{137}Cs in northern Alaskan ecosystems have been continuously monitored since 1959, and since 1962 these studies have included comprehensive evaluation of the lichen-caribou-Eskimo food chain at Anaktuvuk Pass. This extensive data base now furnishes an effective record of the impact of ecologic, economic and cultural factors upon the Eskimo people as they undergo culture change that has been accelerated by resource developments in northern Alaska. Measurements of ^{137}Cs concentrations in the Anaktuvuk Pass residents this year (Table 5) showed that the average adult during 1979 received a radiation exposure of about 10 mrem. This is 2 mrem lower than last year's exposure and about one-fifteenth that received from the relatively high body-burdens observed during the years (1964 through 1966) of maximum fallout deposition and greater dependency upon caribou for food. An unusual decline in the summer exposure as compared to winter exposure was apparently due to 1) laws restricting caribou hunting to periods when the animals of the spring migration were unavailable and 2) constant employment of most adults in village school construction, which restricts hunting and provides money to buy processed food that is low in fallout radioactivity.

TABLE 5. Concentrations (nCi/kg body wt) of ^{137}Cs in Anaktuvuk Pass, Alaska, Residents During January and June 1979

Category	Age in Years	January			June		
		----- ¹³⁷ Cs Concentration (nCi/kg)-----					
		n	$\bar{X}$	s_x	n	$\bar{X}$	s_x
Adults	>21	43	0.76	0.43	38	0.53	0.27
Minors	15-21	8	0.43	0.12	10	0.17	0.13
Children	<15	12	0.29	0.12	6	0.32	0.13

3 Shale Oil

SHALE OIL

• Terrestrial Effects of Oil Shale Development

Vast domestic resources of oil shale may provide a long-term alternative to petroleum-derived fuel products. Methods proposed for obtaining shale oil from its deposits range from conventional mining and aboveground retorting technologies to *in situ* retorting. Although the technical and economic aspects of this wide range of options have been generally studied, the environmental impacts must be assessed because questions of siting, control technology, licensing and regulation can strongly affect the best production strategy.

Three major segments of the aboveground shale oil production cycle have potential for environmental consequences. These include mining of the shale, extraction of the shale oil and disposal of processed shale. In the mining process, aquifer disruption, runoff and movement of process residuals through the soil profile to ground waters may result in impact on streams and rivers. During the extraction cycle (crushing, retorting and refining), the major impact will result from gaseous emissions and liquid effluents containing organic, macro-ion, and trace metal residues. Disposal of retorted shale presents problems in loss of recreation land, in reclamation of land occupied by retorted shale, and in environmental and human exposure resulting from resuspension of particulates and leaching of pollutants to ground and surface waters. The use of pyrolytic methods, together with a relatively high concentration of organic nitrogen and sulfur in oil shale, can lead to the formation of heterocyclic nitrogen components, possibly resulting in environmental problems not ordinarily encountered in other energy industries.

The current PNL program focuses on the chemical characterization of effluent residuals (see PNL-3300, Part 4, Physical and Technological Programs), the potential toxicity of effluent residuals (see PNL-3300, Part 1, Biomedical Sciences), and the chemical and microbiological processes leading to mobilization and plant uptake of inorganic and organic residuals (this report). Supporting research on the feasibility of revegetation in arid climates and application research on a water-harvesting approach are described in the Land Use section of this report, because they are of primary importance to surface mining of coal.

The laboratory and field studies reported in this section concern chemical and biochemical transformations in retorted shales and soils and their relationship to water quality, viability of green plants, and human food chains. This is a multitask, interdisciplinary research approach which draws upon PNL capabilities in hydrocarbon chemistry, soil science, microbiology and plant physiology. Principal objectives of the project are to 1) develop a basic knowledge of important environmental and extraction processes influencing the form, mobility, chemical and microbiological stability, plant availability, and toxicity of trace elements and organic residuals in retort waters and retorted shales, 2) model and validate, through field monitoring,

the movement to ground water of trace elements and stable organic residuals in soil and retorted shale, and 3) develop a rational basis for selection and subsequent laboratory and field testing of proper retort conditions/soil conditioners, irrigation waters and native or agricultural plant species to physiochemically stabilize and revegetate retorted shales for permanent, safe restoration of shale lands. The studies are being closely integrated with field investigations by other federal agencies and universities. Together, these programs should provide a basis for assessment of the potential terrestrial effects of waste disposal and for viable restoration of shale lands.

• Terrestrial Effects of Oil Shale Development

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The potential development of the oil shale industry requires the concomitant development of methodology to evaluate and ameliorate the environmental impact of ground disposal of wastes. Therefore, our research programs have emphasized 1) development of sampling protocols with industry, collection of samples from a range of retort processes, and physicochemical characterization of retort waters, retorted shales, retorted shale leachates, ground waters and soils of the oil shale region, 2) column and field studies to determine the chemistry and microbiology controlling the composition of leachates from retorted shale, 3) the hydrologic factors governing transport to ground waters, 4) validation and modeling of the movement of inorganic and organic residuals to ground and surface waters at aboveground, modified *in situ*, and *in situ* retorting sites, 5) the plant availability and effects of organic and inorganic residuals in retorted shale and retort waters, and 6) genetic selection of native plant species tolerant to effects over the long term.

Field studies of the stability and movement of trace metals and organic residuals in retorted shale have been underway since 1977 at the Department of Energy's Rifle Oil Shale Facility, operated by Development Engineering, Inc. at Anvil Points, Colorado, the Occidental Oil Shale Corporation Site at Logan Wash, Colorado and in the Piceance Creek Basin, Colorado, in conjunction with Colorado State University. Planning is underway with DOE and industry to develop similar studies on sites in Colorado and Utah where modified *in situ* and *in situ* methods are being used. The results of these investigations will be used to validate laboratory models of the mobility of residuals in the oil shale region. The results of laboratory and field studies have led to new concepts of the long-term nature of leachates from retorted shales.

The program has provided technical and administrative support to current programs in integrated assessment of oil shale development, assessment by the National Academy of Sciences of redistribution of accessory minerals as a result of mining and extraction of oil shale, the Oil Shale Environmental Development Plan, Oil Shale Commercialization Environmental Readiness Document and to the DOE Task Force on Oil Shale Development.

Paraho Aboveground Retort

Percolates were collected over a two-year period from lysimeters at Anvil Points, Colorado, and in nonirrigated field plots containing Paraho retorted shale in the Piceance Creek Basin, also in Colorado. These were analyzed for major cations, anions, trace elements and dissolved organic carbon, providing definitive information on the chemical and microbiological effects of weathering on leachate composition. This year, hydrologic evaluations were begun to augment these ongoing chemical and microbiological studies and to provide a basis for evaluation of effects of disposal on surface and ground waters.

In the study area is an intermittent stream, West Sharrard Drainage, which flows at the bottom of a canyon. The retorted shale and raw shale fines have been disposed in this canyon since the research on retorting methods was begun by the U.S. Bureau of Mines in the 1940's (Figure 1). Existing wells in the canyon above and below the disposal site were used to measure the quality of the ground water. Quarterly sampling of both the stream and the wells, and subsequent analysis of the waters, has been conducted since mid-1974 by Development Engineering, Inc. Analysis indicated that the presence of the pile had little or no effect on stream quality.

FIGURE 1. Oil Shale Field Study Area

The lysimeter studies by PNL indicated that chemical equilibrium between water and the disposed shale was a slow process (requiring longer than two years) and that major changes occurred in the composition of percolates as a function of the applied water volume and the water residence time in the shale. Of primary interest were observed decreases in pH (Figure 2). The studies indicated that recarbonization of the surface horizons with atmospheric CO_2 and the activities of microorganisms in surface and subsurface horizons contributed to the changes in the chemistry of the shale on weathering. However, laboratory and field studies indicated that the principal mechanism responsible for the observed decreases

FIGURE 2. The pH of Percolate Waters Collected in 1977 and 1978 from Lysimeters Containing Retorted Oil Shale

in pH of retorted shale percolates was the conversion of major quantities of sulfide in the retorted shale to sulfate. Furthermore, the trace element content of the lysimeter percolates revealed that elevated lithium content was an accurate indicator of waters leaching a zone of retorted shale and was present in concentrations 50 to 500 times greater than in the lithium leachates from the surrounding soils. These observations were verified at the field disposal site. Well waters below the pile in the fall of 1978 and the spring of 1979 contained higher lithium concentrations than did well water above the pile, indicating that the ground water contained retorted shale leachates.

During the winter of 1978 and 1979, contact of heated retorted shale with raw shale fines resulted in a fire and initiated re-torting in the fines pile. The heavy snow-fall that winter resulted in an above-normal quantity of water in the pile as the snow melted. Water and some oil flowed from the pile into the creek. Sediment samples taken from the head-waters to the mouth of West Sharrard Drainage were found to contain significant and consistent levels of hydrocarbons. However, the major source was not the oil from the pile but the normal background of raw shale fines that were present in the sediments from the natural weathering of the watershed. Further identification of organic components specific to the product oil has facilitated the differentiation of product oil in the waters from background levels as a result of the fire.

Analysis of the stream water samples taken in 1979 showed a definite effect of the processing and waste disposal activities on the surface water quality. This effect (Figure 3)

FIGURE 3. Lithium Concentration in West Sharrard Drainage

terly samples did not reveal this effect is partly that the location of the sample point was above the majority of the seeps and partly that there were large diurnal fluctuations and seasonal decreases in water flow. The variation in the stream flow on a seasonal and diurnal basis is shown in Figure 4. The seasonal flow shows a steady decrease in flow rate from the spring through September. Maximum flows probably occurred before the June melt in 1979 as a result of the snow melt in the early spring and cloudbursts in the area during the late spring. The diurnal variation shows a constant flow in the morning with a marked decrease beginning about noon and continuing through the afternoon, coinciding with maximum transpirational demand of the surrounding vegetation. Water quality during the rapid drop in flow rate

FIGURE 4. 1979 Change in Flow Rate to West Sharrard Drainage

is exemplified by the lithium concentrations at different stream bed distances. The sharp rise in concentration at the bed distance +20+00 is due to the presence of disposal site seep water flowing into the stream at this point. The reason that previous quar-

did not change (indicating that evaporation is not the primary control) in salt content above the seepage area, but salt concentrations increased rapidly in the water below the seepage area, reflecting the greater contribution of the high salt content seep

water to total stream flow. In fact, in September the stream above the seepage area ceased to flow in late afternoon and the total stream flow was due to seep water.

Although hydrological studies to define the extent and magnitude of the contamination in the ground water from which the seeps originated are still in progress, several preliminary conclusions are possible. Of those elements which were originally thought to pose problems when water leached retorted shale, there is no increase in concentrations of boron, molybdenum or fluorine in seep waters and no change in pH. These observations are consistent with the data obtained on leachates from the lysimeters after one year of weathering. Dissolved organic carbon levels were elevated in the seep water, contrasting with the lysimeters, in which one year of weathering reduced the levels significantly. This may be due to either the sorption of the organic material on movement through the subsoil or to the fact that organic levels in the seep water originated from a different source than the retort shale, such as leaching from the oil produced during the fire. These studies will be continued in an effort to better understand the important chemical, physical and microbiological mechanisms which control the mobility of important residues from wastes disposed during the production of shale oil.

Occidental Modified In Situ Retort

Retorted shale samples from a coring of experimental retort 3E at the Logan Wash, Colorado, test site were provided by Occidental Research Corporation for study. Room 3E was subjected to modified in situ retorting, exhausted, cooled and sampled by drilling. Samples received included subsamples (in some cases, composites) of core increments over a depth of 135 to 157 m. It has been suggested that oil shale retorting at high temperatures over long heating periods would decrease the inorganic and organic residuals subject to leaching by water intrusion due to the formation of refractory minerals from the decomposition products of thermally unstable minerals originally present in the raw shale. The PNL studies were designed to evaluate the process of residual decrease and to determine the variation in concentration and leachability of residuals in the retort with depth as these may influence leachate mutagenicity. The results of these preliminary studies were subsequently used to evaluate the influence of solid-phase composition and retort variables on leachate composition, and to establish critical retorted shale properties and environmental conditions for more extensive studies.

A total of 10 to 20 g of each core segment was received; this small sample quantity necessitated the development of unique analytical methods and experimental procedures. Two sets of columns were prepared, with the Paraho retorted shale serving as a control. Leaching experiments were performed under aerobic and anaerobic conditions.

The loss on ignition, the weight percentage of carbonate and the mineralogy indicate a rough break of the core into three regions. This probably reflects the temperature regime of the retort. The areas experiencing the highest temperatures or longest periods of heating are characterized by the absence of carbonates, quartz and feldspars, and the presence of the metamorphic silicates: akermanite, diopside, kalsilite and monticellite. In the lower temperature zones, calcite, quartz and feldspars remain, while silication reactions have been minimal. The absence of dolomite suggests that all retort 3E segments experienced higher temperatures than the Paraho retorted shale.

Leachates collected from each column were split into two fractions: one for mutagenic testing and the other for chemical analysis. The atmospheric conditions under which the leaching occurred did not significantly effect the chemical composition of the leachates. Maximum concentrations of all organic and inorganic species were associated with the Occidental core segments; however, leachates with a higher pH were obtained from the Paraho retorted shale. These species were leached from the core segments in a pulse which occurred at $v/v_0 < 0.5$. Relatively high concentrations of soluble organic carbon, in comparison to Paraho, were observed. The pH, conductivity and concentrations of cations and trace metals in the leachates from the individual column segments did not exhibit consistent trends. In general, the cation concentrations and the conductivities of the leachates approximate the mineralogical record and carbonate analysis, with increased levels of soluble materials in the lower zones of the retort where accumulations of gases and liquids might be expected.

Only five of the core segments produced leachates which exhibited significant mutagenicity. Values approximated those of a water extract of Occidental crude oil and did not appear to be related to the measured properties of the core increments.

This preliminary study has suggested that the refractory silication reactions which occurred during in situ retorting did not reduce the quantity of soluble residuals. Room 3E cores contained greater quantities of

soluble material than did surface retorted shale used for comparison. A likely explanation of this apparent contradiction is that a much greater quantity of soluble materials condensed on the surfaces of the in situ retorted shale after the flame front was extinguished. However, drilling may have required the use of some liquids for materials consolidation. This information should be available in the near future and may provide further insight into the differences observed. Investigations are continuing to clarify the effect of silication reactions and retorting parameters on the chemistry of resulting leachates.

Mineralogy of Retorted Shale from Aboveground and Modified In Situ Processes

Previous field studies have indicated that Paraho (aboveground) retorted shale produces leachates which differ significantly from those of raw shale. These leachates are initially saline (0.1 - 0.2M Na₂SO₄) and of high pH. They contain elevated concentrations of lithium, dissolved organics and reduced sulfur species in comparison to local surface waters. The pH, ionic strength, and soluble organic carbon of the leachates decrease after a single winter weathering season. Precipitation reactions may partly account for the observed changes.

Detailed mineralogical studies have been initiated on raw and retorted shales from both aboveground (Paraho) and modified in situ (Occidental) retorting processes to enable the assessment of the role of mineral dissolution and precipitation reactions on the solution chemistry of retorted shale leachates. Specific objectives included 1) the evaluation of mineralogical changes induced by retorting, 2) isolation and quantification of sulfur-bearing minerals and 3) determination of the mineralogical residence of lithium.

Temperatures reached during the Paraho direct mode process (550-600°C) were sufficient to induce 1) partial decarbonization of dolomite to calcite and MgO, 2) decarbonization of nahcolite to Na₂O, 3) partial reduction of pyrite to pyrrhotite, 4) dehydration and partial decomposition of analcite and 5) collapse of illitic phyllosilicates. Inorganic sulfur in raw shale was found to reside primarily in pyrite. Alteration to pyrrhotite during retorting increases the potential solubility of reduced sulfur forms in the retorted shale. The mineralogical residence of lithium is still uncertain. Although normally found in phyllosilicates, the positive correlation of lithium with 1) magnesium and calcium, which occur as carbonates and 2) dolomite and nahcolite decomposition, suggests the possible occurrence of lithium as a carbonate.

Although mineralogical studies on modified in situ retorted shale have just begun, the following generalizations can be made from the Occidental Room 3E core. Temperatures and/or durations of heating are considerably greater and more variable in the modified in situ retorts. Thus, a core through an abandoned retort (at the present state of technology) characteristically displays zones which have experienced differing temperature regimes. These zones can be tentatively identified by the persistence or absence of thermally reactive minerals (calcite, dolomite, and quartz) and the extent of silication reactions. Simplistically, as temperatures approach 500°C dolomite decomposes to calcite and MgO. Increasing temperatures encourage MgO, CaO, and SiO₂ (quartz) silication to produce akermanite, Ca₂(MgSi₂O₇), and forsterite, Mg₂(SiO₄). As temperatures approach and exceed 1000°C diopside predominates, with the mixture fusing into glass at approximately 1200°C. It has been suggested that these silication reactions would limit the subsequent solubility of inorganic species in retorted shale leachate.

Future mineralogical studies will emphasize the mineralogical controls on leachability focusing on 1) the identification of lithium-bearing minerals and 2) the quantification of the major sulfur forms (sulfate, sulfide, organic and elemental) in both raw and retorted shales. In addition, emphasis will be placed on the identification of thermal decomposition products and refractory minerals formed during in situ retorting.

Microbiology of Retorted Shale

Previous studies have shown that the total heterotrophic bacterial colony-forming units (CFU's) were similar ($\sim 3 \times 10^6$ CFU/g dry wt) in soil and Paraho retorted shale samples obtained in June 1977 from the lysimeters at Anvil Points, Colorado. However, the bacterial community of the retorted shale sample was dominated by a single organism which composed 30% of the total CFU. This organism has subsequently been identified as a member of the genus Micrococcus by comparison with organisms isolated in laboratory studies. The relative total heterotrophic bacterial CFU's of retorted shale and soil samples obtained in April 1978 and April 1979 were again similar but were not dominated by a single microorganism. Micrococcus sp. were essentially absent from these communities.

Laboratory studies have indicated that several factors may be important to the initial colonization of retorted shale by Micrococcus sp. These studies were conducted with liquid cultures which contained retorted shale as a source of carbon and soil as the

source of bacterial inoculum. When no more amendments were added, the shale was not colonized until the seventh week, and the bacterial community which developed was heterogeneous. The addition of inorganic amendments such as phosphate or bicarbonate stimulated the colonization of the shale by *Micrococcus* sp. This is best illustrated by the addition of phosphate where colonization occurred within the first week of incubation and the bacterial community was composed entirely of *Micrococcus* sp. Similar results were observed with bicarbonate; however, colonization did not occur until the third week of incubation. The effect of phosphate was concentration-dependent. The rapid colonization of retorted shale by *Micrococcus* sp. was only observed at concentrations greater than 4 mM. The addition of a complex organic media (casamino acids, yeast extract, and glucose; 0.5, 0.1 and 0.2 g/100 ml, respectively) to cultures containing phosphate did not alter the rate, of colonization, but did change the bacterial community to a complex mixture of organisms absent of *Micrococcus* sp. The addition of only glucose and phosphate produced results identical to those found with only phosphate. These results would suggest that in the presence of added phosphate *Micrococcus* sp. can rapidly colonize the retorted shale in deference to other organisms because of its ability to utilize the organic residuals present and the probable absence of organic growth factors. This is in contrast to the situation in which phosphate was not added to the culture medium containing retorted shale or soil. Bacterial competition for available nutrients led to the formation of a heterogeneous community absent of *Micrococcus* sp., although at a much slower rate.

This work is being expanded to determine the types and numbers of additional microorganisms that develop on retorted shale with environmental weathering and aging. Studies will also be initiated to measure the rate and extent of microbial decomposition of representative water-soluble residual organic compounds in retorted shale leachates.

Microbiology of Retort Water

One of the principal factors influencing the persistence, toxicity and mobility of organic residuals in retort water and retorted shale disposal sites will be the activity of the indigenous microorganisms. As previously reported, soil bacteria and fungi in liquid cultures containing retort water (4% v/v) as sole carbon source were able to decrease the dissolved organic carbon content of the cultures 33 and 21%, respectively, within the first 25 days of incubation. No further decrease in dissolved

organic carbon was observed up to 55 days when the cultures were terminated by filtration through 0.22- μ filter membranes.

In a continuation of this study, organic acids in filtrates obtained at 0 and 55 days have been partially characterized by a combination of chemical esterification with BF_3/MeOH and capillary gas chromatography. This methodology has been applied to monitoring the fate of organic acids in biological systems. Analysis of initial samples indicated the presence of a homologous series of mono- and dicarboxylic aliphatic acids which ranged from pentanoic to decanoic acid and butanedioic to dodecanedioic acid, respectively (Figure 5). Most of these components were absent from analyses of 55-day samples of bacterial and fungal culture medium. Apparently these components were either used for growth or converted to less polar compounds.

The ability of microorganisms to degrade or alter organic compounds within a given environment is dependent on the organic material as well as the effect of the environment on the physiology of the microorganisms present. The effect of oil shale retort water on several physiological properties of soil microorganisms was therefore investigated. The growth of soil-derived mixed cultures of bacteria in liquid media containing 10 mM glucose plus increasing concentrations of retort water was only effected at concentrations of retort water greater than 2% (v/v). The mineralization of glucose by soil microorganisms was 50% inhibited by 2% (v/v) retort water. The percentage of a soil bacterial population able to grow on nutrient media plus retort water decreased at concentrations of retort water greater than 6% (v/v). However, the survival of soil bacteria in this growth media was markedly affected only at concentrations of retort water greater than 12% (v/v). The formation of fungal schlerotia, a characteristic of mycelial differentiation, was inhibited by retort water concentrations greater than 0.5% (v/v). At a concentration of $\approx 2\%$ (v/v) retort water, 50% inhibition was observed. Aged retort water samples obtained from liquid cultures containing 4% retort water as sole source of carbon and inoculated with soil derived bacteria and fungi (above) were found to inhibit schlerotial formation to a greater extent, decreasing the concentration required for 50% inhibition to $\approx 1\%$ (v/v).

These studies are continuing and will be expanded to include studies on the degradation of additional soluble organic compounds identified in retort water.

FIGURE 5. Gas Chromatograms of Aliphatic Methyl Esters of 4% (v/v) Retort Water Cultures Inoculated with Soil Bacteria. A, pentanoic acid; B, hexanoic acid; C, heptanoic acid; D, octanoic acid; E, internal standard; F, nonanedioic acid; G, decanedioic acid; H, hendecanedioic acid; I, dodecanedioic acid.

Plant Studies

Since vegetation is likely to be used frequently for surface stabilization of disposed retorted shale, it is important to examine plant availability and toxicity of inorganic and organic residuals in retorted shale. Provided the plant does not discriminate against an element, increases with solubility in the μM to mM range normally result in increased plant uptake. However, it is difficult to relate solubility to uptake in the initial phases of a field study because chemical equilibrium is not established. The elemental composition of alfalfa grown in the lysimeter containing Paraho retorted shale was determined for the 1978 season. There were significant differences in the concentration of several elements in plants grown on retorted shale and soil. The most notable difference was the increased levels of magnesium (four times) in the plants grown on retorted shale. It is not known if these differences will continue in subsequent harvests. The most important information obtained from these analyses is that the chemical form of a major fraction of the arsenic and fluorine in plants grown on the retorted shale may be different than that in plants grown on soil overburden. This is based on a differential loss of arsenic and fluorine on drying of plants grown on the retorted shale, whereas drying had no effect

on arsenic and fluorine concentration of plants grown on soil overburden (Table 1). As expected, drying the tissue resulted in losses of selenium and mercury, reducing concentrations to nondetectable levels regardless of substrate. Laboratory studies to define the kinetics of uptake, translocation, form and toxicity of soluble inorganic and organic residuals are underway.

TABLE 1. Effect of Drying Alfalfa Tissue on the Concentration of Arsenic and Fluorine in Plants Grown on Retorted Shale and Soil

Treatment	Assayed Fresh		Assayed Dried (60°, 24 hr)	
	Arsenic	Fluorine	Arsenic	Fluorine
	$\mu\text{g/g}$			
Retorted Shale Only	2.7(a)	85(a)	0.84(c)	35(c)
Plus 20 cm soil	0.10(b)	7.2(b)	0.08(b)	11(b)
Plus 40 cm soil	0.05(b)	6.4(b)	0.10(b)	10(b)
Plus 60 cm soil	<0.05(b)	7.3(b)	<0.05(b)	5.5(b)
Plus 80 cm soil	0.08(b)	6.3(b)	0.07(b)	6.0(b)
Soil Only	0.22(b)	7.0(b)	<0.05(b)	6.1(b)

(a,b,c) Each value is the average of four determinations. Values for the same element with different letters are different at 0.01 level of significance.

4 Synfuels

SYNFUELS

In his May 1977 environmental message, the President of the United States declared a national commitment to reducing reliance on foreign oil by increasing use of our own vast coal reserves. He admonished, however, that we were to do this in a way that protects the quality of our environment. To meet this challenge, government and industry have entered into an unprecedented series of cooperative efforts to develop new energy technologies. Their purpose is to convert coal to oil substitutes and alternate fuels that burn more cleanly than their parent coals. Among these coal conversion technologies are those in which slurried coal is subjected to high heat and pressure, causing the coal to liquefy.

Of the three most promising coal liquefaction technologies, Solvent Refined Coal (SRC), H-Coal, and Donor Solvent, SRC is expected to reach the demonstration stage of development first. Design of the demonstration facility will derive partly from experience gained at a 50-ton-per-day SRC pilot plant at Fort Lewis, Washington. The demonstration plant will be located near Morgantown, West Virginia, where site evaluations are in progress under the cognizance of DOE's Oak Ridge Operations Office.

The work described herein is PNL's part of a larger research program on coal synfuels being implemented by DOE. To allow us to obtain maximum return on investment over the whole program, the ecological characterization task is being coordinated with complementary programs in other departments in PNL and at Oak Ridge National Laboratory.

During FY 1979, PNL was asked by the Assistant Secretary of Environment (ASEV) to develop a comprehensive plan for research needed to assure the environmental safety of an SRC technology. The result was a six-year research plan involving four PNL research departments. The various departmental responsibilities include acquisition, characterization and disbursal of test materials (Physical Sciences Department), biomedical research (Biology Department), ecological research (Ecological Sciences Department) and atmospheric monitoring (Atmospheric Sciences Department). A draft of these research recommendations was forwarded by Dr. Harvey Drucker, PNL's program coordinator, in December to ASEV for DOE review and comment.

The PNL research program was expanded in FY 1979 to include evaluation of the most likely avenues of environmental change from solvent refining of coal, with particular reference to the Morgantown location. An important element of this expansion was identification of the source materials and variations of source materials needing generic ecological investigation. This required increased collaboration with technology developers and coordination through DOE's Committee on Coal Liquefaction and Commercialization.

Research during FY 1979 was concentrated in three principal areas: 1) developing suitable bioassay methods for reference organisms in each of the major trophic levels, 2) gathering data on chemical partitioning for chronic exposure approaches in aquatic systems and 3) gathering data on leaf and root contact toxicity using appropriate aerosol and lysimeter approaches, respectively. Only a limited number of SRC materials were available for these studies. However, we expect more materials to be available during FY 1980. Several exploratory studies were made on the solid waste problem during FY 1979. These studies included evaluation of factors affecting the long-term fate of SRC products in soils, evaluation of the hydrological and chemical variables at the demonstration site, and evaluation of certain standard methods followed under the Resource Conservation Recovery Act.

In October 1979, sponsorship of a portion of our Synfuels research program was transferred to the Environmental Protection Agency, although the work was substantially carried out under DOE direction. These tasks are indicated by asterisks in the following pages.

• Synfuels

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Synfuels ecological research tasks are organized under aquatic and terrestrial effects, with aquatic effects being further subdivided into acute and chronic categories. The rationale, methods and first-year results of each task are briefly described.

Acute Screening of Solvent Refined Coal Materials*

This task quantifies the acute relative toxicity of a complex hydrocarbon product to representative aquatic organisms. The derived data provides a rational basis for designing studies of chronic and subacute toxicity, bioaccumulation, biodegradation and other aspects of aquatic hazard assessment.

Acute toxicity testing with aquatic organisms is one of the initial steps taken to assess materials that could potentially be released to surface waters during manufacture, transportation and use. Such tests with an unknown complex industrial material are usually conducted under static conditions with invertebrates and, depending on biochemical characteristics of the material, may be extended to flow-through conditions for vertebrate species such as fish.

The three organisms we used in acute screening of SRC materials were the fathead minnow Pimephales promelas (a vertebrate consumer), the zooplankter Daphnia magna (a primary and secondary consumer), and the midge Chironomus tentans (a benthic detritivore). Tests were routinely conducted under standard reference conditions. However, tests under other conditions were included

in experimental protocol to explore the effect of different water quality features (environmental variables) on toxicity of SRC materials. Standard tests used a temperature of 20°C, pH at 7.3 to 8.5, hardness of 65 to 90 ppm and dissolved oxygen (DO) near saturation (9 to 12 ppm). The other tests used temperature, pH, turbidity and hardness levels that deviated from standard.

In general, procedures adopted for acute testing were those applied by lead government and industrial agencies. Concentrations of SRC complex materials, which consisted primarily of hydrocarbons, were routinely measured by total organic carbon (TOC) analysis. Water quality (DO, pH, temperature) and chemical parameters (TOC, total phenols, ultraviolet and infrared carbon analysis) of stock and exposure solutions were also monitored.

The endpoint of acute studies was death of the test organism. Results were quantified by calculation of median lethal concentrations (LC₅₀), confidence limits and the lowest TOC level producing a toxic response ($\alpha = .05$). Daphnids were exposed to the SRC materials for 48 hr. Minnows and midges were exposed for 96 hr. Mortality was checked daily. After exposure, organisms were transferred to uncontaminated water for 24 hr to determine delayed mortality.

We began acute tests with a blend composed of 2.9 parts SRC liquid product (SRC II) middle distillate to one part heavy distillate.

*Portions of these efforts were transferred to the Environmental Protection Agency on October 1, 1979.

This material, of pilot plant origin, may not be representative of products eventually produced by an SRC demonstration or commercial facility. This series of tests is continuing. Some data are now available on toxicity of the mixture to the three test organisms under standard conditions and under high and low temperature regimes. Stock solutions were prepared identically for each test according to information developed by delivery system experiments. Thus, results of tests conducted simultaneously and sequentially are intended to be comparable.

The relative toxicity of the 2.9:1 blend to *Daphnia*, midge larvae and fathead minnows in one test is shown in Figure 1. *Daphnia*

duct, is considerably higher than that of crude oils, and roughly comparable to other refined oils. Our use of TOC values in water-soluble fractions to determine exposure concentrations holds several advantages, particularly since only part of the blend is water-soluble. However, our data based on TOC cannot be compared directly with data on crude and refined oils in the literature because they are based largely on percent dilutions of oil with water.

One acute test was conducted with Prudhoe Bay crude oil as a reference toxicant. The stock solution, prepared in the standard manner, produced a water-soluble fraction with 19 ppm TOC (compared to the 900 to 1100 ppm

FIGURE 1. Acute Relative Toxicity of SRC Blend (2.9 parts middle distillate to one part heavy distillate) to *Daphnia*, Midge Larvae and Fathead Minnows

were most susceptible, and midge larvae least. However, response varied between tests, even when exposures were to identical TOC concentrations. Thus, a series of tests is required to accurately pinpoint LC₅₀ limits and confidence intervals.

Results of several acute tests under standard conditions with best fit to probit analysis assumptions are now available. Median lethal concentrations (LC₅₀), determined by probit analysis at the 95% fiducial level were 2.0 (1.8 to 2.3) ppm TOC for *D. magna*, 8.3 (7.9 to 8.7) ppm TOC for fathead minnows, and 10.0 (4.8 to 20.5) ppm TOC for midge larvae.

The acute toxicity of the water-soluble fractions of the 2.9:1 blend, a refined pro-

duct produced by the 2.9:1 blend). Results of the acute test showed no mortalities of fathead minnows, daphnids or midge larvae at TOC levels producing total mortality from the 2.9:1 blend. Therefore, on an identical TOC basis, the blend is more toxic than Prudhoe Bay crude. Based proportionately on water-soluble fractions, the blend is at least 50 times more toxic than Prudhoe Bay crude. The difference in toxicity may reflect higher concentrations of water-soluble phenolics in SRC preparations.

We found that test solutions in aquaria stocked with fathead minnows were subject to bacterial contamination at 20 and 25°C, presumably because of nutrients provided by phenolic and other components of the test

material. We measured high bacterial populations in 24 hr, and even higher concentrations at 48, 72 and 96 hr, even though solutions used to expose minnows were replaced each 24 hr to avoid low DO levels. Bacterial growth is characteristic of acute toxicity tests with phenol. Future tests are planned to explore this feature and to determine biodegradation products.

Chronic Effects of Solvent Refined Coal Materials

This task evaluates potential sublethal and chronic effects of SRC materials on several life stages of freshwater organisms. Species selected for study include a green alga (*Selenastrum capricornutum*), a zooplankter (*Daphnia magna*), two benthic macroinvertebrates (*Chironomus tentans* and *Tanytarsus dissimilis*) and a fish (*Pimephales promelas*). These species represent various trophic levels and are found in a range of freshwater habitats in the eastern United States near the proposed demonstration facility site. Life-cycle tests conducted in FY 1979 consisted of exposing organisms to sublethal concentrations of toxicant. Population growth (cell numbers) of algae during and after exposure and survival, growth and reproduction of *Daphnia* and midges were studied.

During the next fiscal year we expect to conduct similar tests with fish and initiate avoidance/attraction studies with fish and midgefly larvae. The latter studies will provide data on behavioral responses of these organisms to toxic materials and aid interpretation of acute and chronic toxicity test results. In environments where exposed organisms can move in and out of contaminated water and sediments, concentrations causing death, retarding growth or limiting reproduction may only be important below the avoidance threshold. During FY 1979, our efforts were primarily directed toward 1) realigning task objectives to coincide with technology development goals (for example, current SRC II demonstration plant design includes a zero-discharge concept, so our research currently focuses on effects of product spills), 2) developing test methods to evaluate potential sublethal and chronic effects of complex SRC materials and 3) initiating studies with a 2.9:1 middle-to-heavy blend of SRC distillate from the pilot plant.

Early design experiments indicated that our method of mixing SRC material with water markedly affected the physical and chemical characteristics and toxicity of the resulting aqueous extract. Moreover, it became apparent that, because of the rapid leaching and

dilution of highly water-soluble components, the solutions used in acute toxicity tests may not be representative of those to which organisms would be chronically exposed after a product spill. Therefore it was necessary to devise a system or apparatus which would produce a simulated weathered oil and which could be used to generate test solutions approximating those which may occur in nature over time.

Two mixing methods were evaluated as potential simulators of natural weathering and dilution. The first system (termed batch or replacement) entailed seven sequential extractions of SRC oil with river water at two-day intervals. Each aqueous extract was chemically characterized and used as a stock solution for acute toxicity tests with *D. magna*. The second method (recycling) was an attempt to automate the batch extraction method. In this system, SRC fuel oil was continuously recirculated in contact with flowing river water, while an aqueous fraction was continuously drawn off and collected for chemical and toxicity testing. Initial results indicated that the batch extractions afforded a more controlled and stable system. Consequently, chemical analyses were focused on characterizing the composition of the seven batch extracts.

The extracts contained a complex mixture of phenols and aromatic and saturated hydrocarbons. Concentrations of total organic carbon, phenols and hydrocarbons decreased with each extraction. We also observed changes in relative concentrations of various compound classes of phenols with each replacement (Figure 2). These changes in phenols were attributed to differences in the oil-water distribution coefficients as the weights of phenolic molecules increased.

Logarithmic depuration was observed for total organic carbon (TOC), total phenols, and phenols of lower molecular weight. Approximately linear depurations were evident for aromatic hydrocarbon classes and phenols of higher molecular weight. Phenolic compounds comprised high percentages of the total organic carbon load of all extractions (Table 1).

A two-day settling time had a negligible effect on phenol concentrations but significantly reduced hydrocarbon concentrations. Results of a rapid-dye photometric phenol assay correlated linearly with the molar phenol concentration after the initial extraction. These chemical data are critical in interpreting chronic toxicity test results. Methods developed will be applied

in planning future chronic bioassay experiments and interpreting the resulting data.

FIGURE 2. Effect of Extractions (Replacement Number) on Percent Composition of Various Phenol Classes in Batch Replacement Test Solutions

TABLE 1. Total Organic Carbon (TOC) Load, Percentage of TOC Represented by Phenols, and Percentage Represented by Aromatic Hydrocarbons in Batch Experiment Extracts

Replacement Number	TOC (mg/l)	Percentage of TOC as Phenols	Percentage of TOC as Aromatic Hydrocarbons
0	809	103.6	1.4
1	324	99.1	3.4
2	161	93.0	4.8
3	89	77.0	12.4
4	57	82.3	15.8
5	46	68.0	17.6
6	45	46.7	19.6

Preliminary life-cycle studies in FY 1979 showed that, with the batch mix (first extraction), extracts of 2.9:1 middle-to-heavy SRC blend inhibited algal growth (Figure 3) during exposure to TOC concentrations as low as 6.4 ppm, but did not inhibit algal recovery until TOC concentrations exceeded 57 ppm. *Daphnia* growth was inhibited at TOC concentrations as low as 0.6 ppm, about 4 to 6 ppm TOC lower than the acute LC₅₀. Preliminary studies with the recirculating mix using SRC material extracted after 48 hr showed that survival and reproductive success of *Daphnia* was reduced at about 1 ppm TOC (Figure 3), 2 to 5 orders of magnitude below

FIGURE 3. Effects of Chronic Exposure to Sublethal Concentrations of 2.9:1 Heavy-to-Middle SRC Blend on a) Cell Growth of *Selenastrum capricornutum*, b) Live Births of *Daphnia magna* and c) Time to Adult Emergence of *Tanytarsus dissimilis*

the acute LC₅₀. Additionally, emergence of midges was delayed at these low concentrations (Figure 3).

Acute toxicity tests conducted with Daphnia during evaluation of the two mixing methods indicated that toxicity to a given TOC concentration remained stable with replacement in the batch mix but increased with time in the recirculating mix. However, the recirculating mix produced an emulsion that "oiled" test Daphnia, causing flotation and death. The recirculating mix and resulting emulsion were considered less representative than the batch mix of what might occur during a spill in the Monongahela River at the demonstration site.

Future life-cycle tests will be conducted with the batch mix (initial and a later extraction) to simulate effects of long-term exposures to sublethal concentrations of acute (relative hydrocarbon concentrations after initial contact with water) and chronic (relative hydrocarbon concentrations after prolonged contact with water) mixes. These tests will span the range of mixes anticipated for a product spill at the demonstration site. Effects of exposure to changing hydrocarbon concentrations will also be evaluated by sequentially exposing organisms to Batches 1 through 5 over their life cycle. Data on chronic toxicity of SRC material will be compared to similar data for a common reference oil.

Behavioral studies (avoidance/attraction) are planned for FY 1980 using test chambers that have been designed and are now under construction. The chambers will allow test organisms to enter or leave water or sediments that have been contaminated with various concentrations of SRC materials. The studies will also evaluate the role of other ecological variables such as shelter, food and temperature in determining organism response.

Comparison of Toxicity Testing Between Two Laboratories

Personnel from the Ecological Sciences Department of PNL and from the Environmental Sciences Division of Oak Ridge National Laboratory (ORNL) collaborated to assess the comparability of acute toxicity data generated in each laboratory using the same toxicant and strain of the test organism Daphnia magna. This task is intended to develop a standardized reference toxicity test which will allow direct interlaboratory comparison of test data from alternative synfuel technologies. The experiment is designed to determine if observed differences are caused

by differences in water quality or in laboratory procedures.

Screening tests with acridine were begun as soon as written testing protocols had been established. ORNL testing generated potentially lower 48-hr LC₅₀ than did PNL testing. The slopes of ORNL toxicity curves also appeared to be less steep than PNL tests. With this information, an exchange of water between PNL and ORNL was arranged. The testing protocols were revised to increase precision on data analysis and to adapt methodologies to maintaining and testing Daphnia with the limited supply of exchanged water.

The initial battery of tests with PNL and ORNL water has been interrupted by the unexplained appearance of "floaters" (daphnids which become trapped at the air-water interface) during tests. Our extensive testing to determine the cause of flotation has proven futile, and we must assume that some aspect of the culturing of Daphnia may be responsible for this phenomenon. We are now conducting a second battery of tests with Daphnia cultured under a more liberal water replenishment schedule. We expect to eliminate the incidence of floaters. Once a complete battery of tests with acridine has been completed and analyzed, tests with H-coal materials will be conducted to determine suitability of the methodology for complex materials.

Terrestrial Plants: Contact Toxicity*

Foliage of terrestrial plants could come into contact with airborne SRC products and wastes through a number of possible mechanisms. These might be routine operation of a facility, transportation of products or wastes, or accidental release. To determine whether atmospheric releases of these materials present any environmental hazard, a series of studies were undertaken to evaluate the effects of chronic exposure of plant canopies to SRC materials.

The exposure system currently in use employs a Wright® dust mill and air sampling devices for the generation and quantitation of airborne particles, and for subsequent controlled contamination of plant canopies. Preliminary studies with bush bean plants showed SRC I solid product to have no harmful effect on either dry matter production or seed yield after 30 days of exposure under either dry (60% relative humidity) or moist (simulated rainfall) conditions. Deposition rates ranged in two experiments from 0.04 to 0.94 mg/dm² · min. This led to mass loadings from 1.3 to 56.4 mg/dm² of leaf surface.

Bush beans will continue to be the major test species for this segment of the program. Others will be studied as experimental results dictate. Subsequent studies will examine the effects of SRC II gasifier bottoms, process water incinerator solids, liquid product and SRC I mineral residues. Transfer of specific inorganic and organic components from foliage to seeds will be determined for these materials. Toxicity effects on dry matter production and seed yield will also be studied.

Seed Germination and Root Elongation Studies*

The purpose of seed germination studies was to determine the toxicity of aqueous extracts of SRC products and residues. Radish (*Raphanus sativa*, Scarlet Globe variety) seeds were used in the experiment. They were put into individual holes in special germination trays constructed of transparent Plexiglas®. An aqueous extract made from SRC I solid product was given to the seeds. Some seeds received a mixture of one part extract to one part distilled water, some a mixture of one part extract to nine parts distilled water, and some, distilled water alone.

The seeds germinated in the dark in a temperature-controlled (25°C) environment. After 48 hr, root elongation was measured to the nearest millimeter. Data on seed germination and root elongation are shown in Table 2. These preliminary data indicate that aqueous extracts of SRC I do not impair either radish seed germination or root elongation.

SRC I product was also introduced to Ritzville soil in mixtures and in layers in field lysimeters. These were planted with barley. After the barley was harvested,

TABLE 2. Radish Seed Germination and Root Elongation after 48 hr in SRC Extract and Distilled Water

Treatment	Percent Germination	Average Length of Root, mm $\pm$ S.D.	n
Distilled water	99	40 $\pm$ 13	270
1 part extract, 9 parts water	99	40 $\pm$ 12	270
1 part extract, 1 part water	99	34 $\pm$ 11	270

leachate was collected from the bottom of the lysimeters through porous ceramic cups. The raw leachate was added to seed germination trays and the seeds in them were tested for germinability and root elongation. The results are summarized in Table 3. Radish seed germinability exceeded 90% in all treatments. Root elongation averaged slightly less in distilled water than in aqueous leachates collected from soil columns in field lysimeters. These data indicate that aqueous leachates of SRC I solid product passed through soil are not deleterious to radish seed germinability or root elongation.

TABLE 3. Radish Seed Germination and Root Elongation after 48 hr in SRC I Solid Product Extract and Distilled Water

Treatment	Percent Germination	Average Length of Root, mm $\pm$ S.D.	n
Soil leachate	94	30 $\pm$ 9	108
SRC mixed in soil	95	29 $\pm$ 12	216
TEST B SRC layered in soil	95	29 $\pm$ 12	216
Distilled water	97	26 $\pm$ 11	108
Soil leachate	95	27 $\pm$ 13	108
SRC mixed in soil	96	27 $\pm$ 13	216
TEST B SRC layered in soil	97	27 $\pm$ 13	216
Distilled water	92	26 $\pm$ 11	108

Field Studies: Lysimeter Screening of Solvent Refined Coal Solid Product Spills

Because SRC solid product (SRC I) is a new material, little is known about its behavior in the environment. Although the solid product will not be introduced to soil or water in normal uses, it could possibly be spilled during transportation. Spills could contaminate surface waters and agricultural, range-land and forest soils, possibly causing acute or chronic harm to plants. This investigation examines the effects of SRC I solid product introduced to soil on barley (*Hordeum vulgare*) in lysimeters.

The solid product, consisting of black, pea-sized, angular pellets, was mixed 1:1 with Ritzville silt loam soil by weight in six different lysimeters, providing a surface layer of soil mixed with SRC product

3 dm deep. In six other lysimeters, a 3-dm-deep layer of SRC was placed 3 dm below the soil surface. Six control lysimeters were filled with soil alone. Water was added to the lysimeters in amounts approximating field capacity. Barley seeds were planted in April and the plants, in their lysimeters, were grown outdoors.

There was no visible evidence that SRC product added to the soil produced lesions or chlorotic or necrotic tissues in barley plants. Culms and seed heads were longest on plants grown in the control lysimeters and shortest on those grown in layered soil (Table 4, Figure 4). Barley plants grown in soil mixed with the SRC product had culms and seed heads of intermediate length. These plants had more flowering culms than either the control or layered treatments.

Barley grown in the SRC-soil mixture produced more dry matter than control plants (Table 5). We attribute the greater production of dry matter by the SRC-soil mixture plants to the production of more culms. The low yield of barley grown in SRC-layered lysimeters we attribute to the inability of plant roots to penetrate the SRC layer. This was clearly observed when lysimeters

FIGURE 4. Production of Barley Plants Grown in Soil Treated with SRC Solid Product, Compared with Production of Plants Grown in Untreated Soil

TABLE 4. Average Length (cm) $\pm$ Standard Error of Flowering Culms and Seed Heads of Barley Plants in Soil Containing SRC Solid Product in Field Lysimeters

Parameter	Soil Mixture								
	SRC and Soil, Layered			SRC and Soil, Mixed			Soil Alone		
	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n
Length of Culms	39 $\pm$ 1.4		9	43 $\pm$ 1.6		14	50 $\pm$ 2.0		13
	35 $\pm$ 1.7		10	42 $\pm$ 1.8		17	52 $\pm$ 2.3		12
	35 $\pm$ 1.9		12	46 $\pm$ 1.4		15	44 $\pm$ 1.3		11
	33 $\pm$ 2.3		7	40 $\pm$ 1.7		17	52 $\pm$ 1.3		12
	41 $\pm$ 1.1		8	40 $\pm$ 1.6		15	42 $\pm$ 1.8		15
	37 $\pm$ 1.4		8	36 $\pm$ 1.8		15	46 $\pm$ 2.1		11
	37		9.0	41		15.5	48		12.3
Length of Seed Heads	5.8 $\pm$ 0.2			6.4 $\pm$ 0.4			7.4 $\pm$ 2.0		
	5.7 $\pm$ 0.4			6.1 $\pm$ 0.3			7.3 $\pm$ 0.4		
	5.0 $\pm$ 0.4			6.9 $\pm$ 0.2			6.8 $\pm$ 0.4		
	5.6 $\pm$ 0.4			6.1 $\pm$ 0.3			7.0 $\pm$ 0.2		
	6.4 $\pm$ 0.3			6.2 $\pm$ 0.3			6.5 $\pm$ 0.3		
	5.0 $\pm$ 0.3			5.4 $\pm$ 0.3			6.8 $\pm$ 0.3		
	5.6			6.5			6.9		

were dismantled at the end of the growing period and roots washed from the soil core.

SRC solid product appears to be a relatively harmless material when introduced to the soil and mixed. The next phase of SRC

TABLE 5. Average Dry Weight of Barley (g) $\pm$ Standard Error (n = 6) Grown in SRC Solid Product and Ritzville Soil in Field Lysimeters

Barley Part	Soil Mixture		
	SRC and Soil, Layered	SRC and Soil, Mixed	Soil Alone
Total	9.18 $\pm$ 0.56	18.17 $\pm$ 1.02	16.98 $\pm$ 0.80
Straw	4.25 $\pm$ 0.27	8.73 $\pm$ 1.20	8.20 $\pm$ 0.55
Heads	4.93 $\pm$ 0.32	9.44 $\pm$ 0.56	8.78 $\pm$ 0.27
Grain	4.10 $\pm$ 0.27	7.92 $\pm$ 0.49	7.62 $\pm$ 0.21

research is to test the effects of residual material from the SRC I process, which produces solids, and the SRC II processes, producing liquids. Some lysimeters will be maintained over several years to determine if aging, weathering and soil microbial activity enhance plant toxicity.

Solid Waste Disposal of Residues From Solvent Refined Coal

The disposal of solid residues from the solvent refining of coal in landfills creates the potential for contamination of surface and ground waters through the leaching of soluble inorganic and organic residues. Evaluation of the potential ecological and health effects associated with disposal requires a complete understanding of the processes which will occur with time and weathering after disposal. The most important variables which will control the mobility of residuals from the disposal site include 1) mineralogy and organic composition of the residues, 2) solubilization as a result of oxidative or reductive reactions or microbial activity, 3) precipitation and absorption reactions between soluble components and the substrata and 4) surface and ground water hydrology.

To effectively evaluate and predict the simultaneous operation of all these variables in controlling the mobility of soluble residues from the disposal site, an interdisciplinary research approach using laboratory and field studies is required. The research program which was initiated this year encompassed the disciplines of mineralogy; soil science; inorganic, organic and biological chemistry; microbiology and hydrology. The studies were conducted in cooperation with toxicological research and provided information on the relative importance and persistence of specific inorganic and organic components which may affect biota adversely.

The initial phase of the study has included 1) the development of analytical techniques to evaluate the chemical and microbial oxidation of reduced sulfur species, which are known to play a key role in controlling the chemistry of waters which contact pyritic residues, 2) evaluation of laboratory extraction and incubation methods for the study of specific chemical and microbiological reactions under controlled conditions and 3) initiation of field studies in sediment and water chemistry and hydrology at the Fort Morgan, West Virginia SRC demonstration site.

Analytical Development Using Ion Chromatography

The role of anions in controlling changes which occur in surface or ground water chem-

istry has long been known, particularly in the sulfur-rich coal regions of the southeast. However, analytical methods available to rapidly differentiate important anionic species are limited by the instability of many of the species being analyzed and chemical interferences in the methods used. Among the most important and well-known problems associated with coal and coal-mining residuals are the production of acid waters and the effects of this on metal solubility and organic degradation pathways through the oxidation of reduced sulfur species. The oxidation of reduced sulfur compounds will probably be important in the disposal of solid residuals from the SRC process.

In FY 1979, ion chromatography was shown to be a sensitive method, relatively free of interferences, for analyzing many anionic species. The technique also requires little sample preparation and allows the simultaneous analysis of several anions. Methods have been developed for the analysis of SO_3^- , SO_4^- , S_2O_3^- , SCN^- , Cl^- , NO_3^- , NO_2^- , and PO_4^- in water samples of widely varying salt content. Investigations into the ability to separate and quantify S_2O_4^- , S_2O_5^- , S_2O_6^- , S_2O_7^- , and S_2O_8^- have met with limited success. Of the latter list, S_2O_8^- has been proven to be a highly reactive oxidant when added to typical solutions, while the other sulfur oxide species are unstable in solution forming SO_3^- and SO_4^- . These methods have been successfully applied to solid waste leachates, surface and ground waters.

Hydrological and Chemical Studies at the SRC Demonstration Site

As a basis for laboratory and field studies of the effects of SRC solid waste disposal, investigations have been initiated at the Gulf Mineral Resources SRC II demonstration site near Morgantown, West Virginia. The site is not pristine, but rather is affected by the surface and subsurface activities of previous coal mining. The site is drained by three runs which flow directly into the Monongahela River. Two of these, Robinson Run and Crafts Run, are typical acid mine drainages; the third, Crooked Run, exhibits better water quality.

Sampling locations on the three runs and the Monongahela River were chosen where a range of water and sediment types could be collected. This range is necessary for developing methodologies applicable to a study of the environmental consequences of solid waste disposal and product spills at

this site. Additionally, hydrological reconnaissance of the site was undertaken and the need for additional characterization will be delineated.

Evaluations of the data collected to date are not complete. However, several areas have been identified for additional study. In general, the surface waters (Monongahela River) and ground waters in the area have low salt contents, are fairly well oxygenated and have low soluble organic carbon levels. Robinson Run and Crafts Run are the only acid waters (pH 2.8 to 4.9) with concurrently high concentrations of soluble iron and manganese. The sediments (surface 10 to 20 cm) of the Monongahela are reduced and have relatively high levels of sulfide (380 to 960 $\mu\text{g/g}$) and organic carbon (2.9 to 3.1%). Robinson Run has low sulfide (4 $\mu\text{g/g}$) but high organic carbon (3.9%) and very high iron levels (14% dry wt).

Baseline levels of phenols and basic, acidic and volatile organics are being determined in the water and sediments collected. This information, in conjunction with the hydrological measurements and laboratory studies of SRC II gasified and nongasified mineral residues, will allow a sampling schedule and specific analyses to be developed that will allow proper pre- and post-operational baseline monitoring and the eventual prediction of the mobility to waters of the important residuals from solid wastes.

Value of Resource Conservation Recovery Act Extracts in Evaluation of Potential Problems with Disposal of Solid Waste Residues*

In an attempt to standardize and evaluate rapidly the potential hazards of disposal of solid wastes for the Resource Conservation Recovery Act (RCRA), a standard method using a batch equilibration (1:16 solids-to-solution ratio by weight) for 24 hr has been advocated by the Environmental Protection Agency. Depending on the pollutant, utilization of this method can seriously overestimate or underestimate problems. As examples of the type of problems involved, retorted oil shale was equilibrated (1:10 by weight) for 96 days. The results for sodium and potassium concentrations in solution are shown in Figure 5. The 24-hr leaching period as advocated for the RCRA extraction is represented by the steep part of the equilibration curve for both sodium and potassium. After two days, the sodium appears to be at equilibrium and changes little through the rest of the incubation period. The potassium, on the other hand, does not appear to be at equilibrium even after 95 days. This behavior in-

FIGURE 5. Effect of Incubation Time on Levels of Potassium and Sodium Solubilized by Water from Retorted Oil Shale

dicates release of additional potassium from minerals with slow solubilization kinetics. If the species were some element other than potassium and were toxic, the 24 hr extraction would have underestimated the potential problem which could have occurred on weathering.

Similarly, short-term extracts or column studies may grossly overestimate potential hazards, as illustrated in Figure 6. In this case, significant quantities of soluble organic carbon were released from retorted shale by leachate water in the first year. However, the same shale which was weathered for an additional year exhibited much lower concentrations of soluble organic carbon. This reduction could have resulted from

FIGURE 6. Soluble Organic Carbon in Percolate Collected in 1977 and 1978 from Retorted Shale

microbial degradation or utilization of the soluble organic carbon or from changes in other conditions (such as a decrease in pH) which enhanced the adsorption to the shale. In either case, the results indicate that an understanding of the mechanisms which are responsible for the solubilization or removal of inorganic and organic residuals on interaction of water with the solid wastes is essential for a valid prediction of potential problems.

Evaluation of Trace Element Effects on Toxicity Tests With SRC II Product

The effects of the inorganic trace ions and macro-ions present in solutions used for

toxicity tests of the SRC II product were evaluated by analysis of untreated and filtered water during the tests (described under the Acute Screening section, above). The data for the duration of the 32-hr bioassay are shown in Table 6.

The results show that there are no major differences in composition between filtered Columbia River water used for the controls and filtered water treated with the SRC II product. The only significant difference was in the increase in zinc by about 10 ppb in the filtered sample (one liter). This increase was due to contamination derived from the filter.

TABLE 6. Trace Element Composition of Columbia River Water and Water Used for Toxicity Tests

Time of Sampling (hr)	Filtered <0.2	pH	Organic Inorganic													
			Cond.	Na	K	Ca	Mg	SiO ₂	SO ₄ ⁻	C	C	F	Mo	Sr	Zn	Ba
			µmho's/cm					µg/ml						µg/l		
0.5	Yes	7.4	155	2.1	1.8	21	4.4	4.6	23	14	8	180	8	99	26	24
	No	7.4	156	2.1	0.8	21	4.6	4.7	31	18	8	180	7	100	16	25
2.0	Yes	7.9	156	2.1	0.8	21	4.6	4.6	28	19	2	180	7	100	21	26
	No	7.9	157	2.1	0.7	22	4.6	4.7	27	24	4	170	7	100	15	26
8.0	Yes	7.9	156	2.2	0.7	21	4.4	4.5	21	20	9	170	7	98	31	24
	No	7.9	156	2.1	0.7	21	4.6	4.7	32	23	8	170	7	100	15	25
24.0	Yes	7.9	156	2.1	0.7	22	4.6	4.7	33	26	14	170	7	100	15	25
	No	7.9	156	2.1	0.7	22	4.6	4.7	23	25	13	170	5	100	19	26
32.0	Yes	7.8	156	2.2	0.7	22	4.6	4.7	26	25	8	170	<5	100	30	25
	No	7.9	156	2.1	0.7	22	4.6	4.7	30	27	10	170	<5	100	17	26
Columbia River	Yes	7.9	156	2.0	0.7	21	4.5	5.0	17	6	5	170	<5	98	30	23
	Yes	7.9	156	2.0	0.7	21	4.6	5.1	19	4	8	170	<5	100	27	24
	Yes	7.9	156	2.1	0.7	21	4.6	5.2	28	2	8	170	<5	100	27	24
Distilled H ₂ O	No	8.6	10	0.8	0.08	0.4	0.09	4.6	0.5	<1	<2	<10	<5	1	<1	5

As, Cd, Cr, Cu, Fe, Pb, Hg, Se, Ag, U, T were below 1 ppb in all samples.
Al, Be, Sb, B, Co, Mn, Ni, Sn, Ti, V, Li were below 10 ppb in all samples.

5 Nuclear Waste: Fission

NUCLEAR WASTE: FISSION

- **Transuranic Behavior in Soil and Plants**
- **Quantitative Aspects of Transuranic Field Studies**
- **Analog Elements for Transuranic Chemistries**
- **Transuranic Weathering in Plants**
- **Radioecology of Nuclear Fuel Cycles**
- **Environmental Behavior and Effects of Technetium-99 and Iodine-129**

One of the highest priorities in the continued development and use of fission as a source of energy is the resolution of questions about the long-term fate and ultimate effects of long-lived radionuclides in the biosphere. This long-term behavior generally cannot be confidently inferred from the short-lived isotopes. The individual studies described in this section range from basic investigations of the behavior and effects of long-lived nuclides at the chemical and cellular level to ecosystems studies which describe inventory, transport, and effects in waste management areas. The investigations described in this section are designed to differentiate and measure processes affecting the biological availability of the radioelement.

In terrestrial studies, investigations of transuranics in soils and plants have demonstrated the importance of valence state, complexation, competing elements, microbial processes, redistribution in the soil profile, and weathering cycles in governing the availability of the transuranic elements ^{129}I and ^{99}Tc to plants and to the consuming animals. In the case of plutonium, it was demonstrated that ingestion of plant tissues containing plutonium may result in greater transfer across the gut compared to gavaging animals with inorganic plutonium solutions. However, technetium in plant tissues is less available to animals. This underscores the importance of detailed studies of soil, plant and animal factors influencing uptake by the ingestion pathway. The importance of the ingestion pathway was also demonstrated in studies of foliar interception of airborne transuranic elements in which plants effectively intercept and retain plutonium containing particles in the respiratory size range. Significant quantities of the intercepted plutonium were transported to roots and seeds. Similar studies on the terrestrial ingestion pathway have been initiated with other actinides, including uranium, americium, curium and neptunium.

Radioecological field studies were directed toward establishment of pertinent ingestion pathways and exposure levels through description of habitat types, population densities, and, in several instances, dosimetry, for major insects, reptiles, birds and mammalian species. These studies were extended to agricultural ecosystems

through definition of the uptake of long-lived nuclides. Studies of actinide uptake by edible fish in a nuclear waste pond indicate that maximum levels of accumulation were insufficient to produce significant radiation doses to hypothetical fish consumers such as local predators and man.

Field inventory studies have provided the bases for appropriate environmental sampling, and in conjunction with basic studies of behavior in soils and plants, have resulted in the development of reliable statistical procedures for estimating the concentrations of transuranic elements in soils. Decisions as to need for land decontamination in former nuclear detonation and waste management areas will be based on this inventory.

The approaches developed in terrestrial and freshwater studies were extended to studies of the marine environment. The marine environment may represent an ultimate repository for the mobile forms of transuranic elements, and results indicate that geochemical and biochemical factors may influence transuranic element mobility and availability to biota. Investigations have provided a basis for description of dispersion mechanisms on the sea floor of transuranic elements arising from nuclear detonations and a bomber crash. In addition to physical dispersal, biochemical factors may influence transuranic speciation and mobilization resulting in substantial changes in biological availability as a function of ocean depth.

This comprehensive program, encompassing basic, as well as descriptive, studies on the behavior and effects of the major long-lived radionuclides, should ultimately provide an integrated knowledge of the extent of engineering sophistication required in the nuclear fuel cycle to minimize risks associated with this important energy source.

● Transuranic Behavior in Soils and Plants

Principal Investigators: R. E. Wildung, T. R. Garland, D. A. Cataldo, J. E. Rogers, K. M. McFadden, V. M. McNair and R. G. Schreckhise

The principal objectives of these investigations are to determine 1) the potential for alteration of transuranic solubility through formation of transuranic complexes in soil and the role of the soil microflora in this process, 2) the extent of uptake and translocation by plants and the sites of plant deposition of transuranics or their complexes, 3) the bond types and chemical forms of transuranics or their metabolites in microbes, plant tissues and soils, 4) the influence of soil properties, environmental conditions and cropping on these processes and 5) the retention of airborne pollutants by plant foliage and their subsequent absorption by leaves and transport to seeds and roots.

Soil, Plant and Animal Studies

Soil, plant and animal investigations have resulted in the development of a model for the chemistry or biochemistry of plutonium in the environment. Present investigations have emphasized the mechanistic approach for developing an understanding of the kinetic aspects of the model controlling long-term chemical behavior and bioavailability. In addition, investigations have been extended to apply this knowledge to other actinide elements.

Modification of the Chemical Form of Complexed and Hydrolyzed Plutonium by Soil Fungi

Microbial studies have been directed toward determining the mechanism for previously observed complexation of plutonium and alteration of its mobility in soil. These research efforts are being continued in an effort to determine the mechanisms of plutonium transport by fungi and the exact function of pH in this process; i.e., is the fungal membrane being affected or is the stability of DTPA or organic ligands of fungal origin being altered? Fifty-nine pure fungal cultures isolated from soil, on the basis of carbon source and resistance to plutonium and other metals, have been tested for their ability to modify the chemical form of plutonium diethylenetriamine pentacetic acid (Pu-DTPA) and $\text{Pu}(\text{OH})_n$. Of these cultures, 56 of the fungi reduced the pH of the culture medium from 6.2 to a value less than 3.4.

Of the remaining three, two did not alter the pH and the third reduced the pH to a level of 4.1.

The observed changes in pH were strongly correlated with the final solubility (<0.01) of Pu-DTPA in solution. Of the 56 fungi which reduced the pH to less than 3.4, 51 reduced the solubility of Pu-DTPA to less than 5% of the controls at the end of growth. The remaining three fungi did not alter the solubility of Pu-DTPA. A similar but less pronounced pH effect was observed with $\text{Pu}(\text{OH})_n$.

The effect of pH was further investigated by electron microscopy autoradiography to determine if the decrease in solubility was caused by 1) transport of plutonium across the fungal membrane at low pH, 2) binding of plutonium to the membrane surface or 3) formation of particulate plutonium. Four fungi were examined in this study: two from the high pH group (*Drechsleria*-352 and *Drechsleria*-369) and two from the low pH group (*Trichoderma*-380 and *Fusarium*-427). These studies were conducted in liquid culture using a standard mineral base media with glucose as the carbon source. The plutonium level was $0.02 \mu\text{g/ml}$ for both chemical forms. After completion of growth, samples of mycelia were removed, fixed, embedded and prepared for autoradiographic analysis. During this process, essentially no plutonium was lost to the solution when added as the DTPA complex. However, when $\text{Pu}(\text{OH})_n$ was used, substantial quantities of plutonium were lost to the fixing and embedding

solutions. This suggests that the $\text{Pu}(\text{OH})_n$ was loosely associated with the fungal mycelia.

Autoradiographs of *Trichoderma*-380 and *Fusarium*-427 inoculated with Pu-DTPA (Figure 1) indicated that essentially all of the plutonium tracks were associated with

FIGURE 1. Electronmicroscopic autoradiographs of *Fusarium*-427 Incubated in the Absence of Plutonium (a), Pu-DTPA (b) and $\text{Pu}(\text{OH})_n$ (c)

the intracellular portion of the mycelia. Similar results were observed with both species when incubated with $\text{Pu}(\text{OH})_n$. However, in the latter case, significant quantities of plutonium were associated with the surface membrane. As expected, the autoradiographs of both *Drechsleria* spp. (352 and 369), when incubated with Pu-DTPA, showed very few plutonium tracks. Those present were associated with the surface membrane. The results with *Drechsleria*-369 and 352, when incubated with $\text{Pu}(\text{OH})_n$, were in sharp contrast, however; numerous plutonium tracks and stars (particles) were observed. Essentially all the plutonium was either associated with the surface membrane or, to a small degree, with extracellular amorphous material. These results suggest that the final pH of the culture medium may play an important role in plutonium uptake by different fungi.

Incorporation and Transport of Plutonium in Plants and Animals

Our plant studies have concentrated on 1) the factors controlling plutonium absorption by plant roots; 2) plutonium mobility within the plant following either foliar or root absorption; 3) the chemical form of plutonium while in transport (plant xylem) and following deposition in leaves and seeds and 4) evaluation of the influence of plutonium incorporation in plants on gut absorption and subsequent food chain transfer.

Studies have recently been completed using ^{238}Pu -DTPA(^{14}C) to evaluate the factors controlling root absorption of plutonium. Analysis of $^{238}\text{Pu}/^{14}\text{C}$ ratios in the root zone and xylem exudates indicates an independent plutonium uptake and not absorption of the intact Pu-DTPA complex. Chemical characterization of plutonium forms in exudates clearly shows that the plutonium ligands are not those of DTPA. This is further evidence for the lack of an intact Pu-DTPA complex following root absorption. This data supports the view that organo-plutonium complexes in soils serve mainly to deliver the metal to the root membrane, and that the ligands are not taken up by the plant stoichiometrically with the metal.

While plutonium mobility in plants has been shown to be acropetal (root >> stems > leaves > seeds), previous studies at PNL, involving both foliar absorption and soil studies using split-root techniques, have suggested the presence of a relatively plant-mobile component capable of basipetal transport (leaves to roots). Recent studies, using bifurcated soybean roots, have shown that ~0.2% of the shoot plutonium is transported

basipetally to the unlabeled root. These results and those relating to transport form of plutonium demonstrate the importance of organic plutonium complexes in controlling root absorption and ultimate distribution within the plant.

Previous studies have shown plant-incorporated plutonium to be substantially more available for gut absorption than either plutonium nitrate or Pu-DTPA following ingestion. To identify the relationship between the fate of plutonium in plants and its bioavailability after ingestion, soybean plants which had accumulated Pu²³⁸ for various periods of time were subjected to chemical fractionation procedures. This was done to determine the chemical fate of plutonium. Leaf, stem and root tissues were homogenized and centrifuged, and soluble components were further characterized by ultrafiltration and gel filtration. After homogenization and centrifugation, 64, 56, and 31% of the plutonium was associated with the soluble fraction of leaves, stems and roots, respectively. Ultrafiltration of the solubles show 94% of the soluble leaf and root plutonium to be associated with proteins having molecular weight >10,000, while stems have a substantial fraction (32%) of the solubles associated with ligands of <500 molecular weight. Gel filtration of the soluble fractions of leaves and roots demonstrate the presence of five stable plutonium-containing ligands varying in molecular weight from 1,000 to >150,000. The data thus far obtained on the chemical behavior of plutonium in soybean plants indicates that the metabolic incorporation of plutonium into plant proteins is not unlike the behavior of many nonnutrient heavy metals, for which mobility within the plant (exudate and stem data) is dependent on the presence and formation of organic complexes of low molecular weight.

An important aspect of these data is the difference between the chemical form of plutonium in stem tissue and in leaf tissue. This difference explains results from animal feeding studies, in which gut absorption values were found to be substantially higher for stem tissues than for leaf tissues. This would appear to be a function of the high percentage of low molecular weight components in stem tissues, and their increased bioavailability for gut absorption. Studies are currently underway to determine the chemical fate of plutonium accumulated in soybean seeds and its comparative availability following ingestion.

Incorporation and Transport of Uranium and Thorium in Plants and Animals

Previous investigations have indicated that plutonium added to soil in the VI

valence state behaved as Pu(IV) in plant uptake, indicating that the Pu(VI) was probably reduced before plant uptake. In an effort to observe the effect of valence without the concurrent problems of oxidation or reduction, uranium and thorium were used as models for the VI and IV valence, respectively. Both valences are relatively stable in aqueous aerobic conditions.

Short-term studies (<24 hr) of the plant uptake of U²³² from nutrient solutions containing DTPA, CO₃²⁻ or Cl⁻ showed a nearly quantitative removal of uranium from solution to the roots but little translocation to shoot tissues. Analysis of the xylem exudates indicated that the primary activity transported to the shoots was not U²³², but Th²²⁸ and daughters. Actual concentrations of U²³² in the tissues are currently being determined. The associated anion had no effect on root uptake or translocation to the shoot tissues.

Studies of the chemical form of uranium in the nutrient solutions using thin-layer electrophoresis and chromatography indicated that the stability of any complexes formed with uranium was too low to remain intact under the conditions of the analysis. Related studies are underway to incorporate sufficient uranium and thorium into alfalfa for longer studies of animal uptake.

Foliar Studies

Evaluations of the interactions of submicronic (0.02-2 μm, count mode) particles with foliage of terrestrial plants have been completed. These studies have shown that the behavior of particles submicronic particles differ markedly from the established behavior of particles 1 to 100 μm. They have also shown that the size aspect for airborne pollutants significantly affects retention on foliar surfaces and the subsequent foliar absorption and transport of surface contaminants to other plant parts such as roots and seeds. The results show the foliar route of plutonium transport to be at least as effective as the soil route for acute exposure (less than one year) situations, if not more so. In the long-term chronic situation, where the soil is the cumulative repository for aerial input, the soil route should become the more important transport route with time.

Among the parameters shown to be important in foliar absorption are the chemical form of the contaminant, relative humidity, rainfall events and their timing and, of course, particle size. Based on the principles developed for submicronic particles, facilities are currently being developed to enable evaluation of a broader range of physical parameters and compound types.

• Quantitative Aspects of Transuranic Field Studies

Principal Investigators: R. O. Gilbert and J. C. Simpson

This project is a continuing investigation into the development and use of appropriate statistical design and analysis procedures in environmental actinide field studies. Significant accomplishments of FY 1979 were continued publication of TRAN-STAT, a periodical on Environmental Transuranic Studies, and initiation of a computer simulation study evaluating estimators of ratios, particularly concentration and isotopic ratios. Other activities included membership on the North Marshall Islands Advisory Group, chairmanship of the Statistics and Modeling Panel at the Office of Health and Environmental Research's Actinide Workshop at Arlie House, Virginia, and improving our expertise and capacity to perform Kriging, a technique for estimating spatial patterns of contaminants. Related activities under separate funding included statistical design and analysis for the Nevada Applied Ecology Group and that in connection with decommissioning and decontamination of nuclear facilities.

TRAN-STAT: Statistics for Environmental Transuranic Studies

TRAN-STAT, a periodical distributed to DOE and DOE-contractor personnel engaged in environmental actinide studies, is intended to communicate statistical design and analysis techniques appropriate for environmental actinide studies. Nine issues have been distributed since September 1977, five of which were published during the past year. Topics discussed in Issues 5 through 9 were nonparametric statistical methods contributed by G. C. White of Los Alamos Scientific Laboratory; introduction to aspects of field sampling design; a review of statistical methods for estimating the mean and standard deviation when negative, unreported or "less-than" concentrations are present; some statistical lessons learned in the cleanup of the Enewetak Atoll (contributed by M. G. Barnes of Desert Research Institute) and a description of our ratio simulation study and the motivation behind it. Efforts are underway to publish some of this material in the open literature. TRAN-STAT will continue to be published during FY 1980.

Ratio Simulation Study

The rationale and methodology for this study are discussed in Issue 9 of TRAN-STAT (October 1979, PNL-SA-8074). The computer simulations that form the bulk of the effort will be completed during the coming year and reported in a future issue of TRAN-STAT as well as in the open literature. Twelve estimators of ratios will be investigated for their ability to estimate accurately

and precisely ratios such as concentration and isotopic ratios. Underlying models (additive and multiplicative errors) and statistical distributions (lognormal, gamma, normal) that are believed to be realistic will be used. Hopefully, those estimators particularly suited to environmental actinide research (plant uptake studies and movement between ecosystem components to man) can be identified.

Kriging

In March 1979, DOE purchased BLUEPACK, a computer code for Kriging operations, and had it installed on the DOE computer in Richland. This significantly increases our capability to evaluate this technique further for estimating spatial contaminant patterns. Kriging was successfully used during the Enewetak cleanup. Our expertise in this area continues to grow. This work is primarily funded by the Nevada Applied Ecology Group.

Other Activities

Our statistical work for the Nevada Applied Ecology Group during the past year centered on planning for cleanup and treatment trials on the Nevada Test Site (NTS) and obtaining estimates of the volume of soil exceeding 160 pCi/g (total transuranics) at selected locations on the NTS and surrounding areas. Under separate DOE funding, we are researching statistical design and analysis methodology for inclusion in a guide on decommissioning and decontamination operations being prepared by PNL.

• Analog Elements for Transuranic Chemistries

Principal Investigators: W. C. Weimer, J. C. Laul, J. C. Kutt and E. A. Lepel

A chemical extraction technique for estimating the biologically available fraction of nonessential trace elements in soils has been developed. This procedure has been used in evaluating the uptake of naturally occurring transuranic analog elements from soils into several foodstuffs. The availability of the natural elements has been compared with the availability of their analog transuranics which have been derived from global fallout.

Estimation of the Biologically Available Pool of Nonessential Trace Elements in Soils

The nonessential trace elements in soils which are ultimately biologically available represent only a fraction of the total elemental content of the soil. We have shown this to be true for the rare earth elements (REE's) based on analysis of bulk soils, of plants grown on them and of chemical extractant solutions.

The total REE content of soils can be thought of as consisting of several distinct but related compartments, some of which are biologically available. The relative quantities of elements biologically available from soils to plants is shown diagrammatically in Figure 2. Presumably, the biologically available or potentially biologically available fraction can be related to that portion which is in true soil solution or which can be released into soil solution. The largest

proportion of the REE's in soils is bound within primary or secondary minerals or otherwise held in a form not biologically available in the short term. This is represented in Figure 3 as the mineral-bound REE. This fraction does not enter into exchange reactions with the REE in soil solution. A part of the REE associated with the surface may also be unavailable to participate freely in exchange reactions because of purely physical constraints. Rare earth element forms in ion exchange, precipitation, adsorption, or absorption equilibrium which are accessible to the soil solution, or which are occluded but still available to soil solution, comprise the total exchangeable REE of the individual soil. In Figure 3, this quantity of REE is denoted by a dotted line and termed "soil solution." It is this portion of the total bulk soil concentration that we also term the potentially biologically available pool in a soil.

FIGURE 2. Elemental Availability from Soils

FIGURE 3. Exchange of Rare Earth Elements (REE's) in a Soil/Soil Solution System

We have quantified the potentially biologically available pools of REE's for individual soils by use of a combination of controlled chemical extraction and isotopic exchange investigations. The isotopic exchange studies were performed at five different controlled pH values (1N HNO₃, pH 2, pH 3, pH 4.5, unadjusted). The purpose of examining this range of equilibrium pH values was to evaluate both chemical leaching and possible dissolution of minerals containing REE's as a function of pH for the various soil types. We have focused primarily on neodymium, the REE analog for transuranics americium and curium. For equilibrium exchange reactions only, altering the pH simply shifts the equilibrium position for both the native stable neodymium and the added ¹⁴⁷Nd radiotracer. The calculated value (from the measured neodymium and ¹⁴⁷Nd distribution between the soil and the extracting solution) for the exchangeable neodymium in a given soil remained nearly constant regardless of the equilibrium pH value chosen. At very low pH values, a portion of the mineral-bound neodymium began to dissolve and an excess of neodymium appeared. Thus, the range of pH values employed provide an excellent quantitative check of possible mineral-bound neodymium dissolution, as well as a qualitative comparison of the neodymium solubility in the various soils examined.

The concentrations of total exchangeable neodymium for five experimental soils that we have evaluated are shown in Table 1. (These data were calculated from the pH 2 soil:solution equilibrium systems.) The percentage of the total bulk soil neodymium concentration that the exchangeable neodymium represents is also given in this table. The range in the values of exchangeable neodymium as the percentage of total soil neodymium, from 2.5 to 40%, is quite

remarkable. While the detailed chemistries and mineralogies of each of these soil types are not yet available, we know that both the Ritzville and the Cape Cod-Environmental Measurements Laboratory soils are very sandy soils with a low clay mineral and low organic matter content. This suggests that these two soil constituents, which have a high exchange capacity, may be quite important in retaining the weathered forms of REE's which are released from primary and secondary minerals. In soils with low clay mineral and organic matter content, a large proportion of the neodymium released from minerals by weathering processes may pass out of the soil. A small proportion of the weathered forms may be retained and available for biological incorporation. Soils with a more significant clay mineral and organic matter content may retain a greater fraction of the weathered neodymium forms. These retained forms may then comprise the principal constituents of the biologically available pool of neodymium.

Availability of Native Rare Earth Elements and Fallout Transuranic Elements from Soils to Plants

Detailed examination of the geochemistries of naturally occurring REE's provides estimations of the ultimate geochemical states and biological availabilities of the selected transuranic elements for which some of the REE's are analogs. The data from isotope exchange studies and estimations of biologically available pools of the REE's are quite useful in identifying potential elemental source terms for biological incorporation calculations. Concentration ratios (CR's) calculated for plant uptake of elements from bulk soil have little meaning because of the large fraction of unavailable elements included in the bulk soil. Concentration ratios do have realistic physical interpretation when they are calculated from the soil pool which represents the biologically available elemental concentration. The CR's for the uptake of the REE's for seven crops from their respective soils are given in Table 2. These data are expressed as average CR's for all of the REE series having concentrations measureable in the biological materials rather than just for neodymium alone, although using neodymium alone does not alter the conclusions. The data are expressed in this manner to represent a "mean" CR for the REE's. Since we have previously demonstrated that there is no significant biological fractionation during plant uptake of the REE, any variation between CR's for the individual REE is probably due to analytical variability. Thus, a mean value for the CR's of the suite of REE's may be considered somewhat similar to a mean value for replicate determinations for a single REE on the same sample.

TABLE 1. Total Isotopically Exchangeable Neodymium in Soils

Soil Identification	Exchangeable Neodymium, ng/g	Percentage of Total Soil Neodymium
Ritzville	860	2.5
Cape Cod-Environmental Measurements Laboratory	1220	4
Wheat	15900	40
Rye	5230	37
Rice Paddy	4570	23

TABLE 2. Plant Uptake of Rare Earth and Transuranic Elements

Plant	Rare Earth Element Concentration Ratios(a)		²⁴¹ Am Concentration Ratios from Bulk Soils(b)
	From Bulk Soil	From Exchangeable Rare Earth Element Pool	
Peas	1.1×10^{-4}	5.2×10^{-3}	6.0×10^{-3}
Potatoes	5.3×10^{-5}	2.5×10^{-3}	6.0×10^{-3}
Corn	8.1×10^{-5}	3.9×10^{-3}	6.1×10^{-3}
Squash	4.5×10^{-4}	2.1×10^{-2}	9.9×10^{-3}
Rye	2.4×10^{-4}	5.3×10^{-4}	$<3.0 \times 10^{-3}$
Rice	9.5×10^{-6}	3.5×10^{-5}	$<7.0 \times 10^{-3}$
Wheat	1.9×10^{-4}	4.7×10^{-4}	2.5×10^{-3}

(a) Data are average concentration ratios for the entire series of rare earth elements.

(b) Data provided by E. P. Hardy of the DOE Environmental Measurements Laboratory, New York.

A comparison between CR's calculated from bulk soil REE concentrations and concentrations of exchangeable REE in the potentially biologically available pool is given in Table 2. When calculated from the exchangeable pool, CR values increase from 2.5 to 47 times, the greater increase being associated with the soils that have a relatively small pool of potentially biologically available REE's. The CR values calculated from the exchangeable REE pools represent realistic CR values for the plant uptake of these non-essential trace elements from that portion of the soil total REE pool that may be considered to be both biologically available and at an environmental equilibrium state. Since we have shown that the short-term biological and physical-chemical behaviors of the analog:transuranic pairs neodymium:americium and neodymium:curium are the same, the CR values for these exchangeable and available REE's should represent CR values for environmentally equilibrated transuranium elements (americium and curium) in these soils.

To compare the predicted CR values for the transuranium elements with data for these same soils and plants, CR values for ²⁴¹Am derived from fallout are also listed in Table 2. The ²⁴¹Am in fallout is considered to be dispersed as single atoms and as such should represent the maximum biological availability that will be found for the

transuranium elements in natural environments (with the exception of those released as water-soluble complex forms). There is a fairly close correspondence between the CR's calculated for fallout ²⁴¹Am and those calculated for the native soil neodymium in the exchangeable pool. The data for the native REE actually suggest that transuranium elements in an environmental equilibrium state will be somewhat less biologically available, by a factor of less than five, than elements in fresh fallout. This same reasoning predicts that fallout will become slightly less biologically available as it equilibrates in the environment. (The CR data for the squash suggest a slight increase in fallout activity with time rather than a decrease. The reasons for this apparent trend are presently unknown).

The CR values for plant uptake calculated from the soil REE pool defined by isotope exchange studies are presented as those representing CR's for the ultimate biological availability for americium and curium in the environment. These CR's will apply for all transuranium source terms other than those which would release americium or curium as soluble, stable complexes. All other source terms and eventually even the complexed forms should ultimately reach the same environmental equilibrium; only the rates of attaining this equilibration may change with the form of the source term.

● Transuranic Weathering in Plants

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Releases of long-lived transuranium elements to ecological systems raise issues, about which data are very limited. Quantitative information is needed on their potential pathways through food chains to assess the potential hazards to man. Of special interest is the effect of time (weathering, aging and associated biological processes in soil) on the change in availability of transuranium elements for uptake by plants from soil. This study provides valuable information on the effects of weathering and aging on the uptake of transuranics from soil by range and crop plants grown under realistic field conditions.

Routine Maintenance

Work on this project during the past year involved primarily the care and harvesting of crops in field lysimeters, routine maintenance of the enclosure around the lysimeters, submission of several samples for radiochemical analyses and completion of a paper to be published in the open literature.

The paper, entitled "Comparative Uptake and Distribution of Pu, Am, Cm and Np in Four Plant Species," will be published in Health Physics, as well as in the DOE-sponsored document Transuranic Elements in the Environment. Briefly, we determined the relative uptake of the transuranics by plants to be $Np > Cm \approx Am > Pu$. The uptake of neptunium was about 4,000 times as great as plutonium, while americium and curium were both approximately 20 times as great as plutonium. The relative concentration of each transuranic in seeds was at least a factor of 10 lower than in the remaining aboveground plant parts.

The summer of 1979 was the sixth growing season for some of the lysimeters. These

are the lysimeters that contain a thin layer of soil contaminated with ^{238}Pu located 10 cm below the soil surface. Samples from the 1977 and 1978 harvest were submitted for radiochemical analysis. When the analyses are completed, we will have radiochemical analyses from this set of lysimeters from five consecutive years. These data will enable us to determine if the phytoavailability of ^{238}Pu for peas (Pisum sativum, var. Blue Bonnet), barley (Hordeum vulgare, var. U. Cal. Briggs) and cheatgrass (Bromus tectorum) changed during the five growing seasons.

Additional samples harvested in 1977 and 1978 from lysimeters prepared and planted initially in 1976 were also submitted for radiochemical analyses. This set of lysimeters contain soil contaminated with ^{238}Pu , ^{239}Pu , ^{241}Am , ^{244}Cm or ^{237}Np in a 20-cm layer located 10 cm below the soil surface. Completion of these sample analyses will provide data from three consecutive harvest years for comparing changes in the plant uptake of the transuranics.

• Radioecology of Nuclear Fuel Cycles

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Radioecology of nuclear wastes was pioneered in the western United States on the Hanford Site during the early 1940's when the Columbia River provided coolant water for production reactors. Ecological studies centered on fish and waterfowl as vectors transporting radionuclides to people. After the shutdown of production reactors, the radionuclide content of Columbia River water declined. Attention was then directed toward radionuclides released into the environment as a result of chemical processing of irradiated fuel and as a result of storage of radioactive wastes.

Sites where radioactive wastes are found are solid waste burial grounds, soils below liquid storage areas, surface ditches and ponds, and the terrestrial environment around chemical processing facilities that discharge airborne radioactive debris from stacks. This study provides information to help assess the environmental impacts and certain potential human hazards associated with nuclear fuel cycles. A data base is being developed to define and quantify biological transport routes which will permit credible predictions and assessment of routine and potential large-scale releases of radionuclides and other toxic materials. These data, used in assessment models, will increase the accuracy of estimating radiation doses to man and other life forms. Information obtained from existing storage and disposal sites will provide a meaningful radioecological perspective with which to improve the effectiveness of waste management practices. Results will provide information to determine if waste management procedures on the Hanford Site have caused ecological perturbations, and if so, to determine the source, nature and magnitude of such disturbances.

Important companion studies also are underway. They are funded through local intercontractor support agreements rather than through the Office of Health and Environmental Research. These studies involve short-term assistance to Hanford plant personnel concerned with characterization of particular landscape subunits impacted by operation of nuclear facilities. They are discussed in the section entitled Hanford Project Support.

Aquatic Radioecology of Waste Management Areas

Actinide Transport Study

U-Pond, a nuclear waste pond on the Hanford Site, has been studied for several years to determine the ecological fate and effects of plutonium and other actinide elements (commonly called actinides) that have been discharged into it. Recently, we have completed an experimental investigation at this site to determine the extent

to which edible fish are able to concentrate actinides.

For this investigation, U-Pond is an ideal experimental environment. It has concentrations of the actinides uranium, plutonium and americium that are ~1,000 times background. Moreover, it is shallow enough for the interaction between biota and sediments, where actinides accumulate, to be maximized to an extreme. It was speculated that U-Pond could represent a freshwater environment having a high potential for producing fish containing

actinides at levels that might pose a risk to a human consumer.

However, it was found that, if a person were to eat as much as one pound of bluegill (*Lepomis macrochirus*) or bass (*Micropterus salmoides*) fillets from U-Pond every day, after 70 years he would only accumulate a radiation dose of not more than 0.12 rem to the total body from the actinides in the fish muscle. The calculated dose to his bone tissue after 70 years of consumption would be no greater than 3 rem. These doses are lower than the maximum permissible radiation doses for an individual (general public), which are 0.5 rem per year (or 35 rem accrued over 70 years) to the total body and 1.5 rem per year (or 105 rem accrued over 70 years) to the bone. Therefore, the hypothetical consumer would accumulate much less than the maximum permissible radiation dose by eating a steady diet of one pound per day of bluegill or bass fillets from U-Pond.

Dose to natural predators was also investigated using actinide concentrations in whole fish. Two predators, heron and coyote, were considered to be the principal consumers of fish from U-Pond. Using typical consumption rates, body weights and life expectancies for these predators, we calculated their lifetime total-body radiation doses. Lifetime doses for each of these predators were found to be less than 0.2 rad. The natural background radiation which these animals receive is about 0.1 rad every year. Hence, the actinide concentrations in U-Pond fish do not constitute a significant risk to either potential predators or human consumers, in spite of U-Pond's high actinide concentrations.

Fate and Effects of Thorium and Uranium in Aquatic Systems

Recent interest in alternative (nonproliferation) fuel cycles has prompted investigation into the fate and effects of thorium and uranium in aquatic ecosystems. Our laboratory experiments on this are designed to test actinide uptake in cases of worst possible environmental mobility; therefore, all tests are initiated with actinides in a soluble state.

Screening Toxicity Tests

Thorium was not observed to be toxic to rainbow trout (*Salmo gairdneri*) fry, *Daphnia magna* instars, or amphipods within the range of 0.1 to 100 mg/l. The lack of toxic effects is most probably related to thorium's strong tendency to form insoluble hydroxide complexes. Uranium was toxic to *Daphnia magna* and midge larvae (*Chironomus* sp.) in the range of 1 to 100 mg/l; however, fathead

minnows (*Pimephales promelas*) exhibited no stressful effects at 50 mg/l or less.

Bioconcentration of Thorium by Rainbow Trout

Rainbow trout were exposed to two concentrations (1.52 pg/l and 614 ng/l total thorium) over a 27-day period with three sampling periods followed by two depuration samples at 5 and 25 days. Thorium was determined as both a soluble fraction and total fraction for both concentrations, the soluble fraction representing 49% of the total at 1.5 pg/l and 45% of the total at 614 ng/l (determined by filtering at 0.3 μ). Trout exposed to 614 ng/l of thorium appear to reach equilibrium at nine days, whereas those exposed to 1.5 pg/l may not have reached equilibrium until 27 days. After five days of depuration, both groups of fish lost over 98% of their total body-burden, indicating that the primary route of bioaccumulation was either epithelial adsorption or gut loading. Concentration ratios based on soluble fractions of thorium were roughly double that of total thorium at both concentrations irrespective of sampling time. Observed concentration ratios appeared to be concentration-dependent; that is, concentration ratios calculated at 614 ng/l were nine times lower than those calculated at 1.52 pg/l. A similar study was conducted over a longer period with uranium. We are awaiting sample analyses from this.

Effects of Uranium on Primary Productivity

Static systems containing periphyton were used to assert the potential effects of uranium on primary productivity. Concentrations between 0.6 and 21 mg/l of uranium did not produce any discernible effect on productivity; however, 47% to 72% inhibition of primary productivity was observed at 44 mg/l of uranium. Some of our data are still outstanding, but from present data, thorium and uranium appear to pose minimal hazards to aquatic organisms. Although toxicity was demonstrated in the range of 1 to 10 mg/l of uranium, these concentrations exceed by two orders of magnitude concentrations encountered in contaminated systems on the Hanford Site. A greater radiological hazard may result from high-level decay chains (²³²U and ²²⁸Th); however, the hazard associated with this is greatly minimized by the inherently low environmental mobility of these actinides.

Biotic Transport Parameters

Simulation of the Long-term Accumulation of Radio-contaminants in Crop Plants

In the past, the fifty-year accumulation method (FYAM) was used to estimate exposures

in situations in which radiocontaminants were chronically discharged from a repository into irrigation water over long periods of time. For this method, it was assumed that the quantities of radionuclides that accumulated in the agricultural soils at any given time were equal to the total quantity deposited within 50 years. However, since it was predicted that some of the radionuclides would be discharged into the biosphere over millions of years, questions arose concerning accumulation patterns of radiocontaminants in the soil over much longer periods of time. A model, named CROPRE, was developed to predict both the long-term accumulation of radionuclides and the concentrations of radionuclides in vegetation that would result. This model was designed to include 1) the chronic input of contaminated irrigation water into the soil compartment and directly onto the surface of the vegetation, 2) the incorporation of radiocontaminants into the soil organic matter pool and their eventual release for re-uptake by subsequent crops, 3) the removal of contaminants during harvesting and 4) the downward movement of radionuclides and their loss from the system by percolation when the crop plants are overwatered.

Both of these models were used to estimate radionuclide concentrations in edible vegetation over varying periods of time. There were significant discrepancies between the estimates of the two models. When the system was not overwatered, estimates of radionuclide concentration in edible vegetation from the CROPRE model generally exceeded the FYAM values by about 88 years from the beginning of irrigation with contaminated water. The maximum estimates from the CROPRE model, which usually occurred at times greater than 1000 years, ranged up to a factor of 120 larger than the corresponding FYAM values. The maximum estimates for radionuclides with relatively small plant uptake parameters exceeded the corresponding FYAM values by greater margins than for those elements with larger uptake parameters. The degree to which an element is sorbed onto soil had the greatest effect on the predictions of the concentrations of radionuclides in edible plant material when the system was overwatered. The FYAM values exceeded the maximum estimates from CROPRE for radionuclides that are slightly retarded from percolating through the soil and are readily taken up by plants. Conversely, estimates from CROPRE exceeded the corresponding FYAM values for radionuclides that are retarded by soil and have relatively small plant uptake values.

Rabbit Studies

Radionuclides entering herbivore food chains may be simply attached to the surface

of foliage or may be incorporated into plant tissues. Incorporated radionuclides are likely to be those that occur in chemical forms most available to plants. They may also be the most available for incorporation and retention by consumer organisms.

Published values for biological half-life and fraction reaching the reference organ in man may be inaccurate if they were derived under one set of experimental contamination conditions and are applied to another. This work was designed as a pilot study to determine the feasibility of examining the effect of alternative environmental pathways (surface contamination versus plant root uptake) on the uptake and retention of selected radionuclides by consumers. In this study, lettuce plants were grown in soil contaminated with $^{137}\text{CsCl}$ while other plants were grown in uncontaminated soil. Those raised in uncontaminated soil were surface-contaminated with $^{137}\text{CsCl}$. Rabbits were fed both lettuce with incorporated ^{137}Cs and that with the surface contamination. The animals were whole-body counted to determine which organs took up and retained plant-incorporated ^{137}Cs and which took up and retained nonincorporated ^{137}Cs . The experimental results are currently being analyzed.

Terrestrial Radioecology of Waste Management Areas

Cesium-137 in American Coots (*Fulica americana*)

Process ponds located on the Hanford Site in southcentral Washington contain low levels of radionuclides. Three ponds currently in use contain mixtures of fission and activation products which include transuranic elements. The ponds presently support a variety of plant and animal life. Waterfowl use the ponds extensively and are capable of transporting radioactive materials offsite. Should water-fowl leave the waste ponds and be harvested by hunters, they may become part of the regional human food supply.

During this fiscal year, studies of waterfowl using the waste ponds involved analyzing data collected in previous years. This was done to evaluate the role of American coots in exporting radionuclides from the ponds and to find the dose to man resulting from ingestion. Although a number of waterfowl species use the ponds, the coot is the most abundant. The breeding population was estimated to hatch as many as 200 young per year, 40 of which may reach flight age. In addition to the breeding population, migrant coots use the ponds during fall and winter. Peak numbers of coots on the ponds have reached nearly 600. Beyond being the most abundant waterfowl species on the ponds,

concentrations of ^{137}Cs in coots' flesh are consistently higher than in other waterfowl.

One- and fifty-year ^{137}Cs total-body dose commitments to an individual from ingesting all edible tissues of one Gable Mountain Pond coot were calculated at 1.9 and 2.1 mrem, respectively. The 50-year population dose from the projected harvest of Gable Mountain Pond coots during one year was estimated to be 0.13 man-rem. This is probably an overestimate because the coots must leave the site to be harvested. Total ^{137}Cs export from Gable Mountain Pond via coots, having an average body-burden of $0.092\ \mu\text{Ci}$, was estimated to be $46\ \mu\text{Ci}$ per year.

Coot Nesting Biology Studies

During FY 1979, the analysis and final report on the nesting biology of the American coot on the Hanford Site (see publications list; Fitzner and Schreckhise, PNL-2462) was completed. The objective of the study was to discover whether any differences exist between the nesting biology of birds on low-

were found from April through mid-June on most ponds. Clutch size showed no significant differences. The average number of eggs per nest for all ponds was 6.7. Egg and chick weights and percent hatching success were similar among coots from both study sites. A general comparison of nesting success for coots from Gable Mountain Pond on the Hanford Site and from other locations is given in Table 3. Feeding habits of the coots from the two sites did show some differences. However, this is probably related to the availability of food items in each pond.

Darkling Beetle Studies

Our study of darkling beetles was conducted as part of a larger program characterizing the biota inhabiting areas near energy-related facilities. Four areas were selected for intensive study. Two were located near the Washington Public Power Supply System's (WPPSS) nuclear power plant sites 1 and 4, in mixed bitterbrush-sagebrush (Purshia tridentata-Artemisia tridentata) and bitter-

TABLE 3. Percentage of Successful American Coot Nests

Nests		Location
Number	% Success	
66	92	Washington
14	100	Gable Mountain Pond
17	89	Royal Slough
13	92	Morgan and Halfmoon Lakes
104	91	Iowa
347	77	Iowa
380	97	Manitoba
161	86	Iowa

level radioactive waste ponds on the Hanford Site and that of nesting coots on control ponds.

Nesting habitats were similar for coots from both the control ponds and those exposed to radionuclides. All coots selected habitats in which the water level was moderate to shallow, and stable. Emergent aquatic plants such as cattail (Typha latifolia) and bulrush (Scirpus acutus) were characteristic of both habitats.

There were no apparent differences in nesting chronology between birds from the different study sites; nests containing eggs

brush communities, respectively. Two other study areas were located in sagebrush-dominated communities with cheatgrass (Bromus tectorum) understories. One of these was located near a low-level radioactive waste burial site known administratively as the B-C Cribs. The other study area was located on the nearby Arid Lands Ecology (ALE) Reserve. The study plot on the ALE Reserve, protected from disturbance, was intended to serve as a control for those study areas located near the nuclear power plants and radioactive waste storage areas.

The beetles were captured within each of the study areas by pitfall trapping. The

traps were set out in a grid system at 5-m intervals in April 1976 and checked at weekly intervals through November when they were closed for the winter. They were opened the following March and checked through October 4, 1977. Captured beetles were removed by hand, counted and recorded, and released near their point of capture.

A total of 7,831 individuals representing 11 different species were collected during this two-year study. Six species accounted for over 90% of the total beetle catch. These included *Eleodes hispilabris*, *E. novoverrucula*, *E. humeralis*, *Eusattus muricatus*, *Coniontis setosa* and *Philolithus densicollis*.

The seasonal pattern of total darkling beetle abundance for each of the study areas is shown in Figure 4. Although there are differences in total beetle numbers for particular sample dates, the same general trends appear to have occurred in all study areas during 1976. Similar trends continued during the early 1977 season, but by June greater catches occurred in the bitterbrush and bitterbrush-sagebrush plots. The catches in the control and B-C Crib plots were similar throughout 1977. Other studies conducted both at Hanford and elsewhere have shown that these kinds of periodic fluctuations appear to be characteristic of darkling beetle populations. Additional analysis concerning the seasonal patterns of distribution for individual species is planned.

Honeybee Studies

Although honeybees are native only to Europe, Asia and Africa, they have been introduced throughout the temperate regions of the world. Honeybees have evolved highly complex foraging patterns and morphological adaptations for collecting and transporting provisions for the colony. While foraging, they may effectively cover a 31-square-kilometer area around the colony and carry back any material present on the surface of vegetation or absorbed in water or nectar. Because of the bees' foraging habits, they are almost certain to return with some amount of any environmental contaminant present. What is not known is how the bees may serve as a magnifying agent for particular materials. Therefore, the purposes of this research are to examine the usefulness of honeybees as a tool for environmental monitoring, and to evaluate the biotic transport potential of wild bee colonies located near waste storage facilities.

This year's effort involved establishing colonies in an area remote from radioactive waste management facilities and collecting baseline samples of pollen, honey, and honeybee carcasses. Twenty hive bodies were assembled from new materials and used to establish 10 strong two-story colonies from bees purchased from a supplier. Pollen and honey samples were collected during September; pollen was collected with traps that fit into the hive entrance, and honey

FIGURE 4. Seasonal Pattern of Darkling Beetle Abundance

samples were collected from freshly capped honeycomb. Next year, colonies will be placed near waste processing facilities to determine the availability of radiocontaminants to foraging bees.

Burrowing Owl Food Habit Studies

Five pairs of burrowing owls (*Speotyto cunicularia*) were selected for a study of feeding strategies on the Hanford Site. The purpose of the study was to determine 1) whether owls of the same species feed on the same prey under relatively equal conditions or manifest behavioral constraints in food preferences, 2) whether seasonal and habitat variations in prey availability are reflected in owl diets and 3) the physical capacity for exploitation of certain size-weight ranges of prey.

The studies that have been conducted on burrowing owl feeding habits in shrub-steppe habitats of northwest United States have listed small vertebrates, primarily rodents, as prey, but no invertebrates. No quantitative food habit studies have been reported for the shrub-steppe region of Washington, nor has an attempt been made to correlate burrowing owl diets to prey availability in a statistical fashion. The preliminary results of the burrowing owl dietary analyses for invertebrate and vertebrate prey are given in Tables 4 and 5.

Preliminary data analyses in this study have provided a great deal of information on feeding habits of burrowing owls and prey

TABLE 5. Abundance and Biomass of Vertebrate Taxa in Burrowing Owl Diets

Taxa	Abundance	Biomass (g)
Reptiles		
Side-Blotched Lizard (<i>Uta stansburiana</i>)	1	3.4
Birds		
Passeriformes	2	40.0
Mammals		
Soricidae		
Merriam shrew (<i>Sorex merriami</i>)	1	3.8
Criceidae		
Sagebrush vole (<i>Lagurus curtatus</i>)	69	1359.3
Deer Mouse (<i>Peromyscus maniculatus</i>)	78	1388.4
Western harvester mouse (<i>Reithrodontomys megalotis</i>)	2	24.0
Heteromyidae		
Great Basin pocket mouse (<i>Perognathus parvus</i>)	726	9994.0
Geomyidae		
Northern pocket gopher (<i>Thomomys talpoides</i>)	53	1855.0
Unknown rodents	124	2356.0
Lagomorpha	3	78.0

availability. Tables 4 and 5 show abundance and biomass data on prey consumed by burrowing owls. A planned open literature report in FY 1980 will examine owl feeding strategies as they relate to behavioral differences among owls and seasonal variation in prey availability.

Lizard Studies

An intensive field study concerning the ecology of the side-blotched lizard (*Uta stansburiana*) and sagebrush lizard (*Sceloporus graciosus*) has been completed and data have been analyzed. We are presently preparing a manuscript for publication in the open literature which will cover the life history of these lizards on the Hanford Site. One aspect of the study has dealt with the food habits of the lizards. Table 6 provides a general listing of the major taxa preyed upon.

TABLE 4. Abundance and Biomass of Invertebrate Taxa Occurring Within Burrowing Owl Diets

Taxa	Abundance	Biomass (g)
Coleoptera	2,707 (64)	77.9 (32)
Diptera	1 (<1)	<0.1 (<1)
Hemiptera	9 (<1)	<0.1 (<1)
Hymenoptera	159 (4)	1.1 (<1)
Neuroptera	1 (<1)	<0.1 (<1)
Orthoptera	822 (20)	129.1 (53)
Siphonaptera	1 (<1)	<0.1 (<1)
Solpugida	48 (1)	3.4 (1)
Scorpionida	207 (5)	16.2 (7)
Araneida	137 (3)	16.1 (7)
Acarina	4 (<1)	<0.1 (<1)
Unknown	127 (3)	not applicable

TABLE 6. Number of Prey Eaten by the Sagebrush Lizard (*Sceloporus graciosus*) and the Side-Blotched Lizard (*Uta stansburiana*)

Taxa	Sagebrush Lizard	Side-Blotched Lizard
Acarina	14	12
Araneida	6	45
Coleoptera	25	22
Collenbola	—	11
Diptera	10	40
Hemiptera	36	64
Homoptera	4	31
Hymenoptera	31	145
Isoptera	7	—
Lepidoptera	10	17
Nemoptera	—	1
Neuroptera	3	4
Orthoptera	8	49
Thysanura	1	—

Small Mammal Report

A research report for Rockwell Hanford Operations entitled Analysis of Small Mammal Populations Inhabiting the Environs of a Low-Level Radioactive Waste Pond (see publications list; Gano, PNL-2479) was published in 1979. This project supported additional interpretation and writing on the data reported in this document to prepare it for open literature publication. The manuscript will be submitted to an appropriate journal for review early in FY 1980.

Studies of Vegetative Recovery After Fire

Vegetation plays an important role in stabilizing soil surfaces against wind erosion. When fire removes most or all vegetation, strong winds can remove the surface soil. The soil surface remains susceptible to wind erosion until vegetation becomes re-established. The rate of recovery, as well as the amount of vegetation growing back, depends upon both the soil and the types of vegetation growing on the plot before the fire. This task examined the rate of vegetation recovery on three soil types in four plant communities following a fire in 1978. The results can be compared with an ongoing study following a fire in 1973.

Fifty 0.1-m² plots, each 1 m apart along a transect, were examined for soil and plant community type. The data obtained included species frequency and cover. Figure 5 shows a plot of the cover data comparing the recovering vegetation with the control vegeta-

FIGURE 5. Vegetative Recovery Following Fire in a Shrub-Steppe. Group I is first-year recovery in low-to-moderate erosion areas. Point II is first-year recovery in high-erosion areas in cheatgrass/mustard communities. Group III is first-year recovery in high-erosion areas in sagebrush/cheatgrass communities. Point IV is recovery in all three types of erosion areas after six years.

tion. Four clusters are apparent, although only two clusters actually have more than one point (which indicates that more data will be required to substantiate any conclusions drawn from this work). Cluster I represents first-year recovery in four communities in low-to-moderate erosion conditions. Recovery is proportional to the amount of vegetation cover before the fire. It averages almost exactly 50%. Cluster II represents a single observation in a highly eroded cheatgrass (*Bromus tectorum*)-mustard community, where the recovery vegetation actually yielded more herbaceous cover than the vegetation in the unburned community. Cluster III represents a single observation of recovery in a sagebrush (*Artemisia tridentata*)-cheatgrass community where erosion is high. The recovery vegetation amounts to only 5% of the control vegetation. The fourth cluster shows vegetation recovery in low-, moderate-, and high-erosion conditions after six years. The recovery vegetation exceeds the control vegetation by a small amount.

These initial observations need to be supported by more extensive data to confirm the results. However, these data consistently suggest that even soils with moderate erosion potential revegetate readily if the vegetation existing before the fire has been sufficiently dense. The data also seem to

imply that, in about six years, recovery is complete and the added soil erosion potential disappears. Only in the first season is the difference between communities clear. If these data reflect a general pattern, rather than merely this particular set of conditions, then fire may be much more hazardous in sagebrush communities than in cheatgrass-mustard communities. This fol-

lows from the very sparse revegetation in the first year in the sagebrush community (Cluster III) compared to the cheatgrass-mustard community (Cluster II). The difference may be due to a greater natural seed reservoir in the cheatgrass-mustard community, a point which needs to be examined next year.

• Environmental Behavior and Effects of Technetium-99 and Iodine-129

Investigators: T. R. Garland, R. G. Schreckhise, R. E. Wildung, L. L. Cadwell,
K. M. McFadden and D. A. Cataldo

The objective of this program is to define the environmental behavior of ^{99}Tc and ^{129}I , two long-lived radioactive by-products of the nuclear fuel cycle that have received little study. The specific objectives are to 1) determine the soil physicochemical and microbiological factors and physiological parameters that govern the mobility and bioavailability of different chemical forms of ^{99}Tc and ^{129}I in terrestrial and aquatic environments and 2) validate and measure food web transport in selected field locations.

The potential for both isotopes to exist in multiple valence states and in various complex chemical species has been documented well. For ^{129}I , the kinetics of equilibration of the source-term species with the indigenous iodine pool, or pools, will define its behavior. Unfortunately, these pools are not well defined. Technetium-99 has no stable element pool and its behavior will depend on the chemistries of several analogs and the general physicochemical parameters of the systems. Previous laboratory studies at PNL have shown ^{99}Tc and ^{129}I to be much more mobile in the environment, and more available to biota in ways not previously recognized, than the more intensively studied long-lived radionuclides (strontium, cesium, plutonium).

The major obstacle to field studies with both technetium and iodine is the expensive analytical methodology required to analyze the existing environmental levels. For ^{99}Tc , the development of a reliable technique for chemical separation and assay by a supporting research program (PNL's Physical Sciences Department; see PNL-3300, Pt. 5) has recently been applied.

Although the research on this program has shown technetium and iodine to be mobile in the environment, adequate assessment of the potential hazards to man requires a more thorough understanding of the mechanisms which may limit technetium effects or reduce the long-term mobility. Research has been initiated in 1) defining the mechanisms of technetium toxicity to plants at the cellular level, 2) evaluating the mechanisms and extent of technetium uptake by aquatic algae and soil microorganisms, 3) determining the chemical form of technetium and iodine in soils, plants and aquatic systems, 4) evaluating the mechanisms responsible for iodine mobility in soil and plant systems and 5) measuring levels of iodine and technetium in soils, plants and animals at specific sites selected for a range of activity and environmental conditions.

Behavior of Technetium and Iodine in Aquatic Systems

Laboratory studies using freshwater algal species have been undertaken to evaluate the absorption and transport aspects of technetium and iodine and to develop an understanding of the long-term fate of these radionuclides in the aquatic environs. Selenastrum capricornutum and Microcystis aeruginosa, two unicellular freshwater algal species, are employed in these studies. They vary morphologically in that Selenastrum has a cellulose cell wall while the cell wall of Microcystis is encapsulated with a muc-

laginous carbohydrate material. This morphological feature is believed to account for the marked differences reported for cation absorption in algae.

In the present studies, TcO_4^- was maintained at 1 ppb and I^- at 250 ppb; uptake was measured at periodic intervals over a 95-hr period and measurements were made of exchangeable ions, irreversibly bound ions and ion absorption. Exchangeable levels of technetium and iodine were minimal as judged from desorption studies in the presence of hundredfold excesses of desorbing ions (TcO_4^- and I^- , respectively), with no significant

differences between *Selenastrum* and *Microcystis*. The component irreversibly sorbed to the cell walls was ~ 0.02 ng/g dry wt of cells for TcO_4^- and ~ 9 ng/g dry wt of cells for I^- . No significant differences were noted for the two algal species. Accumulation rates for TcO_4^- were 0.11 and 0.08 ng Tc/g dry wt cells/hr for *Microcystis* and *Selenastrum*, respectively. Calculated concentration ratios (Tc activity/g dry wt cells $\div$ Tc activity/mL solution) for cells grown for 24 hr in 1 ppb solution of TcO_4^- are ~ 2 . Accumulation rates for I^- were 1.0 and 0.8 ng I/g dry wt cells/hr for *Microcystis* and *Selenastrum*, respectively. Concentration ratio values calculated for cells grown in solutions containing 250 ppb I^- for 24 hr were ~ 0.16 , an order of magnitude less than that for TcO_4^- .

^{129}I and ^{99}Tc in Soils and Vegetation Around a Hanford Fuels Reprocessing Site

Although active fuels reprocessing on the Hanford Site was stopped in 1972, ^{99}Tc or ^{129}I might still be present in the soils or vegetation in the area. To investigate this, samples were taken within the 200 West Area boundaries in the vicinity of UO_3 -Plant. These sites were chosen as a result of the estimation that 20% of the total technetium would be in the recovered uranium which would be reprocessed to uranium oxide at UO_3 -Plant. It is unknown what fraction of technetium could have been released as the volatile Tc_2O_7 during conversion of the nitrate solutions to oxides; however, it was assumed that this would be one of the most likely routes of technetium release to the environment via an atmospheric route. On the other hand, most ^{129}I was primarily released during the dissolution of fuel elements.

The results (Table 7) are consistent with the expectations of environmental levels, considering the ^{235}U fission yields ($^{129}\text{I} = 0.9$ and $^{99}\text{Tc} = 6.1$): that technetium is taken up by plants to a greater degree than ^{129}I and that more ^{129}I was released (via atmospheric routes) than technetium.

The behavior of ^{129}I after deposition is seen to be different from that of the mineral iodine present in the soil, especially in the soil and vegetation around U-Pond. Calculated concentration ratios in the vegetation for ^{129}I (using average concentration of 0 to 25 cm) varies from 0.43 to 2.7 where ^{127}I was 0.068 to 0.32. Iodine also appears as a relatively enriched layer in the litter layer.

Technetium levels in the soil northeast of U-Pond were below detection except in the

TABLE 7. Levels of ^{129}I , ^{99}Tc , ^{137}Cs and ^{127}I in Samples in the Vicinity of a Nuclear Fuel Reprocessing Plant

Site I, Sagebrush-Wheatgrass Community East of UO_3 Plant				
Sample	^{129}I	^{99}Tc	^{137}Cs	^{127}I
	pg/g			$\mu\text{g/g}$
Sagebrush (<i>Artemisia tridentata</i>) leaves	8.5	39.2	0.023	0.46
twigs	6.9	—	0.039	0.15
Litter	205.	13.2	0.21	1.3
Soil 0-5 cm	30.0	5.4	<0.0038	0.15
Soil 6-15 cm	16.0	<4.9	<0.0005	0.31
Soil 16-25 cm	4.0	<1.8	<0.0005	0.34
Soil 26-35 cm	2.5	<1.3	<0.0005	0.58
Site II, Willow-Cottonwood Community Northeast of U-Pond				
Sample	^{129}I	^{99}Tc	^{137}Cs	^{127}I
	pg/g			$\mu\text{g/g}$
Cottonwood (<i>Populus trichocarpa</i>) leaves	22.4	6.0	1.84	0.081
bark	14.0	7.2	1.01	0.026
wood	13.7	5.0	0.45	0.0073
Asparagus (<i>Asparagus officinalis</i>)	57.2	22.5	0.28	0.13
Russian knapweed (<i>Centaurea repens</i>)	59.7	700.	1.03	0.076
Tumble mustard (<i>Sisymbrium altissimum</i>)	9.5	15.0	0.0012	0.027
Willow (<i>Salix</i> sp.) leaves	7.3	26.5	1.87	0.069
litter	488.	183.	13.9	1.2
Soil 0-5 cm	38.6	10.3	25.4	0.48
Soil 6-15 cm	16.2	<4.9	0.22	0.36
Soil 16-25 cm	11.8	<4.6	0.066	0.35
Soil 26-35 cm	5.8	<1.5	0.095	0.23

litter layer and in the 0-to-5-cm layer. These results were not unexpected, especially in light of laboratory and field studies which demonstrated low K_d levels and high affinity of plants for the TcO_4^- anion.

The relative differences in concentrations between plants in the same area were surprising. These data may reflect differences in root depth, the physiology of the particular species or simply leaf surface absorption of iodine from air.

Physiological Aspects of Technetium Behavior in Plants

Technetium, while present in relatively low concentrations in the environment, is receiving considerable attention because of mobility in soils and availability to plants. Understanding technetium behavior in plants is essential for a complete knowledge of overall long-term behavior of technetium in the environment. Studies underway deal with specific aspects of technetium behavior in plants, including 1) root absorption behavior of TcO_4^- ; 2) interaction of nutrient and nonnutrient analogs; 3) metabolic aspects of technetium toxicity as it relates to the chemical behavior of technetium in plants and 4) clarification of the chemical fate of technetium in plants.

Previous studies with TcO_4^- have demonstrated that technetium is efficiently accumulated by plants over a concentration range of 0.03 ppt to 80 ppb, suggesting that TcO_4^- may be chemically similar (with respect to bioaccumulation) to an essential element required by plants. Thus, the reported concentration ratio values of 20 to 1000 are explained. Competition studies, while preliminary, showed H_2PO_4^- , MoO_4^{2-} , SO_4^{2-} , IO_4^{2-} , and ReO_4^{2-} to inhibit TcO_4^- transport. Detailed kinetic analyses of ion interactions showed H_2PO_4^- , SO_4^{2-} , ReO_4^{2-} , CrO_4^{2-} , and MnO_4^- to compete with TcO_4^- for transport sites. While many of these anions are demonstrated transport analogs of each other, their metabolic roles and interactions are markedly different. This would suggest that the transport behavior of technetium, with respect to chemical analogs, may not be related to the radionuclide's metabolic behavior and effects. This view is supported by the fact that technetium toxicity in plants can only be demonstrated at germination and early developmental stages of growth, but not after approximately eight days of age. While previous studies failed to demonstrate any pronounced effect of technetium on photosynthesis, respiration or nitrate reduction, a closer evaluation shows a transient reduction in nitrate reduction following three days of pretreatment with technetium with a corresponding recovery at seven days. These data suggest that, metabolically, technetium may be either partially functional as an analog or be involved in a system with a rapid turnover of functional protein. This

hypothesis is supported by chemical characterization data which shows $\geq 70\%$ of the technetium in plants to be associated with ligands of >5000 molecular weight.

Absorption and Chemical Fate of I^- in Plants

Although the environmental behavior of radioiodine has been studied extensively over the past 30 years, little information is available on the chemical and biochemical behavior of radioiodine in the environment. Recent field and laboratory data suggest a significant gas phase transfer of radioiodine between soils, plants and air; by studying the chemical behavior of radioiodine, we hope to better understand the role of metabolically incorporated iodine in the transfer of iodine from plants to animals.

Plant root absorption, tissue distribution, and the chemical fate of $^{125}\text{I}^-$ was determined using soybean plants. Root absorption was shown to be concentration-dependent and biphasic over the concentration range of 0.1 to 25 ppm, with uptake ranging from 0.032 to 3.3 $\mu\text{g } ^{125}\text{I}^-/\text{g dry wt root/hr}$. Approximately 20% of the root-absorbed iodine was transferred to the shoot during the 2-hr uptake period, demonstrating the relatively high mobility of iodine compared to other fission products. Analyses of the chemical fate of $^{125}\text{I}^-$ following absorption shows 75% of the iodine content of roots to be associated with the insoluble residues, while only 10% is in the soluble fraction. Ultrafiltration of the latter shows the iodine to be distributed between the <500 and $>10,000$ molecular weight fractions (66 and 31%, respectively). Similar analyses of leaves show 75% of the iodine to be in the soluble fraction, while only 20% is associated with the insoluble residues. Of the solubles, 90% of the iodine is associated with species of low molecular weight (<500) and the remainder with species of $>10,000$. Gel filtration chromatography of the $>10,000$ molecular weight fraction demonstrates the presence of four components of high molecular weight with 92% of the ^{125}I activity contained in only one. The role of plant metabolism of elements such as iodine is currently being investigated with respect to its effects on food chain transfer following ingestion by animals of plant-incorporated iodine.

6 Marine Research Programs

MARINE RESEARCH PROGRAMS

- **Effects of Energy Systems Effluents on Coastal Ecosystems**
- **Bioavailability of Energy Effluent Materials in Coastal Ecosystems**
- **Marine Chemistry of Energy-Generated Pollutants**
- **Iron-55 Phenomenon**
- **Long-Term Effects of Petroleum Hydrocarbons from Water on Marine Organisms***
- **Long-Term Effects of Petroleum Hydrocarbons from Sediments on Marine Organisms***
- **Fate and Effects of Petroleum Hydrocarbons in the Coastal Marine Ecosystem***
- **Effects of Petroleum Hydrocarbons on Behavior of Marine Organisms***
- **Environmental Impacts of Outer Continental Shelf Petroleum Development**

The use of the ocean by energy technologies has been increasing over the past decade and will continue to increase in the foreseeable future. As the human population along our coast increases and our traditional energy sources decline, the demand for the resources available from the ocean will increase. This demand will create conflicts between various user groups which will need to be resolved in a manner causing minimal environmental damage while maximizing the utilization of the resources. Energy technologies will use the ocean in three principal ways: 1) as a source of energy, such as ocean thermal energy conversion (OTEC) and oil extraction, 2) for transporting energy resources (shipment of oil, coal, solvent refined coal, liquified gas and coal, and products from OTEC plants, and 3) disposal of wastes such as heat from steam electric stations, chemicals from coastal technology facilities, and nuclear waste, which is disposed in the deep seabed.

The DOE marine research programs at PNL address questions about how energy technologies will impact the marine ecosystem and about the consequences of these impacts. The research is based at the Marine Research Laboratory, Sequim, Washington. They are multidisciplinary efforts that integrate information from all aspects of energy technology-marine ecosystem interactions to study problems relevant to the use and development of existing and future technologies.

At this time, research is being conducted in three major areas: marine chemistry, biological availability of contaminants and the biological effects of contaminants. The objectives of the tasks within each of these areas relate to understanding

the impact of energy recovery, transportation, conversion and waste disposal. The marine chemistry studies attempt to define the quantities and forms of material that will be generated by energy technologies and to compare these to the quantities and forms that exist naturally. This research includes development of methods for analysis of effluent material in sea water, sediment, and biological tissues. These efforts provide data needed for biological studies and for the assessment of potential impact.

The biological availability studies are concerned with determining what portion of the materials released are biologically active and what factors control the bioavailability. Knowledge about a contaminant's bioavailability and the factors controlling it is critical to predicting the fate, effects, and ultimate consequences of the materials released. The research tasks within this area address these problems through state-of-the-art analytical and biological effects methodologies.

The biological effects studies are investigating the impact that a particular contaminant has on a particular organism and, in turn, on the population and the community. To determine this requires an understanding of the pathways of the contaminant through the organism and its impact on the organism's ability to survive and reproduce. To obtain this understanding, we are studying the impact of contaminants on processes that are common to a wide spectrum of marine organisms. This information will then be related to the effects these responses have on the organism's ability to remain viable in the ecosystem.

Marine research programs sponsored by the Environmental Protection Agency (EPA) support oil and gas technology. They are designed to determine the threshold level of petroleum hydrocarbon contamination in marine waters and sediments which will not significantly disrupt the balance of marine communities. As part of this research, we are determining the extent of impacts from single large releases of oil and the rate at which organisms and the environment recover after such a perturbation. The information generated will provide a strong data base for specifying the degree of environmental control technology needed at refineries and offshore well-heads. In addition, the knowledge gained will allow us to predict the potential ecological impacts of accidents and other spills, including the duration of such impacts in different marine habitats.

Current research programs are designed to address a range of questions regarding the fate and effects of petroleum. The programs use integrated approaches and expertise in ecology, environmental physiology and analytical chemistry. Hypotheses formulated from results of either field or laboratory investigations are verified by testing in both types of systems, so that the significant variables are defined and the rates and magnitudes of effects are evaluated under natural conditions.

*These programs were transferred to the Environmental Protection Agency in October 1977. During the reporting year, this work was performed under DOE direction.

- o Intralaboratory work order

• Effects of Energy Systems Effluents on Coastal Ecosystems

Principal Investigators: G. Roesijadi and J. S. Young

This program investigates the potential effects of contaminants released by energy technologies into the marine environment. The primary goal is to identify and evaluate meaningful measures of the effects which contaminants produced by energy systems have on marine animals.

The present research emphasizes the role of subcellular binding and storage mechanisms for trace metals with respect to onset of toxic responses and possible detoxification. Mercury, copper, cadmium and zinc are the trace metals under investigation. Elevated levels of these metals on high molecular weight protein fractions have been associated with cytotoxic effects of the metal. Metallothionein and other low molecular weight, metal-binding proteins have been reported to serve a protective function by sequestering potentially toxic metals and preventing them from binding to inappropriate subcellular sites. Additionally, sequestration of trace metals by lysosomes and labilization of lysosomes have been associated with the toxic effects of those metals.

Effect of Copper on the Littleneck Clam

Littleneck clams (*Protothaca staminea*) were exposed to a range of copper concentrations of 0.35 (control), 7, 18, 39 and 82 $\mu\text{g}/\ell$ for 30 days. Mortalities were monitored during the exposure. The results indicated that copper concentrations of 39 and 82 $\mu\text{g}/\ell$ were extremely stressful, with only 14 and 3%, respectively, surviving after 30 days. In groups exposed to 7 and 18 $\mu\text{g}/\ell$, survival was slightly lower than in the control group. Analyses for tissue copper concentrations showed that the gill was the primary organ for the concentration of copper and that it exhibited a linear relationship between its copper content and the exposure concentration. Effects of copper on the gill were demonstrated by a disruption of sodium and potassium regulation at 39 $\mu\text{g}/\ell$ and increases in acid phosphatase activity at lower exposure concentrations. The former was associated with high mortalities, while the latter was considered to be a sublethal cytotoxic response. Examination for low molecular weight, copper-binding proteins in organs of control clams and those exposed to 7 and 18 $\mu\text{g}/\ell$ indicated the presence of such substances in all three groups of clams. Two low molecular weight, copper-binding proteins could be distinguished by differences in their eluting properties. In gill and muscle, copper was associated with a protein of approximately 14,000 daltons, while zinc was associated with a protein of approximately 10,500 daltons. In viscera and kidney, copper and zinc co-eluted on the smaller of the

two proteins. In addition, considerable amounts of cadmium were detected on the kidney protein of low molecular weight. The estimated molecular weight of the smaller protein and its ability to bind copper, zinc and cadmium are similar to properties described for metallothionein. In gill, muscle and viscera, exposure to excess copper resulted in the binding of copper to higher molecular weight proteins (>70,000 daltons), which would include enzymes. Toxicity of copper may have been related to the presence of copper on sensitive subcellular sites such as enzymes.

Effects of Copper on the Polychaete *Eudistylia vancouveri*

The relationship between copper accumulation and injury to the gills of the sabellid polychaete *Eudistylia vancouveri* was investigated. Worms were exposed to 10, 15, 20, and 40 $\mu\text{g}/\ell$ copper and were sampled daily during the first five days of treatment and thereafter at 8, 11, 15, 19, 26, 33 and 65 days. The time course for copper accumulation by the gills indicated a rapid initial accumulation that gradually plateaued after 11 days.

Tissue injury occurred during the period of rapid copper accumulation and was grossly characterized by sloughing of epithelial cells from gill projections called pinnules, by clubbing of the pinnule tips and by their gradual erosion to a premorse condition. Gills were shed from worms in the 20 and 40

µg/l treatments. The rate of progression and extent of injury depended on the strength of the copper dose.

Examination under an electron microscope revealed the following phenomena inside the injured cells: irregular microvilli; formation of electron-dense bodies, inclusions or vesicles; fragmented membranes; swollen and disrupted mitochondria; and lysed and necrotized cells. Eventually the tissue was completely disrupted.

X-ray microanalysis showed that copper was located intracellularly in small, dense membrane-bound vesicles that may be secondary lysosomes. If so, necrosis may have been due to labilization of lysosomes as a result of excess intracellular copper. Gills of worms sampled after 11 days began to show signs of regeneration. Mitosis was visible. Between 33 and 65 days, regeneration was often complete, especially in lower copper treatments. This phenomenon coincided with the decrease in the copper accumulation rate. Copper was distributed throughout the cell organelles and not bound in cytoplasmic vesicles. A preliminary experiment also indicated the induction of a low molecular weight, copper-binding protein during exposure. Such proteins may be involved in the detoxification of copper.

Low Molecular Weight, Mercury-Binding Proteins in the Marine Mussel

Mussels (*Mytilus edulis*) were exposed to 1 µg/l mercury for 90 days. Following exposure, mussels were transferred to aquaria containing mercury as $^{203}\text{HgCl}_2$ or to ^{14}C -cysteine for 24 hr. The influence of pre-exposure to mercury on the ability of the mussels to take up and incorporate ^{203}Hg and ^{14}C into different organs and into soluble, low molecular weight proteins was tested in this study.

Gills were identified as the primary organ for the short-term incorporation of inorganic mercury. Relatively high concentrations were also measured in digestive glands when compared to other organs. Pre-exposed mussels had lower levels of ^{203}Hg than control mussels.

Examination of ^{203}Hg elution profiles identified four peaks: I, high molecular weight proteins (>70,000 daltons); II, 20,000 daltons (observed in viscera only); III, 11,000 daltons and IV, low molecular weight pool (<3,000 daltons). The position of Peak III corresponded to that of the metallothionein-like, cadmium-binding protein previously reported for *Mytilus edulis*. Relatively high concentrations of

^{203}Hg were associated with this protein in gills and digestive glands.

^{203}Hg profiles of gill tissue indicated considerable differences caused by prior treatment in the subcellular distribution of mercury among high molecular weight proteins (Peak I), mercury-binding proteins (Peak III), and the low molecular weight pool (Peak IV). In gills of pre-exposed mussels, ^{203}Hg in the soluble fraction was associated mainly with the mercury-binding protein and, to a lesser extent, with components in the low-molecular-weight pool. Only a small amount was associated with the high molecular weight proteins. This was not the case in the control mussels, in which the amount of soluble ^{203}Hg present on the high molecular weight proteins was about twice that on the mercury-binding protein. Such observations suggest that the mercury-binding capacity of the mercury-binding protein of control gills was not as well-developed as in pre-exposed gills. Less pronounced differences in ^{203}Hg profiles were observed between pre-exposed and control digestive glands. A relatively large degree of incorporation of ^{14}C into the mercury-binding proteins of gills of pre-exposed mussels provided evidence for the de novo synthesis of those proteins during the short-term experiment. An induction of mercury-binding proteins in gills of pre-exposed mussels may provide a basis for the differences in the mercury-binding capacities of mercury-binding proteins of pre-exposed and control gills. In control gills and other organs for all mussels, ^{203}Hg may have bound to existing metallothionein-like proteins.

Mercury in Mussels of Bellingham Bay, Washington

Bellingham Bay, Washington, is known to be contaminated with mercury from discharge of mercury-laden waters from local industry. The status of mercury taken up by mussels (*Mytilus edulis*) of Bellingham Bay was evaluated by the examination of mercury incorporation into different organs and into the soluble, cytoplasmic fraction of those organs. Mussels from Bellingham Bay were analyzed for total mercury in the gills, digestive glands and the whole animal and for the distribution of mercury in the soluble fraction of the gills and glands. Values were compared with those from mussels of Sequim Bay, Washington, which is not contaminated with mercury. Whole mussels, gills and digestive glands of Bellingham Bay mussels exhibited higher levels of mercury than those from Sequim Bay but lower values than those reported previously. Whole animals from Sequim Bay had a mean of 9 ng/g (ppb) mercury, and those from Bellingham Bay

averaged 34 ng/g. Soluble mercury in gill tissue was associated primarily with high molecular weight proteins and low molecular weight pool components. Little detectable mercury was associated with low molecular weight metallothionein-like proteins. In digestive glands, mercury on metallothionein-like proteins was enhanced. However, the main mercury peak was associated with the low molecular weight pool of materials less than 3,000 daltons. The distribution of

mercury in gills and digestive glands of Bellingham Bay mussels was unlike that in the same organs of laboratory-exposed mussels, in which considerable mercury-binding was observed in metallothionein-like, low molecular weight proteins. Mussels in Bellingham Bay probably were not exposed to sufficiently high levels of mercury to induce synthesis of the high levels of mercury-binding proteins observed in laboratory exposures.

• **Bioavailability of Energy Effluent Materials in Coastal Ecosystems**

Principal Investigators: E. A. Crecelius, C. I. Gibson, J. T. Hardy, J. E. Rogers and R. L. Schmidt

Other Investigators: J. M. Gurtisen and C. W. Apts

This program attempts to provide an understanding of the processes involved in the cycling of materials resulting from energy technologies in the marine coastal ecosystem. The primary goal of the program is to enable prediction of the fate and ultimate ecological consequences of materials produced by energy technology in the coastal ecosystem.

One of the major problems limiting prediction of effects of a contaminant in the marine environment is the inability to relate an analytically defined fraction of the total amount of material in the environment with a biological response. This problem is particularly limiting for long-term effects where chemical modification is likely to occur because of chemical, physical and biological influences.

This year our work has continued in 1) the development of accurate methods for measuring the bioavailable fraction of a contaminant in the marine ecosystem; 2) the development of clam gill bioavailable bioassay, and 3) determination of how natural changes, and those induced by energy technology, affect the surface neuston communities, water column and sediments.

Evaluation for Methods of Measuring Bioavailable Trace Metals

Changes in physical and chemical properties of the water can affect the concentration of bioavailable metals in the marine environment. In order to investigate the importance of specific parameters, measurement techniques that are sensitive to the bioavailable form of the metal are needed. Measurements of electroactive copper using differential pulse anodic stripping voltammetry (DPASV) in conjunction with a titration procedure have been found to correlate reasonably well with biological uptake. This year we have evaluated two alternate methods of measuring bioavailable trace metals.

The copper ion-selective electrode exhibits a potential response change relative to the concentration of Cu^{++} in solution. Plots of Cu^{++} ion titration normally follow a 29.3 mV/decade slope. However, in sea water we observed a high degree of slope variability from sample to sample. Equilibrium time necessary to reach steady-state potentials was unacceptably long. The electrode was incapable of returning to a steady-state potential upon sample replication.

Suspected electrode instability and the effect of chloride complexation in sea water samples has negated the usefulness of the copper ion-selective electrode.

Another technique studied employs a particulate (MnO_2) as an ion-exchange resin in the seawater medium. The binding constant between Cu^{++} and MnO_2 is weak. It therefore avoids competition for Cu^{++} already in established seawater complexes (organic and inorganic). After the equilibration of Cu^{++} in sea water, MnO_2 can be added. Free Cu^{++} ion will associate itself with the MnO_2 resin, which can then be separated from the parent solution by filtration. Advanced application of these principles has resulted in the determination of a binding constant for copper-organic ligands, $\log K = 5.92$, and the concentration of ligand, $0.95 \times 10^{-6}\text{M}$ copper equivalent.

Complexometric titration of Cu^{++} using DPASV has resulted in the determination of a binding constant of $\log K = 7.63$ and a ligand concentration of $0.25 \times 10^{-6}\text{M}$. The agreement which exists between numbers generated by these differing techniques is considered reasonable.

Development of a Bioavailability Bioassay Technique

During the past year we have developed a faster, more efficient bioassay technique for determining the processes that control the bioavailability of trace metals in the marine environment. Freshly excised gills from the littleneck clam (*Protothaca staminea*) were exposed to trace metals in sea water. Gill accumulation was considered indicative of bioavailable metals. By manipulating the character of the metal or the physical content of the sea water, we were able to see substantial differences in gill uptake in less than 24 hr. These differences greatly enhance our understanding of the mechanisms that govern bioavailability. The gill respiration rate after such exposures is similar to that of freshly excised gills, indicating that the gills are still viable and healthy. This has enabled us to reduce our exposure time from one month to one day. The use of excised gills has also eliminated secondary pathways of uptake, such as digestion, excretory products which can change the complexing capacity of the sea water, and the ability of the organism to shut the gills off from the outside environment. All these cause changes in the rate of metal uptake and variability in the data. The elimination of testing whole organisms also allows us to use slow-flowing or even static exposure systems, an important consideration when hard-to-find or expensive materials are tested.

The first series of experiments using excised gills was conducted with a 50 ppb copper-sea water mixture in a flowthrough system. In one test, the copper-sea water mixture flowed directly into an exposure tank. In another, the mixture was aged for 72 hr by first passing through a retention reservoir. Samples were taken at the end of a 1, 2, 4, 8, 12 and 24 hr with significant uptake after only 8 hr in the non-aged exposure. Gills exposed to aged copper showed no uptake during the entire experiment, indicating that the sea water removes the bioavailable copper within a matter of hours (Figure 1).

A second series of experiments was conducted statically with a 10 μ M copper-sea water mixture. Five organic complexers (tris, sodium citrate, histidine, glycine and cysteine) were individually added to the copper-sea water mixture in one-liter beakers. Each complexer was tested at 10 mM, 10 μ M and 10 nM concentrations. The 10 μ M and 10 nM concentrations had little effect on gill uptake. However, all complexers at the 10 mM concentration drastically reduced copper accumulation. These results led us

FIGURE 1. Uptake Rate in Excised Littleneck Clam Gills Exposed to 50 ppb Copper

to believe that soluble organic complexers were responsible for the reduction in bioavailable copper seen after aging. To test this theory, we mixed organic-rich bottom sediment with sea water. The effort produced a concentrated soluble organic solution. The solution was filtered through 0.2- μ membrane filters; copper was added and the solution was then aged for 72 hr. Gills exposed to this solution, however, showed no reduction in metal uptake. This seems to indicate that soluble components from sediments may not alter bioavailability copper.

A series of experiments has also been conducted with ^{203}Hg . In these tests, cysteine was added to a 1-ppb ^{203}Hg solution in 1-l beakers. Cysteine concentrations of 10 mM, 10 μ M, and 10 nM were tested. Mercury 203 -uptake in excised gills was reduced at the 10- μ M and 10-nM concentration, but it increased at the 10-mM concentration. Of interest is the fact that a 10-mM cysteine concentration increases the bioavailability of ^{203}Hg but decreases that of copper. This means that individual trace metals must be tested with each new factor that is found to have a controlling influence on bioavailability.

Effects of Coal Fly Ash on Metal Uptake and Primary Production Rates of Littleneck Clams

Coal fly ash may interact with coastal water for a number of possible reasons, including deposition of fly ash from the atmosphere, the use of coal combustion solids for the construction of artificial reefs, the use of fly ash to control phosphate release from sediments in estuaries or the disposal of fly ash at sea. Preliminary experiments testing the uptake of metals by littleneck clams (*Protothaca staminea*) exposed to coal fly ash and the effect of soluble fly ash on the primary production rate of coastal phyto-plankton have been completed. Research

conducted at this laboratory on the solubility in sea water of metals in coal-fired power plant fly ash has shown that several metals, including copper, chromium, arsenic and selenium, are soluble in sea water.

The body burden of metals in littleneck clams was measured after their exposure to a flowing seawater system to which fly ash was continuously added for approximately one month. The dosing rate of fly ash to sea water was 0.9 g/l. Copper was the only metal significantly elevated in the exposed group. Copper concentrations in the gills of exposed clams were approximately 15 ppm compared to controls of approximately 6 ppm. These copper levels in gills are approximately those that would be predicted, knowing the copper concentration in sea water and using gill concentrations measured in dissolved copper clam exposure systems at this laboratory.

The effects of coal fly ash on the ^{14}C uptake rate was measured on coastal phytoplankton that were exposed to different concentrations of the seawater soluble fraction of coal fly ash. These measurements were made off the Washington coast during August, 1979. All phytoplankton samples were collected in surface water up to 10 m deep. All five stations studied exhibited moderate-to-high primary production rate. The addition of soluble coal fly ash material had no effect on the primary production rates; it neither stimulated nor suppressed primary production as compared to control samples. These results are rather surprising because research at other oceanographic laboratories has shown certain phytoplankton species to be extremely sensitive to low concentrations of certain heavy metals. We plan to conduct experiments on the effects of soluble coal fly ash and soluble atmospheric particulates on primary production rate in coastal waters. Research efforts will be focused on the effect of phytoplankton during periods of stress, such as is imposed by limitation of light or nutrients.

Artificial Production of Neuston Communities

The sea-surface microlayer (upper 1 mm or less) often serves as a habitat for a diverse and abundant biological community--the neuston. Studies from different locations indicate that marine neuston communities generally contain much higher densities of microalgae and bacteria than water only a few centimeters below the surface. Chemical analyses by others have shown that heavy metals, petroleum hydrocarbons, plastic particles, pesticides and polychlorinated biphenols all occur at much higher concentrations in the surface microlayer than in water only a few centimeters lower.

We have undertaken studies to determine whether or not naturally occurring phyto-neuston communities can be duplicated in a laboratory microcosm. Such artificially produced communities would be useful in investigating the mechanisms of pollutant transfer through the microlayer.

Field neuston and plankton samples were collected from Sequim Bay, Washington, by submerging a glass plate (20 x 20 cm) vertically into the water and slowly withdrawing it. The depth sampled was $56 \pm 2 \mu\text{m}$. Neuston and plankton samples from duplicate tanks in our laboratory microcosm were collected in the same manner as the field samples were.

The microcosm consisted of 10-gallon outdoor aquariums fitted with elbow outlets to minimize surface disturbance, and fed with sea water from a head tank. The flowthrough rate was a constant 10 ml/sec. Relative species abundance similarities between phyto-neuston communities and phytoplankton were determined using statistical tests. Based on their rank order of species abundance, phytoneuston and phytoplankton communities of Sequim Bay are taxonomically different from each other.

The density of phytoneuston in the surface microlayer of Sequim Bay is generally two to seven times greater than the phytoplankton 10 cm below. Phytoneuston densities in sheltered waters differ greatly, both spatially and temporally. Significant differences in density can occur even hourly.

In the flowthrough microcosm tanks, the number of individuals per square centimeter of phytoneuston increased much more rapidly than the phytoplankton. After one week of incubation, the tank neuston community was between 600 and 6000 times denser than the field community. The rank order of species dominance in the tank neuston was not significantly different when compared to any of the field neuston samples.

Neuston communities produced artificially in tanks are more abundant than the naturally occurring populations of Sequim Bay, Washington, but they are essentially similar in species composition (rank dominance). Thus, it will be feasible to use artificially produced neuston communities to represent natural populations in pollutant studies.

Effects of Bacteria on Bioavailability and Mobility of Trace Metals in Marine Sediments

The mobility and bioavailability of trace metals within marine sediments are dependent on a number of interrelated physiochemical and biological processes. Microorganisms

are known to convert organic substrates to numerous primary and secondary metabolites from stable complexes with trace metals. Many such metabolites have been shown to reduce the bioavailability of trace metals. In addition, certain bacterial species have been shown to reduce inorganic ions. An example of this is the reduction of sulfate to sulfide by *Desulfovibrio* species. These ions can then combine with trace metals to form soluble complexes or insoluble precipitates. The effects of these organic and inorganic compounds of microbial origin are dependent on the types and activities of microorganisms in marine sediments.

A number of bacterial types have been measured in Sequim Bay sediments. Sediment samples were taken to examine depth and seasonal distribution of aerobic, anaerobic and sulfate-reducing bacteria. Samples were taken at different locations within the bay to examine the effects of different sediment types. Currently, the data obtained are being examined with regard to several sediment properties such as sediment organic carbon, dissolved organic carbon, soluble trace metals and sediment trace metals. This is being done to determine if the distribution of trace metals is significantly correlated with the bacterial types enumerated.

Future studies will involve laboratory experiments to examine in more detail the effects of bacteria on the mobility and bioavailability of trace metals in marine sediments. Factors considered will be organic amendments which can be readily metabolized by bacteria, changes in redox potential and pH, and the addition of trace metal amendments at concentrations representative of polluted sediments.

Enrichment of Trace Metals in Sea Water and Sediment

Samples of sea water, suspended particulate matter, interstitial water and sediment were collected from Sequim Bay and analyzed for trace metals, organic carbon and bacteria. Analyses of variance indicate that significant changes ($P < 0.05$) in mean concentration of several components occurred between November 1978 and May 1979. As shown in Figure 2, pH, dissolved organic carbon and suspended particulate matter increased significantly. With these changes, the mean concentrations of particulate copper, sediment organic carbon, sediment iron, sediment copper and sediment zinc also increased significantly.

The extent of the seasonal changes in trace metal concentration can be illustrated by examining the enrichment factors of cop-

FIGURE 2. pH, Dissolved Organic Carbon, Suspended Particulate Matter Concentrations and Enrichment Factors (EF) for Sea Water, Suspended Particulate Matter and Interstitial Water in Sequim Bay, November 1978 to May 1979

per and zinc in water and suspended particulate matter in relation to sediment. The enrichment factor (EF) for this study was defined as

$$EF(X) = \frac{(X/Fe)_1}{(X/Fe)_2}$$

where $(X/Fe)_1$ and $(X/Fe)_2$ are the concentration ratios of element X to iron in water or suspended matter, and in the top 24 cm of sediment, respectively.

As shown in Figure 2, the EF values for soluble copper increased by about twofold in the surface waters and about eightfold in the bottom waters during the period of measurement. The EF value for soluble zinc in surface waters showed little variation; however, in the bottom waters it increased by greater than tenfold. These changes may indicate a decrease in iron solubility, which may be caused by increased pH or uptake by phytoplankton, and/or an increase in soluble copper and zinc concentration.

Since the enrichment of copper and zinc in suspended particulate matter also increased by greater than tenfold during this period, suggesting uptake of soluble forms of these elements by phytoplankton, it appears that there is a source of significant amounts of copper and zinc to the waters of the bay. Although atmospheric and fluvial sources are not precluded, the greater enrichment of copper and zinc in the interstitial and bottom waters during this period indicates that increased microbial activity, possibly due to decomposition of organic detritus, was affecting the release of soluble and potentially bioavailable forms of these elements from the sediment.

- **Marine Chemistry of Energy-Generated Pollutants**
- **Iron-55 Phenomenon**

Principal Investigators: E. A. Crecelius, D. E. Robertson, K. H. Abel and W. C. Weimer

These programs are designed to increase our understanding of the biogeochemical and physical processes that control the fate of energy-generated pollutants that enter the marine environment. The increased energy needs of our country and increased utilization of the coastlines for siting energy-generating facilities and related industries have resulted in the introduction of energy-related pollutants to the oceans. The information found by this research is vital to the U.S. Department of Energy's interests in understanding 1) the natural origins, distributions and concentrations in baseline data of trace metals and other contaminants in the oceans; 2) the input rates and mixing rates of pollutants introduced to the oceans; 3) the behavior and fate of the anthropogenic pollutants entering the oceans from the atmosphere and the continents and 4) an assessment of the potential environmental impact of energy-generated pollutants on the marine environment.

Presently, the Marine Chemistry program is concerned primarily with concentrations and processes affecting the fate of pollutants entering the ocean. The Iron-55 Phenomenon program, closely related to the Marine Chemistry program, is examining the chemical speciation of soluble air particulates. Specific tasks pursued during the last year include 1) intercalibration of mercury analysis technique; 2) determination of uptake rate of mercury vapor from air into sea water; 3) measurement of atmospheric input of combustion organics; 4) development of nickel and cobalt analysis in biological materials; 5) development of vanadium and manganese analysis in sea water; 6) determination of the solubility of air particulates in sea water and 7) analysis of ^{55}Fe in marine samples.

Intercalibration of Mercury Analysis Technique

During the last year we have continued to improve our methods for the collection and analysis of total mercury in sea water. We participated in a comparison of seawater-mercury analyses with two other marine laboratories that have experience in mercury analysis (Skidaway Institute of Oceanography, and Marine Institute, University of Connecticut). Sea water samples were prepared for distribution at the Battelle Marine Laboratory. Sequim Bay sea water was poured into Pyrex® bottles, acidified with concentrated sulfuric acid at the ratio of 10 ml acid per liter of sea water and stored for one month before shipment or analysis. The results from all three laboratories averaged $12 \pm 2 \text{ ng/l}$. This value is for what we would term "total mercury"--mercury which has undergone chemical digestion. If sea water is analyzed immediately after collection without some type of chemical digestion, the mercury values are two to ten times lower.

We feel the agreement for this intercomparison is excellent and indicates that mercury samples can be stored and shipped if proper care is taken. There are several advantages in collecting sea water samples for mercury analysis in Pyrex bottles and storing them acidified. First, only a small amount of reagents are involved; i.e., the acid used to acidify the sample contributes a reagent blank of approximately 1.5 ng/l . Second, contamination of the stored sea water by the container or air appears to be extremely small over a storage period of at least three months ($<0.5 \text{ ng/l}$). Another important advantage is that, during the storage of the acidified sea water, the acid apparently releases mercury that may be complexed or absorbed to dissolved and particulate matter in sea water, so that total mercury may be determined in sea water without the use of additional oxidizing reagents or digestion of the sample at elevated temperatures. This greatly reduces the reagent blank. Mercury storage tests using radio-labeled mercury tracer indicates that loss of dissolved mercury to the walls of the

Pyrex container is minimal (<1%) at the 5 ng/l level over a one-month period.

Determination of Mercury Vapor Uptake Rate by Sea Water

We have initiated some laboratory experiments designed to determine the uptake rate of elemental mercury (Hg^0) vapor by sea water. Elemental mercury vapor is released to the atmosphere by processes such as coal combustion, geothermal power plants, oil shale retorting and smelting. The importance of the ocean in the geochemical cycle of Hg^0 vapor is not known. Man's activities are estimated to release Hg^0 vapor to the atmosphere in a quantity equalling approximately 25% of the natural atmospheric global mercury cycle.

In our laboratory experiments, Hg^0 vapor was labeled with ^{203}Hg tracer and introduced in the elemental vapor form to Pyrex glass bottles containing sea water and air. The mercury concentrations in the air ranged from 100 to 4000 ng Hg/l . Air and water mercury concentrations were monitored with time, using a sodium-iodide gamma-ray counting system. The uptake rate of mercury from air into sea water required approximately 5 hr for 50% of the mercury in the air to enter the sea water. Cooling the sea water increased the uptake rate slightly; mixing increased it dramatically. Once Hg^0 vapor dissolves in sea water, it undergoes changes in chemical and physical form. After Hg^0 vapor in air had been in contact with sea water for 24 hr, 40% of the mercury was as Hg^0 vapor dissolved in sea water, 20% was dissolved in sea water in nonvolatile form, 20% on particulates ($>0.4 \mu\text{m}$) and the remaining 20% was in air or on the walls of the container. As the experiment continued, Hg^0 vapor decreased and nonvolatile dissolved and particulate mercury increased. When ionic mercury (HgCl_2) was added to sea water, a small amount of volatile mercury was present in air above the water after several days, and much of the mercury was associated with particulates. These experiments have shown that mercury can be transferred in either direction across the sea surface. Further experiments are planned to determine the dominant process in air-sea transfer and the role of the surface microlayer in the transfer.

Measurement of Atmospheric Deposition of Organic Combustion Products

This task will estimate the types and quantities of organic pollutants that enter Pacific Northwest marine waters from atmospheric fallout. Data collection and analysis are being conducted jointly with the

University of Washington. During calendar year 1979, monthly air particulate samples were collected at Seattle, Sequim and Quillayute.

Analysis is still in progress; however, preliminary data show a large number of polynuclear aromatic hydrocarbons (PAH) and aliphatic hydrocarbons from C_{15} to C_{35} . Table 1 lists five of approximately twenty PAH's that have been measured in air particulates collected at the three sites during January 1979. As was expected, the concentrations in urban Seattle are much higher than in rural Sequim and Quillayute. Using deposition velocities measured at these three sites, the atmospheric input rates of these organic compounds will now be estimated and compared with the rate of these compounds accumulating in the sediments. Rates of accumulation of organic compounds in sediments of the Pacific Northwest have been measured at the University of Washington.

TABLE 1. Concentrations of Particulate Polynuclear Aromatic Hydrocarbon Compounds in Pacific Northwest Air, January 1979 (ngm^{-3})

Compound	Location		
	Seattle	Sequim	Quillayute
Benzopyrene	2.1	0.25	0.16
Benzofluoranthene	1.8	0.76	0.55
Fluoranthene	1.5	0.15	0.16
Phenanthrene	0.34	0.067	0.077
Pyrene	2.7	0.082	0.15

Development of Nickel and Cobalt Analysis in Biological Materials

Cobalt and nickel concentrations in various deep-sea biological materials have been determined by neutron activation and subsequent gamma-spectrometric analysis of the n-gamma and n-proton products ^{60}Co and ^{58}Co , respectively. Nickel analysis via the n-proton daughter ^{58}Co is not routinely sensitive enough for determination of the low concentrations generally found in marine biological materials. Therefore, several steps were taken to increase both production of the ^{58}Co daughter via the reaction $^{58}\text{Ni}(n,p)^{58}\text{Co}$ and also enhance the sensitivity of subsequent measurement of the ^{58}Co decay photopeak. These steps included 16-hr irradiation instead of the normal 8-hr exposure, and irradiation in the core position

rather than in the normal fringe location. In the core, the neutron flux was higher by a factor of 2 to 3, and the fast neutron component of the neutron flux was greater than in the more thermalized fringe area in the 1MW Triga reactor employed. Finally, gamma-spectrometric analysis was conducted using an anticoincidence shielded Ge(Li) diode system which enhanced the sensitivity of detection. The diode itself was an intrinsic germanium diode of 132 cc and was 32.5% efficient relative to a 3 x 3 sodium iodide crystal. Resolution of the diode was 2.13 keV full width at half maximum (FWHM) at the 1332 keV ^{60}Co photopeak and 1.02 keV FWHM at 122 keV as determined by ^{57}Co . Peak-to-Compton ratio for the diode was 48.4 to 1. This system gave a separation of non-coincident events, such as those associated with ^{58}Co , ^{51}Cr , ^{85}Sr , and ^{65}Zn , from coincident gamma-ray decays, such as those associated with ^{152}Eu , ^{48}Sc and positron decay. Sensitivity for ^{58}Co , ^{65}Zn and ^{85}Sr is increased significantly since interfering coincident Compton events are reduced and nearby coincident peaks at 1121 keV and 511 keV are separated from the noncoincident ^{65}Zn and ^{85}Sr peaks at 1115 keV and 514 keV, respectively. Other elements including iron, chromium, scandium, europium, silver, selenium, antimony, strontium, rubidium, tantalum and zinc can also be determined during the gamma-spectrometric analysis.

Nickel and cobalt concentrations have been determined in amphipods and starfish with mean cobalt concentrations of 0.233 and 0.140 ppm dry weight, respectively. Nickel concentrations were somewhat higher with a mean of 1.1 and 1.3 ppm dry weight.

Development of Vanadium and Manganese Analysis in Sea Water

The marine geochemistries of vanadium and manganese in coastal environments may potentially be affected by energy-generating technologies. Vanadium is present in relatively high concentrations in crude oil and may be released to the marine environment in oil spills. Manganese is a major constituent in sediments and ferromanganese accretions, and pollution processes which result in reducing environments can mobilize manganese from marine sediments.

Because of the interest in these two metals, analytical techniques have been developed which provide very accurate and precise measurements, near or below the ppb range, of their concentrations in sea water. These techniques employ preconcentration by either coprecipitation or solvent extraction, followed by neutron activation in a ^{252}Cf - ^{235}U fueled, subcritical neutron multiplier facility.

Vanadium concentrations in sea water (acidified to pH = 1.5 with high-purity HCl) were determined by adding carrier-free ^{48}V tracer to 1 l sea water and equilibrating overnight. High-purity iron carrier was then added and the pH adjusted to 8.0. The $\text{Fe}(\text{OH})_3$ was centrifuged, washed, dissolved and transferred to irradiation vials. The vials and appropriate standards were irradiated for 10 minutes in the neutron multiplier assembly and then counted on a Ge(Li) gamma-ray spectrometer. The samples were recounted the following day in a NaI(Tl) well-crystal to measure the ^{48}V tracer for determining the yield. The yields were greater than 90%, and vanadium could be measured with a precision of 4% at the 2σ confidence level. The results indicate that the vanadium distribution is extremely uniform at an average concentration of 1.69 $\mu\text{g/l}$. It is obvious that vanadium is not cycled in the oceans by the same biogeochemical mechanisms that affect the nutrients and many other trace elements. Thus, the homogeneous distribution of vanadium will make it easier to identify possible impacts from vanadium added by oil spills.

Manganese concentrations in sea water (acidified to pH 1.5 with high-purity HCl) were measured by adding carrier-free ^{54}Mn to sea water, adjusting to pH 8.0 and extracting into 0.1 M 8-hydroxyquinoline in chloroform. The chloroform extract was washed twice with high-purity water, and the manganese was then back-extracted with high-purity 0.1N HNO_3 . The HNO_3 extract was evaporated to 1 ml and transferred to irradiation vials. It was irradiated overnight (~15 hr) in the neutron multiplier and counted for 20 min the next morning on a Ge(Li) gamma-ray spectrometer to measure the ^{54}Mn (2.58 hr) activation product and the ^{54}Mn tracer. Yields were greater than 90%, and the ^{54}Mn could be measured with counting statistics of 10 to 20% at the 2σ confidence level. The manganese concentrations at Pacific Geosecs Station 322 are similar to the very low values recently reported by investigators at the University of Rhode Island and Woods Hole Oceanographic Institute.

Manganese was also analyzed in coastal sea water samples collected in Teflon®-lined bottles during October, 1977, from Puget Sound, from the Strait of Juan de Fuca, and off the Washington coast. The water samples were immediately filtered by pressurizing the bottles with filtered N_2 gas. The filtered water was acidified to pH 1.5 with hydrochloric acid and stored in carefully precleaned polyethylene bottles. The analytical procedure was similar to that described above, except that the samples were irradiated in the Washington State University

Triga reactor and counted directly on a Ge(Li) gamma-ray spectrometer.

The results of these analyses are presented in Table 2. The manganese concentrations are highly variable but show some systematic trends. It is apparent from the high manganese concentrations at Stations 13 and 16 that the Columbia River plume was moving northward and was encountered at these stations. Columbia River water contains dissolved manganese concentrations normally ranging from about 0.3 to 10 $\mu\text{g}/\ell$. This observation is consistent with the seasonal fluctuations of the Columbia River plume, which begins moving northward in the fall. At Stations 8 and 9, extremely low manganese concentrations were observed. The reason for these low concentrations is unclear, but they could represent an open ocean water mass intrusion that has been scavenged of its manganese content.

TABLE 2. Soluble Manganese Concentrations in Washington Coastal Waters, October 1977

Station Number	Depth (m)	Manganese Concentration ($\mu\text{g}/\ell$)
1	150	0.015
5	500	0.003
6	Surface	0.033
7	40	0.023
8	Surface	<0.001
9	Surface	0.003
9	400	<0.001
10	Surface	0.072
10	100	0.025
11	Surface	0.027
12	Surface	0.012
12	90	0.062
13	Surface	0.120
13	100	0.090
13	150	0.072
14	Surface	0.044
14	5	—
16	Surface	0.160
16	50	0.010
17	Surface	0.042
17	20	0.038

Determination of Solubility of Air Particulates in Sea Water

Elemental solubilities into sea water from aerosol matter collected at Point Barrow, Alaska; Quillayute, Washington and Colstrip, Montana, were determined by leaching neutron-

activated filters. The leaching characteristics were determined by agitating the neutron-activated filters containing aerosol matter in sea water for 1 hr and then refiltering the sea water through a 0.4 μm filter. The percentages of each element leached were determined.

Samples from the two coastal locations (Point Barrow and Quillayute) had significantly higher solubilities of sodium, bromine and potassium, reflecting their primary origin in these locations as evaporated sea salt. Also noteworthy in the data are the much higher solubilities of chromium, iron, cobalt and scandium in samples from Quillayute and Point Barrow. Two possible explanations are 1) that the increased solubilities reflect the great predominance of the marine source over the terrestrial source during this winter period or 2) that very fine (less than 0.4 μm) are present in these winter aerosols. Arctic haze layers have been noted during late winter which are thought to originate in the high deserts of Asia. This lends credence to the conclusion that the increased solubility may be due to very fine terrestrially derived matter.

Samples from Point Barrow show a decrease in solubility percentage for sodium, potassium and bromine during the summer months, evidently reflecting a decrease in the marine-derived aerosol fraction. The aerosols in Colstrip reflect their continental origin by showing much lower solubilities of sodium and potassium, although the solubility of bromine remains extremely high (97%). The solubility of arsenic seems to vary minimally in samples from the three locations, being somewhat greater than 50% soluble at all sites. Selenium appears somewhat more soluble at Colstrip than at either Quillayute or Point Barrow, although the difference may not be statistically significant. Zinc appears somewhat more soluble in the coastal locations, perhaps reflecting a marine source.

Analysis of Iron-55 in Marine Samples

Aerosol and zooplankton samples were collected from two stations on the continental shelf of the northeast Pacific Ocean to compare the levels of ^{55}Fe and stable iron in each of these two compartments. Previous studies in our laboratories have established that 1) ^{55}Fe in oceanic aerosols is in a chemical form much more soluble than the majority of the stable iron in the aerosols and 2) several species of salmon have consistently shown higher ^{55}Fe /stable iron ratios (^{55}Fe specific activities) than ocean aerosols or surface water samples collected

at the same time. This present sampling was conducted to determine whether the enhancement of ^{55}Fe specific activity occurs at the lowest food chain levels. The data for the specific activities of two marine aerosol samples and three zooplankton samples are given in Table 3. These data show that the specific activities of the marine aerosols are approximately 25 times higher than the zooplankton specific activities and suggest that zooplankton may actually discriminate against uptake of ^{55}Fe forms. However, analysis of corresponding water samples is necessary to provide a complete description of air-to-organism transfer of ^{55}Fe and stable iron forms.

TABLE 3. Iron Specific Activity from Washington Coastal Samples

Sample	^{55}Fe dpm/mg Fe
Aerosol #1	88
Aerosol #2	82
Zooplankton, >500 μ , outer drift station	2.6
Zooplankton, 243 μ -500 μ , outer drift station	1.6
Zooplankton, 243 μ -500 μ , inner drift station	5.2

- **Long-Term Effects of Petroleum Hydrocarbons from Water on Marine Organisms***

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A carefully controlled and characterized flowing hydrocarbon exposure system, using extracts of Prudhoe Bay crude oil, has been used to determine threshold levels for the survival of three representative marine crustaceans. Mortality can be interpreted as the product of concentration and exposure time, which allows prediction of impacts during both acute and chronic exposures in the field. Differences in mortality rate have been observed between species and between seasons, and feeding rates of shrimp and fish have been found to decrease at exposures below the lethal range.

Flowing Exposure System

We have earlier reported on extensive data concerning the hydrocarbon components in sea water extracts of Prudhoe Bay crude oil (PBC), on our delivery system and its ability to maintain consistent concentrations in all tests, and on the levels of PBC required to kill several representative Pacific Northwest marine species. We have shown that the mortality is cumulative and may be expressed as straight lines on log-log plots of exposure time and concentration. The slopes of these lines were not significantly different, but the curve for the most sensitive species, a mysid (*Neomysis awatschensis*), was shown to be different from two shrimp species (*Hippolyte clarkii* and *Pandalus danae*). During the past fiscal year we have examined the seasonal variability in sensitivity of *Pandalus danae* (coon-stripe shrimp) to hydrocarbons.

During late spring and summer, water temperatures increase and these animals become sexually mature. Our testing has shown that they are most vulnerable during this period; the toxicity index decreases from a mean of 16 ppm-days to 4 ppm-days. This means that if a spill were to occur in summer, the animals could only tolerate 25% of the exposure concentration for the same time period as shrimp exposed in the winter.

At some of our higher exposure levels in tests with *Pandalus*, we obtained tissue samples at the time of death of the last few animals producing the 50% mortality.

Analyses of these shrimp and those of the surviving 50% showed that differences between the tissues of dead and living organisms were generally not large. In analyses of tissue from both *Hippolyte* and *Pandalus*, most (75% or greater) of the contamination was in the form of naphthalenes. Tissues of *Pandalus* exhibited a trend toward increasing proportions of phenanthrenes with increased exposure time. The 8-, 26- and 74-hr exposures produced 5, 8 and 20% phenanthrenes, respectively. Since the phenanthrenes are of a higher molecular weight than the naphthalenes, it is not surprising that they are retained longer under constant exposure conditions. The pattern of accumulation contradicts the assumption that, during constant exposure, tissues consistently accumulate petroleum hydrocarbons. The pattern is not related to the time of exposure but, rather, to the level of exposure concentration.

Another aspect of our research with the flowing hydrocarbon exposure system is the measurement of abnormal physiological responses resulting from exposure to nonlethal (sublethal) levels. An example of this research is the measurement of the feeding rate of shrimp (*Pandalus*) and flatfish (*Parophrys vetulus*) exposed to sublethal concentrations of PBC extracts. Shrimp were fed littleneck clam meat on every third day for 14 days, and the amount consumed by shrimp exposed to six concentrations (including controls) was determined. Figure 3 provides an overview of the experiment; the means and standard deviations for food consumed is plotted against the exposure concentrations. Feeding among

* Environmental Protection Agency transfer

FIGURE 3. Mean Food Consumptions of *Pandalus* Exposed to Various Concentrations of Hydrocarbons. Bars represent standard deviations with $n = 30$ for each concentration.

animals at the lowest concentration was 15% greater than that of controls, but the higher exposure groups decreased in feeding activity from 9 to 54%. Analyses of the data by analysis of variance testing showed that exposure concentrations produced a significant effect at either the 0.01 or 0.05 level, depending on the number of feeding periods examined. The difference between feeding activity within concentrations at various intervals was not significant.

The results of the flatfish tests demonstrated several interesting phenomena (Figure 4). First, those at the lowest concentration feed more actively than controls; this response is the same as that of the shrimp. Second, the fish initially exhibiting suppressed feeding recovered when the exposure concentration decreased somewhat,

demonstrating that the stress is reversible if not too harsh. Finally, when the treatment concentration is too high, the reduced feeding is not reversible and mortality appears to be imminent. The only points in Figure 4 that are not statistically different from one another at the 0.05 level are the control values and those of the lowest two concentrations. More important is a consistent pattern of decrease in feeding as concentrations increase for both species of marine animals studied. This approach provides information on the stress of hydrocarbons, which is not apparent from mortality data.

FIGURE 4. Mean Amount of Food Consumed by *Parophrys* at Each Hydrocarbon Concentration. Bars indicate standard deviation, $n = 30$.

• Long-Term Effects of Petroleum Hydrocarbons from Sediments on Marine Organisms*

Principal Investigators: J. W. Anderson, R. G. Riley, R. M. Bean and J. E. Rogers

Technical Assistants: S. L. Kiesser, B. L. Thomas and S. W. Li

Analyses of sediment cores from exposure trays where oil was layered on the surface showed that the depuration of oil in the beach environment was quite rapid. Detailed analyses of these cores, as well as past analyses, have provided a detailed description of the rates of specific component losses with time in the natural intertidal environment. To aid in explaining the causes of oil component changes in the field, microbial studies are underway which will describe the rate of CO₂ production from bacterial degradation of hydrocarbons in sediments and the rate of conversion of parent compounds to metabolic products.

Recovery of Hydrocarbons from Different Sediment Types

We have developed methodology for the isolation and quantitation of volatile saturate and aromatic hydrocarbons from different sediment types. The method employs a simplified calibration by which three preselected hydrocarbons can be used as external standards for correcting raw data for recovery.

To demonstrate the general applicability of the procedure, a coarse sediment, a fine sediment and a lagoon mud, representative of sediment types found around Sequim Bay, were amended with known amounts of a mixture of saturate and aromatic hydrocarbons (0.38 to 0.92 ppm, wet wt sediment). The concentrations of hydrocarbons amended to the sediment were selected as representative of intermediate concentrations expected in sediment samples taken from long-term field or laboratory exposure experiments. Extracts prepared from sediments were analyzed by capillary gas chromatography. The recoveries of individual saturate and aromatic hydrocarbons from each sediment type are reported in Table 4. Average recoveries of individual saturate and aromatic hydrocarbons ranged between 55% and 87% for the three sediment types. Few differences were found in recoveries of hydrocarbons from different sediment types. The hydrocarbons used for the simplified calibration (2-methylhexadecane for saturates, biphenyl for naphthalene through phenanthrene and pyrene for the methyl and dimethylphenanthrenes) were recovered from the

TABLE 4. Percentage of Hydrocarbon Recoveries from Coarse, Fine and Muddy Sediments

Compound	Coarse % Recovery ± s.d.	Fine % Recovery ± s.d.	Muddy % Recovery ± s.d.
C ¹²	64.4 ± 1.4	60.8 ± 1.8	56.1 ± 2.9
C ¹³	64.9 ± 2.6	60.8 ± 1.7	56.2 ± 2.7
C ¹⁴	64.3 ± 1.6	61.9 ± 2.1	57.9 ± 2.3
C ¹⁵	66.6 ± 1.6	64.6 ± 2.6	61.9 ± 2.4
C ¹⁶	67.4 ± 0.9	65.2 ± 2.7	63.0 ± 2.9
2-MHD ^(a)	68.8 ± 0.9	67.8 ± 2.8	63.8 ± 3.6
C ¹⁷	71.2 ± 1.3	70.1 ± 2.7	67.2 ± 4.1
C ¹⁸	75.2 ± 0.9	74.1 ± 3.0	70.4 ± 5.2
C ¹⁹	80.7 ± 1.2	78.1 ± 4.3	75.7 ± 7.5
C ²⁰	85.5 ± 1.5	81.2 ± 6.5	81.4 ± 10.7
C ²²	84.7 ± 0.9	78.1 ± 8.4	87.5 ± 12.1
C ²⁴	79.7 ± 2.1	77.0 ± 4.7	78.6 ± 5.7
Naphthalene	59.0 ± 3.5	60.7 ± 1.0	55.2 ± 6.3
2-MN ^(b)	59.8 ± 2.7	62.2 ± 1.1	57.0 ± 5.5
1-MN	60.2 ± 2.4	62.3 ± 0.9	58.5 ± 6.5
Biphenyl	61.3 ± 3.5	63.8 ± 0.09	59.9 ± 6.3
2,6-DMN ^(c)	58.0 ± 3.2	61.6 ± 0.7	57.8 ± 6.4
1,3-DMN	58.3 ± 2.6	62.1 ± 1.2	59.3 ± 8.4
2,3-DMN	58.1 ± 2.6	61.8 ± 0.7	59.7 ± 6.9
2,3,6-TMN ^(d)	56.9 ± 4.4	60.7 ± 0.7	61.2 ± 6.9
Phenanthrene	58.6 ± 4.9	68.2 ± 1.5	61.3 ± 6.1
1-MP ^(e)	63.6 ± 8.6	66.0 ± 2.8	70.9 ± 8.1
2-MP	61.1 ± 6.5	68.7 ± 2.1	65.7 ± 7.7
3,6-DMP ^(f)	67.2 ± 8.1	73.0 ± 4.7	66.6 ± 4.6
Pyrene	67.5 ± 7.3	75.8 ± 7.2	62.3 ± 5.7

(a) MHD = methylhexadecane

(b) MN = methylnaphthalene

(c) DMN = dimethylnaphthalene

(d) TMN = trimethylnaphthalene

(e) MP = methylphenanthrene

(f) DMP = dimethylphenanthrene

* Environmental Protection Agency transfer

sediments at levels similar to the amended hydrocarbons, making them good representative recovery standards.

The method was further tested by analysis of a sediment core obtained from a long-term recruitment experiment conducted last year at Sequim. The hydrocarbon concentrations in this core were compared to the hydrocarbon concentrations in a core taken from the same sediment tray as had been analyzed a year earlier using a hexane-sodium sulfate procedure. The concentrations were very similar, indicating quantitation consistency with methods previously used.

Persistence of Petroleum Hydrocarbons in Sediment as a Function of Depth

In previous studies on the recruitment of benthic animals as a function of petroleum hydrocarbon concentration in sediment, we observed changes in the hydrocarbon ratios of n-C₁₇/pristane and n-C₁₈/phytane after exposure of oiled sediment in the intertidal zone for six to eight months. We attributed these changes to biodegradation. To obtain additional information on the distribution profile of persistent hydrocarbons in these sediments and on n-C₁₇/pristane and n-C₁₈/phytane ratios, cores from these sediment systems were analyzed for concentrations of hydrocarbons as a function of depth using the method described above. Duplicate sediment cores, taken 13 months after installation of a field study, containing sediment layered with oil were analyzed for changes in hydrocarbon concentration and n-C₁₇/pristane and n-C₁₈/phytane ratios as a function of depth.

No saturate and aromatic hydrocarbons could be detected in either sample core to a

depth of 6 cm, measuring at 1-cm intervals. These results were to be expected, because analysis of a core taken six months earlier revealed individual hydrocarbon concentrations at 0.3 ppm or less. Examination of sediment cores taken from a field installation in which the oil was mixed into the sediment produced different results (Figure 5 and Table 5). Samples analyzed 3½ and 9½

FIGURE 5. Persistence of saturate hydrocarbons in sediment as a function of time and depth. Error bars indicate ranges for duplicate analyses.

TABLE 5. Ratios of n-C₁₇/Pristane and n-C₁₈/Phytane in Sediment as a Function of Time and Depth

Age of Sample	Depth (cm)	n-C ₁₇ /Pristane	n-C ₁₈ /Phytane
3½ Months	0-1	1.69 ± 0.01	2.26 ± 0.00
	1-2	1.67 ± 0.06	2.24 ± 0.00
	2-3	1.67 ± 0.06	2.14 ± 0.07
	3-4	1.68 ± 0.03	2.26 ± 0.01
	4-5	1.62 ± 0.02	2.12 ± 0.21
	5-6	1.49 ± 0.07	2.03 ± 0.11
9½ Months	0-1	1.12 ± 0.53	0.83 ± 0.25
	1-2	1.00 ± 0.36	0.76 ± 0.21
	2-3	0.68 ± 0.17	0.62 ± 0.00
	3-4	0.55 ± 0.15	0.66 ± 0.01
	4-5	0.57 ± 0.14	0.67 ± 0.03
	5-6	0.61 ± 0.01	0.65 ± 0.09

months after deposition in the field revealed substantial differences in saturate and aromatic hydrocarbon content. After 3½ months, total saturate hydrocarbon concentrations were about 50% less in the top centimeter than deeper in the sediment. Saturate hydrocarbon concentration appeared to be constant at depths of 3 to 6 cm. After 9½ months, total saturate hydrocarbon concentrations in the top centimeter and in the second centimeter were 68% and 89%, respectively, less than the concentrations present at 3½ months. Furthermore, persistence of saturate hydrocarbons increased at depths below 2 cm, with 47% remaining at 9½ months at a depth of 5 to 6 cm (Figure 5). Specific aromatic hydrocarbons behaved similarly; those deeper in the sediment were more persistent (Figure 6).

FIGURE 6. Persistence of Aromatic Hydrocarbons in Sediment as a Function of Time and Depth. Error bars indicate ranges for duplicate analyses.

Table 5 lists the $n\text{-C}_{17}$ /pristane and $n\text{-C}_{18}$ /phytane ratios at different sediment depths for the same core samples. At 3½ months, the ratios were constant at depths above 4 cm. Small but significant decreases occurred in both ratios at depths greater than 4 cm, indicating the preferential persistence of the branched hydrocarbons, and signaling hydrocarbon degradation. Greater decreases in the magnitude of these ratios were observed

TABLE 6. Mineralization of ^{14}C -Phenanthrene by Microorganisms of Sequim Bay Sediment

Incubation Time, days	^{14}C -Phenanthrene Mineralized/g Wet Sediment ^(a) , n Mole	Percentage of ^{14}C -Phenanthrene Mineralized ^(a)
t = 0	0.0018 ± 0.0007	0.0167 ± 0.0065
t = 15	1.8783 ± 0.5615	17.4887 ± 5.2284
t = 30	2.0683 ± 0.2037	19.2572 ± 1.8968
t = 45	2.1141 ± 0.4557	19.6845 ± 4.2425
t = 57	2.0026 ± 0.5159	18.6460 ± 4.8036
	Average 2.0158 ^(b)	Average 18.7691 ^(b)

(a) Mean ± s.d., N = 3.

(b) Average value of data from t = 15, 30, 45, 57 days.

after 9½ months at all depths. Higher ratios still remained in the upper 2 cm of sediment.

Retention of Hydrocarbons in Sediments from a Short-Term Recruitment Field Experiment

In a previous long-term field study of the recruitment of benthic animals as a function of petroleum hydrocarbon concentrations in sediment, few significant differences in organism populations in clean and oiled substrate were observed. In a system in which oil was layered on the sediment surface, rapid depuration of the oil from the sediment could have been a factor in these observed results. Some differences in recruitment did occur in the field study at some time interval up to two months during which the oil concentrations were higher. Population data were not available for this point in the experiment, so a short-term field experiment was conducted this year to fill this gap and to provide short-term recruitment and chemical data.

The concentrations of individual saturate and aromatic hydrocarbons from sediment cores derived from this experiment as a function of time were determined. As in the previous study, docosane, naphthalene and phenanthrene were selected as representative saturate and aromatic components of oil, and their rate of depuration in the sediment was determined over a period of two months. Results indicated the losses of these components to occur at about the same rate as previously reported in the long-term field study, with only 3.7, 0.7 and 0.9% of the original docosane, naphthalene and phenanthrene, respectively,

remaining in the surface portion of the sediment after 29 days of exposure in the beach environment. Similar trends were found for three representative compound classes; only 3.0, 2.3, and 3.2% of the saturates, methyl-naphthalene and methylphenanthrenes remained after the same period of time (Figure 7). The results were in excellent agreement with observations of depuration from total oil analyses conducted on the same samples.

In order to obtain information on the ability of microorganism populations residing in Sequim Bay sediments to degrade aromatic hydrocarbons, we conducted a laboratory experiment employing C_{14} -phenanthrene amended to sediment. The experiment was designed to provide preliminary information on the possible formation of metabolic products and the extent of mineralization of aromatic hydrocarbons.

FIGURE 7. Concentration of compound classes in sediments as a function of depuration time. Standard deviations indicate analytical procedural error.

Mineralization of C_{14} -phenanthrene by Microfauna in Sequim Bay Sediments

The time required for a marine community to recover from the impact of an oil spill depends on the level of activity of various dissipative factors. These factors include evaporation, photo-oxidation and biodegradation. A factor influencing the recovery of oil-contaminated sediments is the ability of the natural population of microorganisms to degrade components of the oil. Aromatic hydrocarbons constitute a significant portion of Prudhoe Bay crude oil and have been shown to produce both acute and chronic effects in marine organisms. Prolonged retention of such components in sediments have the potential for producing deleterious effects on benthic species.

The extent of mineralization of C_{14} -phenanthrene by microorganisms from Sequim sediment samples is shown in Table 6. These data indicate that the production of CO_2 during the experiment was essentially complete within 10 to 20 days after initiation of the experiment. The mean average conversion of C_{14} -phenanthrene to $^{14}CO_2$ was $18.68\% \pm 3.65$. Control experiments demonstrated a substantial loss of C_{14} -phenanthrene through volatilization in the first 24 hr (60 to 75%). Assuming that 50% of the phenanthrene was metabolized by microorganisms, and taking into account the loss of phenanthrene in the first 24 hr, the recovery of $^{14}CO_2$ in this experiment fell within that expected for microbiological degradation.

C_{14} activity associated with the ethylacetate:acetone phase of the 15-day sample was shown to be entirely C_{14} -phenanthrene. However, analysis of the water phase revealed the presence of two major metabolic products. The relative abundance of these components appear to change with time (Table 7). Other metabolic products appear to be present, but in much lower concentrations. The chemical structures of metabolites A and B have not been determined; however, they are probably of CIS-dihydrodiol or phenol types found for bacterial strains capable of co-metabolizing phenanthrene. Attempts will be made in the future to identify these components.

We are currently developing a static system which would maintain a constant concentration of phenanthrene in the reaction vessel, thereby minimizing volatilization losses of phenanthrene. Two seven-day experiments employing the above system are planned for the future, using C_{14} -phenanthrene with and without Prudhoe Bay crude oil. Rates of mineralization will be determined as well as the rates of formation of major metabolic products. Comparisons of the rates between these two systems will be made in order to determine the effect that the oil has on the rates of metabolism of C_{14} -phenanthrene.

TABLE 7. Distribution of ^{14}C Activity Between Ethylacetate:Acetone Phase and Water Phase of Incubated Samples

Activity	Day 15	Day 30
^{14}C Activity (DPM) in Ethylacetate:acetone phase(a)	219,544 $\pm$ 42,822	152,969 $\pm$ 31,235
^{14}C Activity (DPM) in Water Phase(b)	154,688 $\pm$ 29,025	85,705 $\pm$ 10,816
Total ^{14}C Activity (DPM) Recovered(c)	374,232 $\pm$ 39,614	238,674 $\pm$ 42,026
^{14}C Activity (DPM) in Metabolite A	83,234 $\pm$ 1,913	40,381 $\pm$ 11,442
^{14}C Activity (DPM) in Metabolite B	21,379 $\pm$ 4,752	22,784 $\pm$ 3,106
A/B	3.89	1.77

(a) This radioactivity is associated almost completely with ^{14}C -phenanthrene.

(b) This radioactivity is associated almost completely with ^{14}C metabolic products.

(c) Total ^{14}C -phenanthrene at initiation of experiment = 1 μCi = 2.22×10^6 DPM.

● Fate and Effects of Petroleum Hydrocarbons in the Coastal Marine Ecosystem*

Principal Investigators: J. W. Anderson, W. H. Pearson and R. G. Riley

It is important to understand the alterations of hydrocarbon component composition of oiled natural sediments as a function of time and depth. We have determined the length of time required to depurate the majority of oil from sediments in the natural environment of Sequim Bay. We have also shown that alterations in component composition, including the ratios of n-C17/pristane and n-C18/phytane, occur more rapidly at the surface. In order to relate the form of hydrocarbon contamination to the effects on marine organisms, we have compared the hydrocarbon uptake rates of a benthic amphipod (*Anonyx laticoxae*) exposed to oil in a static water system, flowing sea water, and oiled sediments. These results provided estimates of hydrocarbon fluxes from the different exposures and, therefore, are indicative of the relative hazards of three types of environmental exposures. Field experiments with the Dungeness crab (*Cancer magister*) and the clam (*Protothaca staminea*) have shown that the clams in oiled substrate do not burrow as deep as those in clean sand. Therefore, they are more readily uncovered and eaten by the crabs.

Methodology for Isolation and Quantitation of Saturate and Aromatic Hydrocarbons from Oil-Contaminated Sediments

A method has been developed for the isolation and quantitation of saturate and aromatic hydrocarbons from oil-contaminated sediments, regardless of sediment type. Average recoveries of selected saturate and aromatic hydrocarbons using the method ranged between 55 and 87% among the three sediment types. The method was applied to the analysis of hydrocarbons at various depths from core samples taken from a field study.

Saturate and aromatic hydrocarbons in oil-contaminated sediment had similar depth profiles after 3½ and 9½ months of exposure to the beach environment. These compounds depurated most rapidly from the upper two centimeters. However, approximately 50% of the total selected saturate and aromatic hydrocarbons present in the sediment after 3½ months of exposure still remained at 9½ months at a depth of 5 to 6 cm. These results suggest that care must be taken in correlating effects of petroleum hydrocarbons with a biological effect such as recruitment if such hydrocarbon concentration gradients are occurring with time within the sediment. The n-C17:pristane and n-C18:phytane ratios decreased with increasing depth, suggesting biodegradation of these

compounds down to 6 cm in these medium-to-coarse sediments.

Effects of Surface-Oiled Sediment on Benthic Recruitment

We conducted a short-term field study to determine the effects of surface-oiled sediment on the recruitment of benthic organisms. The depuration of petroleum hydrocarbons occurred at rates similar to those reported for hydrocarbons in a long-term field study. Only about 4% of the original oil (studied by infrared analysis) remained in the surface portion of the sediment after 29 days of exposure. This finding and those on the retention of hydrocarbons as a function of sediment depth show that it would be possible for organisms to colonize this substrate as early as one month after a spill. It is not surprising that surface oiling did not interfere with recruitment during a long-term study. When oil is mixed into sediment, the surface layers also lose hydrocarbons rapidly, so recruitment might be dependent upon the depth at which organisms reside.

Comparative Uptake of Naphthalenes by Benthic Amphipods

An important aspect of tissue hydrocarbon accumulation is the uptake of hydrocarbons under different exposure

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conditions, including exposure to oiled sediment. The data generated in this research relate to the prediction of fluxes of specific hydrocarbons in the water column, sediments and tissues after releases of oil to the marine environment. It is important to understand the magnitude of uptake by organisms from various routes as well as the retention time of contaminants in tissues and sediments. The exposures used in our experiments simulated three different types of environmental conditions which organisms might encounter. The static system produced an initially high level of exposure which decayed with time as a result of differential solubilities and volatilities (spill situation). Flowing exposures to low concentrations might replicate conditions in certain areas following a large spill or, more likely, simulate chronic releases from refineries or platforms. Sediment exposures could result from either specific large spills or the buildup of contamination in harbors from multiple sources.

Table 8 compares the results of the several experiments conducted on the amphipod *Anonyx laticoxae* used as food by fish. In the static system, those amphipods exposed for relatively short periods (5 to 30 hr) contained 30 to 50 times the concentration initially in the water. A portion of the contamination was due to naphthalene and the methylisomers. Presumably, as the amounts of these more volatile components in the

water decreased with time, the tissue eventually contained only dimethylnaphthalenes and, thus, the magnification factor decreased. These results differ with data on tissue content of the specific compounds under constant exposure conditions. Analyses conducted at PNL showed that the delivery system contained naphthalene at a level approximately equal to the total methylnaphthalenes and also equal to dimethylnaphthalenes. If partitioning of hydrocarbons from water to tissues were not dependent on chemical size and structure, then equal magnification of the parent compound and the two groups of isomers would be expected. However, the data show that there are greater contributions from the alkyl naphthalenes and particularly the dimethylisomers. There is strong evidence that under constant exposure an equilibrium in influx and efflux of the naphthalenes is reached at about seven days. The alkyl naphthalenes were the primary contributors to this threshold of contamination, which represented a magnification of about 1000 times.

Regarding sediment exposures, it is not possible to relate our findings to many other studies since few have incorporated detailed analyses of sediment, tissues and water. It is clear that we still have much to learn regarding the interaction of oil-contaminated sediment with benthic marine organisms. Actual bioavailability of petroleum hydrocarbons directly from sediment or detritus particles has not been definitively

TABLE 8. Comparative Amphipod (*Anonyx laticoxae*) Tissue Magnification from Exposure in Three Different Systems

Conditions	Concentration (ppm)		Time	Tissue Uptake		Primary Contaminants	Comments
	TH(a)	TN(b)		TN	Magnification Factor		
Static Water System	6	0.2	96 hr	2	10	DMN	
			5-30 hr	6-10	30-50	N,(c) MN,(d) DMN(e)	At early stages N and MN are still present.
Flowing System	.507	.031	96 hr	2	~100	MN, DMN	Where there is constant input some N and more MN are found in tissues.
	.507	.031	120 hr	2.5	~100	MN, DMN	
	.367	.022	7-27 days	20	~1000	MN, DMN	
Sediment Within Above	300	2	96 hr	8	4	MN, DMN	By 18 days only DMN present.
	(300)	(2)	96 hr	4	2	MN, DMN	

- (a) TH = total hydrocarbons
(b) TN = total naphthalenes
(c) N = naphthalene
(d) MN = methylnaphthalenes
(e) DMN = dimethylnaphthalenes

shown. There is no doubt that hydrocarbons from sediment reach organisms (magnifications of 2 to 4 times, Table 8) and, in some cases, produce mortality. At this point, it appears that waterborne hydrocarbons are a far greater hazard than those which are sorbed to sediment. When oiled sediment releases hydrocarbons to the interstitial water and the dilution factor is low (as in shallow water or an intertidal zone), the water column can sufficiently contaminate organisms to kill them or to cause tissue accumulation. Oiled sediments will have the most dramatic and long-lasting effects in marshes, shallow estuaries, and regions with slow flushing rates. A great deal of information remains to be gathered regarding the retention times and bioavailability of specific petroleum hydrocarbons sorbed to sediments during spills.

Effects of Oiled Sediments on Benthic Predator-Prey Relationships

Field experiments on behavior were designed to assess the effects of oiled sediment on the predator-prey relationship between the Dungeness crab (*Cancer magister*) and the littleneck clam (*Protothaca staminea*). The rate at which crabs consumed buried clams

was measured in field enclosures. In a shallow subtidal area of Sequim Bay, four trays of oiled sand (1500 ppm) and four of clean sand were set into the substrate and covered with cages. After clams marked for size had buried, one crab was placed in each enclosure. Divers observed daily the numbers and sizes of clams dug up and consumed. The first of two experiments ran 10 days; the second, 30 days.

The crabs dug up more clams than they consumed. They consumed more small clams (25 to 45 mm) than large ones (46 to 65 mm), and two to three times the number of clams in oiled sand as in clean sand. The clams buried shallowly in the oiled sand, and they tended not to rebury quickly after being unburied; this behavior made them easier prey.

One obvious implication of this shift in the predator-prey relationship is that oiling of the sediment would probably make littleneck clams, particularly small ones, much more vulnerable to crab predation. In addition, crabs consuming easily obtained clams from oiled sand would probably take up more petroleum hydrocarbons, and their meat could possibly be tainted.

• Effects of Petroleum Hydrocarbons on Behavior of Marine Organisms*

Principal Investigators: W. H. Pearson and S. E. Miller

The behavioral effects of petroleum hydrocarbons have been studied in the blue crab (*Callinectes sapidus*) and the hake (*Urophycis chuss*). The blue crab detected the water-soluble fraction of Prudhoe Bay crude oil at 10^{-6} mg/l, a concentration well below those expected in oil spills. For the blue crab and the hake, the extent of avoidance of oiled sediment appears to be a function of life stages, social density, oil concentration, extent of weathering and other factors. Hake will enter highly oiled areas to obtain shelter.

This study is conducted in cooperation with Bori L. Olla, Chief of Behavioral Investigations at the National Oceanographic and Atmospheric Administration's National Marine Fisheries Service Sandy Hook Laboratory in Highlands, New Jersey. We are studying the effects of petroleum hydrocarbons on the behavior of the blue crab (*Callinectes sapidus*) and the hake (*Urophycis chuss*). Initial work was on chemoreception and the ability of the organisms to detect hydrocarbons. We are presently investigating the factors influencing avoidance of oiled sediment.

The antennules are the site of distance chemoreception in crustaceans; detailed observation of the blue crab's antennular behavior was therefore used to measure threshold concentrations for detecting petroleum hydrocarbons. The blue crab detected naphthalene at 10^{-7} mg/l and the water-soluble fraction of Prudhoe Bay crude oil at 10^{-6} mg/l. In comparison, the crab detected a clam extract at 10^{-12} mg/l. The water-soluble fraction detection threshold was well below the concentration of petroleum hydrocarbons found during an oil spill and in chronically polluted areas. The crab therefore appears quite capable of detecting oil at real-world concentrations. A baseline study with the hake has established behavioral criteria for detection and determined the detection threshold for a clam extract to be below 10^{-8} mg/l.

Long-term effects of oil spillage on organisms will probably derive from their contact with oiled sediment, and the blue crab and hake were used to compare behavioral responses to oiled sediment of a burrowing

crustacean and a sheltering demersal fish. Testing methods for both species were similar. In large chambers, the animals were presented with both clean sand and sand oiled with one of three concentrations (nominally 10, 100 and 1000 ppm). With the hake, shelter was variously arranged. The amount of time which the animals spent in various activities (such as walking or burying) in both clean and oiled areas were measured 24 hr a day for five days.

With the blue crab, the occurrence and extent of avoidance of oiled sediment varied with the animal's life stage, with the oil concentration, and perhaps, with weathering. Adult female crabs did not avoid oiled areas, but juveniles avoided oiled areas at concentrations higher than 10 ppm. Extent of avoidance appeared to decline with time, perhaps because of weathering of the oil.

Avoidance of the oiled areas by hake depended on sediment type and level but most importantly on shelter arrangement. When shelter was available in both oiled and clean areas, hake avoided oiled areas (Table 9). When shelter was available only in oiled areas, the fish either sheltered themselves in the oiled areas or swam almost continuously.

One major implication of the results of the avoidance studies is that unsophisticated avoidance experiments may not give answers applicable to real-world situations. Behavioral and ecological factors such as life stage, shelter requirements and social density can not only greatly influence avoidance but can in some cases reverse it.

* Environmental Protection Agency transfer

TABLE 9. Percentage of Fish-Hours Spent by Hake in Oiled Areas. Nominal oil concentration was 1000 ppm. Total fish-hours of observation was 160 per replicate. There were two replicates. Percentage of fish-hours swimming was for both clean and oiled areas and night and day. Values less than 50% in oiled areas indicate a preference for clean areas, with or without shelter.

Shelter Arrangement	Percentage of Fish-Hours in Oiled Areas			Percentage of Fish-Hours Swimming
	Day	Night	Day and Night	
Shelter in Both Clean and Oiled Areas	36.4	47.6	41.8	87.1
	41.2	37.5	39.6	47.2
Shelter in Clean Areas Only	4.5	7.0	5.8	53.8
	23.2	31.2	27.5	66.7
Shelter in Oiled Areas Only	72.6	84.9	78.3	56.5
	22.7	19.0	20.9	90.6

o Environmental Impacts of Outer Continental Shelf Petroleum Development

Principal Investigators: P. J. Mellinger, J. W. Anderson and J. R. Vanderhorst

Significant changes in the patterns of the nation's production and consumption of energy are likely to occur during the remainder of this century. The potential environmental impacts of these changes will vary from region to region and from site to site within regions. Mitigating strategies aimed at reducing adverse impacts may be implemented more efficiently and effectively when federal, state and local officials understand the magnitude and significance of these changes. This study, sponsored by the Technology Assessment Division of DOE's Office of Technology Impacts, is intended to influence decision-making and policy formulation.

In general, published information was collected, assembled and reviewed for this study. These were our observations and conclusions:

- Components of the routine discharges to the marine environment from outer continental shelf (OCS) development include metals in produced water, drilling muds, and borehole cuttings and chemicals in drilling mud. Hydrocarbons and oils are discharged to the oceans from ships' bilges and produced water and to the atmosphere as combustion products from ships and platform power generation sources and as evaporation products from

offshore and land-based storage tanks. The number of discharged species is large and they occur as dissolved species, or as suspended or settleable soils.

- Offshore technologies do exist to assure that the concentrations of discharged pollutants to the oceans and atmosphere be kept to a level that complies to regulatory limits.
- Where sufficient effluent data exist, we conclude that long-term ecological effects from routine discharges have not been detected and appear not to occur.

7 Statistical Development of Field Research

STATISTICAL DEVELOPMENT OF FIELD RESEARCH

- **Methodology for Collecting and Interpreting Ecological Data***

During recent years, litigation during environmental impact proceedings, the enforcement of existing environmental standards, and the need for additional environmental regulation has shown that present techniques for collecting and interpreting ecological data are not supported by adequate sampling and statistical methodology. The need for quantitative methodology suitable for assessing the fate and effects of environmental contaminants has become self-evident. Moreover, the myriad of environmental impact studies now extant clearly indicate that existing quantitative methodology is not useful in detecting population effects. Our project contains both basic and applied research; we evaluate available data and develop additional quantitative methodology for environmental regulatory functions and for setting environmental standards.

Our research on environmental sampling has focused principally on the analysis of variance in which log-transformed data obtained from inappropriately-designed studies are applied to detecting the impact of an electrical power plant. This extension of our previous work seems an effective way to detect impacts on organisms of lower trophic levels. We have proposed a plan for quantitative and qualitative studies on the more difficult task of assessing future population effects on upper-trophic-level aquatic organisms. In a related effort, we published our findings on how estimates of the variability for indices of population abundance can be used to calculate sample sizes for field studies dealing with environmental impact. Both line- and strip-transect methods were investigated to provide other quantitative tools.

Future research in support of the environmental regulatory process will be initiated to improve the efficiency of field measurements required for enforcement and thereby reduce costs. A second aim is to improve the accuracy and reliability of field data so that they are scientifically acceptable, and useful in the decision-making process. To accomplish this the following three tasks have been identified: 1) to document sampling designs whereby the levels of contaminants in biological systems can be better quantified, 2) to improve techniques for measuring the abundance of natural populations and 3) to develop more effective methods of assessing environmental contamination in free-living populations and the surrounding abiotic environment. Special emphasis will be on quantitative procedures for studying patterns of contaminant distribution, for comparing different contaminated areas and for fitting models to field data.

*This effort was transferred to the Environmental Protection Agency on October 1, 1979.

• Methodology for Collecting and Interpreting Ecological Data

Principal Investigators: L. L. Eberhardt and J. M. Thomas

Technical Assistant: M. I. Cochran

The main goal of this program continues to be the development of quantitative ecological methods suited to real-world applications. Since the broad scope of energy technologies and associated regulatory processes tend to involve a wide range of species and environments, we have attempted to consider a similarly wide range of quantitative methodology. We consider several major topics, described in the paragraphs below. Detailed results for FY 1979 appear in fifteen recent publications.

One of our long-term projects has had to do with the statistical aspects of environmental contamination. We have developed a framework that encompasses four areas of sampling: 1) descriptive (largely the methodology of sample survey textbooks), 2) analytical (statistical analysis and comparisons of data), 3) sampling for spatial pattern, and 4) sampling for modelling. Much of the focus in the current year's effort has been on analytical sampling and has considered the necessary statistical basis for a field approach to comparisons between affected sites, use of control sites, and so on.

We have continued a long-term study of the problems of animal censusing. Several recent publications have been concerned with that problem, with a particular focus on the utilization of line- and strip-transect methods. Some firsthand censusing experience has been incorporated by participation in design and analysis of a large-scale aerial survey conducted by the National Marine Fisheries Service, and our further advice on aerial survey work has been sought by the National Academy of Sciences, through participation in a National Research Council committee.

We have made a significant entry into research on animal population regulation. This is one of the more difficult problems facing population ecologists and environmental "managers," in that the regulatory mechanism may well be able to compensate for some degree of damage due to environmental impacts of some installation or pollutant. Thus, responses may be nonlinear and very

difficult to detect and quantify. We have published a number of papers on these issues and participated in an international symposium in which several aspects of the problem were addressed. We have also participated in an ad hoc review of the issues bearing on the recently started harvest of krill (*Euphasia superba*) in the Antarctic, and note that the affected region encompasses about 10% of the earth's surface.

Various opportunities to study issues having to do with the subjects described above have come through our work on the Scientific Advisory Committee of the U.S. Marine Mammal Commission. Also, Dr. Eberhardt has recently been appointed to a National Academy of Sciences Committee concerned with problems closely comparable to those discussed above.

Our research on quantitative issues associated with environmental impact has identified the following six areas which need additional research (we have published, prepared, or submitted papers in the first five areas):

- improved methods of field design and statistical analysis
- the use and basis of simulation models for assessing ecological effects
- the need for effects assessments based on field data, at least for lower trophic levels
- methodology appropriate for assessing future population effects, particularly at higher trophic levels (i.e., salmon, striped bass, clams, and oysters)

- the use of monitoring data in impact assessment
- the place and use of quantitative data in the judicial, hearing, and intervenor process as related to environmental impacts.

In order to begin addressing the future population effects question, we have devised

a preliminary plan to assess the biological effects of power plants on fish populations. The essence of the plan is outlined in Figure 1. It entails the derivation of a model which is periodically modified during the course of field monitoring studies initiated during a preconstruction or preoperational period. A negotiating committee will assure good quality and appropriateness of the studies.

FIGURE 1. A Procedure for a Quantitative/Qualitative Assessment of the Effects of Waste Heat on a Fishery

The advantages of our plan are sixfold:

- 1) Field monitoring will be terminated after an agreed time unless studies that have been conducted are inadequate.
- 2) Imaginative scientists, currently supervising routine monitoring, can design and conduct studies that investigate key questions in population ecology.
- 3) Both statisticians and modelers have the opportunity to suggest or institute new field work.
- 4) The use of litigation to establish and assess the adequacy of current monitoring programs, where the burden of proof is on the utility, is by-passed. The aim is to obtain a "reasonable resolution" on a site-by-site basis.
- 5) Regulators evaluate more meaningful data and may participate in the research process.
- 6) The public interest is protected.

Specific features considered in this plan include 1) the amount of population decline which might be ecologically acceptable 2) the problems in defining precision needed for measuring crucial parameters, 3) the problems which could result during frequent meetings of a negotiating team over many years, 4) equalizing monitoring costs among power plant sites and 5) the inclusion of fossil-fueled utilities and other users of a common resource in the cost-sharing.

The plan and ideas concerning its implementation are preliminary. The purpose of the plan is to initiate a dialog to begin a change in how power plant monitoring programs are now conducted. The practicality of some of our suggestions may be questionable. Clearly, the constraints from legal and interest groups on any change are real. However, change is needed. The direction of any change can be debated. The aim of our plan is to initiate the debate.

We have also begun a small project designed to assess the utility of multiple regression as a quantitative tool for analyses of ecological data sets. We plan to attack the problem via computer simulation and have completed a portion of the programming this past fiscal year. In another computing

effort, we are attempting to develop an algorithm which allows the analysis of laboratory studies via "randomization tests." This intuitively simple but time-consuming procedure does not require the restrictive assumptions of most statistical procedures. Finally, we have succeeded in modifying input routines for a file manipulation system (PDP 11/70) which allows us to accomplish information retrieval of specialized quantitative bibliographic files without the aid of a skilled system programmer. The results of this work are being presented at the Fall (1979) DECUS Symposium.

Two papers have been submitted as a result of our collaborative efforts this past year, both of which can aid in the regulatory process. The topics of uptake and retention of heavy metals by aquatic plants and the retention and translocation of plutonium in terrestrial plant systems were addressed.

In collaborative work with the Biology Department, we investigated the similarities and difficulties in the standards-setting process (Figure 1) or accidental release situation (Figure 2) for protection of both the ecosystem and human populations. In both figures, similarities are depicted in heavy-bordered boxes, while light-bordered boxes illustrate places in each process where major differences exist. The two most striking differences evident when standards are set for ecological protection compared to biomedical effects are 1) the wide range of organisms which must be tested during acute and subchronic evaluation *vis a vis* the laboratory rat and mouse and 2) the complexity of extrapolating laboratory toxicity results to an interacting ecosystem. To assess the probable effects of an accidental release (Figure 3), the data and process for devising standards are used in conjunction with computer models of various transport processes. An added complexity for estimating environmental effects is the necessity to devise a scheme to assess effects on subsequent populations of various species. The experience of resource managers suggests that this is most difficult to assess for hunting, fishing and other kinds of harvests or uses of "environmental resources." The effects of pollutants on the future of the same systems will be difficult if not impossible to assess. Other aspects of environmental protection were discussed in June during the meeting of the Western North American Region of the Biometrics Society.

FIGURE 2. Assessment of Acceptable Biomedical and Ecological Effects (Standards)

FIGURE 3. Calculation of Likely Environmental or Human Effects (Accidental Release)

8 Nuclear Fusion

NUCLEAR FUSION

- **Ecological Effects of Lithium and Beryllium on Aquatic Communities**
- **Sublethal Effects of Tritium on Aquatic Systems**
- **Teratological Effects of Low-Level Magnetic Fields**

It is possible that, with increasing applications of nuclear energy, greater quantities of potentially harmful radionuclides will be released to the environment. This may be particularly true in the use of advanced reactor designs such as the fusion reactor. In this design, radionuclides, principally tritium, may escape from the plant in both gaseous and liquid effluents in quantities significantly greater than for present pressurized-water reactor or boiling-water reactor designs. Foreseeing such contingencies, the research programs described herein respond to the need to measure the potential radiation effects of tritium releases on individuals and ultimately on populations and biotic communities. As a first approach, our efforts are directed toward determining effects of low-level chronic exposures on developing embryo and larval stages, which are clearly the most sensitive.

The anticipated increase in the release of beryllium and lithium from mining, refining and fabrication of materials used during construction of fusion reactors may also have adverse effects on the environment. Accordingly, our fusion-related research during FY 1979 included efforts to study the metals in living organisms and to determine at what levels toxicity may be expected. The effects of lithium were assessed in embryo and larval stages of rainbow trout (*Salmo gairdneri*) as well as on the production and biomass of benthic microflora and macrofauna communities.

Our fusion-related research also included preliminary experiments on the effects of low-level magnetic fields. It is conceivable that magnetic fields of 70 to 450 gauss will be encountered by attendant personnel working in the transport and hot cell areas of fusion reactors. Also, personnel assigned to the areas immediately surrounding the reactor may be exposed for substantial durations to field strengths of 1 to 70 gauss. The current PNL program is aimed at developing different measurement end-points for the early detection of developmental and adult chronic effects. Work involving mammalian tissue and cell systems is reported in PNL-3300, Part 1, Biomedical Sciences; work involving nonmammalian animal systems is reported here. Dr. Dennis Mahlum is coordinator for the comprehensive program. The approaches described here utilize embryo and larval stages of aquatic organisms which should provide meaningful data applicable to ultimate assessment of effects in life processes of higher vertebrates.

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- Work order agreement

- **Sublethal Effects of Tritium on Aquatic Systems**
- **Ecological Effects of Lithium and Beryllium on Important Aquatic Organisms and Associated Communities**
- **Teratogenic Effects of Low-Level Magnetic Fields**

Principal Investigators: J. A. Strand and R. M. Emery

Other Investigators: M. P. Fujihara, T. M. Poston, D. C. Klopfer, R. G. Genoway, J. R. Skalski, C. S. Abernethy, J. C. Montgomery and W. G. Woodfield

Continuing studies of the sublethal effects of tritium on freshwater species emphasize the potential for genetic transmission of suppressed immune competence in offspring of parental rainbow trout (*Salmo gairdneri*) exposed to tritium (0, 0.04, 0.4, 40.0 rads) during embryogenesis. We plan to determine the relative biological effectiveness of tritium beta irradiation when compared to ⁶⁰Co gamma irradiation using the relatively radiosensitive immune process of rainbow trout. During FY 1979, we concluded genetic effects studies and prepared a manuscript for open literature publication summarizing FY 1977 and FY 1978 studies on the permanence of suppression of the primary immune response in rainbow trout sublethally irradiated during embryogenesis.

We are also studying the potential effects of beryllium and lithium on aquatic systems. Because of mining and refining subsequent to the use of these metals in the construction of fusion reactors, increased levels of each are likely to be encountered in surface waters. Studies included an evaluation of potential toxicity of lithium on embryological life stages of rainbow trout and an assessment of fate and effects in artificial stream habitats. Levels of lithium necessary to cause an observed effect would have to be at least three orders of magnitude above observed background.

Studies initiated in FY 1978 include evaluation of effects of low-level magnetic fields on embryologic development of rainbow trout. The objective of these studies to provide data useful in assessing potentially harmful effects of low-level magnetic fields encountered by attendant personnel working in the transport and hot cell areas of fusion reactors. This approach is less costly than using mammalian systems, provides large numbers of experimental organisms for meaningful statistical analysis and permits examination of potential latent effects in a representative vertebrate.

SUBLETHAL EFFECTS OF TRITIUM ON AQUATIC SYSTEMS

Genetic Effects Studies

Genetic effects studies first undertaken in FY 1978 were directed toward determining the potential for genetic transmission of suppressed immune competence to offspring from parental rainbow trout sublethally exposed to ³H₂O (0, 0.04, 0.4, 4.0, and 40.0 rads) over 21 days of embryogenesis. We had previously demonstrated that the primary im-

mune response of parental fish was significantly suppressed and permanently altered at doses as low as 4.0 rads.

Offspring of each test cross (all test crosses were conducted within respective treatments) at five months of age were intraperitoneally injected with 0.1 ml of heat-killed antigen (1.8×10^8 cells/ml *Flexibacter columnaris* in 25% Freund's incomplete adjuvant). A 0.1-ml sham vaccination (saline adjuvant) was similarly administered to a second group of control fish.

One week before vaccination, three weeks after vaccination, and at bimonthly intervals thereafter, a standard tube agglutination test for the specific antigen of vaccination was performed on the serum from 20 fish from each of the control and treatment test groups. Additional agglutination tests were performed when offspring were 12 months old.

Tritium-irradiated and control treatment groups were arranged among the available compartments of three concrete troughs according to a completely randomized block design

(three blocks, five treatments). The data were treated by analysis of variance. All experimental groups sampled one week prior to vaccination failed to demonstrate positive agglutinins to *F. columnaris* at the lowest test dilution.

A clearly observable and statistically significant change in mean titer values occurred during the 11-week sampling period following vaccination (Figure 1). However, significant differences in mean titer values among the five groups of offspring at each

FIGURE 1. Mean Titer Values of Offspring for Specific Agglutinins to *Flexibacter columnaris* at Each Sampling Interval

sampling were not demonstrated. Serum agglutinins in sham-injected control fish were mostly nondetectable. Although a small percentage of the sham-injected fish demonstrated a positive titer, mean values of all positive titers were significantly lower than mean values of all positive titers in the control groups injected with antigen and adjuvant.

All experimental groups sampled 12 months after vaccination still demonstrated positive agglutinins to *E. columnaris*; but, as before, statistically significant differences in mean titer values among the five groups of offspring at each sampling were not detected. Accordingly, it could only be concluded that under the described experimental conditions suppressed immune competence as mediated by early radiation injury was not genetically transmitted.

Publications Activity

A manuscript for open literature publication summarizing studies on the permanence of immune suppression in rainbow trout following early radiation injury was prepared. Upon completion of internal review, it will be forwarded to Radiation Research.

Future Studies

In FY 1979 a proposal was prepared and forwarded to the Division of Health Effects Research with the objective of determining the relative biological effectiveness (RBE) of tritium beta irradiation when compared to ⁶⁰Co gamma irradiation applying the relatively radiosensitive immune process of the rainbow trout.

Much experimental work has been devoted to determining whether the high linear energy transfer of the tritium beta particle is reflected in an increased RBE when compared with gamma- or X-irradiation. The International Commission on Radiological Protection recognizes the RBE for tritium to be unity. More recently, however, there has been evidence to suggest a higher value is justified. Of particular note are the studies at Lawrence Livermore Laboratory on the survival of female germ cells in juvenile mice exposed to a continuously maintained level of tritium oxide in body water^(a,b). These studies would indicate an increasing RBE with longer exposure, with the suggestion of an RBE of 3 or 4 at very low doses (<20 rad). This question is of practical importance because tritium is, and probably will continue to be, a major contributor in the effluents from nuclear power plants, both fission and fusion designs. The radionuclide is not easily removed by

conventional waste treatment practices. There is, therefore, increasing concern as to the ultimate concentration in, and the effect upon, biological systems. Furthermore, likelihood of tritium having increased biological effectiveness, particularly over extended low-level exposures, warrants careful examination.

ECOLOGICAL EFFECTS OF LITHIUM AND BERYLLIUM ON IMPORTANT AQUATIC ORGANISMS AND ASSOCIATED COMMUNITIES

The purpose of this study is to determine the ecological significance of elevated concentrations of lithium and beryllium in fresh water. The objective for this year was to identify parameters in freshwater biota that are perturbed by lithium and to relate the perturbations to the concentrations at which they were observed.

Observations to date indicate that lithium concentrations of >100 mg/l reduce fertilization of rainbow trout (*Salmo gairdneri*) eggs by >50%, completion of embryogenesis by >95%, and hatching success by >95%. Lithium concentrations of >10 mg/l caused >95% mortality in rainbow trout sac fry exposed during embryogenesis. Concentrations of >5 mg/l caused 95% mortality in trout alevins after four days of exposure, and >3 mg/l caused 95% mortality in rainbow trout fingerlings after the same exposure period. For juvenile rainbow trout, we observed that >2 mg/l caused >90% mortality after 10 to 30 days of exposure.

In 14-day exposures of naturally occurring Columbia River periphyton communities to lithium, we observed that >25 mg of Li⁺/l reduced primary production by >95% and total biomass by >50%. In addition, the habitation of chironomid (midge) larvae living among the periphyton was reduced by >80% at lithium concentrations of >60 mg/l.

A review of the literature indicates that natural background ranges of lithium in most freshwater systems occur from 0.0001 to 0.001 mg/l, but may be as high as 0.1 mg/l in lakes and streams with higher salinity, and sometimes exceeding 1.0 mg/l in hot

- (a) Dobson, R. L., J. H. Arrington and T. C. Kwan. 1975. Tritium Toxicity: Increased Relative Biological Effectiveness of ³HOH with Protraction of Exposure. DOC. UCRL-76558.
- (b) Dobson, R. L., and T. C. Kwan. 1976. "The RBE of Tritium Radiation Measured in Mouse Oocytes: Increase at Low Exposure Levels." Radiation Research 66:615-625.

springs and salt lakes. Thus far, investigations lead us to a tentative conclusion that lithium concentrations in most fresh-water systems would have to be increased by at least three orders of magnitude to cause biological effects.

TERATOGENIC EFFECTS OF LOW-LEVEL MAGNETIC FIELDS

Influence of Magnetic Fields on Development of Rainbow Trout (*Salmo gairdneri*)

Previous studies suggested that fertilization, development and hatchability of rainbow trout eggs could be affected by exposure to magnetic fields. Additional studies were performed to confirm these results and to examine the relative susceptibility of sperm and egg to magnetic fields. Preliminary results and their interpretation are presented here for two different experimental approaches.

In the first approach, previously used, approximately 4,500 eggs were exposed from fertilization through 21 days in an oscillating field (2,800 to 11,200 gauss within a 1-min period) of a Varian Model V-3603 electromagnet. An additional 4,500 eggs maintained outside the magnetic field served as controls.

Incubation chambers for exposed and control embryos were identical. Subsampling of 150 embryos from both exposed and control chambers was conducted on days 7, 14, and 21. At 21 days (eyed stage), the embryos were transferred to standard drip incubators where hatching occurred in 28 ± 1.5 days.

The fry were transferred to standard hatchery troughs two weeks after hatching to detect potential latent mortality and impaired growth. A chi-square test statistic was applied to each index of effect to determine differences between exposed and paired control groups. Evaluation of each index was conducted "in the blind" to decrease the possibility of investigator bias.

Table 1 presents results for three distinct stages of development, which include 1) fertilization, 2) hatching at 28 days, based on fertile embryos and 3) survival of hatched fry through 10 weeks. Fertilization success was higher in the exposed group than in the control group. No significant differences were detected for either percent hatchability or percent survival of hatched fry between exposed or control groups.

Results of subsampling are presented in Table 2. Combining all data for 7, 14 and 21 days indicated no significant difference in fertilization success between the exposed and control group, a result that contradicts enumeration of nonsubsampled embryos. Subsampling on day 7 revealed a higher incidence of abnormal embryos in the control group; however, this observation was not confirmed by subsampling at 14 and 21 days.

A second approach was designed to examine the fertilization process itself and to determine the relative susceptibility of egg and sperm to magnetic field effects. Accordingly, approximately 5,000 eggs and 50 ml of sperm were separately exposed in a homogeneous 11,200-gauss magnetic field for 1 hr. A similar number of eggs and aliquot of sperm were maintained outside the magnetic

TABLE 1. Effect of Magnetic Exposure on Early Life Stages of Rainbow Trout

Condition	Number of Eggs	Number Sub-sampled	Percent Fertile	χ^2	Level	Percent Hatched	χ^2	Level	Percent Survival at 10 Weeks	χ^2	Level
Control	4511	754	85.23	—	—	99.31	—	—	99.40	—	—
Exposed	4388	749	89.42	29.28	(a)	99.48	0.72	(b)	99.72	3.76	(b)

(a)Significant χ^2 at 0.05 level

(b)Nonsignificant χ^2 at 0.05 level

TABLE 2. Effect of Magnetic Exposure on Incidence of Abnormal Embryos

Condition	Time, Days	Number Subsampled	Percent Fertile	χ^2	Level	Percent Abnormal	χ^2	Level
Control	7	149	93.29	—	—	10.79	—	—
Exposed	7	150	88.67	1.94	(b)	1.50	10.00	(a)
Control	14	155	87.74	—	—	0.74	—	—
Exposed	14	150	90.67	0.67	(b)	0.74	0	(b)
Control	21	150	84.00	—	—	0	—	—
Exposed	21	150	87.33	0.67	(b)	0.76	0.96	(b)

(a) Significant χ^2 at 0.05 level

(b) Nonsignificant χ^2 at 0.05 level

field. After exposure, eggs and sperm were placed in standard hatchery drip incubators in the following combinations: 1) egg unexposed, sperm unexposed; 2) egg exposed, sperm unexposed; 3) egg unexposed, sperm exposed and 4) egg exposed, sperm exposed. Subsampling was again performed on days 7, 14 and 21. Hatching occurred in 28 ± 1.5 days and fry were transferred to standard hatchery troughs two weeks after hatching.

Results (Table 3) suggested that there was a significant difference in fertility among the four treatment groups; however, again the lower percentage was encountered in the control group. While no significant difference in hatchability was found, these data suggested that survival through 10 weeks

was significantly greater in the control group. A homogeneity χ^2 test of all data ($p = 0.05$) supported these interpretations.

Results of statistical analyses to determine the relative influence of magnetic exposure on egg or sperm are shown in Table 4. Although these data suggested that magnetic exposure of either egg or sperm significantly influenced fertility and survival, the finding of a significant interaction diminished interpretation of main effects; that is, an additional influence on fertility and survival occurred that was not attributable to magnetic exposure of egg and sperm alone.

The findings reported in Table 5 suggested that a significant increase in the incidence

TABLE 3. Effect of Magnetic Exposure on Early Life Stages of Rainbow Trout

Condition	Number of Eggs	Number Subsampled	Percent Fertility	Percent Hatched	Percent Survival at 10 Weeks
Eggs/Sperm	2491	300	85.53	92.42	97.06
Eggs*/Sperm	2417	300	95.56	92.09	95.33
Eggs/Sperm*	2338	299	88.72	92.70	93.80
Eggs*/Sperm*	2509	300	95.29	92.16	95.77

*Gamete exposed to magnetic field

TABLE 4. Relative Influence of Magnetic Exposed Gamete on Fertility, Hatchability and Survival

	Fertility			Hatchability			Survival at 10 Weeks		
	d.f.	χ^2	Level	d.f.	χ^2	Level	d.f.	χ^2	Level
Main Effect, Eggs*	1	187.78	(a)	1	1.82	(b)	1	0.01	(b)
Main Effect, Sperm*	1	7.57	(a)	1	0.09	(b)	1	7.52	(a)
Interaction, Eggs*/Sperm*	1	8.44	(a)	1	0.02	(b)	1	8.34	(a)

(a)Significant χ^2 at 0.05 level

(b)Nonsignificant χ^2 at 0.05 level

*Gamete exposed to magnetic field

TABLE 5. Effect of Magnetic Exposure on Incidence of Abnormal Embryos, Day 21

Condition	Normal Development	Abnormal Development
Eggs/Sperm	60	4
Eggs*/Sperm	70	4
Eggs/Sperm*	61	2
Eggs*/Sperm*	63	11

*Gamete exposed to magnetic field

TABLE 6. Relative Influence of Magnetic Exposed Gamete on Incidence of Abnormal Embryos, Day 21

	d.f.	χ^2	Level
Main Effect, Eggs*	1	1.32	(b)
Main Effect, Sperm*	1	2.83	(b)
Interaction, Eggs*/Sperm*	1	4.10	(a)

(a)Significant χ^2 at 0.05 level

(b)Nonsignificant χ^2 at 0.05 level

*Gamete exposed to magnetic field

of abnormal embryos occurred in subsampling the group in which both egg and sperm were exposed to the magnetic field. A homogeneity χ^2 test of all data ($p = 0.05$) supported this interpretation. Although not shown, analyses of subsamples collected on days 7 and 14 revealed no significant differences among the four treatment groups. As shown in Table 6, there was also no effect on development from exposing egg or sperm separately.

9 Pumped Storage and Hydroelectric Development

PUMPED STORAGE AND HYDROELECTRIC DEVELOPMENT

- **Effects of Hydroelectric Generation on Riverine Ecology**

This section describes ongoing research on the effects of peaking power generation by hydroelectric facilities on aquatic ecosystems. Hydropower is attractive for peaking generation because 1) turbine output may be quickly adjusted to match demand; 2) smaller amounts of fuel are required to maintain a standby turbine than to maintain a “spinning reserve” at a thermal power plant; 3) hydropower is relatively inexpensive and 4) no severe penalty is imposed on station equipment by frequent cycling, as is the case for thermal plants. However, frequent adjustments in power production are reflected in fluctuating water flows below the station.

Changes in physical characteristics of aquatic habitats caused by fluctuating flows may impact production of downstream biota. The objective of the study reported here is to evaluate factors influencing the effect on ecosystem structure and production.

• Effects of Hydroelectric Generation on Riverine Ecology

Principal Investigators: C. D. Becker and D. H. Fickeisen

Other Investigators: D. A. Neitzel, J. C. Montgomery and S. A. Barraclough

NORCUS Appointee: J. Nelson

Hydropower generation of peaking electricity requires frequent changes in the amount of water released through dam turbines. The resultant variations in flow rate are directly related to the daily cycle of electrical demand. Changes in amount of water released at dams cause water levels to fluctuate downstream and affect water temperatures.

The objectives of this program are to examine effects of water level changes on downstream biota and to quantify ecological impacts to the fullest possible extent. Fluctuating water levels cause changes in wetted habitat areas. Ponds and pools in the shore zone are alternately flooded and drained; backwater slough areas are subject to a sudden influx of water as river levels rise.

Aquatic species dependent on shallow water habitats during part of their life cycles are impacted by hydropower generation. For example, the area available for spawning by fish that build redds or nests in shallow water (e.g., salmonids and centrarchids) may be limited. Draining habitats containing incompleting fish nests may discourage spawning above the minimum water level; should spawning take place at higher elevations, eggs and larvae may be lost as a result of dessication or because of adults' inability to guard nests from predators. Nursery grounds for many fish species (salmonids, centrarchids, catostomids, cyprinids, ictalurids) are located in shallow water where juveniles can be trapped in ponds as the water level falls. Subsequent draining results in death of fish from dessication or predation. Shallow ponds not drained may be heated by the sun above the upper tolerance limit of entrapped fish. Shallow-water benthos may also be affected by dessication, resulting in a limited supply of food organisms and, indirectly, reduced fish growth.

Our studies examined the reproductive success of smallmouth bass (*Micropterus dolomieu*) populations in the Hanford area of the Columbia River. We are planning comparable studies of chinook salmon (*Oncorhynchus tshawytscha*) spawning.

Water Level Monitoring

Water flow rates in the Hanford Reach are controlled by releases from Priest Rapids Dam and vary seasonally from a licensed minimum flow of 36,000 cubic feet per second (cfs) to a typical peak flow of 120,000 to 180,000 cfs. Vertical water level fluctuations up to 2 m occur daily in the river. We have obtained a mathematical model that predicts water level from releases at Priest Rapids Dam and accounts for bank storage. We plan to refine the model for application to slough areas that are important spawning and nursery habitats for fishes.

To estimate parameters and validate the model, we monitored water levels in the mainstem of the river and in sloughs. Monitoring was done with pressure-sensitive underwater radiotransmitters that report water depth data to a shoreline receiving station. We are now analyzing continuous recordings made at three stations throughout the spawning and larval development period of smallmouth bass (*Micropterus dolomieu*) in 1979. Separate instruments provide continuous temperature records at the same sites. Results indicate that solar insolation and water level fluctuations interact to produce daily temperature fluctuations of

about 10 C in slough areas during the late spring and summer. Surveys were conducted to establish elevations of the sloughs and calibrate depth data, and to estimate slough volume in order to model temperature fluctuations.

Smallmouth Bass Recruitment

Smallmouth bass (*Micropterus dolomieu*) spawn in sloughs along the Hanford Reach. Previous studies with tagged bass indicated that adults disperse widely, particularly downstream, after spawning. Thus, the sloughs may represent important habitats for the regional smallmouth bass population, and recruitment to the sport fishery may be largely dependent on spawning success of fish collected seasonally in the slough. Smallmouth bass nests were surveyed in their principal spawning sloughs; although many nests were constructed, relatively few eggs were found per nest. Daily temperature fluctuations from hydropower generation may restrict bass spawning. After a period of stable water levels in the slough, significant spawning of bass occurred. A few nests produced bass fry, many of which survived.

To assess effects of temperature fluctuations on development of bass larvae, we developed a laboratory unit to simulate the temperature fluctuations found in slough

spawning areas. We also developed methods for collecting viable bass eggs and transporting them to the laboratory. Eggs gathered from natural nests were hatched under constant temperatures. Eggs will be cultured under fluctuating temperatures next year to quantify effects of power generation accurately.

Salmon Redd Studies

We plan to begin experimental studies to clarify the effects of water level fluctuations on intragravel development of salmonid eggs in FY 1980. Our experimental design requires simulation of salmon redds in troughs filled with a mixture of sand and gravel and supplied with flowing Columbia River water. Fertilized eggs and/or fry will be inserted beneath the gravel via a standpipe and reared under varying drainage regimes.

Gas Bubble Disease Publication

We edited a special session for the Transactions of the American Fisheries Society to bring together recent work on effects of dissolved gas supersaturation and to update our 1974 symposium. About 20 submitted papers have been reviewed and submitted to the journal editor for publication.

10 Pathways Modelling, Assessment and Hanford Project Support

PATHWAYS MODELLING, ASSESSMENT AND HANFORD PROJECT SUPPORT

- o Waste Isolation Safety Assessment Program**
- o Hanford High-Level Waste**
- o Waste Management Safety Studies**
- o Assessment of Environmental and Safety Aspects for the Enhanced Recovery of Gas from Unconventional Sources**
- § Rockwell Support Studies**
- § Decommissioning and Decontamination (Burial Ground Stabilization) Studies**
- § Service Assessment Studies**

This section reports on related environmental studies organized in support of nuclear waste management responsibilities of the Rockwell Hanford Operations (RHO), the Office of Nuclear Waste Isolation (ONWI) or the Decommissioning and Decontamination program under direct DOE funding. These studies are designed to 1) ascertain the potential for uptake and transport of waste radionuclides away from shallow low-level waste burial grounds located on the Hanford Site, 2) to develop techniques for guarding burial grounds against biotic penetration, 3) to monitor over long intervals the several most important animals likely to be impacted by industrial development of the Hanford Site and 4) to provide dose calculations for safety assessment purposes for various options under consideration in nuclear technology development.

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- o Work performed by Ecological Sciences Department under intralaboratory work order**
 - § Intercontractor support study**

o Waste Isolation Safety Assessment Program

Principal Investigators: J. K. Soldat, B. A. Napier, D. L. Strenge and E. C. Watson

The Waste Isolation Safety Assessment Program (WISAP) is managed through PNL's Water and Land Resources Department and funded through the Battelle Office of Nuclear Waste Isolation (ONWI). The Ecological Sciences Department was involved in a subtask under the Release Consequence Analysis Task and two subtasks under the Dose Assessment Task.

Dose Calculation Methodology for Application to Site Analysis

During FY 1979 several computer codes available for calculation of aerial radiation doses to people were evaluated and those suitable for the WISAP project were modified and converted to the new DOE/RL 1100/44 computer. The codes now available are listed in Table 1. All of them have been documented except PABLM. The first draft of the documentation for this code was prepared in FY 1979.

The codes were used to calculate potential doses from consumption of salt obtained by solution mining of a salt dome. The potential radiation doses were significantly high and could be especially important for consideration in the ONWI program. A careful quality-assurance review of these results is planned for FY 1980. Our recommendation that doses not be calculated beyond 1000 years was accepted by WISAP and by ONWI. This recommendation concurs with current Environmental Protection Agency standards.

TABLE 1. Computer Codes for Calculating Aerial Radiation Doses

Code	Calculation
KRONIC	External exposure to gaseous effluent plumes: chronic releases
SUBDOS	External exposure to gaseous effluent plumes: accidental releases
DACRIN	Inhalation of airborne radionuclides: chronic or accidental releases
PABLM	A combination of the capabilities of two older codes: <ol style="list-style-type: none">1) ARRRG — doses from water pathways of drinking water, aquatic food and aquatic recreation2) FOOD — doses from terrestrial foods contaminated by deposition of either airborne or waterborne radionuclides

o Hanford High-Level Waste

Principal Investigators: J. K. Soldat, D. L. Strenge, B. A. Napier,
M. G. Zimmerman and W. E. Kennedy, Jr.

PNL is assisting Rockwell Hanford Operations (RHO) to prepare a programmatic environmental impact statement for management of Hanford defense waste. The Ecological Sciences Department is leading the task on calculation of radiation doses from a large matrix of potential routine and accidental releases of radionuclides. Included are releases at Hanford, releases at an offsite disposal site and releases during transportation. The FY 1979 work was organized into two phases—high-level waste and low-level waste. The principal effort in FY 1979 was on high-level waste.

High-Level Waste Dose Assessment Task

Methodology was developed for estimating the amount of land which might require control or decontamination because of contamination above specified limits following accidental releases of radionuclides at Hanford. Provisional limits were derived by calculating potential doses via food pathways and external exposure. The results of the calculations were published in a preliminary draft report. The report also included the results of calculations of potential doses from onsite and offsite transportation accidents.

Computer codes were modified to permit calculation of doses from many scenarios for onsite and offsite, chronic and accidental

releases. In addition, unit dose factors (rem/Ci released) for all pathways combined were calculated for use by RHO and HEDL with releases from waste processing alternatives.

Low-Level Waste Dose Assessment Task

Preliminary planning and dose code modification work was accomplished in FY 1979. Most of the low-level waste work was postponed until FY 1980 to allow more effort to be devoted to the higher priority high-level waste work. In FY 1980, several individual tasks will each require calculation of potential radiation doses; such as retrieval of waste from burial grounds, transport and processing of low-level waste, and potential future doses incurred by leaving the waste where it is.

o Waste Management Safety Studies

Principal Investigators: P. J. Mellinger, G. R. Hoenes, L. W. Brackenbush and J. Greenborg

Work conducted by the Ecological Sciences Department for the Waste Management Safety Studies consisted of a task on the management of ^{85}Kr .

Krypton-85 Management Trade-Offs: A Perspective to Total Radiation Dose Commitment

Until now, all of the ^{85}Kr from nuclear fuel reprocessing has been intentionally released to the atmosphere; however, federal regulations for commercial fuel irradiated after 1982 will require collection and long-term storage of about 85% of the ^{85}Kr previously released during reprocessing.

In this project, we compare individual and population radiation doses from the presently accepted practice of total release of ^{85}Kr with doses resulting from the operation and maintenance of krypton recovery, transportation and storage operations required to comply with the forthcoming federal regulations. Results indicate that it makes little difference to the world population dose whether ^{85}Kr is captured and stored or chronically released to the environment.

o Assessment of Environmental and Safety Aspects for the Enhanced Recovery of Gas from Unconventional Sources

Principal Investigators: E. F. Riedel, D. L. Brenchley, P. J. Mellinger, L. J. Ethridge

The purpose of this project is to identify and evaluate known, potential, and heretofore unknown environmental and safety concerns associated with the recovery of natural gas from unconventional sources (GUS). Results of this study will be used by DOE to evaluate the commercialization of this important resource.

Efforts during FY 1979 were focused on tight western sands and methane associated with coal seams. We assessed the environmental, health and safety problems of the commercialization of obtaining natural gas from these unconventional sources. We found environmental, health and safety problems to be minimal. They will present no obstacles

to the commercialization of these resources. The major environmental concerns are road construction, well pad construction, drilling pits and diesel engine exhaust. The most serious occupational safety problems are noise (in excess of 100 dBa at 4.5 m) and accidents from use of heavy equipment.

§ Rockwell Support Studies

Principal Investigator: L. L. Cadwell

Other Investigators: F. G. Burton, D. A. Cataldo, J. F. Cline, R. E. Fitzner, K. A. Gano, L. E. Rogers, R. H. Sauer and W. E. Skiens

Technical Assistants: M. A. Combs, C. A. Lee, M. J. Harris, L. F. Nelson, S. L. Owzarski and M. E. Thiede

Consultant: N. E. Woodley

Studies performed for the Rockwell Hanford Operations (RHO) were designed to either 1) identify the role of biota in the uptake and transport of radionuclides from low-level waste management areas or 2) design and/or evaluate methods for reducing biological transport of radionuclides away from waste management areas. The completion and publication of documents reporting the results of previous studies was also emphasized this fiscal year. Field studies were designed to characterize insect and plant communities occupying low-level waste management areas and to evaluate pond ecosystem response to herbicide application. Laboratory studies involved the examination of 1) germination response in plant species proposed for surface stabilization on burial grounds, 2) a synthetic polymer matrix as a carrier and delivery system for phytotoxins to act as a barrier to plant root penetration of shallow-land low-level waste burial sites and 3) factors responsible for differences in radionuclide availability to plants resulting from the chemical equilibration of radionuclides occurring under field conditions.

Characterization Studies: Plants

During the summer of 1979, previously developed and standardized methods were applied to characterize the vegetation of three RHO low-level radioactive waste management sites. The sites included S-17, a decommissioned pond site; Z-8, a French drain; and the Z-19 ditch, an open ditch approximately 810 m long. Most of the effort was directed toward S-17, where data on canopy cover, biomass, and basal area of Siberian wheatgrass (*Agropyron sibericum*) were collected in six treatments. At Z-8, cover and biomass data were collected. A preliminary estimate of vegetative cover was made at the Z-19 site.

At S-17, the biomass data are quite variable, ranging from 397 gm/m² to 13. The canopy cover, however, is less variable, with a mean of 34%. At Z-8, the biomass data are again quite variable (90 ± 27 gm/m²). This reflects the spotty growth pattern of Russian thistle (*Salsola kali*)

and other species (84 ± 19 gm/m²). The data at the Z-19 ditch were taken by measuring consecutive 30-m lengths along the ditch. In each length, the species present were estimated to be in one of the standard cover classes established by Daubenmire.^(a) Vegetative composition along Z-19 consisted mostly of cattail (*Typha latifolia*) at 25%, herbs at 31% and willow (*Salix amygdaloides*) at 17%. Sedge (*Carex* spp.) and poplar (*Populus* spp.) make up the small remainder of the vegetation. Radiocontaminants in the ditch required that observations be made from some distance; this introduced a measure of inaccuracy because some plants growing inside the ditch could not be clearly seen. In spite of these restrictions, the method appeared adequate to describe the general composition of the ditch.

(a) Daubenmire, R. 1959. "A Canopy-Coverage Method of Vegetation Analysis." Northwest Science 33:43-64.

Characterization Studies: Invertebrates

This year's invertebrate studies supported by Rockwell concentrated on the S-17 decommissioned pond site. The various habitat types present at this site were sampled. These included Siberian wheatgrass (Agropyron sibericum), bare areas among the wheatgrass (this included sampling of cheatgrass, Bromus tectorum) and air volume above the vegetation, which was sampled for flying insects. Special techniques were employed to sample grasshoppers and harvester ants (Pogonomyrmex owyheeii).

Results are shown in Table 1. The invertebrates associated with the bunchgrasses contributed the most in numbers and biomass. There were relatively few individuals associated with bare areas and even fewer flying insects. Results of the grasshopper census indicated that there were 0.97 individuals per square meter. The average biomass was 6.9 mg/m². This estimate is much lower than estimates of density from other Hanford locations.

Harvester ants are abundant on 200 Area soils, but they are often hard to sample because most colony members remain below ground. Harvester ant density and biomass values were estimated by counting the number of colonies within specified plots at the S-17 site and then multiplying by an estimate of the number of ants per colony. Ant numbers were determined by excavating nearby colonies. Harvester ants inhabiting the S-17 site were estimated at 15.5 ± 4.4/m². Estimated biomass was 46.4 ± 13.1 mg/m².

Combining estimates from individual strata and including the estimates of grasshopper and harvester ant abundance resulted in an overall estimate of 10,242.2 ± 3318.4 invertebrates per m².

tebrates per m². Total biomass was 402.8 ± 238.6 mg/m². This estimate does not include other ant species, belowground insects, nematodes or acarids that may be present.

Characterization Methods

As a part of a broad program to characterize waste management sites, RHO requested a summary of standard methods currently available within PNL's Ecological Sciences Department for sampling and analyzing selected biological components of both aquatic and terrestrial ecosystems. PNL publications were reviewed and investigators consulted in order to assemble a report on the methodological considerations for biotic characterization of Hanford Area Waste Management Sites. In the report, important biological parameters were identified and methods were described for field sampling, laboratory analysis and treatment of the data. This synthesis included other important factors such as manpower, sample size and statistical considerations.

Pond Herbicide Study

A pond herbicide study is currently underway to evaluate the effectiveness and ecological impact of applying an experimental herbicide (Sonar®) to emergent vegetation in radioactive wastewater ponds. The objective of the herbicide treatment is to reduce biological transport from ponds by removing vegetation and thereby decreasing the pond's attractiveness to waterfowl and other wildlife.

The study site is a small equalizing pond connected and adjacent to Gable Mountain Pond, a 29-ha impoundment containing low-level radiocontaminants. Surveys of bird and mammal species occupying the experimental site were made before the herbicide was applied. Follow-up surveys will be conducted to ascertain the success of herbicide treatment in controlling the emergent vegetation and to document the response of bird and mammal populations to the treatment.

Germination Studies

We investigated the germination potential of six plant species that may occur naturally or be seeded on Hanford waste management sites. This study supports RHO's effort to determine what plant species may be used successfully to stabilize the disturbed soil surface over low-level waste burial grounds. The effects of three environmental factors, soil moisture, soil temperature and planting depth, were studied for these six common

TABLE 2. Invertebrate Density and Biomass Contributions from Major Habitat Types at the S-17 Study Site

Habitat Type	Density/m ²		Biomass, mg/m ²	
Bunchgrass	10,051.1 ±	3256.5	307.3 ±	182.0
Bare areas among bunchgrass	166.6 ±	30.4	36.4 ±	4.2
Air above vegetation, measured for flying insects	8.0 ±	2.0	5.8 ±	2.8
Total	10,225.7 ±	3313.1	349.5 ±	207.0

plant species growing on the Hanford Plateau.
The results are given in Table 2.

These data indicate that 1) revegetation
should include a combination of Siberian

TABLE 3. Effect of Soil Moisture, Soil Temperature and Planting
Depth on Six Plant Species

Percent Germination ($\bar{X} \pm 1$ S.D.) with Varying
Soil Moisture at 20°C

Species	Soil Moisture (bar)			
	0	-0.3	-1.0	-5.0
Siberian wheatgrass (<i>Agropyron sibericum</i>)	86 $\pm$ 4	60 $\pm$ 4	24 $\pm$ 4	23 $\pm$ 22
Thickspike wheatgrass (<i>Agropyron dasystachyum</i>)	96 $\pm$ 3	70 $\pm$ 2	0	0
Indian ricegrass (<i>Oryzopsis hymenoides</i>)	4 $\pm$ 2	0	0	0
Russian thistle (<i>Salsola kali</i>)	90 $\pm$ 4	85 $\pm$ 8	68 $\pm$ 11	47 $\pm$ 19
Sand dropseed (<i>Sporobolus cryptandrus</i>)	1	1	0	0

Percent Germination ($\bar{X} \pm 1$ S.D.) with Varying Soil
Temperature at -0.3 Bar Soil Moisture

Species	Soil Temperature				
	1°C	5°C	10°C	15°C	20°C
Siberian wheatgrass	0	62 $\pm$ 3	79 $\pm$ 2	72 $\pm$ 3	64 $\pm$ 3
Thickspike wheatgrass	0	53 $\pm$ 3	93 $\pm$ 3	86 $\pm$ 9	61 $\pm$ 6
Indian ricegrass	0	0.2 $\pm$ 0.1	1.6 $\pm$ 1.1	1.8 $\pm$ 1.4	0.4 $\pm$ 0.6
Cheatgrass	0	59 $\pm$ 5	79 $\pm$ 6	86 $\pm$ 2	78 $\pm$ 3
Russian thistle	25 $\pm$ 1.3	27 $\pm$ 2	31 $\pm$ 17	37 $\pm$ 5	37 $\pm$ 7
Sand dropseed	0	0	0	0	1.6 $\pm$ 1.3

Percent Germination ($\bar{X} \pm 1$ S.D.) with Varying
Planting Depth at -0.3 Bar Soil Moisture
and 20°C Soil Temperature

Species	Planting Depth, in. (cm)			
	0	1 (2.5)	2 (5.1)	3 (7.6)
Siberian wheatgrass	66 $\pm$ 8	21 $\pm$ 11	3 $\pm$ 2	0
Thickspike wheatgrass	57 $\pm$ 5	51 $\pm$ 19	14 $\pm$ 11	2 $\pm$ 1.6
Indian Ricegrass	11 $\pm$ 1	0	0	0
Cheatgrass	82 $\pm$ 4	15 $\pm$ 10	1 $\pm$ 1.7	0
Russian thistle	32 $\pm$ 2	3 $\pm$ 2	0	0
Sand dropseed	3 $\pm$ 2	0	0	0

wheatgrass (*Agropyron sibericum*), thick-spike wheatgrass (*Agropyron dasystachyum*) and cheatgrass (*Bromus tectorum*), and 2) optimal germination will be achieved by shallow planting, maintaining high soil moisture (-0.3 bar or above) and seeding during the season when soil temperatures are 5°C to 20°C. A mulch to maintain soil moisture during seed germination may be necessary.

Polymer/Incorporated Herbicide Studies

This work was designed as a feasibility study to test the concept of using synthetic polymers with incorporated herbicides for controlling unwanted vegetation on low-level waste management sites. The delivery system was designed to act as a reservoir for the phytotoxic compound, prevent excessive loss and/or degradation of the compound over a prolonged period and regulate delivery of the compound at a suitable rate to prevent root growth through the application zone. This study was designed to ascertain a broad range of application for seven selected phytotoxins and determine which are most suitable for release from selected polymeric delivery vehicles. No attempt was made to analytically determine and optimize release rates or release periods in this work. Specific optimization of polymeric systems, prolonged life, particular phytotoxins and release levels can be ascertained through additional proposed efforts beyond the scope of this feasibility study.

Seven herbicides were selected for this study based on their mode of action and soil sorption behavior. These included Treflan®, Surflan®, Bromoxynil®, Dinoseb®, Paraquat®, bromoxynil octanoic acid ester and TBA. The herbicides were incorporated into silicone polymer sheets at ~10% (wt/wt). Control treatments consisted of sheets of silicone rubber without incorporated herbicide. The sheets were cut to fit the lysimeter. They were perforated to determine if the herbicide would move beyond the sheet and effectively prevent root penetration through voids or breaks in normal sheet biobarriers. Lysimeters contained a 15.2-cm layer of soil, the treated or control polymer sheet and an additional 35.56-cm layer of soil. The lysimeters were then planted with Russian thistle (*Salsola kali*) and placed in a controlled environmental growth chamber with 16 hr of light per day and temperature settings of 20°C day and 12°C night.

Table 3 shows the shoot and root dry weights for each of the treatments. Root development appears to have proceeded normally until the roots penetrated to within 2 cm of the polymer containing herbicide. Subse-

TABLE 4. Shoot and Root Weights (g/lysimeter) of Russian Thistle Plants Grown in Herbicide-Treated Lysimeters

Herbicide Treatment Number	Shoot Yield (Leaf and Stem Tissue)	Root Yield
1. Treflan	7.30	4.75
2. Surflan	5.56	4.76
3. Dinoseb	1.62	1.54
4. Bromoxynil	2.41	0.53
5. Paraquat	1.32	5.48
6. Bromoxynil, Octanoic Acid Ester	1.68	5.45
7. TBA	1.80	3.77
8. Control	6.61	9.15

quent root growth was confined to the soil horizon above the barrier in treatments 1, 2, 5, 6 and 7 (Table 3). Root growth was severely reduced in treatments 3 and 4 (Table 3). The herbicides in the latter treatments (Dinoseb and Bromoxynil) were more water-soluble and exhibited lower soil sorption capacity than the other herbicides tested and therefore diffused further into the soil profile. This accounts for the reduced root growth. Analyses of the shoot dry matter production shows the Treflan and Surflan treatment to be comparable to the controls, except that in the Surflan treatment a noticeable chlorosis was evident early in the growth period. This suggests some degree of plant mobility of the herbicide. Each of the other herbicides tested resulted in phytotoxic symptoms to the shoots, indicating root absorption and translocation of the herbicide to the shoot. It is worth noting that shallow-rooted cheatgrass (*Bromus tectorum*), which germinated in each of the treatment containers, was unaffected by the herbicide treatment.

These preliminary data demonstrate the versatility of the polymeric barrier systems in waste management application. Careful selection of the proper herbicide and polymer may, in effect, provide a stabilizing ground cover of either shallow-rooted vegetation alone (using a translocatable herbicide), a combination of shallow-rooted grasses and deep-rooted forbs (with root depth penetration limited to the soil horizon above the barrier zone by a nontrans-

locatable herbicide) or a totally vegetation-free surface soil (using a soil-diffusible nonspecific herbicide).

Plant Uptake Studies

Studies are presently underway to determine how chemical equilibrium of radiocontaminants in soils (presumed to be related to the residence time of radionuclides in those soils) influences radionuclide availability and uptake in plants. The plant uptake studies used soils from field plots that were amended with ^{90}Sr and ^{137}Cs approximately 20 years ago. Recently, ^{85}Sr and ^{134}Cs were added to the soil in order to compare plant uptake of strontium and cesium that have had no opportunity to reach chemical equilibrium. Endogenous stable cesium and strontium pools were also analyzed.

Another part of this study examines the relationship between plant age (stage of maturity) and uptake of strontium and cesium. Preliminary data from this part of this study are presented in Table 4.

TABLE 5. Concentration Ratios for Barley at Various Maturity Stages Grown in Soil Containing Both Aged and Recently Amended Strontium and Cesium

Radionuclide	Plant Age at Harvest (Months)		
	1	2	3
^{90}Sr (aged)	1.63	0.52	not available
^{85}Sr (recent)	2.69	1.54	0.51
^{137}Cs (aged)	0.004	0.005	0.003
^{134}Cs (recent)	0.016	0.006	0.005

Pond Management Alternatives

An evaluation of pond management alternatives proposed by RHO was completed late in FY 1979. We examined several methods by which wastewater ponds on the Hanford Site may be managed to decrease transport of radionuclides via several distinct biological components. The primary objective of the evaluation was to predict the effectiveness of each proposed management alternative.

Previous baseline ecological studies, supported both by RHO and DOE's Office of Health and Environmental Research, provided necessary data upon which to base the evaluation.

The analysis used index values associated with four basic parameters (biomass, organism mobility, radionuclide concentration and route to man) for defining status quo conditions. Levels of impact were defined and all alternatives were evaluated for each biological component. The analysis showed that waterfowl comprise the biological component with greatest biotransport potential. Terrestrial mammals were of second greatest importance, followed in order by invertebrates, fish, terrestrial plants, aquatic plants, birds other than waterfowl, semi-aquatic mammals, reptiles and amphibians. Among the alternatives analyzed, exclusions (fences and mesh top coverings) appear most capable of reducing biotransport. Habitat modification, including recontouring the pond margins and destroying vegetation in and around the pond, were estimated to provide the second greatest reduction in biotransport. Treatments of herbicide only were next, followed by wildlife deterrents and finally increased monitoring and surveillance.

Document Preparation

Five PNL documents reporting on research sponsored wholly or in part by RHO were published during the fiscal year. Additional support for the jointly sponsored studies was provided by OHER. The subject matter of these documents included analysis of small mammal populations near a radioactive waste pond, behavior of technetium and iodine in Hanford soils, comparative availability of cesium and strontium for plant absorption from a Rupert soil, the nesting biology of the American coot (*Fulica americana*) on Hanford waste ponds and radioactivity associated with the biota of a crib that had received liquid radioactive wastes.

Progress was made on five additional documents that are currently nearing completion. These report on the breeding ecology of the Swainson's hawk (*Buteo swainsoni*) in southeastern Washington, use of the Hanford Site by hawks and owls, the occurrence and relative abundance of shrub-inhabiting insects on the Hanford Site, the diets of deer in the vicinity of waste management areas and an evaluation of the effectiveness of proposed RHO management alternatives for reducing biotransport of radionuclides from waste ponds.

§ Decommissioning and Decontamination (Burial Ground Stabilization) Studies

Principal Investigator: J. F. Cline

Other Investigators: K. A. Gano and L. E. Rogers

Technical Assistants: H. A. Sweany, M. E. Thiede and W. J. Christian

The decommissioning and decontamination of retired Hanford facilities and the future use of surrounding landscapes require isolation of contaminated wastes from the biosphere. Burial ground stabilization studies were conducted to determine the effectiveness of physical barriers for isolating contaminated wastes in shallow-land burial sites from plants and animals.

Deep-rooted plants such as tumbleweed (*Salsola kali*) and gray rabbitbrush (*Chrysothamnus nauseosus*) have, on occasion, penetrated the soil covering Hanford shallow-land burial sites. Burrowing animals have also dug through the soil layer over low-level wastes and brought contaminants to the surface. These incidents have prompted investigations into the use of barriers to isolate wastes in shallow-land sites from the biota.

This study was undertaken to determine the effectiveness of using a layer of loose rock between the waste and the surface soil covering to prevent both plant root and animal penetrations. During earlier years of this study, a simulated shallow-land burial facility was constructed. It consisted of a trench partially filled with soil to simulate waste, then covered with approximately 1.2 m of loose rock and finally covered with an upper layer of topsoil about 1 m deep. The facility was enclosed with a fence to retain small mammals and burrowing mice, and harvester ants (*Pogonomyrmex owyheei*) were introduced. The area was seeded with cheatgrass (*Bromus tectorum*), and naturally occurring plant species, including tumbleweed, were allowed to grow on the simulated burial plot.

During this fiscal year, portions of the plot were excavated and examined for evidence of biotic penetrations through the loose stone barrier. Figure 1 shows a diagram of the rooting of tumbleweed and of the observed burrowing pattern of harvester ants and Great Basin pocket mice (*Perognathus parvus*). In general, the ants burrowed through the 1 m

FIGURE 1. Diagram of Rooting of Tumbleweed (*Salsola kali*) and of Observed Burrowing Pattern of Harvester Ants (*Pogonomyrmex owyheei*) and Great Basin Pocket Mice (*Perognathus parvus*)

of soil above the stone layer but did not penetrate it. Mouse tunnels and chambers also were constructed only above the stone. Tumbleweed roots turned and grew horizontally along the upper edge of the stone barrier until they encountered columns of soil that had sifted between the rocks and formed vertical channels. These observations suggest that loose stone barriers can be effective for preventing plants and animals from penetrating to buried waste. Improvements in the barrier design should incorporate an additional (graded) layer of gravel above the stone to keep soil from sifting in between the stones and reducing the barrier effectiveness.

§ Service Assessment Studies

Principal Investigators: D. G. Watson and W. H. Rickard

Service Assessment Studies, supported through the Richland Operations Office, involve the long-term ecological monitoring of several important animals likely to be impacted by the industrial development of-km² Hanford Site. This report concerns spawning of chinook salmon (*Oncorhynchus tshawytscha*) and the nesting of Canada geese (*Branta canadensis*). Observations on these species are now in their third decade.

Fall Chinook Salmon Spawning Near Hanford, 1978

Studies begun in 1947 of fall chinook salmon (*Oncorhynchus tshawytscha*) spawning in the Hanford reach of the Columbia River were continued in 1978. Estimates of the relative fall chinook spawning population are made annually by several aerial surveys of the Columbia River between Richland, Washington (river km 542) and Priest Rapids Dam (river km 634). Information from these studies initially was related to the operation of the Hanford plutonium reactors. Since closure of all but one of these reactors, the surveys have been continued for baseline ecological information useful for estimating the impact of industrial development in the Hanford area (e.g., nuclear power plant construction by the Washington Public Power Supply System). These data are also used by resource management agencies, such as the State of Washington Department of Fisheries.

In the fall of 1978, salmon surveys were made on October 9, 16 and 26 and on November 6 and 11. The total number of salmon redds (nests) observed during this time was 3028, slightly more than the 16-year average of 2618. The start and peak of spawning appeared to be one to two weeks later than usual.

Based on a redd-to-fish ratio of 7, the estimated fall chinook spawning population near Hanford was 21,196. This was about 48% of the adult fall chinook that passed over McNary Dam (river km 467), the nearest downstream dam to the Hanford area. The 1978

fall chinook passage up the Snake River, which enters the Columbia between Hanford and McNary Dam, was only about 4% of the McNary fall chinook count, and the run up the Columbia River above Hanford was about 15% of the McNary count. About one-third of the fall chinook run passing McNary Dam was unaccounted for. A small number of the fish unaccounted for spawn in the Yakima and Walla Walla Rivers, which are tributaries to the Columbia River downstream of Hanford. The Hanford reach of the Columbia River continues to support a substantial population of spawning fall chinook salmon. In 1978 it accounted for nearly half of the fall chinook spawning in the middle Columbia River drainage.

Canada Goose Nesting, 1979

Annual goose nesting surveys were conducted in the spring of 1979. Islands in the free-flowing stretch of the Columbia were surveyed bimonthly and the number and status of goose nests adjacent to the Hanford Site were recorded. During the surveys, 126 nests were found, and of these, 107 were successful in hatching eggs. The average clutch size was 5.73 eggs per nest. Thirty-two percent of all nests occurred above the Hanford townsite. Of these, 10% were unsuccessful in hatching eggs. Nineteen percent of the nests on the lower river islands (between the Hanford townsite and Richland) were unsuccessful. Continuing studies are planned which should provide information on the causes of these differences. Table 5 provides a detailed record of the nests found on each island on 1979.

TABLE 6. Goose Nesting Data, Hanford Area 1979

Island Number or Location	Number of Nests	Number of Eggs	Number of Eggs Hatched	Nest Deserted and/or Destroyed
1	22	101	93	2
2	7	33	33	1
White Bluffs	3	?	?	1
5	1	7	6	
7	1	6	0	1
9	6	32	31	
11	3	17	5	2
12	25	149	112	6
15	5	32	30	
17	21	126	99	2
18 upper	5	29	23	1
18 lower	2	12	12	
19	22	136	100	3
20	6	39	21	2

11 Electric Field and Microwave Research

ELECTRIC FIELD AND MICROWAVE RESEARCH

- **Biological Studies of a 1200-kV Prototype Transmission System Located near Lyons, Oregon**
- **Investigation of Microwave Effects on Terrestrial Ecosystems (Proposed)**

Meeting future power demands will require innovative approaches to generation and transport of electrical power. Two such approaches are described here. One approach is to place solar collector satellites in outer space and transmit electrical power to earth via microwave beams. The second approach involves transmission of power between regional power generation centers and population centers via ultrahigh-voltage (higher than one million volts) transmission systems. While both approaches are technically feasible, serious questions have been raised about their environmental acceptability. In both cases, substantial land areas either along transmission line rights-of-way or near microwave receiving stations would be exposed to electric energy fields higher than background levels. Ecological effects possibly associated with a 1200-kV power transmission system are currently being investigated under sponsorship of the Bonneville Power Administration (DOE). A proposed research program, presently being considered for sponsorship by DOE, is designed to evaluate any ecological effects associated with receiving-antenna facilities used in conjunction with a satellite power system.

• **Biological Studies of a 1200-kV Prototype Transmission System Located near Lyons, Oregon**

Principal Investigators: L. E. Rogers

Other Investigators: K. A. Gano, R. O. Gilbert, S. L. Owzarski and J. L. Warren

Consultants: M. Burgett, J. Hedlund and D. Lavender

Since 1976, PNL has been investigating possible ecological effects of ultrahigh-voltage transmission (1200 kV) at a test line in Lyons, Oregon. The test line is in an area representative of the types of habitats commonly found west of the Cascade Mountain Range, including forests, meadows and agricultural lands. Study areas are located both near or beneath the power line (treatment areas) and at some distance away from the line (control areas).

Previous studies have identified some ecological effects. Continuing research will document the geographical extent and magnitude of changes observed. The general research protocol involves assessing the potential environmental impact of the system on native and domestic plants and animals. Specific research topics are pasture vegetation and cattle, honeybees, crops and pollination, trees and wildlife.

Cattle and Pasture Vegetation Studies

Although the data gathered for the 1979 study period has not been fully analyzed, data from previous seasons did not reveal any change in pasture usage in response to the electrical fields. This year the maximum field strength is to be increased to between 13 and 14 kV/m within the pasture area, a higher level than is expected to normally occur in the environment. Cattle behavior, as reflected in movement patterns, remain the primary focus of the study.

In 1980, measurement of pasture vegetation will be expanded to include a larger number of sample points in relation to distance from the line. Ten sampling points will be selected at increasing distances from the line. The first point will be beneath the line with a field strength of about 14 kV/m. The other points will be at intervals farther from the line, and field strength will decrease by about 2 kV/m at each point.

Vegetation in five quadrats will be sampled at each point. Our previous studies indicate that this level of effort will pro-

vide adequate estimates of the study parameters (leaf length, seed head length and culm length). Regression techniques will then be used to relate plant growth responses to the field strengths at the sampling points. Regression techniques have not been employed previously during data analysis since only three sample points were available. This change in design is prompted by insights gained during earlier analysis and will enable more sensitive analysis of the response of grasses to electric field exposures.

Honeybee Studies

Studies during 1978 and 1979 clearly showed that honeybee colonies placed within 12 kV/m electric fields suffered adverse effects. We plan now to document threshold levels and investigate possible mitigating procedures. Recent research, both in studies at PNL and in studies in Illinois sponsored by the Electric Power Research Institute, indicates that colony effects may be related to the short-circuit current flowing through the hives. This means that the effects may be related not only to field strength but also to such influences as hive height,

vegetation, and soil conductivity, so hive currents need to be documented in future studies.

Our 1980 research design will employ a total of 18 colonies, each comprised of three deep brood chambers and one foundation chamber. This will provide a total hive height of 1.22 m. Three colonies will be placed within the most intense electric field, where hive currents are about 40 μ A; three where hive currents are approximately 30 μ A; and three with 20 and <10 μ A hive currents, respectively. In addition, three colonies shielded with hardware cloth will be placed within the treatment area (intense field strengths) and three within a control area (0 field strength) to test this type of shielding as a mitigating procedure.

The actual placement of hives in relation to electric field strengths and short-circuit currents is determined experimentally on site. The amount of variation in hive currents is not known but may vary substantially depending upon weather conditions. A data-logging system is currently being developed to be used with the bee studies, permitting computer sensing and recording of actual current flows every 15 minutes. This will permit an assessment of those physical parameters most important in predicting potential impact on honeybee colonies located near electric power lines.

Crop and Pollination Studies

Crop growth studies are conducted using small weighing lysimeters employed during previous years. This approach permits control of microenvironmental factors in both treatment and control lysimeters. During previous seasons, both barley and pea plant growth responses were tested. During 1980, however, only barley plants will be included in the study design. Specific study parameters include dry weight of vegetative matter, dry weight of seeds, shoot elongation (culm length), leaf length, seed head length and seed germination. Phenological observations are noted but not included in the quantitative assessment.

A study designed to assess the effects of electric fields on insect pollinators will be added to the 1980 studies. Insect pollinators service at least 90 crops in the United States. Monetary estimates of the value of pollination vary widely, but when all fruit-, vegetable- and seed-producing crops are considered, it is usually estimated at several billion dollars.

This study investigates the effects of the electric field on pollination by deter-

mining whether seed production is equivalent in crops grown in treatment and control areas. The study crop will be ladino clover, provided that a preliminary growth chamber test proves satisfactory. Seed yield is measured in terms of numbers and biomass per lysimeter.

The first year's results have provided an estimate of the variability in the crop plant study parameters. The results have also allowed us to re-evaluate required sample sizes. In this case it is desirable that the parameter estimates be correct to within 10% of the true mean. There is no guarantee that the estimated parameters will be correct to within this limit, but probability statements can be written to describe the chance that the estimated value does, in fact, lie within the desired limit. In this case a 95% probability is assumed to be adequate so that sample size can be estimated from

$$n = \left[\frac{Z_{.95} (S/\bar{X})}{\text{Limit } (\%)} \right]^2$$

where S = A measure of the variability about the mean ($\bar{X}$), and Z value is a table value ($\alpha = 0.05$) representing the Z distribution. Overall, this technique indicates that a total of 10 lysimeters in both the treatment and control areas is adequate for determining parameter estimates for both the barley and pollination studies.

Tree Studies

Studies of the growth of conifer trees and saplings are continuing. The annual growth of the trees and saplings located in treatment and control areas is measured and related to electric field strength levels to establish thresholds for effects. Final analysis will include integrating these data with the data collected during previous years to identify any subtle long-term trends. Trees purposefully left growing near the line have clearly showed adverse effects. Those growing within about 16 m have displayed visible corona discharge and a reduction in the annual growth of apical terminals.

Wildlife Studies

Small mammals and birds are studied using well-documented sampling techniques. These include live-trapping (capture, mark, recapture) of small mammals and transect counts of birds. In 1980, the sampling periods will be fewer than in past years. Past studies have shown a small but consistent reduction in the number of birds observed in treatment transects, as compared to those in

the control transects. Bird studies are now being conducted for three consecutive mornings during the months of May, June, July and August rather than on a quarterly basis. This will permit quantitative comparisons with data from both pre- and post-energization periods censused during May through August in the study years 1976 through 1979, while reducing the overall level of effort.

The mammal studies have also shown differences between mammal populations in treatment and control areas. Populations studied in forested areas appeared equivalent during the pre-energized period in 1976, but the

numbers from the treatment area have consistently been lower than for the control area all through 1977, 1978 and most of 1979. There was some suggestion that we were approaching equivalency again during the July and August 1979 study periods. This was not true of the small mammals occupying shrub study areas. They were much more abundant in the treatment area during the 1976 pre-energization period, but the number occupying this area steadily declined after energization until about August 1978, when both treatment and control populations appeared to be stabilized at about the same low level.

- **Investigation of Microwave Effects on Terrestrial Ecosystems (Proposed)**

Principal Investigators: M. L. Warner, L. E. Rogers, B. E. Vaughan and R. D. Phillips*

To study microwave irradiation, we have proposed a research program on the ecological impacts of satellite power systems. The purpose of the study proposed is to identify any changes in organism populations or in whole ecosystems continuously exposed to the microwave frequencies and the power density levels that are anticipated in or near an operating satellite power system receiving-antenna system.

PROPOSED INVESTIGATION OF MICROWAVE EFFECTS
ON TERRESTRIAL ECOSYSTEMS

The Department of Energy, in cooperation with the National Aeronautics and Space Administration, is currently conducting a concept development and evaluation program for the Satellite Power System (SPS). A major portion of this program is devoted to assessment of the environmental effects of implementing the SPS concept. The transmission of energy from orbiting satellites to earth via microwaves would expose significant portions of the biosphere to potentially hazardous levels of non-ionizing radiation. In past years, PNL has participated in studies of possible radiofrequency interference and biological effects of microwaves at the SPS frequency and the laboratory has conducted some planning studies related to the ecological effects of the microwave beam.

The possibly harmful effects of microwaves from radar, communications and other familiar sources have been studied for the past 25 years, but no studies have yet attempted to assess the impacts of microwave irradiation on the structure and function of intact ecosystems. Impacts on whole eco-

systems are of special importance to the SPS concept since an area greater than 125 km² would be continuously irradiated at each receiving-antenna facility. It is expected that whole-ecosystem studies similar to those proposed by PNL during 1978 will be required if examination of the feasibility of the SPS continues. The purpose of the study proposed by PNL is to identify any changes in organism populations or in whole ecosystems continuously exposed to the microwave frequencies and the power density levels that are anticipated in or near an operating SPS receiving-antenna system. This information is necessary for the environmental assessment of the SPS concept and for planning and locating receiving-antenna sites. The initial planning effort conducted by PNL confirmed the technical feasibility of constructing an adequate field exposure system and it identified a battery of possible ecological studies that would be carried out using this exposure system. The information to be developed will aid in specifying the extent of buffer zones around the receiving antenna system. Further development of this potential study will occur at the request of the SPS Program Office in DOE's Office of Energy Research.

12 Energy Research for Other Agencies

ENERGY RESEARCH FOR OTHER AGENCIES

Nuclear Regulatory Commission

National Oceanographic and Atmospheric Administration

**National Oceanographic and Atmospheric Administration/Marine
Ecosystem Analysis**

Electric Power Research Institute

National Institute of Environmental and Health Sciences

The purpose of this section is to correlate energy research conducted for other sponsors to U.S. DOE energy programs where interagency and other mutual agreements exist to support work on a nonduplicative basis. The research topics identified in this section are complementary to work discussed in other sections of this report. The data bases being developed provide a unique long-term reference for environmental and energy assessment in the arid West. Work transferred to the Environmental Protection Agency in October 1979 is discussed in the sections entitled *Synfuels*, *Marine Research Programs*, and *Statistical Development of Field Research*. During the reporting year, this work was performed under DOE direction.

Nuclear Regulatory Commission

Biocide By-Products in Aquatic Environments (Principal investigators: D. R. Anderson, R. M. Bean and C. I. Gibson; other investigators: D. C. Mann, K. M. McFadden, R. G. Riley, R. E. Shirmer and B. W. Wilson; specialists: C. S. Abernethy, K. Hanson and E. W. Lusty)

Chlorine is used as a biocide at many nuclear power plants. Reactions of chlorine and naturally occurring compounds in the power plant cooling water can create by-products of the chlorination. The objective of this research program is to identify chlorination by-products and their toxicity and bioaccumulation in freshwater and marine biota.

Studies of chlorine chemistry of natural waters at 10 locations in the continental United States demonstrated that trihalomethanes (THM's) are the most predominant organohalogen product. During low-level chlorination (2 ppm total residual chlorine), THM's form as chloroform in fresh waters and as bromoform in sea water. THM concentrations are usually found below 100 ppb. Other lipophilic organohalogen products including phenols are also formed, but in smaller quantities than the THM's.

Acute toxicity studies with chloroform produced 96-hr LC 50's of 18, 18, 52 and 75 ppm for rainbow trout (Salmo gairdneri), bluegill (Lepomis macrochirus), largemouth bass (Micropterus salmoides) and channel catfish (Ictalurus nebulosus), respectively. Chloroform was found to accumulate in tissues of these species between one and two orders of magnitude above the water concentration. Bromoform acute toxicity studies were conducted with brown shrimp (Penaeus aztecus), eastcoast oyster (Crassostrea virginica), quahog (Mercenaria mercenaria), little-neck clam (Protothaca staminea) and menhaden (Brevoortia tyrannus). The organisms showed LC 50's of 26 ppm, >40 ppm, >40 ppm, >40 ppm and 12 ppm, respectively. In general, bromoform was not found to bioaccumulate in tissues and levels significantly higher than surrounding water concentrations. Both chloroform and bromoform induced behavioral changes in the species tested. Future studies will be conducted at nuclear power plants to measure chlorination by-products produced with different cooling system designs and water quality.

Environmental Standard Review Plans for the Environmental Review of Construction Permit Applications for Nuclear Power Plants (Principal investigator: D. G. Watson)

For several years the Ecological Sciences Department, along with several other PNL departments and with other national laboratories, has been assisting the Nuclear Regulatory Commission (NRC) in the development of Environmental Standard Review Plans (ESRP's). This project concluded with NRC's publication of the plans this year.

The ESRP's are a series of instructions for the NRC staff's environmental review of applications for nuclear power plant construction permits. They describe the proper procedures for environmental reviews, the manner in which judgments are reached on impacts caused by nuclear power plant construction and operation, and specific methods for determining the significance of these impacts. Use of the ESRP's will help insure that 1) data essential to decision-making and to a specific environmental review will be supplied and reviewed, 2) consideration is given to appropriate Federal and state requirements, 3) analysis and evaluation review procedures are standardized, 4) each impact assessment will concentrate on the review of only potentially significant environmental impacts and irrelevant data and insignificant impacts will be ignored, 5) the methods of analysis and judgment are objective and based on sound analytical procedures.

Although the ESRP's were prepared with instructions in mind from NRC's Regulatory Guide 4.2, Preparation of Environmental Reports for Nuclear Power Stations (U.S. NRC; NUREG-0099; 1976), these instructions did not constrain development of the ESRP's. The ESRP's will, in fact, provide the basis for an NRC revision of this guide.

Application of Fisheries Management Techniques to Assessing Impacts (Principal Investigators: D. H. McKenzie and J. R. Skalski)

This project investigates the design and conduct of ongoing fisheries monitoring programs at nuclear power plant sites. These monitoring programs attempt to detect large-scale or catastrophic departures from predictions in associated environmental impact statements. During our first year in this study we reviewed and evaluated fisheries management techniques and case histories of their application, and determined their statistical properties and their ability to predict or document changes in fish populations. During FY 1979 we reviewed fish and shellfish monitoring programs at operating nuclear power plants.

In this review we examined fishing gear combinations for specific fish species in order to develop guidelines for evaluating monitoring programs. We conducted statistical investigations to determine the inherent sampling and year-to-year variability as well as the relationships among the different fishing gears employed at a power plant site. A major goal for the project was to identify monitoring and assessment methods that are independent of population simulation models and absolute abundance estimates. Consequently, our efforts have focused on indices of population abundance. These investigations have indicated that catch per unit effort and hydroacoustical techniques are potential candidates for the envisioned monitoring programs. Creel censuses, age and growth parameters, spawner-recruit relationships and morpho-edaphic indices were evaluated as having low potential for detecting population changes.

We have developed guidelines to aid in selection and deployment of fishing gears to meet site-specific nuclear power plant fisheries combinations, and we have provided evidence that these can lead to more efficient and better designed monitoring programs. Statistical analysis of data from existing monitoring programs has identified the magnitude of the variability that can be expected in such monitoring programs. These efforts have also identified the need for development of guidelines to aid in the analysis and interpretation of the data accumulated by ongoing monitoring programs. Such guidelines will be the focus of ongoing project efforts and will aid regulators and utilities planners in assessing the changes in fish and shellfish populations in the vicinity of nuclear power plants.

Quantitative Assessment of Aquatic Impacts of Power Plants (Principal investigators: D. H. McKenzie, J. R. Skalski, D. H. Fickeisen and D. G. Watson)

This project has evolved from earlier reviews by PNL, Oak Ridge National Laboratory and Argonne National Laboratory of the current status of aquatic nonradiological monitoring at nuclear power plant sites. From these earlier studies, it was generally concluded that definitive quantitative statements could not be made about the existence or magnitude of impacts. Concurrent studies at the University of Washington showed that simulation models were not capable of adequately predicting aquatic environmental impacts. Subsequent efforts have been devoted to improving the bases for the two approaches to impact assessment, i.e., field measurements and simulation modelling. Improvement of the interchange between the two approaches has also been addressed. Improved statistical designs have been explored and recommended as bases for establishing and conducting monitoring programs. Model investigations have been conducted to evaluate the state of the art of environmental modelling and its application to impact assessment.

During the past year we have collaborated with staff from the University of Washington working on a companion NRC project. Together we are considering the roles of modelling and monitoring in impact prediction and assessment for fish populations. The earlier studies, which focused on lower trophic levels, are being extended to include fish and benthos.

We selected 14 of these models for inclusion in the University of Washington simulation library, and intensively reviewed them and their underlying biological assumptions. Now we are testing the validity of these assumptions with existing field and laboratory data. We are also investigating the adequacy of existing data sources for estimating model parameters. The preliminary results of these efforts are being published by NRC.

Ongoing work with the University of Washington is directed toward implementation of a generalized simulator AEGIS (Aquatic Ecosystem General Impact Simulator) at the University facility. Extending the past work, we are comparing existing fish and benthos simulation models, developing criteria for assessing system behavior during sensitivity analysis, investigating important (for example, compensatory) mechanisms, and reviewing, evaluating and recommending roles

of combined monitoring programs and modelling efforts in impact assessment and licensing proceedings.

Safety and Costs Related to Decommissioning of Light Water Nuclear Reactors

Safety and Costs Related to Decommissioning of Nuclear Fuel Cycle and By-Product Utilization Facilities (Principal investigators: E. C. Watson, W. E. Kennedy, Jr., R. B. McPherson and B. A. Napier)

NRC is developing a more explicit policy for decommissioning commercial nuclear facilities. Current NRC efforts address policy questions in several important areas. To assist in this, PNL is conducting generic studies for the NRC to conceptually determine the safety impacts and costs of decommissioning. The nuclear facilities under consideration in these generic studies to date include a fuel reprocessing plant, a pressurized water reactor, a small mixed-oxide fuel fabrication facility, a low-level waste burial ground and a boiling water reactor. Our generic studies cover the following topics:

- residual radionuclide inventory identification at various process locations within the facilities studied and on their sites
- generic site descriptions
- acceptable residual contamination level determination both within the facilities and on their sites
- atmospheric radioactivity release determination resulting from routine decommissioning operations
- accident scenario postulation resulting in atmospheric releases of radioactivity
- radioactive waste transportation analysis
- public radiation dose calculations for both routine and accidental radiation releases
- public safety impact analysis associated with the inventory of chemicals present during decommissioning.

The Use of Reconnaissance-Level Information for Environmental Assessment (Principal investigators: R. F. Foster, W. H. Rickard, J. A. Strand and M. L. Warner)

The objective of this program was to develop guidelines for using reconnaissance-level information (RLI) in comparing environmental features of candidate nuclear power plant sites, and in classifying each site according to potential levels of concern. RLI is here defined as data that can be obtained from published reports, public records, public and private agencies, site visits, persons knowledgeable about a special subject and area, and from brief investigations. It is information that is already available and thus differs from information obtained by new research and collection of raw data. Although our study focused on a particular stage of the site selection process, RLI is important to all stages of power plant siting and also for guiding plant design, surveillance programs and impact assessments.

Since the quantity of information available about a region containing a potential power plant site can be voluminous and often irrelevant to the questions being addressed, we developed guidelines to narrow the search to only that data relevant to site evaluation and comparison. In the case of the specific site to be proposed for licensing, we used only data that may influence major design features. Specifically, we identified dominant concerns accompanying each of several site characteristics associated with potential environmental or societal impacts. These include impacts on aquatic ecosystems, terrestrial ecosystems, land use, water use, socioeconomics and institutional (statutory) requirements. We defined the concerns to make it possible, using RLI, to classify site characteristics according to potential levels of concern. While in some cases "gating criteria" were suggested for distinguishing between levels of concern, no attempt was made to develop weighting factors or to suggest a "point system" for scoring the concerns to identify some "superior" site.

Analysis of Effects of Severe Natural Phenomena on Existing Plutonium Fabrication Facilities

Environmental Consequences of Postulated Plutonium Releases from Commercial Plutonium Fabrication Facilities as a Result of Severe Natural Phenomena (Principal investigators: R. B. McPherson and E. C. Watson)

This NRC project is conducted at PNL as a subcontract from Argonne National Laboratory. The objective of this project is to estimate the potential dose to people from postulated plutonium releases from six existing commercial plutonium fabrication facilities following severe floods, tornados, high winds or earthquakes. The project uses both primary data generated by the contractor and data found in literature. Dose to the individual receiving maximum exposure and to a definitive population via selected environmental pathways will be estimated from accident scenarios, associated source terms and air/water concentration data supplied from other studies in this project. The dose contributions from individual plutonium radioisotopes and ^{241}Am will be accounted for, and doses will be calculated for the total body and for several organs of reference. The association of these doses with environmental pathways will be determined. The organs to be considered will include the bone, kidneys, liver and lungs.

Modeling of Environmental Exposure Pathways and Consequent Radiation Doses in Reactor Accident Situations (Principal investigators: D. L. Strenge, E. C. Watson, J. K. Soldat and D. A. Baker)

The purpose of this project is to develop a set of mathematical models and computer codes for use in performing detailed environmental assessments for reactor accidents of all magnitudes. Results of this study will be used by NRC to facilitate a decision on whether to revise or reissue as a regulatory guide a proposed annex to the Code of Federal Regulations, 10 Energy, Part 50, Appendix D (Office of the Federal Register, National Archives and Records Service, General Services Administration, 1978). During FY 1979, mathematical models were selected, and preparation of the computer program was initiated.

Population Dose Commitments Due to Radioactive Releases from Nuclear Power Plant Sites in 1976 and User's Manual (Principal investigators: D. A. Baker, J. K. Soldat and E. C. Watson)

Population radiation dose commitments have been estimated from reported radionuclide releases of 58 commercial power reactors operating at 43 sites during 1976. Fifty-year dose commitments from one-year exposure were calculated from both liquid and atmospheric releases for four population groups--infant, child, teenager and adult--residing between 2 and 80 km from each site.

The total dose commitment from both liquid and airborne pathways ranged from a high of 160 man-rem to a low of 0.0002 man-rem. The arithmetic mean was 11 man-rem. The total population dose for all sites was estimated at 470 man-rem for the 90 million people considered at risk.

The codes and data files used for the estimation of these doses were transferred and converted to run on the Nuclear Regulatory Commission (NRC) Eclipse computer to enable NRC personnel to continue these studies in the future. The codes and procedures used have been documented in a draft user's manual not yet published.

Environmental Statement Concerned with the Exemption of Low-Enriched Uranium and Technetium-99 as Residual Contamination in Smelted Alloys (Principal investigators: D. A. Baker, B. A. Napier and S. A. Smith)

A preliminary draft of an environmental statement (see Publications list; Draft Environmental Statement, NUREG/CR-0518) has been developed concerning the environmental and economic costs and benefits of exempting from licensing the handling and distribution of metal alloys slightly contaminated with low-enriched uranium and technetium-99. These metals (iron, copper and nickel), which come from the scrap generated by the DOE gaseous diffusion plant upgrading program, will be smelted to reduce any uranium contamination to less than 17.5 ppm and the technetium-99 to less than 5 ppm. This alloy will then be distributed in the commercial market without special licensing requirements. Both individual and population doses resulting

from contact with the smelted alloy were estimated for various scenarios of the distribution process and for various end-products of the alloy.

National Oceanographic and Atmospheric Administration

Accumulation of Organic Constituents and Heavy Metals from Petroleum-Impacted Sediments by Marine Detritivores (Principal investigator: J. W. Anderson)

This research complements research programs conducted at our marine laboratory under U.S. DOE/Environmental Protection Agency (EPA) funding because it relates to bioavailability of hydrocarbons from sediment (see "Marine Sciences Programs," above). While the techniques and organisms differ, the findings will help us to evaluate the fate and effects of petroleum hydrocarbons in marine organisms and the marine environment. Research on the bioavailability of hydrocarbons and trace metals from oil-impacted sediments during FY 1978 and FY 1979 has resulted in publication or submission of the reports and articles listed below:

Anderson, J. W. 1979. "An Assessment of Knowledge Concerning the Fate and Effects of Petroleum Hydrocarbons in the Marine Environment." In Marine Pollution: Functional Responses, eds. W. B. Vernberg, A. Calabrese, F. Thurberg and F. J. Vernberg, pp. 3-22. Academic Press, New York.

Anderson, J. W., J. M. Augenfeld and E. A. Crecelius. 1979. Research to Determine the Accumulation of Organic Constituents and Heavy Metals From Petroleum-Impacted Sediments by Marine Detritivores of the Alaskan Outer Continental Shelf. Annual Report to NOAA, Juneau, Alaska.

Anderson, J. W., J. W. Blaylock and S. L. Kiesser. 1979. "Comparative Uptake of Naphthalenes from Water and Oiled Sediment by Benthic Amphipods." In: Proceedings of the 1979 Oil Spill Conference, pp. 579-584. API, Washington, DC.

Augenfeld, J. M. 1980. "Effects of Prudhoe Bay Crude Oil Contamination on Sediment Working Rates of Abarenicola pacifica." Marine Environmental Research (in press).

Augenfeld, J. M., J. W. Anderson, D. L. Woodruff and J. L. Webster. 1980. "Effects of Prudhoe Bay Crude Oil-Contaminated Sediments on Protothaca staminea (Mollusca:Pelecypoda): Hydrocarbon Content, Condition Index, Free Amino Acid Level." Marine Environmental Research (submitted).

Riley, R. G., B. L. Thomas, J. W. Anderson and R. M. Bean. 1980. "Changes in the Volatile Hydrocarbon Content of Prudhoe Bay Crude Oil Treated Under Different Simulated Weathering Conditions." Marine Environmental Research (in press).

Roesijadi, G., and J. W. Anderson. 1979. "Condition Index and Free Amino Acid Content of Macoma inquinata Exposed to Oil-Contaminated Marine Sediments." In Marine Pollution: Functional Responses, eds. W. B. Vernberg, A. Calabrese, F. Thurberg and F. J. Vernberg, pp. 69-84. Academic Press, New York.

Effects of Petroleum Hydrocarbons on Chemoreception in Marine Organisms: I. Dungeness Crabs (Principal investigator: W. H. Pearson)

In cooperation with Bori L. Olla, Chief of Behavior Investigations at the National Marine Fisheries Service's Sandy Hook Laboratory, we are investigating the effects of petroleum hydrocarbons on chemoreception in marine organisms and the associated behavioral effects. Threshold concentrations at which the Dungeness crab (Cancer magister) detects the water-soluble fraction of crude oil and naphthalene have been measured. Exposure to oil-in-water dispersions in continuous-flow systems has been found to impair detection of a food extract. Studies on avoidance of oiled sediment and the effects of oiled sediment on the ability of the crab to find buried prey are in progress.

A report and a journal article have been published this year from this research:

Olla, B. L. and W. H. Pearson. 1979. The Effects of Petroleum Hydrocarbons on Chemoreception in Marine Organisms. I. Dungeness Crab, Cancer magister. 1979 Progress Report to NOAA.

Pearson, W. H., P. C. Sugarman, D. L. Woodruff and B. L. Olla. 1979. "Thresholds for Detection and Feeding Behavior in the Dungeness Crab, Cancer magister (Dana)." Journal of Experimental Marine Biology and Ecology 39:65-78.

National Oceanographic and Atmospheric Administration/Marine Ecosystem Analysis (MESA)

Effects from Prudhoe Bay Crude Oil on Faunal Recovery Following Contamination by Prudhoe Bay Crude Oil on the Strait of Juan de Fuca (Principal investigator: J. R. Vanderhorst; other investigators: J. W. Blaylock and P. Wilkinson; technical assistants: J. Webster and M. T. Wilkinson)

This research is sponsored directly by the NOAA-MESA Puget Sound Project with funds provided by EPA. Its purpose is to document effects on recovery rate and identify important recovery mechanisms for intertidal fauna of the Strait of Juan de Fuca following experimental contamination with Prudhoe Bay crude oil.

The first phase of the work considers intertidal infaunal communities. We have completed our first year of study in this phase. Effects of the oil, sediment type and field site on recovery rate for early colonizers during a fall colonization period were evaluated. The effects of oil and tide level on recovery rate by early colonizers during a spring colonization period and effects from oil and sediment type after one year of recovery is also being studied. The second phase of this work expands the general methodology for use in the rocky intertidal habitat and a commercial clam bed on the Strait of Juan de Fuca. This work has been underway for approximately one month.

Electrical Power Research Institute

Long-Term Effects of Temperature and Chlorine Stress on Communities of Attached Marine Organisms in Flowing Water Systems (Principal investigator: J. R. Vanderhorst; other investigator: P. Wilkinson; technical assistant: J. R. Bridge)

This research is being carried out to determine the effects of residual chlorine oxidant in low parts-per-billion concentrations on floral and faunal colonization in continuous and intermittent chlorination regimes. The study involves monthly comparisons of biota growing on experimental substrates. These organisms receive continuous treatment of 10 and 50 ppb chlorine-produced oxidant and intermittent treatment of 10 and 50 ppm (30 minutes three times daily); control substrates receive no treatment. The projected two-year exposure will allow comparison of treatment effects over a series of community successional stages.

The treatment apparatus has been completed and tested and a statistical evaluation of system performance and reliability of nondestructive sampling techniques has been reported to the sponsor. The formal exposure has been underway for approximately one month.

National Institute of Environmental and Health Sciences

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

The program underway for the National Institute of Environmental Health Sciences is directed toward understanding the influence of soil, microbial and plant physiological processes on the form and behavior in soils and plants of trace metals arising from energy production. Trace elements may enter the environment as a result of extraction, combustion, upgrading and refining of fossil fuels, production of synfuels, and the utilization of geothermal energy. Little is known of the effects on the environment and on human health resulting from alteration of the sensitive concentration balance of trace metals in the environment, because investigations of these phenomena are relatively recent. However, a background of information to predict the behavior of these elements is available from studies of radionuclides. Furthermore, use of radiotracers is essential to studies of trace metals where problems of contamination and detection required rapid, sensitive and specific detection methods.

The studies are providing an integrated view of the potential for trace metal-organometal complex formation in soil, uptake by plants, localization in edible tissues and gastrointestinal

transfer in animals. This information has provided a basis for assessment of problems associated with entrance of trace metals into the environment as a result of energy production and a unique insight into the effect of similar transformations on the long-term behavior of radionuclides. Nuclear science has, in turn, provided the tools and a part of the background necessary to conduct experiments appropriate to development of this understanding.

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- Anderson, D. R., and E. W. Lusty. 1979. Toxicant Delivery System for Liquid Chlorinated Organics. PNL-3164 (in review).
- Anderson, D. R., E. W. Lusty and C. S. Abernethy. 1979. Chronic Effects of Chlorination By-Products in Rainbow Trout (*Salmo gairdneri*). PNL-3045 (in review).
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Organization Charts Distribution

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