**Pacific Northwest Laboratory
Annual Report for 1976
to the
ERDA Assistant Administrator for
Environment and Safety**

Part 2 Ecological Sciences

May 1977

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

May 1977

**Battelle
Pacific Northwest Laboratories
Richland, Washington 99352**

PREFACE

Pacific Northwest Laboratory's 1976 Annual Report to the ERDA Assistant Administrator for Environment and Safety is organized by major program categories into five Volumes, or Parts. Parts 1-4 are oriented to particular segments of the program supported by the Division of Biomedical and Environmental Research. Part 5 includes reports on projects supported by the Division of Safety, Standards and Compliance; Division of Technology Overview; Division of Environmental Control Technology; and the Office of Environmental Policy Analysis. Each Part consists of project reports authored by scientists from several PNL research departments, reflecting the interdisciplinary nature of the research effort. The Parts of the 1976 Annual Report are:

Part 1	Biomedical Sciences Program Manager - W. R. Wiley	R. C. Thompson, Report Coordinator D. L. Felton, Editor
Part 2	Ecological Sciences Program Manager - B. E. Vaughan	B. E. Vaughan, Report Coordinator J. L. Helbling, Editor
Part 3	Atmospheric Sciences Program Manager - C. L. Simpson	J. M. Hales, Report Coordinator E. L. Owzarski, Editor
Part 4	Physical and Technological Programs Program Manager - J. M. Nielsen	J. M. Nielsen, Report Coordinator G. M. Garnant, Editor
Part 5	Control Technology, Overview, Safety, and Policy Analysis Program Managers - N. E. Carter D. B. Cearlock J. C. Fox D. L. Hessel H. V. Larson	W. J. Bair, Report Coordinator L. C. Counts/R. W. Baalman, Editors

Activities of the Environmental and Safety Research Program at PNL are broader in scope than the articles in this report indicate. PNL staff members participate throughout the year in many projects not funded through specific 189s. These efforts include overall program planning, response to requests for information from the ERDA Assistant Administrator for Environment and Safety and other officials, participation on national and international standard-setting committees, and public education. During 1976, staff of PNL's Environmental and Safety Research Program:

- prepared two volumes (Fission and Fusion) of ERDA's Balanced Program Plan for Biomedical and Environmental Research;
- contributed to a number of environmental assessments and impact statements, including the Generic Environmental Statement for Mixed Oxide Fuels and the Clinch River Breeder Reactor Statement, and participated in hearings on nuclear issues;
- sat on National Academy of Sciences/National Research Council Committees, the National Council on Radiation Protection and Measurements, and the International Commission on Radiological Protection;
- compiled and prepared the final reports for two of these committee efforts; and
- provided, in response to requests from the Assistant Administrator for Environment and Safety, interpretations of research results to clarify information given to the public on a number of issues. These included the Hanford Mortality Study findings, faulty published interpretations of data on mortality in plutonium workers, and several other issues concerned with nuclear power.

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

Previous Reports in this Series:

Annual Report for

1951	HW-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
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
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1971	BNWL-1650, Vol. 1, Pt. 1-2, BNWL-1651, Vol. 2, Pt. 1-2
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1973	BNWL-1850, Pt. 1-4
1974	BNWL-1950, Pt. 1-4
1975	BNWL-2000, Pt. 1-4

FOREWORD

In Part 2 of these volumes, all work funded in the Ecological Sciences by DBER has been brought together. This covers effort in budget activity categories RT-02-01 (Land and Fresh-water Environmental Sciences) and RT-02-02 (Marine Sciences). For convenience, organization charts for the several participating departments and sections of PNL will be found at the back of the report.

During the past reporting year, major developments have taken place in research programs needed to deal with the problem of environmental information or amelioration of environmental consequences for the new energy technologies. Several non-nuclear programs had commenced shortly after ERDA was established and at the time of the last annual report about two-thirds of our research program lay either in non-nuclear or multitechnology areas (e.g., marine effects of oil pollution and aquatic ecological impacts of steam electric plant operation).

For this annual report, we have revised the format, separating research projects (schedule 189s) according to the energy technology area they primarily support. Schedule 189s are indicated at the top of each blue divider page by boldface dot and title followed by a description of how these efforts are important to technology considerations. In the abstracts that follow, principal investigators are listed, who may be contacted for further information about individual projects. While this format represents a wide departure from our past format, (i.e., reporting progress by organizational component), we wanted to make the report useful not only to DBER but to managers of technology programs as well. We hope the technology manager can go to an appropriate blue section of this report, then find immediately those ecological problems PNL is working on that are pertinent to his concerns. In several instances, e.g., shale oil program, coordinated efforts lie across biomedical and organic chemistry areas outside the purview of this report. We have cross referenced to appropriate parts of the four volume Annual Report, in those cases where companion work exists in another volume.

The projects described herein were, of course, commenced before implementation of AEC's Environmental Development Plans. The projects arise, nevertheless, out of PNL's direct experience with industry and other agencies concerned with energy installations, including the Nuclear Regulatory Commission, Electric Power Research Institute, U.S. Army Corps of Engineers, American Petroleum Institute, private utility companies, and the Environmental Protection Agency. In numerous instances, opportunities for PNL to contribute to the preparation or review of environmental impact assessments have provided valuable insight into research needs and many of those needs have, indeed, been implemented with DBER's support in the programs reported here. Several studies were funded also by other divisions of ERDA as interdigitating effort with the DBER program, notably work for the Division of Magnetic Fusion Energy (DMFE) on tritium, lithium and other fusion products. This includes, also, work for the Division of Nuclear Fuel Cycle and Production (DNFCP) funded through intercontractor agreements with the Atlantic Richfield Hanford Co. (radioecological problems concerned with fuel reprocessing and waste sites).

In ongoing discussions with technology representatives, a frequent misunderstanding turns up over measurement levels suitable for environmental acceptability, as regards energy residuals. In most cases, any such numbers are highly provisional and cannot be applied generally to different landscape or other habitat types--a point not generally recognized. Much of the data in existing ecological data bases deal with environmental processes, whereas the measurement of technological impact will usually require data on interacting populations rather than individual species. Also, organisms of the natural environment show extremely wide range in their susceptibility to damage depending on climatic, ecosystems, and geologic factors of a variable nature. For these reasons, impact assessment and optimal management strategies must be site specific. In research reported here, those investigations concerned with underlying environmental processes are reported in the Multitechnology Section. Investigations that have been systematized well enough to treat technology problems directly have been reported in an appropriate technology section.

During the past reporting year, also, extensive preparations were made for dedication of the Hanford site as a National Environmental Research Park (NERP). Dedication took place on March 18, 1977, shortly after this report was prepared. In establishing the Hanford NERP, major segments of the 570 square mile site have been set aside for applied research in ecology, open to all qualified research scientists. Charter intent is to stimulate: 1) baseline observations, 2) field studies of response to man-made disturbances, 3) predictive modelling of impacted ecosystems and, 4) public demonstration of alternate land use at the energy activity sites. Use policy and procedures have evolved from those developed earlier on the Arid Lands Ecology (ALE) Reserve. Within the Hanford NERP, the ALE Reserve (110 square miles) remains a

protected area intended for long range modulation studies (i.e., nondestructive studies) of grasslands and desert steppe shrub ecosystems. A brochure has been prepared describing background characterization of the NERP.^(a) Related publications will be found in another report.^(b)

Among other changes that took place during the past reporting year, ERDA work for EPA ("pass-through" projects) was incorporated into primary DBER programs in several cases. Dr. Raymond E. Wildung, PNL, was appointed to the NAS/NRC Committee on Accessory (Trace) Elements, with membership in three subpanels, Coal, Oil Shale and Uranium. Dr. Richard F. Foster, PNL, assumed responsibility to develop Environmental Standard Review Plans. This project is designed to bring to better focus proper environmental issues, to carry out environmental analysis with scientific rigor, and to provide consistency both in approach and criteria, for assessments in support of license application.

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Subprogram Manager
Ecological Sciences

(a) National Environmental Research Park, Hanford (Brochure)

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(b) A Bibliography of Environmental Research

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1.0 LAND USE AND COAL TECHNOLOGY

- **TERRESTRIAL ANIMAL ECOLOGY**
- **TERRESTRIAL PLANT ECOLOGY**
- **ALE ADMINISTRATION**
- **RESTORATION OF SURFACE-MINED LANDS**
- **EFFECTS AND BEHAVIOR OF FUEL EFFLUENTS IN FRESHWATER ECOSYSTEMS**
- **EFFECTS OF COAL UTILIZATION ON FRESHWATER BIOTA**

Ecological research is underway relating to several energy technologies and their impact on land use in the western United States; this is the primary focus for PNL terrestrial and freshwater studies described here. Current research efforts are directed toward land restoration and toward aquatic studies that concentrate on biotic effects of coal technology effluents.

Coal is currently regarded as the most dependable interim source of energy in the United States. To meet the expected demands coal will need to be mined in large quantities. Often the most economical way to extract coal is through surface mining. Much of the low sulfur, surface mineable coal is located in arid and semi-arid regions of the Western United States. It is expected that following coal extraction the pits will be filled with overburden, graded to approximate original contour, native topsoil applied to prescribed depths and planted with climatically adapted herbs, shrubs or trees. Because primary productivity in arid regions is characteristically low, it is realistic to expect that if the above procedure is followed (as sometimes recommended by law) that the revegetated surfaces will also produce little phytomass in the years following restoration.

Research on land restoration at PNL is aimed at purposeful modifications of topography to collect and direct rainfall (water harvest) and to determine the best arrangement of suitable soil in an engineered design to produce a more favorable environment for plant growth. A better plant growth environment permits a wider selection of plant species some of which could be harvested as food. The idea is to provide a way to grow useful plants on reclaimed surface mines in arid regions without a need for irrigation. A description of the proposed experimental design and initial plantings is included in this section of the annual report.

Coal conversion is designed to eliminate or greatly reduce the noxious elements of conventional coal combustion that are released as waste by-products. Aquatic effluents from coal conversion may, however, have deleterious environmental impacts or they may have effects on aquatic biota inhabiting natural lakes and ponds. Conventional coal combustion steam-electric stations characteristically release small amounts of sulfur and trace metals into the atmosphere which present certain long-term problems.

Current ecological effort is designed to ultimately provide a rational basis for amelioration of certain long-term environmental/ecological consequences of modifications consequent on the operation of combustion and conversion technologies. This section is divided into two segments. The first deals with land use in arid regions concerning threatened species, baseline data, natural and man-induced stresses and ecosystems analysis. The second part concerns land restoration and the effects of coal and coal by-products on freshwater ecosystems.

LAND USE

- **TERRESTRIAL PLANT ECOLOGY**
- **TERRESTRIAL ANIMAL ECOLOGY**
- **ALE ADMINISTRATION**

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One of earth's most important natural resources is the land. Historically ERDA's land holdings have been associated with nuclear energy facilities and among these is the Hanford Reservation. Justification for large land holdings has centered around a need for security and also as a buffer zone in the event of accidents. In recent years the importance of these large land holdings have become nationally recognized as highly desirable locations for ecological research concerning the function and structure of terrestrial ecosystems and as places to investigate the response of terrestrial ecosystems to long-term man-imposed environmental stresses.

Some land areas also need to be set aside as control areas and protected as much as possible from man-induced stresses so that ecosystem functions can be studied and used as baselines for comparison purposes. Because certain ecological trends occur slowly it is necessary to monitor ecosystem dynamics over extended periods of times, 10 years or more. Carefully selected and protected land areas exist on the 110 square mile Arid Land Ecology Reserve (ALE) at Hanford, and are available for this purpose. The Reserve is also a dedicated Federal Research Natural Area.

The projects described here provide supporting research for several applied projects that deal with environmental impact and land restoration, described immediately following. Information gained from this research has wide use and applicability to all kinds of energy technologies centered in the semi-arid shrub-steppe region of the northwestern United States. In the supporting research, three standard types of ecological information are being developed: 1) Biotic characterization, including description of major habitats and endangered or threatened species. A small university-related effort is included here also; the latter effort is an addition to the characterization of ancillary species; 2) Performances of native

plant species. This includes determination of growth habits, nutrient requirements and productivity; 3) Mineral cycling: This includes particularly the estimation of availability and behavior of airborne deposits to green plants.

Endangered and Threatened Species

Scientific and public interest in preserving and protecting representative habitats of native plants in the United States is growing as the demand for converting wild acreages to agricultural, residential, and industrial use increases. The protection of wild habitats on federally-owned lands in the Pacific Northwest for scientific and educational purposes has been pioneered by the United States Forest Service (Franklin et al., 1972). There is also a renewed interest in providing protection for plant species whose populations are so low that they are in danger of becoming extinct. One of the initial steps to promote action is the preparation of a list of species in need of protection. Such a list has been published by the Smithsonian Institute (1974) and shows two Washington shrub-steppe species, Balsamorhiza rosea Nels. and Macbr. (Compositae), and Eriogonum thymoides Benth. (Polygonaceae) as being "threatened."

This paper provides recent information concerning the occurrence of these plants in Benton County, Washington, a rapidly developing area for both irrigated agriculture and industry.

The first list of vascular plant species of Benton County was published by St. John and Jones (1926). These authors cite herbarium specimens from the Rattlesnake Hills for both E. thymoides and B. rosea.

Hitchcock et al. (1955-69) described the distribution of B. rosea from only 3 localities, all in southcentral Washington, and the type locality is indicated as the Rattlesnake Hills. Eriogonum thymoides is described as being widely distributed in the shrub-steppe region east of the Cascade Mountains in Washington and Oregon and extending eastward into Idaho.

Weber (1946) collected several specimens of B. rosea from a rocky hilltop southwest of the town of Touchet in adjacent Walla Walla County.

The locations of known plant populations in Benton County are shown in Figure 1.1. Eriogonum thymoides is probably restricted to the ridge crest habitats on Emerson Nipple, Yakima Ridge, and the Rattlesnake Hills. Balsamorhiza rosea occurs on the Rattlesnake Hills, Jump Off Joe Butte, Badger Mountain, and Red Mountain; but it

was especially abundant in the Horse Heaven Hills. Eriogonum thymoides was less widely distributed than B. rosea, and it was also less abundant when the two species occupied the same habitat. For example, a site in the Rattlesnake Hills showed that B. rosea plants were 45 times more numerous than E. thymoides (density of 18/m²) compared to 0.4/m²).

FIGURE 1.1. Map of Benton County, Washington, Showing Prominent Ridges Searched for Populations of Balsamorhiza rosea and Eriogonum thymoides

The highest elevation observed to support B. rosea colonies was 1093 m and the lowest 425 m (Table 1.1). Generally, B. rosea extended to lower elevations on steep north-facing slopes than it did on steep south-facing slopes. The population of B. rosea is clearly disjunct with the largest stands in the Horse Heaven and Rattlesnake Hills. Some colonies comprising a few acres or less were located on the summits of Red Mountain, Jump Off Joe Butte, and Badger Mountain.

Balsamorhiza careyana grows on deep soils at the lowest altitudes and extends to the highest altitudes as long as the soil profile remains at least partly stone-free. Balsamorhiza careyana often grows within a few meters distance of B. rosea habitat. When this occurs, plants that appear to be hybrids sometimes occupy a narrow zone between the two distinctive populations.

At maturity, B. rosea is a much smaller plant than B. careyana (Figure 1.2).

TABLE 1.1. Occurrence of *B. rosea* and *E. thymoides* in Benton County, Washington

Location	Elevation (Maximum) Meters	Taxa	
		<i>B. rosea</i>	<i>E. thymoides</i>
Rattlesnake Hills	1093	+	+
Emerson Nipple	977	-	+
Jump Off Joe	923	+	-
Johnson Butte	623	-	-
Horse Heaven Hills	624	+	-
Yakima Ridge	508	-	+
Badger Mountain	458	+	-
Red Mountain	428	+	-
Gable Mountain	339	-	-

+ Present
- Not observed

FIGURE 1.2. Photographs of *B. rosea* (B), *B. careyana* (A), and Suspected Hybrid Plants (C). A leaf of *B. careyana* is shown with *B. rosea* in Plate B as an indication of relative plant size.

Balsamorhiza rosea is dark green in leaf color as compared to light green for *B. careyana*. At maturity, the petals of *B. rosea* take on a reddish coloration that is absent in *B. careyana*. The leaves and peduncles of *B. rosea* tend to be prostrate while those of *B. careyana* are more upright. Tracings made from pressed leaves of *B. rosea* and *B. careyana* are shown in Figure 1.3 along with data showing the number of leaves per plant, number of peduncles per plant, number of flowering heads per plant, and the length of the largest leaf. The suspected hybrid is mostly intermediate in regard to these measurements.

FIGURE 1.3. Leaf Outlines of *B. rosea* (B), *B. careyana* (A) and Suspected Hybrid (C) With Associated Measurement Showing Range and Mean Values and the Standard Error of the Mean, $n = 10$.

Balsamorhiza rosea populations occupy relatively little total acreage and thus are vulnerable to extirpation or perhaps extinction by man's activities. However, it seems to be aggressively invading disturbed ground,

such as that associated with scraping of roadsides. This aggressiveness could enhance its ability to survive future soil disturbances. Eriogonum thymoides did not seem to have this same ability, but the opportunities to observe seedling establishment of E. thymoides were also much less frequent.

The most obvious man-induced disturbances presently being imposed on ridge crest plant communities today are the installation of radio and television communication towers, their supporting buildings, and the service roads leading to the facilities (Figure 1.4). Although the acreage occupied by such facilities is not very large, neither is the total acreage occupied by B. rosea and E. thymoides. Even when small parcels of land are preempted, the population on a particular site can be substantially reduced. At this time, there is no special protection provided for ridge crest habitats except in the portions of Rattlesnake Hills located within the boundaries of the Arid Lands Ecology (ALE) Reserve, a tract of land maintained in a wild condition by the Energy Research and Development Administration as a site for scientific and educational study.

FIGURE 1.4. Communication Facilities Located on the Crest of the Rattlesnake Hills. These kinds of facilities pre-empt scarce ridge crest plant habitat.

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An Authentic Record of Elk in the Shrub-Steppe Region of Washington

In the judgment of wildlife biologists, the American elk (Cervus canadensis L.) did not occur in the treeless interior region of Washington within recent history. Since the turn of the present century, elk have been restricted to the Cascade Mountains and other mountainous forested areas. During winter months, elk regularly move into the bordering, nonforest areas to avoid deep snow and seek forage. They then retreat into the forests in the spring to bear young. Over the past 100 yr, the Columbia Basin has been greatly modified by agricultural activities. Only three sizable land tracts still support native shrubs and grasses. These are the Yakima Indian Reservation, the U.S. Army's Yakima Firing Range, and ERDA's Hanford Reservation. Of these, only the Hanford Reservation is not subjected to livestock grazing. The portion of the Hanford Reservation that includes the Rattlesnake Hills contains some of the most extensive and least disturbed stands of the Artemisia tridentata/Agropyron spicatum association remaining in the Pacific Northwest.

Although closely watched by aerial surveillance since 1943, elk were not present on

the Hanford Reservation until the tracks of a single animal were reported in 1972 (Rickard et al., 1974). In the early autumn of 1974, routine aerial surveillance revealed a group of about 14 elk using the ALE Reserve. Since then, elk have been present year-round (Figure 1.5). It is suspected that calves were dropped in the spring of 1975, but young calves were not actually seen. In the autumn of 1975, much rutting activity was observed as the bulls rubbed velvet from hardening antlers on available trees. On 15 June 1976, two cows, each with a newborn calf, were observed during a special helicopter flight over especially rugged terrain.

It is believed that the original group of elk wandered onto the ALE Reserve from the Cascade Mountains more than 60 miles to the west. The protective isolation, abundant forage, and drinking water available at several small natural springs now make the treeless ALE Reserve acceptable as year-round elk habitat.

In this instance, changes in land use from predominantly livestock grazing to industrial (large buffer zone around ERDA's Hanford facilities) has encouraged the self establishment of a large native ungulate to a historically unoccupied range.

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A Census of Ring-Billed Gull, California Gull and Forster's Tern Nesting Colonies

Two nesting colonies of gulls, composed of ring-billed (*Larus delawarensis*) and California gulls (*L. californicus*) and one colony of Forster's terns (*Sterna forsteri*) presently are established on certain islands in the Columbia River. The gull colonies are located on Islands 18 and 20 (Hanson, 1971) while the tern colony is situated on the upper extension of Island 18.

In May 1976 four transects were established on Island 18 and all nests lying within the prescribed area were tallied; the number of young and eggs per nest were also recorded. The densities of nests on both Islands 18 and 20 were approximated by application of the densities recorded in the belt transect to the total areas available for nesting. Tern nesting density was determined by direct count. The census results are shown in Table 1.2, while clutch sizes, egg weights, and weights of 1-day-old young are presented in Table 1.3.

On the gull nesting islands, both species nested in well-defined areas. California gulls used higher ground near the center of the islands and ring-billed gulls used the periphery. Forster's terns did not nest among the gulls; they used a portion of Island 18 which was separated from the remainder of the island. Gulls nested in areas where lupine (*Lupinus* sp.) and eriogonum (*Eriogonum compositum*) predominated as

FIGURE 1.5. Bull Elk on the ALE Reserve in July 1976

TABLE 1.2. Number of Nests of California and Ring-Billed Gulls and Forster's Terns on the Hanford-Richland Reach of the Columbia River

	Total Number of Nests	Area in Hectares Used by Nesting Colony
Island 18		
California Gull	213	0.91
Ring-billed Gull	863	3.69
All Gulls	1076	4.60
Island 20		
California Gull	386	1.65
Ring-billed Gull	339	1.45
All Gulls	725	3.10
Upper end of Island 18		
Forster's Tern	25	3.0

ground cover (Figure 1.6), while terns nested in areas where eriogonum was dominant.

In 1961, Hanson (1963) conducted a census of nesting gulls in this same reach of the Columbia River and found two gull colonies, one at Coyote Rapids and one near Ringold (Hanson, 1971). Since Hanson's census both colonies have disappeared. The Ringold colony was destroyed by domestic pigs in 1970 and has not reestablished itself. The fate of the Coyote Rapids colony is unknown. Hanson (1963) reports that a total of 2,191 gull nests (ring-billed and California gulls combined) were present in 1961 and that the average clutch size was 2.7 at Ringold and 2.6 at Coyote Rapids. Our findings of 1,801 nests in total and an average clutch size of 2.5 for California gulls and 2.8 for ring-billed gulls compares with Hanson's findings.

TABLE 1.3. Clutch Size, Egg Weights, and Weights of 1-Day-Old Young of Ring-Billed and California Gulls and Forster's Terns

	Clutch Size		Egg Weight		1-Day-Old Young Weight (grams)	
	N	$\bar{X} \pm SE$	N	$\bar{X} \pm SE$	N	$\bar{X} \pm SE$
Ring-billed Gull	45	2.94 ± 0.13	21	49.89 ± 0.98	12	41.17 ± 1.04
California Gull	32	2.50 ± 0.11	46	75.63 ± 0.8	---	---
Forster's Tern	14	2.79 ± 0.13	---	---	---	---

FIGURE 1.6. Gulls Nested in an Area Where Lupine (*Lupinus* sp.) and Eriogonum (*Eriogonum compositum*) Predominated as Ground Cover

We feel, however, that a more precise census is needed on Island 20 since we visually estimated over 4,000 birds nesting on the island. Nests may be packed closer on Island 20 than on Island 18 and our application of density estimates made on Island 18 to Island 20 may not be entirely valid.

Hanson (1963) did not report on Forster's tern nesting density or clutch size. Apparently, our data are the first reported for the Hanford area.

The high nesting densities of gulls and terns in the Priest Rapids Dam-Richland stretch of the Columbia River is conducive for further studies. These fish-eating birds can be valuable in examining environmental contamination (Vermeer, 1971; Fimreite et al., 1971). By analyzing eggs plus bird tissues for pesticide, heavy metal, and radionuclide content, coupled with yearly data on population productivity, environmental contaminants can be monitored from year to year.

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Phenology Studies

Observations on plant phenology were continued for the fourth consecutive year on the ALE Reserve with the objective of providing a long-term data set to determine the response of plants to annual variations in weather. Each week 143 individual plants

are scored for current phase of development. The nine phenophase stages are: new growth, immature leaves, mature leaves, senescent leaves, dead leaves, floral buds, open flowers, maturing fruit, and dispersing seeds.

These data are punched on data cards and processed via a special data management system. The data system displays for each species all phenophases observed for all observation dates. Years are displayed one over another to facilitate visual comparison of yearly differences in phenological progression.

Gypsum blocks were buried at two locations in January, 1975, to measure soil water potential at the following depths: 5, 10, 20, 30, 40, 50, 60, 70, 80, and 90 cm. The data have been compared to standard gravimetric data and the soil blocks are performing very well. The gypsum soil blocks determine soil water potential from a measurement of electrical conductivity between two stainless steel screen electrodes embedded in gypsum. They were used in this study because weekly gravimetric analyses in the relatively small area would unnecessarily disturb the otherwise pristine soil and vegetation.

An interactive computer program was developed to compute soil water potential from the conductance measured for each soil moisture block. Each block was a unique calibration curve relating soil water potential, percent water, and conductance. The computer program calculates soil water potential from conductance, then percent water from soil water potential, using the block identification number to obtain the calibration coefficient from the storage array.

In addition to the above, a continuous record of soil temperature at 5 and 10 cm depths, and weekly accumulations of net solar radiation were started in October 1976. These data will be regressed with other data from the ALE Reserve and the Hanford Meteorological Tower to construct a continuous record of these variables back to the start of the phenology data set. These weather data are required to identify relationships between changes in weather and changes in rate and timing of plant phenology (Sauer and Uresk, 1976).

Literature Cited

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Second Year Plant Recolonization of a Plowed Cheatgrass Community

A portion of an abandoned agricultural field dominated by cheatgrass (*Bromus tectorum*) which has not been disturbed for 30 yr was plowed again in the fall of 1974. The purpose was to provide a new site to study the natural process of plant succession during the early years.

Phytomass estimates were obtained on the plowed and unplowed (control) sites by clipping circular plots each 0.0324 m². During the second year, productivity on the plowed site was lower than that associated with the unplowed control, 118 g/m² as compared to 177 g/m², respectively (Table 1.4).

TABLE 1.4. Dry Weight of Live and Dead Plants on Second Year Recovery Plowed Ground (plowed) and 30-yr-old Plowed Ground (control) During May 1976

----Taxa----	Plowed		Control	
	---Live (green)---		Tissue----	
Annuals				
<u>Bromus tectorum</u>	47.6 ±	7.0	169.3 ±	11.0
<u>Sisymbrium altissimum</u>	12.8 ±	6.4	0	
<u>Descurainia pinnata</u>	51.9 ±	11.7	0	
<u>Amsinckia lycopsoides</u>	6.1 ±	2.7	1.0 ±	1.0
<u>Holosteum umbellatum</u>	Trace			
Perennials				
<u>Microseris lanciniata</u>	0		4.5 ±	4.5
<u>Poa sandbergii</u>	0	1.9	1.9	
Total Live Phytomass	118 ±	12	177 ±	11
--Category--	-----Dead Tissue-----			
Standing Dead Stems	27.4 ±	9.9	42.6 ±	22.4
Flat Litter	15.6 ±	5.0	312.0 ±	18.4

The 2-yr-old plowed ground had only 16 g/m² of litter as compared to 312 g/m² for the 30-yr-old plowed ground. By sampling these study sites throughout the year, the seasonal

dynamics of plant growth can be determined in relation to variable weather patterns and possible pest outbreaks (grasshoppers). By sampling over a period of years, the dynamics of litter accumulation can be established.

The importance of natural litter in plant communities in providing an environment for seed germination and seedling growth is appreciated by field ecologists, but the dynamics of litter disappearance and accumulation have not been quantitatively described. Continued studies at this site are designed to yield this latter kind of information.

Factors in Relation to Growth of a Cheatgrass Community

The production of cheatgrass (*Bromus tectorum*) varies in time and location. However, the dynamics of cheatgrass growth as related to factors associated with western rangelands have not been fully investigated.

The study site is an abandoned field that has supported a more or less uniform stand of cheatgrass with several minor species, e.g., tansy mustard (*Descurainia pinnata*) and tumble mustard (*Sisymbrium altissimum*) for more than 30 yr without additional stresses imposed by grazing livestock.

Harvest data of aboveground live phytomass were combined over 3 yr (1971, 1972, and 1973) for the regression analyses shown in Table 1.5. Only replicate means of herbage by harvest dates were used in these analyses. Analyses were separated into two periods. First, regression equations were developed from the beginning of growth to peak biomass of cheatgrass. Secondly, regression analyses were determined from peak biomass to the last sampling period for live herbage. The dependent variable ($\bar{Y}$) in all of these analyses was the daily change in herbage. Independent variables tested in these analyses included herbage wt (WT), soil temperature (T) at 4 dm depth, nitrogen (N), phosphorus (P), potassium (K), and soil water (W) at 1 dm increments to 8 dm in depth. The independent variables were used to predict the daily change in herbage weight (at a maximum of 3-week intervals from date a to date b). Only those independent variables that contributed significantly to the dependent variable (daily change in biomass) are presented.

The growth of cheatgrass usually begins with seed germination in late October or early November when moisture first becomes available; the changing biomass of herbage is presented in Table 1.6. Nitrogen content in the live herbage ranged from 0.40% to a high of 2.60%. Nitrogen content was highest in herbage early in the growing season and it decreased as the cheatgrass matured. Soil temperatures at a 4 dm depth ranged from

TABLE 1.5. Regression Analyses Showing the Influence of Abiotic Factors on Growth Rates (Y) of Cheatgrass

	Equation	R ²
Increasing Period		
1	$\hat{Y} = -0.971 + 0.393 (0.114) T$	0.66 ^a
2	$\hat{Y} = 5.61 + 0.345 (0.077) T - 3.211 (0.726) N$	0.88 ^a
Decreasing Period		
3	$\hat{Y} = 13.86 - 1.209 (0.3887) T$	0.81 ^a

T = Soil temperature at 4 dm
N = Nitrogen content of plant tissue
^aSignificant at P ≤ 0.01.

3.9°C to 21.0°C during the 1971 to 1975 growing seasons (Table 1.6). During late fall each year, after adequate moisture is stored in the soil, temperature appears to regulate plant growth. Soil temperatures may be the best single factor to estimate rates of herbage production until 11°C is reached at the 4 dm soil depth (Equation 1). Nitrogen then begins to limit plant growth as temperatures continue to increase (Equation 2). Equation 2 was used to estimate plant growth until a temperature of 15°C was obtained or approximately 0.5% nitrogen was present in the cheatgrass tissues. The decrease in the rate of live herbage production was a function of soil temperature (Equation 3). Soil temperature was the most highly correlated independent variable of all parameters associated with the deaccelerated growth rates of cheatgrass. Soil water content in the upper meter of soil profile did not contribute significantly during the period of deaccelerated growth.

TABLE 1.6. Seasonal Values of Herbage Yield, Nitrogen, and Soil Temperature During 1971-1975 for a Cheatgrass Plant Community

MONTH-DAY	HERBAGE (g/m ²) $\bar{X} \pm SE$	NITROGEN (%) $\bar{X}$	SOIL TEMPERATURE, °C 4 dm
<u>1971</u>			
3-08	35.5 ± 2.9	1.95	3.9
4-01	59.6 ± 5.7	1.56	8.5
4-19	126.9 ± 7.8	1.30	11.2
5-07	198.4 ± 11.7	0.95	15.4
5-28	124.9 ± 11.1	0.57	21.0
11-10	47.5 ± 5.9	2.08	7.5
<u>1972</u>			
3-14	56.3 ± 4.1	2.31	9.8
4-03	76.4 ± 2.6	1.48	10.3
4-13	131.7 ± 6.6	1.11	10.5
4-25	126.2 ± 5.5	1.00	13.2
5-15	112.1 ± 20.2	1.03	19.0
<u>1973</u>			
3-06	32.0 ± 3.2	2.25	7.8
3-26	36.4 ± 2.3	2.10	9.5
4-06	70.4 ± 5.2	2.14	12.8
4-23	115.1 ± 11.3	1.83	15.5
5-07	165.8 ± 11.2	1.18	17.3
5-18	62.2 ± 6.8	1.70	16.5
<u>1974</u>			
3-13	78.0 ± 4.7	2.04	7.0
4-11	143.6 ± 7.5	1.23	8.5
4-28	253.1 ± 14.1	0.64	13.0
5-22	328.0 ± 16.1	0.40	17.2
<u>1975</u>			
3-20	60.0 ± 6.8	2.60	4.8
4-28	193.0 ± 7.6	1.13	10.0
5-13	294.5 ± 18.6	0.54	14.1
6-06	221.4 ± 15.4	0.40	21.0

Figure 1.7 shows the relationship of predicted and actual biomass values for 1974 and 1975 based on equations validated in 1971, 1972 and 1973. Changes in herbage rates were predicted at weekly intervals and these values were summed to obtain total biomass at date b. Close agreement is shown between predicted and actual values.

The growth period of cheatgrass is comprised of three soil temperature categories. Growth was possible but slow at temperatures between 2.5-11°C. The optimum period of growth occurred between 11°C and 15°C. Above 15°C, the growth rate decreased.

Invertebrate Density and Biomass Distribution in a Cheatgrass Community

Density and biomass of invertebrates were estimated in a community vegetated primarily with cheatgrass to determine the taxonomic and trophic distributions of density and biomass values.

Although cheatgrass communities are now an important component of the ecology of the grassland regions of this area and serve as the pioneer stage in the secondary succession of disturbed areas, little is known about their associated animal populations. An abandoned wheatfield last cultivated in 1943

FIGURE 1.7. Comparison of Plant Growth in a Cheatgrass Community as Affected by Abiotic Factors During the 1974 and 1975 Growing Seasons

served as the study site. Cheatgrass is the dominant plant species but others such as yellow salsify (*Tragopogon dubius*), lanceleaf microseris (*Microseris lancinata*), jagged chickweed (*Holosteum umbellatum*), and tumble mustard (*Sisymbrium altissimum*), are common. The old field is about 400 ha in size and is located at an elevation of 533 m.

Invertebrates were sampled at 1 month intervals from April to August using a drop trap technique. Collected plant and invertebrate material was taken to the laboratory and the invertebrates extracted into 75% ETOH using a Berlese funnel technique. Specimens were sorted by hand, measured, and identified with a dissecting microscope. Weight estimates were calculated for individual specimens using length-weight regression equations previously developed. Average weights were determined by drying and weighing representative specimens where regression equations were not available.

The seasonal distribution of estimated density and biomass values are shown in Figure 1.8. The highest density values were obtained in April. A steady decline in density progressed into the summer months, reaching a low point during August. Estimated biomass followed a similar trend, peaking in May and declining through summer with the minimum value recorded in August. These patterns approximate the live plant biomass trend; peak biomass values for green vegetation occur during April or May and then decline with the onset of summer drought conditions. Only a small amount of green vegetation remains after the first of July. This implies that the dominant invertebrate groups, in terms of biomass and density, are correlated with the green growth period of early spring.

FIGURE 1.8. Seasonal Distribution of Estimated Density and Biomass Values

The taxonomic composition of the invertebrate fauna is shown in Table 1.7, expressed as percentages of density and biomass. The most abundant invertebrates were mites, beetles, and thrips. The mites were largely members of the family Tetranychidae, a group that feeds on green vegetation. Their life cycle would be expected to be closely attuned to the availability of host plants. There was one abundant beetle family present, the Tenebrionidae or darkling beetles. They are generally regarded as omnivores and probably do not require a diet of green vegetation, being able to feed on detritus. Darkling beetles were most abundant during the months of May and June, with 54 and 34 individuals per m² respectively--mostly larvae. The Thysanoptera (thrips) were the third largest contributor to total invertebrate density (Table 1.7). Thrips are small, frequently overlooked insects. Most are phytophagous and occur on the foliage and in flower heads of various plants.

Two taxa, beetles and grasshoppers, accounted for 71% of the total estimated invertebrate biomass (Table 1.7). None of the other 10 taxa represented over 7% of the total biomass values. Grasshoppers are occasionally very abundant in this type of community, but were present in low numbers during this study. Their relatively large size, however, made them a significant biomass contributor.

An analysis of the trophic distribution of invertebrate density and biomass is shown in Table 1.8. The herbivore and omnivore groups were the two most important trophic levels, in terms of both density and biomass. The tissue and sap feeders comprised 28 and 24% of the total herbivore density, while pollen and nectar feeding insects consisted of only 4%. There were few flowering plants other than grasses inhabiting the cheatgrass community, so the paucity of pollen and nectar

TABLE 1.7. Taxonomic Distribution (%) of Invertebrate Density and Biomass

TAXA	DENSITY		BIOMASS	
	-----%		-----%	
COLEOPTERA (BEETLES)	18		51	
DIPTERA (FLIES)	4		6	
LEPIDOPTERA (BUTTERFLIES AND MOTHS)	<1		7	
ARANEIDA (SPIDERS)	1		3	
HYMENOPTERA (ANTS, BEES, WASPS)	8		7	
ACARI (MITES)	37		<1	
HOMOPTERA (CICADAS, APHIDS, ETC.)	1		<1	
HEMIPTERA (BUGS)	8		3	
THYSANOPTERA (THRIPS)	15		1	
PSOCOPTERA (BOOK LICE)	7		<1	
ORTHOPTERA (GRASSHOPPERS)	<1		20	
CHILOPODA (CENTIPEDES)	<1		<1	

TABLE 1.8. Trophic Distribution (%) of Invertebrate Density and Biomass

Trophic Level	Density	Biomass
	-----%	
Herbivore	56	47
Tissue	28	24
Sap	24	4
Pollen & Nectar	4	19
Predator	8	4
Omnivore	34	48
Fungivore	<1	<1
Unknown	<1	<1

foragers was expected and is consistent with our field observations. The percent biomass value for pollen and nectar feeders was higher (19%) than the density value, mainly due to the presence of a few large adult moths.

Conclusions from these data show that in an old field cheatgrass community: 1) invertebrate populations reach maximum density and biomass early in the season (April, May) correlated with peak biomass of green vegetation; 2) the most abundant invertebrate taxa are mites, beetles, and thrips; 3) beetles and grasshoppers are the largest contributors to invertebrate biomass and 4) herbivores and omnivores are the largest trophic groups in terms of both density and biomass.

ELM Model

The Grasslands Biome simulation model ELM, which simulates dynamics in primary producers, consumers, N, P, decomposers, plant phenology, and abiotic factors, has been installed on the Richland computer. A reliable weather data set for 1 October 1970 to 30 September 1975, has been compiled as input to ELM. Primary producer data for the corresponding years has been organized and loaded into the model for simultaneous plotting with simulation output. The immediate goal is completion of the modeling chapter of the Grassland Biome Synthesis Volume. Later, ELM will be modified and used to study the Hanford Reservation in more detail, rather than be restricted to the vegetation around the Grasslands Biome study site.

Plant Uptake of Minerals from Soil

The steppe region of south-central Washington has remained relatively free from man-induced trace metals and sulfur released into the local atmosphere by coal-burning industrial facilities. Most of the electrical

energy used in the region is produced by hydroelectric dams and by nuclear fuel.

The study site is a greasewood community located on the Hanford Reservation in south-central Washington (Rickard and Keough, 1968). Three greasewood shrubs were selected for study. Leaves were hand-picked in early autumn just prior to leaf fall. At the same time, dead leaves were hand-picked from the ground beneath the same shrubs. A sample of mineral soil (a 1 in. deep layer) was collected from beneath the mat of dead leaves. Three other soil samples were taken from the open ground beneath the widely-spaced shrubs.

The results of chemical analyses are summarized in Table 1.9. Sulfur was abundant in fresh leaves but old leaves had only about one-half the sulfur content. The other elements tended to increase in concentration in old leaves as compared to fresh leaves or remain unchanged.

The soil beneath greasewood shrubs had greater concentrations of sulfur, manganese, zinc, and selenium than did the adjacent nonshrub affected soil but copper was less abundant in the soil beneath shrubs.

The data indicate that natural plant communities supporting widely-spaced, long-lived individual plants present different kinds of sampling strategies than do closed canopy forest or grassland communities. When taking soil samples from desert, shrub-steppe, or savannah communities for chemical analyses it is desirable to collect from between and beneath shrubs and/or trees to help reduce the variability and therefore analytical costs of environmental monitoring programs.

A consistent sample taking strategy is particularly important for long-term environmental monitoring programs designed to detect increasing levels of airborne environmental contaminants.

As a general rule, the trace elements measured in greasewood leaves are not high

TABLE 1.9. Average Content (ppm dry wt) of Greasewood Leaves, Litter, and Soil - 1975

ELEMENT	LEAF	LITTER	SOIL	
			UNDER	OPEN
SULFUR	8700 ± 1500 ^a	3700 ± 30.0	30 ± 2.0	5 ± 0.58
MANGANESE	100 ± 21	370 ± 73.0	15 ± 2.7	7 ± 0.58
ZINC	8.3 ± 2.2	17 ± 4.8	0.90 ± 0.20	0.23 ± 0.03
COPPER	4.3 ± 0.73	13 ± 1.1	0.53 ± 0.03	0.93 ± 0.09
SELENIUM	<0.10	<0.10	<0.017	<0.007
LEAD	0.2 ± 0.2	0.3 ± 0.3	0.67 ± 0.07	0.67 ± 0.07
CADMIUM	<1.2	<1.2	0.03 ± 0	0.03 ± 0
*MERCURY	0.23 ± 0.13	0.31 ± 0.07	51 ± 22.0	63 ± 33

*PARTS PER BILLION

^a MEAN ± MINUS STANDARD ERROR, n = 3

as compared to crop plants. Cline and Rickard (1974) added isotopic tracers to greasewood-affected soil and compared plant uptake with adjacent soil not affected by greasewood. The isotope of sulfur was not so readily taken up by plants grown in the sulfur-rich soil beneath greasewood shrubs as it was in the sulfur-poor open soil. However, an isotope of zinc was more readily taken up by plants when grown in the greasewood-affected soil as compared to the open soil. The inference is that soil chemical analyses do not necessarily provide insight as to the behavior of mineral elements added to the endogenous levels in the soil and uptake by plants. Also, under actual field conditions the input of external deposition of elements via foliar uptake has not usually been separated from those elements incorporated within plant tissues by way of root uptake processes. However, in this Northwest greasewood community, the external deposition of mineral elements could provide only a small part of the measured element content of leaves and soil because the region is not a heavy user of coal. In future years coal combustion may become more important as the demand for electrical energy increases, and recent study indicates that sulfur concentrations in air if less than 300 µg/m³ will likely be beneficial to native plants (Sauer, 1976).

A large coal-fired steam-electric station is presently under construction at the Portland General Electric Company's Carty site near Boardman, Oregon. From an ecological point of view, baseline data should be collected for a variety of plant species and habitats to provide a basis to determine if the operation of coal-fired stations can substantially change the mineral content of plants and interfere with plant growth. Several recent studies show that airborne deposits, if small, are very efficiently retained by green plant leaves, contrary to widely held common belief (Vaughan et al., 1976; Cataldo and Vaughan, 1977). Also, elsewhere in this report plutonium aerosols are shown to be as available or more available to seed from leaf deposition than from soil deposition via root uptake (see Section 4, pp. 4.1-4.13). It is likely that this phenomenon is not restricted to plutonium, and it may in fact be general to many of the heavy metal elements present in air as submicronic, or "respirable," particles.

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Airborne Contaminants and Accumulation by Plants

Cesium-137 is a radioactive constituent of fallout debris that over the past 30 yr has become ubiquitously distributed over the earth's surfaces as a result of testing nuclear devices. It is strongly absorbed by soil so that its downward migration in the soil profile is slow. It is also only sparingly taken up from soil by the plant root system (Cline and Rickard, 1972).

In some cases, direct interception from the air, by green plants, dominates soil-to-root uptake (Vaughan et al., 1976 cited above). There is a need, also, to continue to document the long-term behavior of persistent, widely-distributed airborne contaminants like cesium in a variety of terrestrial ecosystems. This investigation describes the behavior of individual clumps of bluebunch wheatgrass, *Agropyron spicatum*, as accumulation sites for airborne ¹³⁷Cs.

The study site is an experimental sagebrush-bluebunch wheatgrass pasture on the Arid Lands Ecology (ALE) Reserve. The pasture has had a long history of freedom from livestock grazing and fire. However, a wildfire burned part of the pasture in August 1973. Controlled cattle grazing was imposed upon a part of the pasture from 1971 through 1975. In the spring of 1976 three clumps of bluebunch wheatgrass were randomly selected for harvesting for radiochemical analysis of ¹³⁷Cs in control, burned, and grazed pastures.

The results of radiochemical analyses are summarized in Table 1.10. These data show that the highest levels of radiocesium are clearly associated with grass crowns. The youngest (uppermost) grass parts are associ-

ated with the lowest levels of radiocesium. The data also indicate that grass clumps can collect airborne debris and dust on aerial parts and direct these materials to the crown tissues. The mechanisms of crown accumulations are not well known, but dead leaves do tend to accumulate in the crown. Leaf decay probably contributes to the increased levels of ¹³⁷Cs as measured in grass crowns.

TABLE 1.10. Cesium-137, pCi/g Dry Weight, in Bluebunch Wheatgrass Clumps and Soil - 1976

PASTURE			
CONTROL	GRAZED	BURNED	POOLED
UPPER CLUMP SAMPLES			
* BDL	0.38	0.60	AVG 0.59
0.026	0.17	0.28	
* BDL	0.60	0.19	
LOWER CLUMP SAMPLES			
1.1	0.57	0.89	AVG 0.59
0.67	*BDL	0.32	
0.70	0.63	0.45	
CROWN SAMPLES			
4.4	3.2	3.9	AVG 4.5
4.4	7.1	5.1	
3.2	5.6	3.8	
SOIL SAMPLES			
2.8	2.5	3.6	AVG 2.7
2.8	1.7	2.6	

*BDL = BELOW DETECTION LIMITS

Cattle grazing upon the upper parts of bluebunch wheatgrass clumps would ingest tissues with small amounts of radiocesium. If cattle grazed the clumps to ground level, they would ingest tissues with slightly greater amounts of radiocesium.

Radiocesium was measured in clumps of cottongrass in northwestern arctic Alaska (Rickard et al., 1975). The aboveground parts of cottongrass, *Eriophorum vaginatum*, averaged 5 pCi per gram dry weight and crowns 9 pCi per gram dry weight. These values are about twice those of similar parts of bluebunch wheatgrass clumps, reflecting higher atmospheric deposition rates in the Arctic or a slower loss rate from cottongrass.

Individual clumps of bluebunch wheatgrass act as points of fallout accumulation. The accumulation is concentrated in the crown tissues and suggests that small invertebrate organisms living and reproducing in the crown environment are exposed to slightly higher doses of exogenous ¹³⁷Cs than those organisms living in or on the adjacent, open soil.

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Strontium and Cesium in Field Plots

Strontium-90 and ^{137}Cs purposefully added to surface soil of small field plots 22 and 14 yr ago, respectively, were measured in tissues of cheatgrass and alfalfa in 1976. The results are compared to those last obtained in 1970 (Cline and Rickard, 1972). As with the Cs studies above, these tracers allow one to compare the long-term soil behavior of these two elements entering the soil as airborne deposits.

Data in Table 1.11 show that the uptake of ^{90}Sr by plants was similar in 1976 to that of 1970. The slightly reduced levels of radionuclides in the plant tissues is due to physical decay (decay factor for 6 yr is 0.86 for ^{90}Sr and 0.87 for ^{137}Cs). The greater reduction of ^{137}Cs concentration in alfalfa is not so clear. Only a few alfalfa plants remain in the plots after 12 yr without care or irrigation, and the few surviving plants were at the edge of the plots exposing their roots to a large volume of uncontaminated soil.

In the 1970 Annual Report it was stated that approximately 2% of the estimated loss could be assigned to plant harvest and cultural practices. During the past 12 yr with

no harvest the uptake of these elements remain steady.

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Cline, J. F. and W. H. Rickard. Radioactive strontium and cesium in cultivated and abandoned field plots. Health Phys. 23:317-324, 1972.

Microclimatology

For 10 years, microclimatology of portions of the NERP has been systematically documented. On the ALE Reserve, for example, isolines for several temperature and precipitation variables may be mapped with statistical variance. These data are computerized and keyed to the 25 year data base obtained at the Hanford Meteorological Station. (Thorp and Hinds, 1977).

A Class A station provides comprehensive data to the NOAA national system. These data provide reference for climatologically-controlled events affecting organic production.

Literature Cited

Thorp, J. M. and W. T. Hinds. Microclimates on the ALE Reserve, 1968-1975. BNWL-SA-6231. Battelle-Northwest, Richland, WA, 1977.

Other Species, Hanford Site

More intensive ecosystems characterization specifically applicable to the Hanford site and its nuclear energy facilities is also underway. This work differs, in focus-

TABLE 1.11. Comparison of ^{90}Sr and ^{137}Cs (nCi/g) in Plants Harvested from the Same Field Plots in 1970 and 1976

ISOTOPE	1970				1976			
	CHEATGRASS		ALFALFA		CHEATGRASS		ALFALFA	
	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW
^{90}Sr	1.77 ± 0.24	0.35 ± 0.08	0.96 ± 0.13	0.15 ± 0.03	1.60 ± 0.37	0.21 ± 0.07	0.81 ± 0.61	0.27 ± 0.18
^{137}Cs	0.03 ± 0.01	-	0.04 ± 0.01	-	0.02 ± 0.003	-	0.01 ± 0.001	-

HIGH SOIL = (1.49 mCi/m^2) ADDED ISOTOPE

LOW SOIL = (0.27 mCi/m^2) ADDED ISOTOPE

ing on food chain considerations rather than on the dynamics of vegetational processes dealt with in this section. For Hanford related data, see Section 4, pp. 4.72-4.136, and Section 8, pp. 8.1-8.43.

UNIVERSITY RELATED STUDIES

Long-Billed Curlew Ecology Study

The long-billed curlew, (*Numenius americanus*), though one of the largest and most conspicuous of the North American shore-birds, has only recently begun to attract scientific research into its biology, ecology, and behavior. This is because it has only recently become economically feasible to convert vast acreages of arid land into high production farm land, and thereby severely reduce or eliminate the preferred nesting habitat of the curlew. This continuing loss of habitat may eventually reduce curlew numbers to the point of rare or endangered status. Consequently, a study considering the ecology and behavior of the species on the Hanford Reservation was begun in January 1976 by Julia Fitzner.

The first curlew to be sighted on the Hanford Reservation this year arrived on March 17; for the next few weeks their numbers increased gradually until 15 pairs and up to 25 "unemployed" individuals were located. Up to 20 curlews were estimated to be using Cold Creek Valley on the ALE Reserve but only a few observations were made there. The majority of the pre-hatching observations were made on curlews utilizing a site adjacent to the 300 Area as this area is crucial curlew habitat and supports the greatest density of curlews on the Hanford Reservation.

Upon arrival, the birds spent the majority of their time feeding in cheatgrass fields and along sandy ridges among the rabbitbrush, antelope bitterbrush, and sagebrush shrubs. Pit traps were established to collect ground-dwelling insects and checked weekly throughout the nesting and hatching phases; fecal material was collected over the same period in hopes of correlating frequency of occurrence data for dietary analysis.

Curlews were observed in detail throughout courtship, egg laying, and incubation. The chicks were weighed, measured, and banded as soon as they hatched. In all, 26 chicks were banded with U.S. Fish and Wildlife Service metal leg bands and with colored plastic leg bands. At least one parent remained with the chicks until they fledged.

Mortality of chicks was high. After the first week, no broods were found with more than two chicks, two broods had only one chick, and eight broods were lost. Suspected predators are magpies, coyotes, gulls, and

raptors; two chicks died from internal disorders. There are about 80-100 long-billed curlews on the Hanford Reservation proper this year, a sizable reduction from the 100 plus nesting pairs found here last year.

In an effort to assemble other information on their life history and status, a survey of western National Wildlife Refuges to locate the birds and follow their movements was made from July 26 through August 20. A survey of other western nesting areas was also included as necessary to evaluate the overall importance of the Hanford Reservation as a curlew breeding ground. Two points are worth noting. The first is that suitable dryland nesting habitat for curlews is rapidly disappearing with the advancement of irrigated farming throughout much of their western range. In other areas, long-standing ranching and farming practices are major factors affecting curlews. Secondly, the National Wildlife Refuge System is, in practice, a duck and goose production system. Refuges are primarily managed for waterfowl and such management does not always benefit other birds. For example, ducks rely on heavy cover to avoid predation during nesting whereas curlews rely on camouflage and require sparse cover; few refuges seek to provide both. Refuges are established around water and often do not include the necessary dry land acreage to provide nesting habitat for other birds.

If the curlew population appears to be declining on the Hanford Reservation, it is important to know why and to maintain an ongoing census of their numbers. The Hanford Reservation is already an island in a sea of irrigated farmland, thereby making it especially crucial habitat to dryland nesting birds such as the long-billed curlew.

Small Birds

The ALE Reserve serves as one of three intensively studied shrub-steppe sites in a National Science Foundation project to Oregon State University. The purpose of the study is to understand the relationship between habitat heterogeneity and avian behavior. The project is directed by Dr. John Wiens and the field studies are performed by graduate students John Rotenberry, Roy Mason, and Robert Pietruszka.

Breeding season studies are aimed at determining nesting bird densities and constructing time and energy budgets based upon observations of bird behavior. Fall and winter studies are largely concerned with detailed measurements of vegetation structure and dispersion. Integration of the data sets should provide information on how adaptive aspects of the behavior of birds are altered in response to environmental heterogeneity.

COAL TECHNOLOGY

- **RESTORATION OF SURFACE-MINED LANDS**
- **EFFECTS OF COAL UTILIZATION ON FRESHWATER BIOTA**
- **EFFECTS AND BEHAVIOR OF FOSSIL FUEL EFFLUENTS ON FRESHWATER ECOSYSTEMS**

Principal Investigators: R. H. Sauer, C. E. Cushing, and D. R. Anderson

Other Investigators: D. H. Fickeisen, C. D. Becker, E. W. Lusty,
D. W. Uresk, J. F. Cline, and J. T. Thorp

Technical Assistants: H. A. Sweany and V. D. Charles, M. A. Combs

COAL EXTRACTION

There is much public concern about the potential for successfully revegetating lands that will be surface mined for coal. The concern is especially valid for landscapes with less than 10 in. of annual precipitation. Topsoiling, fertilization, seeding and surficial irrigation can, in most instances, provide a vegetative cover that provides protection against wind and water erosion. One aim of revegetation is to provide a self-sustaining vegetative cover that is resistant to herbivory as imposed by wildlife, insects or grazing livestock for long periods of time.

PNL's research in revegetation of surface-mined lands is centered upon topographic modification and water harvest with the aim of combining available soil with rainfall harvesting to provide soil and water conditions that are suited to the growth of plants of known economic value.

Because cattle grazing is an important economic use of western rangelands PNL has researched the impact of cattle grazing on the growth and reproductive performances of cattle on ranges in pristine condition so that these values can be used as baseline conditions to evaluate revegetation performances in semi-arid regions.

PNL's coal extraction studies are complementary but not duplicative of studies being conducted in western coal fields by Argonne National Laboratory, U. S. Forest Service, U. S. Bureau of Mines and others.

COAL COMBUSTION AND CONVERSION

PNL's research efforts in coal technology in freshwater ecosystems centers on two areas: 1) Food chain implications of energy residuals, especially metals and organic compounds finding their way into fish directly or indirectly via subsurface and surface waters; 2) the environmental implications of the creation of new aquatic habitat. Newer gasification systems will require, for example, as much as 6 pounds of process water for each pound of coal converted. To support these requirements some form of multiple use will almost certainly be required. In turn, specific research data are needed for the optimal management of such aquatic impoundments; e.g., characterizing effects on aquatic organisms of the combined action of metals and organic compounds; and the preliminary assessment of potential impact on aquatic habitat, based on such considerations as load ratio and toxicity of mixed pollutants. For a given degree of effluent water cleanup, and, at a sufficiently large load ratio, it is quite likely that a well established, attractive aquatic/riparian ecosystem would be maintained, suitable for recreational use.

Current investigations concern the disposal of potentially toxic metals and organics to surface waters associated with coal combustion and coal conversion plants in the western United States. The studies are broadly based and include quantitative ecological characterization of coolant ponds with long operational histories in Utah and Colorado, trace metal transfers between ecosystem compartments, water, sediments and biota in both field and laboratory experiments, immediate effects of process effluents on aquatic organisms, especially fish, and the chemical characterization and bioassay studies at an operational pilot coal-solvent plant.

Restoration of Surface-Mined Lands by Water Harvest

One of the important ecologic concerns expressed about surface mining is the potential for establishing a plant cover to prevent soil erosion and to provide a new substrate suited for the long-term growth of plants. To facilitate the prompt establishment of vegetative cover, surface-mined lands are legally required to approximate original topography with the placement of topsoil over the spoil in layers of sufficient depths to provide soil water storage and an adequate supply of available mineral nutrients.

Most ecologists regard arid landscapes as among the most difficult to restore to plant productivity. This is because of a very stressful plant environment characterized by soil drought and poor soil nutrition, especially low nitrogen. Sometimes soil salts may be present in quantities large enough to inhibit the growth of plants.

The purpose of the project restoration of surface-mined lands conducted on the semi-arid Hanford Reservation is to use modern machinery such as that used in removing the

overburden from coal seams to create a new environment more favorable to plant growth than conventional land leveling and topsoiling (Figure 1.9). The project consists of two parts: 1) modifying the topography to accumulate precipitation (water harvest) and 2) the placement of topsoil in sufficient depth to provide soil water storage and mineral nutrition to enable the growth of plants with higher economic value than those plants that can be sustained under conventional land restoration practices.

Two sets of mound rows resembling spoil banks of strip mines are planned. The first set, perpendicular to our strongest winds, has been completed. The other set, Figure 1.10, parallel with our strongest winds, is only partially complete due to a work strike. The mounds are 97 m long, 17 m apart (crest to crest) and 2.9 m high. A strip of topsoil 4 m wide is placed between the mounds for crop cultivation. The ratio of peak-to-peak distance to topsoil width, 17/4, will deliver approximately a fourfold increase in precipitation, if water runoff efficiency from the slopes is 100%.

Runoff efficiency will be tested in three experimental treatments: butyl rubber sheeting, no treatment (control), and

FIGURE 1.9. Operating Dragline During Construction

paraffin. In addition, a level ground treatment has also been prepared. The butyl rubber has been installed, Figure 1.11, and the paraffin treatment, Figure 1.12, is waiting for equipment to spray melted paraffin on the slopes.

The mound rows perpendicular to the strongest prevailing winds are completed. A cross section showing topsoil placement is shown in Figure 1.13. The rainfall magnification factor is approximately 4. Thus, the meager 15 cm of annual precipitation expected at this location can be magnified to as much as 61 cm.

Three plant species will be used to test the effectiveness of the slope treatments: winter wheat, Siberian wheatgrass and grapes. These plants are expected to respond to a wide range of soil moisture levels and thus make good indicators of effectiveness of each rainfall harvesting technique. The wheat and wheatgrass have been planted. The grapes will be planted next spring.

The wheat, "Nugains," a soft white winter variety planted extensively in eastern Washington in regions receiving precipitation as low as 26-31 cm annually, was planted October 20, 1976. The soil was prepared by making furrows with a disc; the seed was

FIGURE 1.10. Mound Rows Arranged to Prevailing Winds

hand broadcast evenly at the rate of 100 kg/ha. The seeds were covered by disking.

Siberian wheatgrass was planted at the same time and in the same manner. The planting rate for wheatgrass was 6 kg/ha. This grass is used as cattle forage, but has not been successfully grown in areas as dry as this study site without irrigation.

The third test species, grape, will be planted in the spring. Three varieties have been suggested by our consultant from Washington State University's Irrigated Agricultural Research and Extensions Center at Prosser, Washington. The varieties, Concord, Chardonnay and Cabernet Sauvignon, were chosen for their cold and drought hardiness. The consultant will assist in the culture and care of the grapes as viticulture is best handled by an expert.

Productivity of wheat and wheatgrass will be determined by harvest methods. Grape production will be estimated from length of new growth and weight of material pruned

FIGURE 1.11. Completed Rubber Sheeting Treatment Showing Tires Needed to Hold the Sheeting in Place in High Winds

FIGURE 1.12. Paraffin Treatment Mounds Ready for Application of Paraffin Spray

FIGURE 1.13. Cross Section Showing Topsoil Placement

away. Fertilizer requirements will be determined from tissue analysis.

Climatological measurements of precipitation, wind speed, and air temperature will be made at the study site supplemented with periodic measurements of soil moisture and temperature.

A fence is being built to exclude rabbits and mule deer. The fence will be about 2 m high. The lower section will be chicken wire. Barbed wire at 3 dm intervals up to 2 m will prevent mule deer jumping over the fence. The fence is needed to protect the test plantings from damage.

Soil

Soil samples were taken from the topsoil (4 depths to 70 cm in center of each valley plus 2 surface samples in each valley end) and "spoil" (surface from center of each slope). The results are given in Table 1.12.

Natural Vegetation of Steep Slopes

Steep slopes are common land forms throughout the semi-arid and arid western United States. Steep south-facing slopes are often nearly devoid of seed plants. The paucity of

plants is usually attributed to a lack of rooting substrate or extreme stoniness and rapid runoff of precipitation with little opportunity for the water to be stored in the substrate for future root withdrawal. South-facing slopes also provide a more stressful microclimate because the more intense solar radiation leads to higher soil temperature and evaporation rates (Hinds, 1975).

It is likely that steep slopes overlie valuable mineral deposits that may be mined in the future. Restoration of steep slopes in arid regions will require an assessment of the original vegetative conditions. It may be necessary and prudent to accept a nearly barren steep slope as vegetatively rehabilitated. This is especially important if the land restoration criteria insist upon a return to original contour.

The regional climate of the study area is characterized by low precipitation, averaging slightly less than 16 cm annually, nearly all of which falls in the cool part of the year. Summers are hot and dry and winters cold to cool and wet.

Ten pairs of contrasting slopes were selected (Figure 1.14). Twenty-three cover

TABLE 1.12. Soil Analyses Results from Restoration of Surfaced Mined Lands Project

	"Spoil" n=6	Topsoil n=18
	$\bar{X} \pm SE$	$\bar{X} \pm SE$
pH	8.05 $\pm$ 0.04	7.74 $\pm$ 0.04
% Organic matter	0.72 $\pm$ 0.06	1.46 $\pm$ 0.08
P ppm	5.83 $\pm$ 0.91	11.11 $\pm$ 0.70
k ppm	114 $\pm$ 11	316 $\pm$ 12
Ca meq/100gm	5.62 $\pm$ 0.43	6.41 $\pm$ 0.11
Mg meq/100gm	0.93 $\pm$ 0.02	0.30 $\pm$ 0.02
N lbs/acre	30.67 $\pm$ 4.50	54.50 $\pm$ 5.06
Cation exchange capacity Meq/100gm	9.58 $\pm$ 0.20	12.11 $\pm$ 0.22
Conductance mmhos/cm	0.37 $\pm$ 0.01	0.61 $\pm$ 0.03
S ppm	2.50 $\pm$ 0.43	4.50 $\pm$ 0.77
B ppm	0.32 $\pm$ 0.02	0.63 $\pm$ 0.02
Zn ppm	0.62 $\pm$ 0.17	0.78 $\pm$ 0.07
Mn ppm	1.67 $\pm$ 0.21	3.06 $\pm$ 0.26
Cu ppm	0.72 $\pm$ 0.02	0.60 $\pm$ 0.00
Fe ppm	8.33 $\pm$ 0.33	10.44 $\pm$ 0.41
% Base Saturation	71.67 $\pm$ 5.17	70.56 $\pm$ 1.04
	"Spoil" n=5	Topsoil n=18
	$\bar{X} \pm SE$	$\bar{X} \pm SE$
% sand	79.20 $\pm$ 1.24	59.75 $\pm$ 1.21
% silt	16.70 $\pm$ 1.19	32.61 $\pm$ 0.91
% clay	4.10 $\pm$ 0.19	7.69 $\pm$ 0.53

estimates and soil depth measurements were made per slope at 0.5 m intervals. Soil depth was estimated by pushing a metal rod vertically into the soil until it met a solid resistance, usually a rock. Vegetative cover was estimated using the Daubenmire (1959) method.

The percent cover on each slope was converted to proportional cover which was used to calculate diversity indices according to Hill (1973):

$$N_a = (p_1^a + p_2^a + p_3^a \dots + p_n^a)^{1/(1-a)}$$

Where N_a is a diversity index, $p_1 \dots p_n$ are the proportional abundances or covers for the n species, and a is the parameter which determined the diversity index calculated:

- $a = 0$ Number of species
- $a = 1$ Exp (H) (H is Shannon's entropy)
- $a = 2$ Reciprocal of Simpson's modified index for $a = 1$,

$$N_1 = \exp - p_i \ln(p_i) = \exp (H) \quad (\text{Hill, 1973})$$

where i progresses from 1 to n .

Wilcoxon's Signed-Rank Test was used to test for differences in percent cover for each species, total cover, and the diversity indices because it assumes only that the samples come from the same but unknown distribution (Woolf, 1963).

Thirty-seven species were recorded from 20 slopes (Table 1.13). Of these, 23 were common to both slopes, 4 were found only on the south slopes, and 10 were found only on the north slopes. The south slopes had much less cover (25%) than the north slopes (72%), due to annual grasses (S: 14%, N: 8%), perennial grasses (S: 0.7%, N: 48%), and annual forbs (S: 3%, N: 10%). Shrubs and perennial forbs had similar cover percentages. Salvia doriai provided most of the shrub cover. On the north slopes, Agropyron spicatum, Poa sandbergii, and B. tectorum provided most of the cover. The diversity indices calculated from these data are shown in Table 1.14. N_0 , N_1 and N_2 were significantly different. The means for N_0 , the number of species found in the transect, were different at $p < 0.05$ (Table 1.14).

FIGURE 1.14. Photographs of Vegetation on Paired Steep Slopes. The top pair are south-facing slopes and the bottom two are north-facing slopes. Each vertical pair is from the same canyon.

TABLE 1.13. Mean Canopy Cover ($\bar{X}$), Standard Errors (SE), and Fraction of Slopes on Which the Species Was Found (F). Wilcoxon signed rank test used to determine differences at $p < 0.05$ (*) and $p < 0.01$ (**). Means and standard errors are descriptive only and were not used in tests for significant differences.

SPECIES	SOUTH FACING			NORTH FACING		
	F	SE	X	$\bar{X}$	SE	F
GRASSES						
PERENNIAL						
** <u>Agropyron spicatum</u>	0.3	0.17	0.20	13.66	1.68	1.0
<u>Poa cusickii</u>	0	0	0	5.98	3.69	0.4
** <u>Poa sandbergii</u>	0.5	0.24	0.50	28.11	4.52	1.0
TOTAL PERENNIAL GRASSES*		0.29	0.70	47.75	6.07	
ANNUAL						
* <u>Bromus tectorum</u> ¹	1.0	4.80	13.40	7.72	2.91	1.0
* <u>Festuca</u> sp.	0.3	0.06	0.09	0.34	0.11	0.5
TOTAL ANNUAL GRASS		4.80	13.49	8.06	2.91	
TOTAL GRASSES*		4.81	14.19	55.81	6.73	
FORBS						
PERENNIAL						
<u>Achillea millefolium</u>	0.1	0.01	0.01	0.65	0.41	0.3
<u>Astragalus purshii</u>	0.3	0.19	0.32	0	0	0
<u>Balsamorhiza careyana</u>	0.1	0.60	0.60	0.39	0.37	0.3
<u>Calochortus macrocarpus</u>	0	0	0	0.23	0.19	0.2
<u>Crepis atrabarba</u>	0	0	0	0.02	0.01	0.2
<u>Delphinium</u> sp.	0	0	0	0.97	0.57	0.4
<u>Fritellaria pudica</u>	0	0	0	0.01	0.01	0.1
<u>Lomatium grayii</u>	0.6	1.03	2.27	0.07	0.07	0.1
<u>Lomatium macrocarpum</u>	0.1	0.01	0.01	0.84	0.41	0.5
<u>Phlox longifolia</u>	0	0	0	0.17	0.15	0.2
TOTAL PERENNIAL FORBS		1.21	3.21	3.35	0.93	
ANNUAL						
<u>Amsinckia lycopsoides</u>	0.6	0.16	0.24	0.12	0.10	0.3
* <u>Cryptantha circumscissa</u>	0.9	0.23	0.90	0.11	0.07	0.4
<u>Descurainia pinnata</u>	0.8	0.18	0.48	0.65	0.21	1.0
** <u>Draba verna</u> ¹	0.1	0.04	0.04	1.15	0.28	0.9
<u>Epilobium paniculatum</u>	0.2	0.01	0.02	0.01	0.01	0.1
* <u>Gilia leptomeria</u>	0.6	0.10	0.30	0.02	0.01	0.2
** <u>Holosteum umbellatum</u> ¹	0.1	0.01	0.01	3.59	1.24	0.8
<u>Layia glandulosa</u>	0.2	0.02	0.03	0	0	0
<u>Lactuca serriola</u>	0.4	0.20	0.24	0.26	0.13	0.5
<u>Mentzelia albicaulis</u>	0.3	0.04	0.07	0	0	0
<u>Microsteris gracilis</u>	0.4	0.11	0.22	0.51	0.16	0.8
<u>Montia</u> sp.	0	0	0	0.10	0.10	0.1
<u>Phacelia</u> sp.	0.4	0.10	0.17	0.01	0.01	0.1
* <u>Plectritis macrocera</u>	0.4	0.20	0.25	2.25	0.57	0.9
<u>Sisymbrium altissimum</u> ¹	0.2	0.01	0.02	0.13	0.10	0.2
TOTAL ANNUAL FORBS*		0.48	2.99	8.91	1.44	
TOTAL FORBS*		1.30	6.20	12.26	1.71	
SHRUBS						
<u>Artemisia tridentata</u>	0	0	0	0.07	0.07	0.1
<u>Chrysothamnus nauseosus</u>	0	0	0	0.30	0.30	0.1
<u>Eriogonum niveum</u>	0.3	0.61	0.84	0	0	0
<u>Eriogonum</u> sp.	0.3	0.30	0.42	0.59	0.59	0.1
<u>Grayia spinosa</u>	0.1	0.01	0.01	0.74	0.49	0.2
<u>Lithospermum ruderale</u>	0	0	0	0.21	0.13	0.3
<u>Salvia dorrii</u>	0.4	1.24	2.62	1.09	1.02	0.2
TOTAL SHRUBS		1.47	4.18	3.00	1.32	
TOTAL COVER ALL SPECIES*		5.15	24.57	71.07	6.30	

¹ ALIEN SPECIES

TABLE 1.14. Percent Cover and Diversity Indices (see test for explanation of N_0 , N_1 and N_2). Wilcoxon signed rank test used to determine differences at $p < 0.05$ (*) and $p < 0.01$ (**). Means and standard errors are descriptive only and were not used in tests for significant differences.

	SLOPE NUMBER										MEAN	STANDARD ERROR
	1	2	3	4	5	6	7	8	9	10		
----- SOUTH FACING SLOPES -----												
PERCENT COVER **	5.7	4.7	58.0	29.0	21.0	29.0	37.0	8.4	26.0	26.0	24.5	5.0
N ₀ *	8.0	6.0	6.0	11.0	13.0	12.0	10.0	11.0	13.0	9.0	9.9	0.8
N ₁	5.0	3.7	1.6	4.2	6.1	5.7	4.1	6.2	3.0	4.1	4.4	0.5
N ₂	4.0	2.9	1.3	3.0	4.7	4.0	2.6	4.4	2.0	3.5	3.3	0.3
----- NORTH FACING SLOPES -----												
PERCENT COVER **	54.5	64.0	88.0	69.0	98.0	98.0	79.0	72.0	45.0	43.0	71.0	6.3
N ₀ *	11.0	13.0	16.0	16.0	12.0	21.0	9.0	14.0	12.0	11.0	13.5	1.1
N ₁	4.0	4.7	7.0	6.5	3.7	8.5	3.8	5.4	3.8	4.5	5.2	0.5
N ₂	3.0	3.1	5.0	4.3	2.4	6.1	2.8	4.0	2.4	3.4	3.6	0.4

Average soil depth (penetrability) (Table 1.15) on the north-facing slopes (32.9 cm) was 3.8 times greater than on the south-facing slopes (8.6 cm) and different at the 5% level.

South-facing slopes were stonier and also had more sand and less silt and clay (Table 1.15). Organic matter content was slightly higher on the north-facing slope and the pH was slightly lower. There was little soluble salt in the surface and electrical conductivity values were similar when compared between slopes.

Diversity is a measure of how many species are present as examination proceeds down to a certain level of rarity (Hill, 1973). It is interesting to note that on the south slopes, B. tectorum was the dominant species

because it alone used most of the environmental resources, whereas on the north slopes, dominance was shared by B. tectorum, P. sandbergii, and A. spicatum, yet the diversity indices N_1 and N_2 were not different. This may mean that the one dominant species on the slope, B. tectorum, is equivalent in community function to the three codominant species on the north slopes. With more consideration of rare species as given in the N_1 diversity values, the north slopes have slightly more (S: 4.4%, N: 5.3%) species though the difference is not statistically significant. Overall, dominance, as indicated by N_1 and N_2 , appears the same.

The contrast in cover and number of species and similarity in numbers of nonrare species may be related to the seasonal progression of microclimate. The region is characterized by hot, dry summers and cool to cold, wet winters. Because few rains occur during the late spring and summer, soil water reserves are those accumulated during the previous winter (Rickard, 1967). The deeper the soil, the more water available during the spring and summer when temperatures are adequate for rapid growth. The south slopes warm faster in spring so plant growth begins earlier. However, the growing season on the south slopes is also shorter because the thin soil stores little water. The difference, then, between the slope aspects is the length of growing season. Perhaps those species found only on the north slopes require a longer growing period to mature. Further speculating, fewer species are adapted to the more stressful environment of the south slopes, hence the difference in number of species.

TABLE 1.15. Soil Properties of Steep North and South Slopes. Significant Differences Based on t-Test $p < 0.05$ (*) and $p < 0.01$ (**).

SOIL PROPERTIES	SOUTH FACING		NORTH FACING	
	STANDARD ERROR	MEAN	MEAN	STANDARD ERROR
*SOIL DEPTH (VERTICAL) cm	1.0	8.6	32.9	7.0
**ROCK %	5.6	53.3	14.5	5.0
SAND %	2.2	64.4	57.2	7.7
SILT %	2.5	30.8	39.0	3.2
CLAY %	1.1	2.8	3.8	1.1
ORGANIC MATTER %	0.1	0.5	1.1	0.1
pH	0.1	7.7	7.5	0.1
CONDUCTIVITY mmhos/cm	0.06	0.34	0.36	0.03

Another factor is the much lower rock content of the soil from the north slope, which suggests this soil is not totally derived from the alluvial parent material but is of aeolian origin. It also seems likely that the more abundant north slope vegetation cover would decrease wind speeds so that dust would be deposited onto the north slopes. In essence, this is a positive feedback system; a more mesic environment leads to more vegetation, soil, more mesic environment and vegetation.

Presently, most of the vegetative cover on south slopes is provided by the alien annual grass, *B. tectorum*, which suggests these nearly barren south slopes were even more devoid of vegetative cover before the introduction of this grass. The data gathered in this investigation show that steepness per se is not the cause of slope barrenness. The soil and vegetative cover data suggest that the interactions between vegetation and microclimate lead to a more favorable environment with more plant cover on the north slopes favoring the deposition of fine-textured soil material to further increase the thickness of soil.

Statistical Design and Analysis for Terrestrial Ecology

These technics are described in Section 4, pp. 4.32-4.41, where they have been tested in another project concerned with the NTS site. Topics covered include the following:

- Validating the use of averages of individual ratios
 $\bar{R}$ versus $\bar{Y} \div \bar{X}$
- Improving the presentation of field data sets
 frequency histograms and order statistics } versus { $\bar{X} \pm S.D.$
- Estimating spatial pattern with error intervals
 contours weighted for correlation structure } versus { contours through "nearest neighbor" data points
 "Kriging"
- Streamlining the measure of biomass production
 double sampling (harvest and girth) } versus { traditional harvesting of total area

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Trophic Transfers of Minerals and Forage to Cattle on Shrub Steppe Pastures

It is economically important to rehabilitate strip mined lands with plant species which can be utilized by livestock. If productivity is to be regulated by natural rainfall and other attendant climatic factors and soil, then it is important to treat the range forage resource with great care, so as to avoid irreversible damages in terms of changes in plant species composition or species dominance of nonpalatable species. Experimental studies such as this one can test the reactions of forage plants to grazing stress and the ability of the forage to provide essential nutrients to livestock.

Quantitative information concerning palatability, consumption rates and nutritional values of livestock forage plants is essential in determining trophic transfers and long-term management of rangeland resources. Such information is important in maintaining long-term self-sustaining plant growth, vigor and reproduction. Generally, in assessing land values the end results are measured in meat products for man, derived through livestock grazing.

Two experimental pastures on the ALE Reserve, each 9 ha in area, were subjected to moderate spring grazing by yearling steers. These pastures are unique in that they have not had a long grazing history and have been protected from livestock for more than 30 yr. During 1971, 15 yearling steers were confined to the pastures for 58 days, alternating between pastures at weekly intervals. The same stocking rate and system of grazing were maintained for 41 days in 1972. However, in 1973 and 1974 grazing was excluded from one fenced pasture. Seven and five steers were introduced on this pasture during 1973 and 1974, respectively. The steers were removed from the pasture when it was visually estimated that about 50% of the new growth of bluebunch wheatgrass had been grazed, which was prior to the root stage of growth. Steers were weighed before and after the grazing season.

Forage intake rates, which included both live and dead plants, were estimated by measuring water consumption, corrected for forage moisture content, evaporation of water and mean daily air temperatures. The identification of forage plants consumed was determined on feces collected from the steers by using the microscopic technique.

Bluebunch wheatgrass and Sandberg's bluegrass (*Poa sandbergii*) were the most abundant grasses in the pastures. Grasses made up approximately 60% of the herbage available to the steers. Hawksbeard (*Crepis atrabarba*) was the most abundant forb, *Erigeron filifolius* was the important half-shrub and big sagebrush (*Artemisia tridentata*) was the only shrub.

Bluebunch wheatgrass was the important plant consumed by steers, comprising 11% to 56% of the total steer diet. Cusick's bluegrass (*Poa cusickii*) was important early in the grazing season when it comprised 47% of the forage intake. However, at the end of the season it comprised only 12% of the forage intake. Other important plants in the diet include Thurber's needlegrass (*Stipa thurberiana*) and hawksbeard. The preferred cattle forage plant was Cusick's bluegrass, followed by Thurber's needlegrass, hawksbeard and bluebunch wheatgrass in decreasing order.

Bluebunch wheatgrass and Cusick's bluegrass made up 36% and 30% of the total steer diet for both years, respectively. Thurber's needlegrass comprised 9% and 18% of the total diet. These four forage species comprised approximately 93% of the total diet during the 2-yr study. Grasses were the major food items, 77%, followed by forbs and half shrubs, 20%, while shrubs and miscellaneous items made up only 3% of the total diet.

Forage intake during the 1974 grazing season ranged from a low of 9.9 kg/head/day to a high of 10.9 kg/head/day. Forage removed by the five steers was lowest during the first 17-day period on the pasture with a total of 0.55 g/m²/day. The amount removed generally increased throughout the season (Figure 1.15) to approximately 0.58 g/m²/day removed during the last two weeks of the grazing season. The steers consumed a total of 325 kg/ha from the 9 ha pasture during the 57-day grazing period and gained a total of 21.6 kg/ha.

Figure 1.15 illustrates the total live herbage present on a shrub-steppe rangeland on the ungrazed and grazed pasture. The herbage ranged from 27 g/m² early in the growing season to a high of 82 g/m² on the ungrazed pasture. The difference between

FIGURE 1.15. Comparison of Living Plant Biomass on Grazed and Ungrazed Pastures Through the Spring Growing Season

the two pastures represents the amount of forage consumed by the steers.

Table 1.16 shows the seasonal trends of herbage, crude protein, phosphorus and calcium during the 1974 growing season. The highest amounts available on the pasture occurred during mid-April. Crude protein averaged 10 g/m² for the growing season while phosphorus and calcium averaged 0.18 g/m² and 0.42 g/m², respectively.

TABLE 1.16. Seasonal Trends of Total Herbage, Crude Protein, Phosphorus and Calcium (g/m²) Present on the Pasture During 1974

	Early March	Late March	Mid April	Early May	Early June
Herbage	27	36	82	78	67
Crude protein	9.0	8.3	13.9	10.4	9.6
Phosphorus	0.09	0.18	0.23	0.22	0.16
Calcium	0.26	0.29	0.57	0.50	0.48

A peak value of 822 kg/ha of available herbage present on the pasture (Figure 1.16a), of which approximately 325 kg/ha was consumed by steers. The net result was 22 kg/ha of cattle weight gain. Expressed in terms of forage, it requires 15 kg of forage to produce 1 kg of live animal gain or 6.7% conversion.

During the grazing season, 139 kg/ha of crude protein was present in forage plants (Figure 1.16b) and the cattle consumed 37 kg/ha of crude protein which was converted into 4.7 kg/ha of live animal gain. Therefore, 8.4 kg/ha of protein consumed by steers produced 1 kg/ha of protein in animal tissues, a 12.7% conversion. The efficiency

of trophic transfers from crude protein present in the pasture to that which is converted to live animal tissue was 3%.

Phosphorus in forage was estimated at 2.3 kg/ha (Figure 1.16c). Cattle consumed 0.6 kg/ha and utilized 0.5 kg/ha in production of live animal tissues. This conversion rate was approximately 83%. The phosphorus intake includes estimates of the amount consumed in the drinking water and by salt licks. Range forages are often deficient in phosphorus and supplementing of this mineral is essential for normal livestock production. This is a conversion of 1.2 kg/ha phosphorus consumed to produce 1 kg/ha phosphorus in the live animal.

Figure 1.16d illustrates the conversion from calcium present in the pasture to that found in the live animal. The pasture contained 5.7 kg/ha of calcium. Cattle consumed 3.9 kg/ha which includes intake through water and converted this to 1 kg/ha of live animal tissues produced, which was approximately 25%. The trophic transfer of converting calcium which was present on the pasture to that found in the live animal was approximately 18%.

FIGURE 1.16. Weight Distributions of Herbage, Crude Protein, Phosphorus and Calcium Present in the Pasture, Ingested by Cattle and Incorporated into Cattle Tissues

Cattle Diets and Selectivity of Plants

Information on cattle diets and selectivity is needed for land rehabilitation practices following surface mining because cattle grazing is an important biologic use of Western rangelands. This study reports on the botanical composition of steer diets while grazing on semi-arid *Artemisia-Agropyron* pastures.

These data indicate more plant species selected by cattle and those that may apply to land restoration practices. By seeding the most palatable and preferred plants, livestock production may be increased.

Sampling for plant biomass consisted of hand clipping 16 one-half square meter circular plots at 3-week intervals throughout the spring growing season.

Ten samples of cattle feces were collected over a 2-day period bi-weekly during the grazing season. These were oven-dried for 48 hr at 65°C and passed through a Wiley mill fitted with a 1 mm screen. The sample was thoroughly mixed and 5 microscope slides were made from each sample. Twenty microscope fields were examined on each slide. The identification of plant fragments was based on cellular characteristics, primarily the epidermal tissues.

Forage preference indices were calculated as outlined by the following relationships:

$$\text{Preference} = \frac{\% \text{ Diet Frequency} \times \% \text{ Diet Composition}}{\% \text{ Range Frequency} \times \% \text{ Range Composition}}$$

The pasture was comprised of relatively few plant species (Table 1.17). Clearly, the abundant grass was bluebunch wheatgrass. Sandberg bluegrass was the second most abundant grass, and small amounts of Cusick bluegrass and Thurber needlegrass also occurred mixed with small amounts of annual fescues.

The most abundant forbs in the pasture were hawksbeard and tansy mustard, and the most abundant half-shrub was erigeron. Big sagebrush was the only shrub species in the pasture.

Bluebunch wheatgrass comprised between 34 and 50% of the total vegetation at various times during the spring grazing season, and the botanical composition of Sandberg bluegrass varied between 2 and 18%. Hawksbeard contributed between 3 and 14% of the herbaceous forage. Big sagebrush ranged from 11 to 30% during the grazing season.

The botanical composition of the collected fecal material is shown in Table 1.18. These data indicate that Cusick bluegrass and hawksbeard were the most abundant plants selected in the early part of the grazing season. These declined in importance as the season progressed because they became less available. However, bluebunch wheatgrass comprised more of the diet as the season progressed. Although Sandberg bluegrass was

TABLE 1.17. Average Botanical Composition of Plants (% $\pm$ SE) on the Shrub-Steppe Pasture During the Spring Season of 1974

PLANT TAXA	APRIL 9	APRIL 15	MAY 8	MAY 29	AVERAGE
GRASSES					
BLUEBUNCH WHEATGRASS (<i>Agropyron spicatum</i>)	50.0 $\pm$ 9.6 ⁽¹⁾	35.1 $\pm$ 4.9	39.8 $\pm$ 5.9	34.4 $\pm$ 5.3	39.8
CUSICK BLUEGRASS (<i>Poa cusickii</i>)	1.9 $\pm$ 0.8	2.0 $\pm$ 1.0	1.4 $\pm$ 0.7	2.2 $\pm$ 1.6	1.9
THURBER NEEDLEGRASS (<i>Stipa thurberiana</i>)	1.1 $\pm$ 1.0	1.0 $\pm$ 0.6	1.1 $\pm$ 0.7	3.3 $\pm$ 2.5	1.6
SANDBERG BLUEGRASS (<i>Poa sandbergii</i>)	1.9 $\pm$ 0.1	9.0 $\pm$ 0.4	18.4 $\pm$ 1.8	17.6 $\pm$ 2.6	11.7
FESCUE (<i>Festuca</i> spp) ⁽²⁾	0.9 $\pm$ 0.8	3.0 $\pm$ 0.4	8.7 $\pm$ 1.8	5.4 $\pm$ 1.5	4.5
TOTAL	55.8	50.1	69.4	62.9	59.5
FORBS AND HALF SHRUBS					
HAWKSBEARD (<i>Crepis atrabarba</i>)	6.1 $\pm$ 1.6	13.9 $\pm$ 4.8	3.0 $\pm$ 1.3	8.0 $\pm$ 2.8	7.8
TANSY MUSTARD (<i>Descurainia pinnata</i>)	2.8 $\pm$ 0.3	6.9 $\pm$ 0.9	4.4 $\pm$ 1.1	0.9 $\pm$ 0.5	3.8
LUPINE (<i>Lupinus laxiflorus</i>)	0.7 $\pm$ 0.7	0	0.7 $\pm$ 0.7	0.2 $\pm$ 0.2	0.4
ERIGERON (<i>Erigeron filifolius</i>)	4.0 $\pm$ 1.4	10.4 $\pm$ 2.8	6.2 $\pm$ 2.0	12.2 $\pm$ 6.4	8.2
PHLOX (<i>Phlox longifolia</i>)	0.7 $\pm$ 0.7	0.5 $\pm$ 0.3	0.8 $\pm$ 0.8	1.5 $\pm$ 1.1	0.9
MISCELLANEOUS	0.1	0.4	4.6	1.5	1.5
TOTAL	14.4	32.1	19.7	23.8	22.5
SHRUBS					
BIG SAGEBRUSH ⁽³⁾ (<i>Artemisia tridentata</i>)	29.8 $\pm$ 6.9	17.8 $\pm$ 4.1	10.9 $\pm$ 2.5	13.3 $\pm$ 3.1	18.0

(1) CALCULATED BY CLIPPED (LIVE + DEAD) HERBAGE VALUES (n = 16)

(2) ANNUAL SPECIES

(3) VALUES CALCULATED ON ONE HARVEST DATE

TABLE 1.18. Botanical Composition (% $\pm$ SE) of Steer Diets During 1974

PLANT TAXA	APRIL 9	APRIL 15	MAY 8	MAY 29	AVERAGE
GRASSES					
BLUEBUNCH WHEATGRASS	11.3 $\pm$ 0.8 ⁽¹⁾	27.1 $\pm$ 10.0	33.7 $\pm$ 1.0	55.7 $\pm$ 4.6	31.9
CUSICK BLUEGRASS	48.2 $\pm$ 1.3	23.7 $\pm$ 1.4	17.1 $\pm$ 0.9	11.7 $\pm$ 0.4	25.5
THURBER NEEDLEGRASS	11.9 $\pm$ 2.2	9.0 $\pm$ 1.6	17.4 $\pm$ 3.1	9.4 $\pm$ 0.8	11.9
SANDBERG BLUEGRASS	1.0 $\pm$ 1.0	0.1 $\pm$ 0.1	0.2 $\pm$ 0.2	0.0	0.3
FESCUE SPECIES ⁽²⁾	3.4 $\pm$ 0.3	0.9 $\pm$ 0.9	6.1 $\pm$ 1.3	5.8 $\pm$ 1.4	4.1
TOTAL	75.8	60.8	74.5	82.6	73.2
FORBS AND HALF SHRUBS					
HAWKSBEARD	23.9 $\pm$ 0.7	33.7 $\pm$ 4.2	19.6 $\pm$ 5.7	12.0 $\pm$ 3.0	22.3
TANSY MUSTARD	0.7 $\pm$ 0.3	0.2 $\pm$ 0.2	3.0 $\pm$ 0.9	1.4 $\pm$ 0.6	0.3
LUPINE	0.3 $\pm$ 0.3	0.0	0.0	0.0	0.1
ERIGERON	0.0	0.7 $\pm$ 0.2	0.1 $\pm$ 0.1	0.9 $\pm$ 0.9	0.5
PHLOX	0.0	1.4 $\pm$ 1.2	1.6 $\pm$ 1.1	1.8 $\pm$ 1.8	1.2
MISCELLANEOUS	0.5 $\pm$ 0.5	0.4 $\pm$ 0.2	1.3 $\pm$ 0.1	1.4 $\pm$ 0.3	0.9
TOTAL	25.4	36.4	25.6	17.5	26.3

(1) CALCULATED BY RELATIVE DENSITY VALUES (n = 12)

(2) ANNUAL SPECIES.

relatively abundant, it was only sparsely represented in the fecal material. Tansy mustard and erigeron were relatively abundant plants on the pasture, but comprised little of the cattle dietary consumption. Botanical composition of steer's diets show that grasses were the major food items followed in importance by forbs and half-shrubs.

Indices of the relative preferences of the important herbaceous species growing in the shrub-steppe pasture are shown in Table 1.19. Clearly, Cusick bluegrass and Thurber needlegrass were the highly preferred grass species. The steers showed no preference for Sandberg bluegrass. Hawksbeard was the preferred forb species.

These data correlate very well with general field observations made as to the plant species preferences of cattle under moderate spring grazing in these same pastures.

TABLE 1.19. Relative Steer Preference Indices of Important Forage Plants in the Shrub-Steppe Pasture at Four Times During the Spring Grazing Season

SPECIES	APRIL 9	APRIL 15	MAY 8	JUNE 6
GRASSES				
CUSICK BLUEGRASS	39.24	16.97	11.69	6.56
THURBER NEEDLEGRASS	24.20	8.79	22.93	2.92
SANDBERG BLUEGRASS	0.02	*	*	0
BLUEBUNCH WHEATGRASS	0.03	0.19	0.23	0.62
FORBS AND HALF SHRUBS				
HAWKSBEARD	1.40	0.91	1.70	0.31
LUPINE	*	0	0	0
TANSY MUSTARD	*	*	0.01	0.10
PHLOX	0	0.48	*	0.34
ERIGERON	0	*	*	*

* < 0.01

Trace Metals from Coal-fired Power Plants

Present effort is directed to the study of leachates from ash and char residues. Earlier studies have examined the terrestrial deposition of trace metals (Vaughan et al., 1975) and of SO₂, sulfates, particulates, and NO_x (Renne and Elliott, 1976) in the vicinity of planned coal-fired electrical generating plants. In the trace metal study cited, an ecological assessment was made for a modern 1400 MW_e installation burning Western Coal and operating to 99% ash removal standards. Cadmium was the only metal element showing appreciable soil buildup over 40 years. Nevertheless, the environmental impact of this operation was not expected to be significant. Combination plants operating at less stringent standards and burning other type of coal might present some degree of concern as regards airborne metal emissions and deposition within 100 km distance of the combustion plant. In the Northwest, sulfur emissions would affect larger areas and might cause more serious impact (Renne and Elliott, 1976; Sauer, 1976).

In current effort, site surveys at existing coalburning power plants in the West were initiated to provide data on: 1) existing levels of trace metals in various components of the freshwater ecosystems, and 2) effluent trace metal content to use in designing laboratory studies to determine transfer coefficients of trace metals in freshwater ecosystems.

Three sites have been visited: 1) Valmont Plant, Colorado, 2) Hale Plant, Utah, and 3) Huntington Plant, Utah. Only the Valmont site uses a cooling pond and it is presently our site of most interest. The Hale Plant employs a "once-through" cooling system and discharges cooling water directly

into the Provo River. The Huntington Plant utilizes a "once-through" cooling system and an evaporation pond. Thus, the Utah sites are of interest primarily in terms of characterizing the composition of their effluents.

The cooling pond system at Valmont uses three lakes: Leggett, Valmont #3, and Hillcrest, in various combinations depending upon ambient water temperatures. The Valmont Plant draws intake water from Hillcrest Lake. Leggett Lake receives both the cooling water discharge as well as overflow from the ash-disposal ponds.

The Valmont site was sampled 26-27 May 1976; results received to date are presented in Table 1.20. Three water samples are being analyzed for trace organics by gas chromatography-mass spectroscopy. Data reveal, in general, that trace metal concentrations in the sediments and biota are high, in most cases, particularly in Fe and Zn. Despite the low concentrations in the water entering Leggett Lake, an obvious build-up of trace elements appears to be occurring in the sediments and biota.

We are hesitant to interpret these data further without additional information, but they do permit partial characterization. Our second sampling of the Valmont site (November 1976) will include water, biota, and sediment from Leggett Lake, Valmont #3, Hillcrest Lake, South Boulder Creek (source of make-up water), and a nearby lake not receiving aqueous effluents but having similar edaphic features. These data will permit a more thorough evaluation as well as provide information on seasonal variations.

During our site visits to the two Utah plants, the Hale Plant was temporarily burning a small allotment of gas, and the Huntington Plant was closed for extensive repairs after a turbine explosion. Thus, the effluent and water samples from Hale are atypical; another set of samples will be obtained when the plant is again burning coal. Samples of coal, fly ash, and water from the evaporating pond at the Huntington Plant were collected. In general, trace metals in the water samples from Hale and Huntington were quite low in concentration. Samples of fly ash and coal from both plants contained significant quantities of most trace metals, especially Fe, Zn, Mn, and Cr. Further data on effluents from these sites will be collected when they return to normal operations. At ERDA's request, more emphases in the project will be devoted in the future to the study of a solvent refined coal (SRC) plant at Fort Lewis, Washington."

TABLE 1.20. Trace Element Concentrations in Valmont
Site Sample, X-Ray Fluorescence

SAMPLE	Mn	Cr	Fe	Ni	Cu	Zn	Pb
WATER SAMPLES ⁽¹⁾							
LEGGETT LAKE							
INLET CANAL	0.0006	0.0004	0.0684	0.0004	0.0018	0.0010	0.0004
MIDDLE OF LAKE	0.0002	0.0003	0.0554	0.0004	0.0021	0.0006	0.0002
VALMONT #3							
SHORELINE	0.0014	0.0000	0.1434	0.0006	<0.0001	<0.0001	0.0009
HILLCREST LAKE							
PLANT INTAKE	0.0012	<0.0008	0.0967	0.0008	<0.0003	<0.0002	<0.0005
CANAL INLET	0.0004	0.0006	0.0581	0.0004	0.0017	0.0004	0.0003
ASH POND #2							
SLURRY ⁽²⁾	0.0963	<0.0340	70.47	0.163	0.6249	0.3415	0.2068
POND	0.0196	0.0036	1.271	0.0010	0.0875	0.0078	0.0050
ASH POND #1							
POND ⁽³⁾	<0.0001	0.0002	0.0124	0.0006	0.0027	0.0015	0.0002
EFFLUENT ⁽⁴⁾	0.0	0.0016	0.0352	0.0009	0.0025	0.0006	<0.0003
SEDIMENTS AND BIOTA ⁽⁵⁾							
SEDIMENT CORE, 18"							
UPPER 6"	581.2	97.78	39100.0	28.8	201.0	275.1	117.5
MIDDLE 6"	546.6	89.71	38500.0	18.0	196.8	301.0	211.6
LOWER 6"	525.8	62.44	31600.0	15.7	194.1	194.1	171.0
SURFACE SEDIMENTS	549.2	126.6	44700.0	22.7	187.9	237.5	96.9
BOTTOM ORGANIC MATTER ⁽⁶⁾	526.0	213.1	30100.0	43.0	165.6	281.2	86.2
SESTON	142.0	28.4	8000.0	40.8	66.8	91.6	19.1
BLUEGILL							
MUSCLE	<1.719	5.30	49.0	0.98	1.39	37.8	1.21
CARCASS	15.0	8.32	229.6	1.59	4.29	117.6	2.07
CARP							
MUSCLE	2.358	5.75	56.1	1.07	3.0	46.6	1.58
CARCASS	81.266	11.40	709.6	2.43	18.9	1431.0	11.4

(1) ppm

(2) ANALYSIS OF DISSOLVED AND FINE SUSPENDED MATTER; LARGE PARTICULATES EXCLUDED

(3) ASH POND #1 NOT RECEIVING EFFLUENTS, ALTHOUGH OVERFLOW WAS ENTERING LAKE

(4) OVERFLOW ENTERING LEGGETT LAKE

(5) ppm/gram, DRY WEIGHT, ALL FROM LEGGETT LAKE

(6) COARSE > 1 mm) ORGANIC MATTER STRAINED FROM SEDIMENTS.

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Shortly after this writing, an independent project was funded at PNL by the Electric Power Research Institute (EPRI), Menlo Park, CA. Concurrently, ERDA effort as described above was shifted at sponsor's request to the study of a solvent refined coal plant at Fort Lewis, WA (see following section). Relative to these projects, a co-joint ERDA/EPRI agreement exists to fund projects on a complementary coordinated basis without duplication of program. The EPRI study (RP 880-1) aims: a) to synthesize existing information on cooling ponds and lakes, b) to analyze existing data for establishing inferences on population and ecosystem effects of cooling water, and c) identify and explain effects that cannot be assessed with existing data. In addition to the above mentioned sites, about 60 additional sites are being evaluated with respect to load ratio, pond surface acreage/MWe output, contaminant analyses and operational factors. A final report of this work will be made publicly available in accord with the cooperative agreements between ERDA and EPRI.

Evaluation of Effluent from a Solvent Refined Coal Plant

Because constituents of liquid effluents from coal processing plants are potential pollutants of aquatic habitats, information on their identity and relative concentration is needed before commercialization of the process is advocated in a broad scale.

Studies were initiated in 1976 at a solvent refined coal (SRC) pilot plant located near Fort Lewis, Washington. The plant is operated by Pittsburgh and Midway Coal Mining Company under an ERDA contract. The new process used by this plant mixes crushed coal with a recyclable coal-derived solvent. When the mixture is heated to about 825°F in the presence of hydrogen at 10,000 psi or greater, the coal swells and dissolves. The solution is filtered to remove the inorganic residue and evaporated. The solidified product is refined coal with less than 1/2% of either sulphur or residual ash capable of providing reduced air emissions during burning.

Liquid effluents from the SRC plant are discharged into a small, semi-permanent pond lacking a surface outlet, and they eventually percolate into the underlying gravel. Prior to discharge, the effluent is subjected to complex treatment based on advanced technology to minimize the presence of potential toxicants and carcinogens. The final treatment includes an oxycontact biodigester unit, followed by sand and activated carbon filtration. Nevertheless, some impurities represented by organic and inorganic fractions remain in the process effluent.

Our research is designed to investigate the behavior and effects of the SRC effluent on individuals, populations and communities of freshwater ecosystems. The specific objectives of the program are to:

- Characterize the organic and inorganic constituents occurring in liquid SRC plant effluent.
- Determine the acute and chronic toxicity of liquid SRC effluent, and of key components of the effluent.
- Measure the accumulation and retention of key SRC effluent constituents in selected freshwater plants and animals.

Effluent Characterization

Permission to collect effluent samples at the Fort Lewis plant was not obtained from Pittsburgh and Midway Company and the Office

of Coal Mining research, ERDA, until mid-1976. Through October 1976, samples were obtained on four occasions from successive locations in the liquid SRC treatment process for component identification: a) untreated effluent leaving the surge reservoir; b) partially treated effluent leaving the ion exchange columns; and c) treated effluent leaving the biodigester and filters, and entering the discharge pond. Inorganic analyses were conducted by x-ray fluorescence, and organic analyses are underway by a combination of nitrogen sparging, gas chromatography and liquid chromatography.

Only data from the first inorganic analysis, collected 21 May, are now available (Table 1.21). The in-plant treatment process appears efficient in reducing most inorganic fractions in the SRC effluent, since concentrations decrease according to the stage of effluent treatment. The liquid leaving the biodigester and entering the discharge pond contains minimal element concentrations. However, until demonstrated otherwise, levels of inorganics (and organics) in the discharge cannot be assumed to remain consistent from one sampling period to the next, due to variations in efficiency of in-plant treatment and/or internal design changes. Also, periodic backflushing of the filters and biodigester will temporarily increase constituent levels, perhaps drastically.

TABLE 1.21. Inorganic Analyses (ppm) of SRC Effluent as Determined by X-Ray Fluorescence, Replicates 1 and 2

ELEMENT	UNTREATED SURGE RESERVOIR		TREATED ALUM. AND POLYELECTROLYTE		TREATED BIODIGESTER (POND EFFLUENT)	
	1	2	1	2	1	2
Ni	<0.01	<0.01	0.001	<0.001	<0.001	<0.001
Cu	0.003	<0.001	<0.001	<0.001	<0.001	<0.001
Zn	0.10	0.13	<0.001	<0.001	<0.001	<0.001
Ga	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hg	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Se	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pb	<0.01	<0.01	<0.001	<0.001	<0.001	<0.001
As	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Br	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Rb	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Sr	0.09	0.09	0.09	0.09	0.09	0.09
Al	0.47	0.32	0.43	0.32	0.23	0.24
Si	5.58	3.19	4.38	2.40	2.55	2.43
P	0.20	0.13	0.10	0.11	0.04	0.04
S	26.37	13.52	6.72	5.90	0.12	0.11
Cl	0.04	0.07	0.03	0.03	<0.01	<0.01
K	0.06	<0.01	<0.01	<0.01	<0.01	<0.01
Ca	0.019	0.014	<0.01	<0.01	0.001	0.001
Ti	<0.01	<0.01	<0.001	<0.001	<0.001	<0.001
V	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cr	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Mn	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Fe	0.46	0.53	0.02	0.01	<0.01	<0.02
Co	<0.01	<0.01	<0.001	<0.001	<0.001	<0.001

Continued sampling and analysis is required to quantify variations in both inorganic and organic fractions of the SRC plant discharge. Unfortunately, analyses of inorganics and organics is a costly and time-consuming operation that limit the number of samples than can be processed under the present budget.

Effluent Bioassays

Three bioassays have been completed with SRC effluent taken at the discharge pond inlet and transported to our Richland laboratories in 12-gal glass carboys. Standard bioassay design involves 12 aquaria, 2 each containing various dilutions, (0, 20, 40, 60, 80, 100%) of SRC effluent with untreated Columbia River water in a randomized pattern, and a 96-hr exposure period. Of necessity, each bioassay was static to avoid further dilution of the effluent.

The first test used juvenile rainbow trout (10/aquaria) at 18.5°C ($\pm 0.5^\circ$) water temperature. The water was not aerated to avoid possible loss of volatile organics. No effluent toxicity was evident, but dissolved oxygen declined to critically low levels and some test fish were eventually lost.

The second test used fingerling large-mouth bass (10/aquaria) at 18.5°C ($\pm 0.5^\circ$) water temperature. The water was aerated to avoid low oxygen stress. Disease accounted for a few late deaths but, again, no effluent toxicity was evident among test species.

The third test used juvenile rainbow trout (10/test) at a 17.5°C ($\pm 0.5^\circ$) water temperature. The water was aerated. All test fish in 100% SRC effluent died in 24 hr and mortality was high in the 80% dilution. No losses occurred at 40% and 20% dilutions or among the controls. Crayfish placed in 100% effluent after 20 hr survived, although the rainbows used initially had died. Higher than "normal" toxicant concentrations were expected in this test because the effluent was collected during backflush operations at the SRC plant. Apparently, the toxic component was a volatile organic that disappeared after 24 hr aeration. The precise organic and inorganic constituents involved will be identified during subsequent chemical analyses.

Continuation of the program will involve additional laboratory bioassays with SRC effluent and *in situ* bioassays conducted with caged fish in the plant discharge pond. This work will be extended to analyses of inorganic fractions in the pond water and sediments, and will track the cycling of trace metals among pond water, sediments and pond biota.

Uptake and Retention of Cu and Zn by Sediments and Macrophytes

Increased demand for electrical energy has resulted in a corresponding increase in concern about potential environmental impacts resulting from discharge of effluents from power-producing plants. A new program was begun in 1975 to examine the behavior and effects of trace metals from coal-burning power plants in freshwater ecosystems. This report presents data on the movement of Cu and Zn, two known components of effluents from coal combustion, between water and sediments and between sediments and macrophytes. Three sites were selected for exploratory studies to determine transfer coefficients of these trace metals. The study sites were: 1) Gable Mountain Pond (GMP), a lake at Hanford that receives low-level radioactive wastes and also contains high levels of trace metals in its sediments; 2) Quarry Pond, an off-site control lake with lower levels of trace metals; and 3) artificial ponds containing Columbia River water and sediments. Table 1.22 presents data on Cu and Zn levels in components of the ecosystems.

TABLE 1.22. Concentrations of Trace Metals in Water, Sediments, and Macrophytes, ppm

	GABLE MOUNTAIN POND		QUARRY POND		COLUMBIA RIVER	
	Cu	Zn	Cu	Zn	Cu	Zn
WATER	0.004	<0.1	---	---	0.002	0.026
SEDIMENTS	80	860	16	59	100	200
POTAMOGETON	17	180	21	40	---	---
MYRIOPHYLLUM	21	330	7	30	---	---

* NOT MEASURED

** NOT AVAILABLE

Two experimental manipulations were done. First, sediments and macrophytes were transferred from Quarry Pond to GMP and sampled over time to provide estimates of rates of change. Second, sediments and macrophytes were transferred from GMP to the artificial ponds and also sampled over time. No transfers were made from GMP to Quarry Pond due to radioactive contamination of sediments and plants from GMP. Two species of aquatic macrophytes were studied, *Myriophyllum heterophyllum* and *Potamogeton richardsoni*.

Sediments

For the sediment transfer experiments, statistical analysis revealed that only the change in Zn concentration of sediments

moved from Quarry Pond to GMP was significantly ($P < 0.05$) greater than zero over the 97-day test duration. Both the upper and lower 5 cm layers of core samples taken exhibited this increase, which suggests the Zn is the more mobile of the two elements. Apparently Cu and Zn are bound strongly within sediments, which is consistent with data from the literature.

Macrophytes

Figure 1.17 shows the actual data, as well as the results of nonlinear least squares fits to the observed data, for the concentration of Cu and Zn in Potamogeton and Myriophyllum. Predicted uptake of both Cu and Zn by Myriophyllum was rapid, with concentrations reaching levels similar to resident plants in about 20 days (initial concentrations are shown in Table 1.22). Loss of Cu and Zn was much slower in Myriophyllum than in Potamogeton (Figure 1.17). Zn uptake by Potamogeton was twice that of Myriophyllum, reaching equilibrium in less than 10 days.

The exponential uptake parameters for Cu and Zn by Myriophyllum were similar (0.146 for Cu and 0.142 for Zn), as were loss parameters (0.006 for Cu and 0.008 for Zn). This represents nearly a two order magnitude difference between uptake and loss exponential parameters. Earlier work in our laboratory with uptake and retention of ^{65}Zn by periphyton, another primary producer, gave a single order of magnitude difference between these parameters (Cushing et al., 1975). The relative magnitude of absorption and adsorption processes for these two primary producers may explain part of the observed difference. The exponential uptake parameters of Zn by Potamogeton was 0.516; no uptake parameter for Cu was obtained. Loss parameters for Cu and Zn were 0.017 and 0.010, respectively.

Calculated half times ($t_{1/2}$) for uptake for Cu and Zn by Myriophyllum were similar, but absolute amounts accumulated showed that 18 times as much Zn as Cu was taken up over the same time. In contrast, the half-time

FIGURE 1.17. Cu and Zn Uptake and Retention in Potamogeton and Myriophyllum (• = observed values, — = predicted values)

for uptake of Zn by *Potamogeton* was four times faster than *Myriophyllum*, but similar amounts of each element were taken up over that time.

The parameter estimates the results of a complex interaction among the roots, shoots, water, sediments, attached microflora, etc., and represents net fluxes or rates. Further work is required to separate net fluxes into their respective components (individual rates).

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Immediate Electrophysiological Response of Fishes to Toxic Materials

The physiological and behavioral responses of fishes to toxicants expected in the effluents from coal conversion processes are being investigated. An electrophysiological technique has been developed which permits recording electrocardiogram (EKG), respiratory rate and activity from a fish without restraining it by attached cannulae or electrodes. Our protocol requires recording the resting levels of heart, respiration and activity rates prior to adding selected toxicants at concentrations increasing with time while simultaneously monitoring the electrophysiological signals.

This is a new task and the major effort has been acquisition of equipment and development of the technique. Procurement included a polygraph to record the electrophysiological data, a digital oscilloscope to monitor the signal, and a recording computer terminal to permit storage of the digitized data and transmission to a remote computer for data processing. Assembly of the electronic equipment and debugging the system proceeded simultaneously with development of the system and testing of various electrode configurations.

We examined several electrode configurations which allow the fish to swim freely in an aquarium during testing. All of the configurations resulted in poor signal to noise ratios and the signal amplitude was sensitive to orientation and position of the fish in the aquarium. Signals generated by rainbow trout swimming activity masked EKG and respiratory signals, although it was possible to obtain heart and respiratory pumping rates with some of the configurations most of the time. Only poor recordings were obtained with black bullhead or pumpkinseed sunfish.

Because lateral suppression of the electrical field around a trout and enhancement of the field dorso-ventrally occurs (D. Webber, personal communication), we modified a tube chamber previously used to record rainbow trout EKG by moving the electrodes from the sides to the top and bottom of the chamber (Figure 1.18). This configuration resulted in very good signal-to-noise ratios with little interference from activity. A typical recording is presented as Figure 1.19.

FIGURE 1.18. Modified Tube Chamber for Fish Physiology Studies

FIGURE 1.19. EKG Signals Generated by Rainbow Trout. Upper curve is EKG. Lower Curve Represents Gill Ventilation Activity.

With the chamber diameter slightly less than the fish's length, the fish may turn around in it easily, but generally remains parallel to the electrodes. Signal amplitudes are not affected by orientation or position of the fish in the tube. Since this apparatus restrains the fish somewhat, it can no longer be considered "free-swimming." However, fish did not appear stressed and were not restrained by electrodes. This chamber also permits better control over the concentration of toxicant reaching the fish. Thus, we have selected it for future testing.

We plan to introduce the toxicant to a mixing chamber and supply the exposure chamber from the mixed water. Adding a constant toxicant concentration to the mixing chamber containing river water, while withdrawing water to supply the exposure chamber, permits an increasing concentration over time and from the initial zero concentration level. A mathematical model of this toxicant delivery system was devised and used to select flow and chamber volumes. We are constructing the exposure system and toxicant mixing and delivery system and plan to gather empirical data to test the model.

Initial testing with phenol (0.8 ppm) indicated a reduction of heart rate of

rainbow trout from 86 to 66 beats/min and a reduction in swimming activity within 10 to 20 min of exposure (Figure 1.20). Bursts of swimming activity caused increases in heart rate as indicated in Figure 1.20.

FIGURE 1.20. Heart Rate and Swimming Activity of Rainbow Trout Influenced by Testing With Phenol Exposure Began at Time 0 (0.8 ppm) and was increased at time 108 to 1.7 ppm. ↑ Indicates Burst of Swimming Activity.

An apparent change in the shape of the QRS complex of the EKG also took place, and we are awaiting computer software availability for confirmation. The change, recorded at toxicant concentrations approximately one order of magnitude below the 96-hr TL_{50} for phenol, might represent a change in blood volume or in tissue electrical conductivity.

Our goal is to determine the threshold of immediate effect for changes in heart rate, activity and respiratory pumping rate for a variety of toxic materials associated with coal utilization.

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2.0 SHALE OIL

- **TERRESTRIAL EFFECTS OF OIL SHALE DEVELOPMENT**
- **DEVELOPMENT OF CHEMICAL REACTIONS, STABILITY AND TRANSPORT MODEL OF OIL SHALE PROCESS WASTES IN SOIL**

The current shortage of domestic energy supplies will lead to increased exploitation of domestic fossil fuels. In addition to the liquefaction and gasification of coal, use of vast domestic resources of oil shale may provide another long-term alternative to petroleum-derived fuel products. Practical processing experience with shale oil is limited in comparison to other fossil fuel production since serious industry consideration of schemes for shale oil production is fairly recent. Methods proposed for winning shale oil from its deposits range from conventional mining and aboveground retorting technologies to in situ combustion methodology. Although the technical and economic aspects of this wide range of options have been generally studied, the environmental impacts must be assessed, as 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 and reclamation of processed shale. In the mining process, aquifer disruption, runoff and movement through the soil profile to groundwaters of process residuals 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, saline, and trace metal residues. Disposal of spent shale presents problems in loss of recreation land, in reclamation of land occupied by spent 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 on organic N in oil shale can lead to the formation of organic components which are unique, thus producing environmental problems not ordinarily encountered in other energy industries.

The current program focuses on the chemical characterization of effluent residuals (see BNWL 2100, Part IV, Physical and Technological Programs), the potential toxicity of effluent residuals (see BNWL 2100, Part I, Biomedical Sciences), and the soil processes affecting plant uptake toxicity and vegetational events. Supporting research on the feasibility of revegetation in arid climates and application of research on a water harvesting approach are reported in the preceding section of this report, where they are of primary importance to surface mining of coal (see Section 1.0).

The specific studies reported in this section concern soil biochemical transformations impacting human food chains and the viability of green plants. This effort represents a multitask interdisciplinary research approach which takes unique advantage of PNL capabilities in hydrocarbon chemistry, soil science, microbiology, and plant physiology. The principal objectives of the project are to: 1) develop a basic knowledge of important extraction and environmental processes influencing the form, mobility, chemical, and microbiological stability, and plant availability and toxicity of organic residuals in retort waters and spent shales, 2) model movement of stable organic residuals in soil and spent shale, and 3) develop a rational basis for selection and subsequent laboratory and field testing of proper soil conditioners, irrigation waters and native or agricultural plant species to revegetate spent shales and result in 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 of waste and for viable restoration of shale lands.

One of the major questions being addressed by PNL scientists, in aquatic projects, is the biological availability and effects of effluents resulting from energy production and use. However, to do this, the entire system we are concerned with must be assessed to identify those factors and processes that have a significant impact on the behavior of a particular material in the ecosystem. We need to establish the baseline conditions, identify the impacts to the ecosystem, understand the processes that take place in the biogeochemical cycling of a material, and be able to accurately measure effluent bioavailability and effects so that the ultimate cost to the ecosystem can be estimated.

Presently, one of man's major concerns is how to best utilize the resources of the aquatic ecosystem. For the freshwater system, this means deciding just how much industrial and domestic waste materials our lakes and streams will absorb and still be desirable for other uses such as domestic water supplies, fisheries and recreation. The same is true for the marine environment, except that the coastal system is receiving pressure as a site for all types of industrial development, particularly, energy recovery and production. Indiscriminate disposal of waste products is not desirable for several reasons, but beyond a certain point, the removal or reduction of materials from an effluent stream requires costly energy. Thus, to conserve our available energy, it is important to determine and understand how to use the natural cycling capabilities of the aquatic systems to the benefit of man.

This section summarizes the research that PNL has undertaken to understand how the ecosystem functions and what impacts are caused by man's development and use of various energy technology. The state-of-the-art varies significantly between the energy areas that are involved in this multidisciplinary effort and, therefore, the studies are directed to establishing an understanding at the level necessary to assess the significance of the environmental impacts.

• TERRESTRIAL EFFECTS OF OIL SHALE DEVELOPMENT

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A key, initial element of newly funded research programs to assess the terrestrial effects of oil shale mining and extraction is the development of appropriate analytical techniques to determine the mobility of organics in spent shales and soils, microbial alteration of these compounds and their plant availability. The results of initial phases of these programs are described below.

Separation of Polar Shale Oil Compounds Using High-Speed Liquid Chromatography

Previous studies of the chemical composition of shale oil have identified major classes of water-soluble, polar organic compounds present in shale oil and spent shales. These classes include polycyclic aromatic hydrocarbons (Schmidt-Collerus, 1974), thiophenes and pyridines (Cook et al., 1968) and other nitrogen bases (Brown et al., 1970; Poulson et al., 1971).

Some compounds from the above classes are toxic and are present as constituents of spent shale or process waters derived naturally or through chemical conversion during the processing of shale oil. These compounds have the potential of being biologically available in surface deposits as well as entering into groundwaters. This year methods were explored for the separation of specific compounds in the water soluble polar organic fraction by high-speed liquid chromatography (HSLC). A detailed report has been published (Riley, 1976).

For this study, a Waters Model 6000 liquid chromatograph was used with solvent programming mode and sample loop injection system (maximum injection volume, 2 ml). Compounds eluting from the liquid chromatographic (LC) columns were detected employing a Shoefel SF 770 UV[®] monitor containing

wavelength monitoring capability between 200 nanometers (nm) and 400 nm.

In the first part of the study, LC conditions were established which optimized resolution and minimized analysis time of an aromatic-PNA hydrocarbon mixture (9 compounds in all). Reverse-phase liquid chromatography was chosen as the mode of separation with a μ Bondapak (Waters Associates) C₁₈ column and acetonitrile-water as the mobile phase. Utilization of solvent programming and two sets of conditions allowed for essentially baseline resolution of 5 of the 9 compounds in the mixture with an analysis time in the range of 18-23 min. A typical chromatographic separation is shown in Figure 2.1. The baseline resolved compounds were naphthalene, phenanthrene, anthracene, pyrene and benzo(α)pyrene.

From the standard, anthracene was selected as representative, and its minimum detectable concentration level at 254 nm (anthracene Σ max at 253 nm $\sim$ 250,000) was determined. A calibration curve for a sample of anthracene (170 ng/ml) was prepared by injecting a range of sample volumes (50 μ l to 1.0 ml) on the liquid chromatograph. The curve showed excellent linearity throughout the entire range (50 μ l to 1.0 ml; 8.5 ng to 170 ng). Injection of 1.0 ml of an anthracene sample (6.3 ng/ml) produced a peak whose height was about 2.5 times the height of noise level.

FIGURE 2.1. Separation of Components of Aromatic-PNA Standard by Reverse-Phase Liquid Chromatography. U.V. at 221 nm.

Assuming the minimum detectable level to be twice the height of noise level, the minimum amount of anthracene that could be detected under these conditions was about 2.5 ng. A minimum concentration range was calculated to include all nine compounds based on a maximum injection volume of 2.0 ml. The minimum detectable concentration range under these conditions was estimated to be 2.5 to 15.5 ppb.

In order to obtain information as to the ability of HSLC to resolve aromatic compounds in shale oil, a hexane soluble shale oil sample^(a) was prepared and analyzed by reverse-phase liquid chromatography. The chromatogram (Figure 2.2) showed a complex of UV absorbing compounds superimposed on a large envelope. Some peaks from an aromatic-PNA internal standard matched closely with peaks in natural shale oil, suggesting the presence of some of these components in the oil. One component was strongly suggested to

be naphthalene because its peak was enhanced by the presence of an internal standard of naphthalene. Based on the assumption that the unknown in shale oil is naphthalene, its concentration was calculated to be 550 $\mu\text{g/g}$ shale oil or 500 ppm (0.055%). A value of 0.10% has been estimated for the concentration of naphthalene in Green River shale oil (Cook et al., 1968).

FIGURE 2.2. Chromatograms of UV Absorbing Components in Shale Oil (1) and Shale Oil Containing Aromatic-PNA Standard (2).

The more polar classes of compounds (thiophenes and indoles) were separated by high-speed liquid-solid chromatography employing a μ Porasil column (Waters Associates). The separation of a mixture of six thiophenes using hexane as the mobile phase is shown in Figure 2.3. Analysis time was approximately 11 min. Thiophene, 2,5-dimethylthiophene and 2,3-benzo-thiophene were baseline resolved whereas 2-methylthiophene and

(a) The shale oil was obtained from the 10-ton simulated in situ vertical retort located at the Energy Research Center, Laramie, WY.

3-methylthiophene were not resolvable and 2-ethylthiophene only slightly separated from the two methylthiophenes. Increased resolution could be achieved by using two μ Porasil columns in series. It is doubtful that such a measure would produce any degree of separation between the methylthiophenes.

FIGURE 2.3. Chromatogram of the Separation of Substituted Thiophenes by HSLC on μ Porasil Column. A) Thiophene, B) 2-Methylthiophene + 3-Methylthiophene, C) 2-Ethylthiophene, D) 2,5-Dimethylthiophene, E) 2,3-Benzothiophene

Similarly, a mixture of indoles was partially separated employing solvent programming with hexane-methylene chloride as the mobile phase (Figure 2.4). Analysis time was approximately 10 min. 1,2-dimethylindole was clearly separated from the other alkylindoles. The 3-methylindole and 2,3-dimethylindole were partially resolved from each other and indole was almost completely resolved from 2-methylindole and 2,5-dimethylindole which were nonresolvable. Here, also, increased resolution could be achieved by placing two columns in series.

Before the described separation procedures can be further utilized, other separation schemes have to be developed which separate shale oil or its waste products into fractions containing the individual compound classes. Therefore, no attempt has been made to identify or quantitate compounds from the above classes in shale oil, especially in the case of thiophenes and indoles. Nevertheless, we have demonstrated that HSLC can be a rapid and sensitive method for the separation of polar compounds from classes recognized to be present in shale oil and its waste products.

FIGURE 2.4. Chromatogram of the Separation of Substituted Indoles by HSLC on μ Porasil. A) 1,2-Dimethylindole, B) 3-Methylindole, C) 2,3-Dimethylindole, D) Indole, E) 2-Methylindole + 2,5-Dimethylindole

Chemical Characterization of Retort Water

These studies dealt primarily with the characteristics and behavior of a single sample of retort waste water obtained from 10-ton simulated in situ vertical oil shale retort located at the Energy Research Center, Laramie, WY. Preliminary studies indicated that the process water contained ~ 40 g/l total organics which were highly polar, hydrophilic, exhibited high N and S content and were poorly extracted into less-polar solvents immiscible with water. Based on the solubility characteristics, results of the Davidson indicator test, the C, N and S content and color, the majority of the components present in the process water were probably represented by one or more of the compound classes which include amino salts of organic acids, polyhydroxyphenols, dibasic

and polybasic acids, aminodisulfonic acids, bisulfates of weak bases, heterocyclic amines, nitrosulfonic acids, cyanosulfonic acids and aminophenols. If these compound classes are, in fact, present in the process water they would also be expected to form complexes with metals, which could either increase or decrease the potential mobility of metals present in the spent shale, the process waters or soils. Therefore, thin-layer chromatographic and electrophoretic studies were initiated to characterize the soluble components of the process water and their interaction with Tl, Cd and Ni, trace metals shown in previous soils studies at PNL to be important in terms of biological availability and effects. Separation of process water components by thin-layer chromatography (TLC) on a wide range of available solid supports (Table 2.1) and with a series of solvents ranging in polarity (dielectric constants from 3.5 to 25) (Table 2.2), was evaluated. Azeotropic solvents were prepared and distilled as suggested by Roder (1971). The process water was filtered (0.45 μ) and several dilutions made with distilled water to result in solutions containing 40,000, 20,000, 8000, 4000, 1600, 1000 and 400 μ g/ml of total organic matter. Aliquots of two or more of these dilutions and 1 of 14 C-labeled benz(a)pyrene, 6-mercapto-purine and 3-amino-1,2,4 triazole were subject to thin layer chromatography. After drying, the plates were viewed under short and long wave UV light and the localization of fluorescent quenching or fluorescence of the separated components was recorded. Localization of the 14 C-labeled standards was by film autoradiography.

TABLE 2.1. Sorbents Used for Thin-Layer Chromatography

Sorbent	Primary Chromatographic Mechanism
Silica Gel	Adsorption
Cellulose	Partition
Kieselguhr	Partition
Aluminum Oxide	Adsorption
Silica Gel w. 0.1% AgNO ₃	Adsorption
Polyamide	Partition
Acetylated Cellulose	Partition PEI
Cellulose	Ion-exchange

Separation of process water components was evaluated for a single solid support-(cellulose) with development in 7 mixed solvents, all of much greater polarity than the azeotropic solvents but, additionally, exhibiting a range of pH values (Table 2.3). Two dilutions, containing 20,000 and 4000 μ g/ml

TABLE 2.2. Azeotropic Solvents(a) Used in Thin-Layer Chromatography

Solvent	Components	Fraction w/w	Boiling Point of Azeotropic Mixture °C (760 mm)	Dielectric Constant
1	Ethyl Acetate Cyclohexane	46 54	7.16	3.95
2	Ethanol heptane	48 52	72.1	9.50
3	Ethanol Toluene	68 32	76.5	17.3
4	Acetone CCl ₄	97 13	56.0	19.3
5	Methanol Acetone	11 89	55.5	22.1
6	Water Ethanol	4 96	78.2	25.4

a. Roder, Azeotropic Mixtures as Chromatographic Solvents in TLC, in Progress in TLC and Related Methods, Vol. 11, pg. 93 (1971), edited by A. Niederwieser and G. Pataki, Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan.

TABLE 2.3. Mixed Solvent Systems and Buffers Employed in Thin-Layer Chromatography and Electrophoresis

Solvent/Buffer	Description	Component Ratio
A	Butanol: Acetic Acid: Pyridine: H ₂ O	600:120:400:485
C	Ethanol: 25 ammonium hydroxide: H ₂ O	726:182:92
F	Chloroform: Acetic acid: H ₂ O	250:255:25
G	water: Acetic acid (pH 3.5)	995:5
K	0.21 M Ammonium acetate (pH 8.5)	1
L	Ethanol: Ammonium hydroxide: acetic acid	700:300:20
M	n-Butanol: 2N HCl: 2 Tartaric acid (Butanol saturated phase used)	400:100:100
pH 3.3	Pyridine: Acetic acid: water	6:60:1734
pH 6.5	Pyridine: Acetic acid: water	100:3.5:1897
pH 8.6	Diethyl Barbituric acid: Diethyl Barbiturate: thimerosal: H ₂ O	0.33g:1.95g:0.02g:120:1

total organic matter, were used in these studies.

Separation of the components in the process water on the basis of charge was accomplished by low voltage thin-layer electrophoresis (TLE) on cellulose plates (0.1 mm) in three buffer systems (Table 2.3). Only the 20,000 μ g/ml dilution of the process water was used for these studies. All spotting and visualization were as described above.

The potential for components in the process water to complex Cd, Ni and Tl was evaluated by incubating (24 hr), with shaking (200 rpm) 10 ml aliquots of each of 2 dilutions (20,000 and 4000 μ g/ml total organic matter) containing added Cd(NO₃)₂, Ni(NO₃)₂ and TlNO₃. Prior equilibration (24 hr) of the metal with radioactive tracers 109 Cd, 63 Ni and 204 Tl allowed simple assay in

solution and localization of the metal on thin layer plates by autoradiography. Evaluation of a change in the form of the added metal was made through solubility measurements (filtering the solution through a 0.01 μ filter) and by subjecting the solution to TLC, using solvents and solid supports described above, and comparing the observed R_f to the metal and tracer in water only. In those cases in which multiple spots were observed by film autoradiography, a preliminary estimation of the fraction of radioactivity (and thus the fraction of metal) in the spot was made by a comparison of the relative densities of film darkening.

The ability to use azeotropic solvents of a given polarity, as indicated by their dielectric constant, would allow the preparation of identical solvents over a long period of time due to the constant boiling nature of the azeotropic mixture. Similarly, separation of solvent components by differences in vapor pressures of components in the developing chamber would be eliminated. However, the results of the separation of components in the process water, using these solvents, were less than satisfactory, since the bulk of the components did not move from the origin for the range of polarities of solvents tested (dielectric constant 3.5-25). Similar behavior was observed for the ^{14}C -labeled standards (Table 2.4). The R_f values were 0 for 6-mercapto-purine in all solvents and solid phases except solvents with dielectric constants > 19.3 on silica gel plates. Some degree of partitioning was observed for 3-amino-1,2,4 triazole in solvents with dielectric constants > 19.3 on thin-layer plates of silica gel, cellulose, polyamide and acetylated cellulose, but no R_f greater than 0.32 was observed with any combination. This reaffirms the very polar character of the components present in the process water. However, a very small fraction of fluorescent material did move, exhibiting R_f values in approximating but not equal to those observed with benz(α)pyrene, a relatively nonpolar compound. Although, for the separation of the majority of components of the process waters, the range of solvents was of little utility, they may be useful at a future date when studies of the components of the oil are undertaken. The results with benz(α)pyrene will also allow evaluation of solvents and adsorbent combinations for separation of these less-polar components.

The more polar solvent mixtures (Figure 2.5) gave good separation of components in the process water. However, it should be noted that the visualization techniques showed primarily fluorescent components, and even assuming these represented the majority of the total organic matter, the complexity of the chromatograms indicates that development in several dimensions or other

TABLE 2.4. R_f Values for C-14 Labeled Standards on TLC Using Several Sorbent and Solvent Systems

Sorbent	Azeotropic Solvent Dielectric Constant	R_f Value for		
		Benz- α -pyrene	6-mercaptapurine	3-amino-1,2,4 triazole
Silica Gel (0.25 mm)	3.95	0.55	0.0	0.0
	9.50	0.75	0.0	0.35
	17.25	0.69	0.0	0.23
	19.30	0.70	0.09	0.07
	22.50	0.71	0.11	0.18
	25.4	0.61	0.37	0.32
Cellulose (0.10 mm)	3.95	0.97	0.0	0.0
	9.5	0.96	0.0	0.0
	17.3	0.90	0.0	0.0
	19.3	0.98	0.0	0.04
	22.5	0.92	0.0	0.05
	25.4	0.87	0.0	0.09
Kjeldose N (0.25 mm)	3.95	0.95	0.0	0.14
	9.5	0.93	0.0	0.0
	17.3	0.92	0.08	0.0
	19.3	0.96	0.0	0.0
	22.5	0.96	0.0	0.0
	25.4	0.88	0.0	0.0
Al ₂ O ₃ (0.2 mm)	3.95	0.62	0.0	0.0
	9.5	-	-	-
	17.3	0.65	0.0	0.0
	19.3	0.70	0.0	0.0
	22.5	0.62	0.0	0.0
	25.4	0.53	0.0	0.10
Silica Gel G w/Ag NO ₃	3.95	0.58	0.0P	0.0P
	9.5	0.67	0.0P	0.0P
	17.3	0.77	0.0P	0.0P
	19.3	0.94	0.0P	0.0P
	22.5	0.78	0.0P	0.0P
	25.4	0.68	0.0P	0.0P
Polyamide (0.1 mm)	3.95	0.68	0.0	0.0
	9.5	0.28	0.0	0.06
	17.3	0.54	0.0	0.0
	19.3	0.52	0.0	0.0
	22.5	0.50	0.0	0.07
	25.4	0.14	0.04	0.14
10% Acetylated Cellulose	3.95	0.80	0.0	0.0
	9.50	0.40	0.0	0.0
	17.3	0.66	0.0	0.0
	19.3	0.62	0.0	0.09
	22.5	0.60	0.0	0.16
	25.4	0.23	0.0	0.32
Cellulose w/PEI, 0.1 mm	3.95	0.94	0.0	0.0
	9.50	0.90	0.0	0.0
	17.3	0.89	0.0	0.0
	19.3	0.96	0.0	0.0
	22.5	0.74	0.0	0.0
	25.4	0.90	0.0	0.0

preliminary separations must be undertaken. Since the ability to detect fluorescent components is quite sensitive, it may be that the total amount of UV visualized components are only a small fraction of the 40-200 μg of material added to the plates (Figure 2.5). At this time, the fraction of total organic matter visualized is unknown.

Although the application of TLC to such a complex mixture of components is probably not feasible for most research purposes, these results do indicate the applicability and required conditions for utilizing this method after other preliminary separations. The major advantage with TLC is the speed and number of samples processing, which could be extremely useful in several future applications where the complexity of components has been reduced. Current capabilities for sample application and solvent development exceed 300 samples per day.

FIGURE 2.5. Separation of UV Visible Components in Shale Oil Process Water by TLC Using Cellulose Plates (0.1 mm), 7 Solvents at 2 Dilutions

Initial separation of components (UV, visible only) in the process water by TLE indicates the presence of both positively and negatively charged components (Figure 2.6). Although the electrophoresis was conducted for only 20 min, relatively large numbers of compounds or compound mixtures were reported. The effect of pH on the separation appeared to be marked, with a pH change from 3.3 to 6.5, resulting either in alteration of the charge of a yellow, very mobile component (cationic to anionic) or the UV visibility. These results also indicate the usefulness and applicability of TLE in future studies, either alone or in combination with TLC.

The incubation of two levels of metal (1 and 50 $\mu\text{g/ml}$) with two levels of total organic matter from the process water (4000

and 20,000 $\mu\text{g/ml}$) show that the components in the process water are reactive with, at least, Cd, Ni and Tl. The observed behavior is explainable on the basis of a series of different ligands present which have quite different stability constants. In addition, those organic components which form the most stable complexes may be present in quite low concentrations (<1 $\mu\text{g/ml}$).

The observed solubility of the three metals was quite different. The Tl, (added as the Tl^{+1}) and Ni (added as Ni^{++}) were mostly soluble in all cases (Table 2.5). The Cd, (added as Cd^{++}) when present at <1 $\mu\text{g/ml}$ level was soluble. However, when the metal level was increased to 50 $\mu\text{g/ml}$, the solubility in the solution containing organic

FIGURE 2.6. Thin Layer Electrophoresis Separation of UV Visible Components in Shale Oil Process Water in 3 Buffer Systems

TABLE 2.5. Solubility ($<0.01\mu$) of Metals (1 and 50 $\mu\text{g/ml}$) Equilibrated with Shale Oil Process Water

Metal(a)	Added Metal Concentration $\mu\text{g metal/ml}$	Metal Solubility in Presence of Process Water Organic Matter Concentrations of	
		4,000 $\mu\text{g/ml}$	20,000 $\mu\text{g/ml}$
Tl	1.0	1.0	1.0
	50.0	50.0	50.0
Cd	1.0	0.80	0.86
	50.0	0.10	22.0
Ni	1.0	0.71	0.73
	50.0	50.0	50.0

(a) Metals added as Tl^+ , Cd^{++} , Ni^{++} .

matter at the 4000 $\mu\text{g/ml}$ level was $< 0.1 \mu\text{g/ml}$ compared to $\sim 22 \text{ g/ml}$ when the Cd was added at 50 $\mu\text{g/ml}$ to the solution containing organic matter at a level of 20,000 $\mu\text{g/ml}$. Although speculation as to the factors which result in factors of greater than 200 difference in Cd solubility (e.g., limiting ligand concentrations, colloid formation) is possible, the major point is the potential difference in behavior which may occur in such a complex system on undertaking simple dilution.

The presence of organic components changed the chemical form of the added metal (Figure 2.7, 2.8 and 2.9). The metal Tl changed in form (different from water control) at all concentrations for solvent L (Figure 2.7). Other solvent systems reported other components but they represented minor fractions of the total. In contrast, changes in both Ni and Cd form were dependent on both the metal and the organic matter concentration (Figures 2.8 and 2.9). This may indicate that there are several components of limited concentration in the process water that formed quite stable complexes, but as the metal concentration increased or the ligands are diluted, saturation of the most stable metal ligands occurred. Since the specific activity (d/m of tracer/ μg metal) is 50 times lower for solutions containing 50 $\mu\text{g/ml}$ metal, the minor components visualized at the 1 $\mu\text{g/ml}$ metal levels (equivalent organic matter concentration) cannot be detected at the higher metal level.

It is apparent that the process waters have the potential for mobilizing trace metals. The extent and importance of this phenomenon will be the subject of future research.

FIGURE 2.7. The TLC Separation of Soluble ($<0.01\mu$) Tl-Ligands Formed on Equilibration of TlNO_3 and Oil Shale Process Water

FIGURE 2.8. The TLC Separations of Soluble ($<0.01\mu$) Cd-Ligands Formed on Equilibrating of $\text{Cd}(\text{NO}_3)_2$ and Oil Shale Process Water

FIGURE 2.9. The TLC Separation of Soluble ($<0.01\mu$) Ni-Ligands Formed on Equilibration of $\text{Ni}(\text{NO}_3)_2$ and Oil Shale Process Water

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• **DEVELOPMENT OF CHEMICAL REACTIONS, STABILITY AND TRANSPORT
MODEL OF OIL SHALE PROCESS WASTES IN SOIL**

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Use of oil shale retort process water for compacting spent shale and soil overburdens to aid in the establishment of surface stabilizing plants has been proposed in environmental impact assessments of oil shale waste disposal. Initial studies at PNL indicated a retort process water from the simulated in situ 10-ton retort at the Energy Research Center, Laramie, WY, has contained a high concentration of very polar, soluble organic compounds. The prediction of the transport of soluble organic components through spent shale and soil-spent shale systems must be predicated on measurements of sorption of these components. Sorption of previously-identified model compounds and the development of techniques for the separation of retort process water soluble organics into compound classes which can be treated as single entities in an analogous manner to model compounds will allow sorption and other organic transport reactions to be defined.

Sorption of collidine on both a soil and spent shale was determined. Collidine is the substituted pyridine found in highest concentration in a shale oil distillation fraction and has a high water solubility (Cook et al., 1968). Sorption of collidine was measured on a Ritzville silt loam soil and a spent shale from the above described retort. Sorption was measured in a batch system with a 4:1 solution to soil ratio. Collidine in the solution phase was measured by gas chromatography using a 4 ft chromsorb 101 column. Sorption was measured by difference between the initial and equilibrium collidine solution concentration.

Collidine sorption was described by a Langmuir isotherm equation for both the soil and spent shale systems. The Langmuir isotherm equation has the form:

$$\frac{C}{X} = \frac{1}{K_m M} + \frac{C}{M} \quad (1)$$

where

C = the equilibrium solution concentration
of collidine (µg/g)

X = the equilibrium solid phase concentration of collidine (µg/g)

K_m = the Langmuir bonding term which is proportional to bonding energy of the sorption reaction (ml/µg)

M = the Langmuir sorption maximum (µg/g)

The Langmuir equation is a linear equation from which parameters K_m and M can be determined by linear regression analysis of the experimental sorption data. For the soil, two energetically different sorption sites were delineated which were designated sites A and B, the r value for both sites was greater than 0.99 showing a significant relationship between $\frac{C}{X}$ and X for the sites. Parameters K_m were 0.59 and 0.040 for A and B, respectively. Since K_m is directly proportional to the bonding energy of the sites, site A is approximately 15 times more energetically favored. Due to this difference in bonding energy, site A can be expected to be filled at equilibrium before site B becomes active. Parameter M was found to be 502 and 1,160 µg/g for sites A and B, respectively, demonstrating site B has a greater capacity by about a factor of 2.

In contrast to the soil, spent shale collidine sorption was found to fit a single Langmuir isotherm equation. The r value for the linear equation was 0.96, again showing a significant relationship of C to X . The Langmuir parameters K_m and μ were found to be 0.039 and 258 $\mu\text{g/g}$, respectively. The agreement between the K_m for site B of the soil and for the spent shale suggests that these sites may be similar in nature and could be related to inorganic fractions of the soil. Site A, in contrast, may be related to the organic fraction of the soil which is largely destroyed by retorting the spent shale at high temperatures. The low capacity of the spent shale suggests that collidine sorption will be low relative to that of the soil.

A preliminary experiment was conducted to determine the extent of sorption of soluble organic material from a retort waste water on a spent shale. These data are required to assure that sorption estimated from integrating the sorption of model compounds and compound classes will predict the sorption soil-spent shale systems in the field.

Sorption was measured in a batch system at 10:1 solution to soil ratio. Total organic carbon (TOC) was used as the measure of soluble organic material after filtration through a 0.01 μ millipore filter. Influent solution had initial TOC values ranging from 21-309 mg/l. Sorbed carbon was measured by difference between influent TOC and equilibrium TOC.

TOC sorbed ranged from 22-40%. Sorption was found to fit a Freundlich isotherm sorption model. The Freundlich equation has the form

$$x = ac^{1/n} \quad (2)$$

where

x = the equilibrium concentration of TOC on the solid phase ($\mu\text{g/g}$)
 c = the equilibrium concentration of TOC in solution ($\mu\text{g/ml}$)
 a = the distribution coefficient (ml/g)
 n = is a system parameter

Both n and a are dependent upon the nature of the sorbate and sorbent. The natural logarithmic form of the Freundlich equation is

$$\ln x = \ln a + \frac{1}{n} \ln c \quad (3)$$

From the slope and intercept of the logarithmic straight line parameter a and n can be determined from linear regression analysis. Parameters a and n were found to be 1.5 and 1.3, respectively. The r value for the regression was $r = 0.98$ showing a significant relationship between $\ln x$ and $\ln c$. The sorption of many organic compounds have been shown to fit the Freundlich equation. Parameter $1/n$ generally has been found to have a value of less than, but approximately, 1.0. The value greater than 1.0 found in this experiment predicts that there is a sorption enhancement at the higher equilibrium concentrations used in this study. This could result from the production of new sorption sites or increased selectivity of present sites by the organics initially sorbed; or, it could be due to the formation of associated organic entities in the more concentrated solutions which are more strongly sorbed than their dilute solution counterparts. In either event, sorption based on the use of model compounds and compound classes will need to be evaluated with care. This may be overcome in the case of the isolation of compound classes if the separations are made at the organic solution concentrations typical of soil-spent shale systems in the field.

This experiment was conducted at a reasonably dilute total carbon concentration compared to the sorption capacity that was determined from studies of collidine sorption on spent shale. In a system which has less than 10-15% of the total sorption sites occupied, it can be shown that the Langmuir isotherm reduces to the Freundlich isotherm for any one compound. Thus, this work does not negate the use of model compound and compound classes to describe sorption.

A literature review entitled "Ground Disposal of Oil Shale Wastes: A Review with Annotated Bibliography" is being prepared as an ERDA report. The annotated bibliography is divided into two sections entitled: 1) Environmental Aspects of the Potential Disposal of Oil Shale Wastes to Ground, and 2) General Oil Shale References. A total of 352 references is included in the review and annotated bibliography. Preparation of the draft is nearing completion. Upon completion of the ERDA report, the review will be modified for submittal to open literature.

PUBLICATIONS AND PRESENTATIONS

PUBLICATIONS

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3.0 OIL AND GAS

- **EFFECTS OF REFINERY WASTES AND OIL FROM TRANSFER**
- **FATE AND EFFECTS OF PETROLEUM HYDROCARBONS IN MARINE COASTAL ECOSYSTEMS**
- **LONG-TERM EFFECTS OF HYDROCARBONS (EPA)**

The major objective of these programs, in support of ERDA's Oil and Gas Technology development, is to establish classes and levels of petroleum hydrocarbons which do not significantly disrupt aquatic ecosystems. This knowledge will provide a strong technical rationale for specifying degree of environmental control technology needed to be applied at production sites, i.e., offshore well head and at the refinery. Additionally, this research provides data input for predicting the potential ecological impact of petroleum hydrocarbons resulting from transportation spills and accidents.

The quantity of petroleum hydrocarbons entering the oceans today has been estimated to range from 5 to 10 million metric tons per annum (mta). The best estimate for inputs are: 1) transportation, i.e., tankers, terminal and ship operations at about 2.1 mta, or 35% of the total; 2) coastal refineries, municipalities and industries at about 13%; 3) offshore production, atmospheric fallout and natural seeps contribute about 21% to the total; and 4) the remaining 1.9 mta, or 31% of the total, comes from urban and river run-off.

Much conflicting laboratory data of the potential biological impact can be attributed to exposure conditions. The development and validation of a flow-through continuous exposure system by PNL in 1974 has resolved many of these problems such that realistic exposure of organisms to the water-soluble compounds can be conducted. Development of sensitive techniques for quantitative and qualitative analyses of water, tissue and sediment is essential for environmental monitoring and determination of hydrocarbon fluxes between biotic and abiotic compartments. While most of the physiological and behavioral studies have to be conducted with individual species, in the real-world situation, whole communities or parts of ecosystems are affected when exposed to oil. Part of our research has been directed towards defining the effects of low-level contamination on discrete communities and indicates that composition of the communities can be altered. Hopefully, we can also demonstrate that there is sufficient resilience in the community or ecosystem for recovery. Field experience would indicate this may be from months to years depending on the degree of the contamination.

Sediment is a sink for hydrocarbon contamination in the marine environment about which little is known. To determine the significance of this source, the degradation rates of specific compounds and their bioavailability to benthic organisms must be investigated.

The research progress of PNL, directed toward ERDA's Oil and Gas Technology, encompasses the above topics and provides better definition of the most significant goals in future research.

• **FATE AND EFFECTS OF PETROLEUM HYDROCARBONS IN MARINE COASTAL ECOSYSTEMS^(a)**

Principal Investigator: J. R. Vanderhorst

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Integrated field and laboratory studies have as their objectives the identification and measurement of effects at marine population and community levels from the oil and gas technologies, including production, transport, and processing. The approach followed in the program is to compare measurement reliability and response sensitivity for: 1) populations of a single commercially important species having cosmopolitan distribution on the Pacific Coast; and 2) groupings of species or "communities" from the intertidal zone. To date, laboratory studies of the community type have received the most attention, but this year a larger part of the effort has gone to field applications of both population and community studies. Chemical methods for better characterization of test solutions are being developed concurrently with the community and population studies. These analytical developments are shared with the program, "Long-Term Effects of Hydrocarbons on Selected Ecosystems and Associated Organisms."

Community Studies

Earlier in the program, a system to continuously deliver petroleum contaminants suitable for long-term exposure of marine colonies and populations was developed and tested. We have published a detailed description of the apparatus and results from application of the apparatus to: 1) short-term single species bioassay, and 2) six-month chronic studies of intertidal colonies consisting of more than 80 species of plants and animals (Vanderhorst et al., 1976; 1977a).

Reported LC₅₀ values from short-term laboratory studies with a No. 2 fuel oil and a single species (*Pandalus danae*) probably do not reflect lethal thresholds, since a marked reduction of LC₅₀ from 24 to 96 hr was observed with intermediate values at 48 and 72 hr. This finding contrasts with results reported by others using static bioassay techniques and is partially explained by an earlier study published on this

program which indicated a rapid loss of toxic substances from No. 2 fuel oil batch-treated systems.

Based on our findings in short-term single species experiments, we applied two low concentrations (0.1 and 0.6 mg/l) of No. 2 fuel oil to marine intertidal colonies on concrete substrates. Preliminary experimentation had indicated that if preselection of field-colonized bricks was based on a full cover of the alga (*Ulva*), homogeneity of randomly selected colonies from this preselection would be sufficient to allow meaningful comparisons for at least some colony parameters (Vaughan et al., 1975).

In chronic exposures from June through December, *Ulva* persisted on all experimental colonies. There was a general decline in biomass over the time period for all colonies, an observation consistent with seasonal distribution in the field. However, in treated colonies, an initial increase in biomass ($P = 0.15$) was followed by a

(a) This report includes material from the associated program, "Effects of Refinery Wastes and Oil Transfer Facilities on Marine Organisms"

significant decline ($P = 0.05$), which was not exhibited by controls. Given the dominant position of *Ulva*, a biomass decline in areas chronically polluted by oil could have important ecological implications.

No. 2 fuel oil treated colonies had a highly significant reduction in species diversity (Shannon Index) and species richness ($P = 0.001$) while relative abundance of individuals (Peilou formula) did not appear to be influenced by treatment. Data for species richness are illustrated in Figure 3.1. These data are representative of the high amount of variability obtained for measured parameters but clearly indicate the difference (significant at $P = 0.001$) between species richness for controls compared to experimentals. Differences for estimated parameters between the two treatment concentrations were not observed.

FIGURE 3.1. Species Richness in No. 2 Fuel Oil Treated and Untreated Colonies

For 36 species in which occurrence was more frequent on control colonies than experimentals, 22 species never occurred on No. 2 fuel oil treated colonies. To statistically describe these occurrence patterns, comparisons paired by month of exposure were made using the chi square statistic. Significant ($P = 0.05$) differences in frequency of occurrence were noted for species among the segmented worms, the clams, the tana-daceans, the amphipods, and the decapods.

Since the frequency of occurrence for individual species extends over substantial portions of the life history for many of the organisms, our data reflect the relative vulnerability of the tested species to the effects of No. 2 fuel oil.

Control colonies identical to treated colonies except for fuel oil treatment were used for comparisons to measure effects from the No. 2 fuel oil. In addition, 18 colonies were maintained outside the laboratory to gain insight as to the effect of natural photoperiod and lighting conditions. While the species compositional data have not been fully analyzed, the total biomass data have. Total biomass was significantly ($P = 0.001$) greater for the colonies maintained outside. Since the increased biomass may be expected to show a greater sensitivity to the effects of treatments, we have planned future community experiments to be conducted outside the laboratory. While the initial experiments were conducted with a No. 2 fuel oil because the oil is less complex than crudes, and because the effects of No. 2 fuel oil have been studied much more intensely in short-term studies, we will be conducting outside studies with Prudhoe Bay crude oil because of its expected dominant position in Pacific coast transport and refining operations.

Field Studies

To complement laboratory studies, a multifaceted field sampling program is being conducted at a refinery site, a proposed oil transfer facility site, and two control sites in the north Puget Sound region. The objectives are to measure parameter magnitude and sampling reliability for estimation of changes in parameter magnitude for intertidal communities related to site. Sensitive parameters, identified in the laboratory studies, will receive emphasis in data analysis.

To define intertidal communities in a manner consistent with the laboratory studies, we have established specific study plots, 100 m in width, within which colonies of *Ulva* are contained. The physical extent of the colonies, as it changes through the season, is established by field measurements and computer mapping. The maps produced are three-dimensional and provide information on vertical as well as horizontal distribution. Species composition and abundance within the *Ulva*-colonized areas are determined by randomized quadrat sampling.

To improve the quality of sampling, because of daylight extreme low tides, and to encompass periods of maximum production and reproduction for most of the species, we have restricted sampling to the months of April through September. A cycle of samples has now been collected and sorted, and data from the initial part of the cycle have received some preliminary analysis. Coefficients of variation (standard deviation/mean $\times 100$) for species richness within stations are about 30%. While the estimates are

tentative, this amount of variation is consistent with a successful sampling stratification based on *Ulva* cover. Previous experience in the same general areas with sampling stratified on tidal height alone indicated coefficients of variation of more than 100% to be common.

We anticipate that the analysis of the complete cycle will provide a basis for defining the necessary sampling intensity for detecting within-year changes in species richness and diversity, as well as frequency of occurrence patterns for species identified in the laboratory studies to be sensitive to the effects of petroleum hydrocarbons.

Population Studies

The use of community health (species diversity, richness, and occurrence patterns) indicators are a necessary part of broadly defining effects from petroleum related activities, but as indicated by the laboratory studies with No. 2 fuel oil, they will not likely provide quantitative information on individual abundances within species. In addition to the studies of communities, there is the need to gain quantitatively reliable data on changes in population abundance which may occur over many generations. We have chosen populations of the native little-neck clam (*Protothaca staminea*) for this intensive study for several reasons: 1) they are primarily intertidal in habitat and essentially nonmobile, making them vulnerable, as the tide shifts, to a broad spectrum of petroleum hydrocarbons; 2) they are cosmopolitan in distribution on the Pacific Coast, i.e., occurring from Alaska to Mexico, and 3) in Puget Sound, they are commercially and recreationally important. Methods for indirectly measuring age, past growth histories, and recruitment have been validated for some regions.

One of the most attractive features of a single species as an indicator is the potential for measuring age, past growth history, and recruitment from current sampling data. We have, therefore, initiated studies to validate the procedures using the little-neck clam at the selected refinery, oil transfer facility, and control sites. Validation involves tracing the growth history of individually-marked clams over a sufficient period of time to determine the relation of clam growth to periodicity in the formation of "checks" or patterns of shell rings. A side product of the validation process is that direct measurements of growth on marked individuals are made and these can be used for an initial evaluation of clam growth at test sites. We have marked in excess of 3,000 clams during the first season. About 2,000 clams have been stocked at known locations (site of capture)

at our primary control site in Sequim Bay. The remaining 1,000 have been stocked at known locations at the secondary control site and two experimental sites. To ensure that we could relocate marked clams and that marking did not cause excessive mortality, and to obtain some estimate of expected summer growth, we sampled marked clams during September which had been marked and stocked in April. A check on the variance of interpretation was accomplished by two investigators who independently aged a sample of individually marked clams. In no case did we find dead or empty marked clams, which would indicate that mortality had occurred. Measurable summer growth for this small sample ($n = 27$ clams) occurred only for clams 43 mm or less in length. In no case did we observe a summer check (other than that associated with marking). This is a positive indication of the potential for future validation of the procedure. It is our estimate that the clams which grew were 5 yr or less in age. In 90% of the cases, ages determined by the two investigators agreed within 1 yr. The discrepancy is thought to relate to erosion of the first "annulus," especially for older clams, and we have, therefore, standardized a convention regarding the first "annulus" for use in our first major recapture effort in the spring.

Additionally, we are using randomized quadrat sampling to directly estimate population abundance, size distribution, and biomass for the little-neck clams at the field study sites. This sampling is conducted on plots used for community evaluation but uses a completely randomized design in lieu of the stratified design used for communities. Different quadrat sizes (1 m^2 , $1/4 \text{ m}^2$, $1/6 \text{ m}^2$, $1/32 \text{ m}^2$) and mesh sizes (13 mm, 7 mm, 3 mm, 1 mm) are being compared. The design will allow evaluation of the influence of quadrat size, mesh size, and spatial relationships on clam abundance. This, in turn, will allow us to choose the most economically efficient combinations for future monitoring at the sites.

To more rapidly accumulate information on the reliability of this sampling approach, we have sampled our Sequim Bay control site at a much greater intensity. More than 200 replicate randomized samples were taken during the summer and the data have been key-punched. Preliminary analysis has allowed construction of size-weight models and the data are being analyzed to determine size class frequency. Sample size requirements for detection of differing percentages of change in abundance, by size class, and for the total little-neck clam stock, are being measured. We believe these data will provide a realistic foundation for evaluating not only the clam sampling at the experimental sites, but also the community sampling program.

From the community studies, we learned that in exposures of 6-months duration (No. 2 fuel oil), the occurrence of some clam species is significantly reduced. Long-term exposure studies of little-neck clams are being initiated using Prudhoe Bay crude oil. This will allow direct evaluation of the effects of crude oil extract (flowing system) on growth rates, mortality and long-term accumulation of petroleum hydrocarbons in tissues.

Analysis of Petroleum Hydrocarbons in Flowing Seawater Systems

Investigations of the toxicity of crude and refined oils in seawater have been underway at the PNL Marine Research Laboratory for several years. A principal means of carrying out these studies has been the use of continuous-flow oil exposure apparatus developed at the PNL Marine Research Laboratory (Vanderhorst et al., 1977b). Early acute toxicity experiments involving oil concentrations on the order of several parts per million (ppm) in sea water permitted the monitoring of concentrations by direct extraction of water samples with carbon tetrachloride and determination of the infrared absorbance. The solvent extracts could also be examined for hydrocarbon component concentrations using gas chromatography (Bean, 1974; Bean et al., 1974).

Present investigations of the toxic properties of petroleum involve lower concentrations of oil in sea water (less than 1 ppm) and are directed toward sublethal effects of oils on intertidal communities. At these concentrations, previous methods for determination of total and individual hydrocarbons in the water column were not effective. Coefficients of variations (CV) for total oil by IR were found to be over 100% (Vanderhorst et al., 1977a). In addition, the sensitivity of the gas chromatographic methodology was not sufficient to determine individual oil components in solvent extracts.

In order to determine total oil concentrations and to quantitate individual hydrocarbons at the concentrations currently being used, two separate analytical techniques have been developed and applied. The first technique involves extraction of petroleum-contaminated seawater samples with helium and injection of the helium extract into a gas chromatograph for determination of volatile hydrocarbon component concentrations. This method is about 3 orders of magnitude more sensitive than conventional methods. It has recently been described in detail (Bean and Blaylock, 1976) and has been applied in a study of No. 2 fuel oil toxicity utilizing oil concentrations of less than 200 parts per billion (ppb) (Vanderhorst et al., 1977a).

A second analytical method developed provides characterization of very low concentrations of oil in sea water and involves the use of resin adsorption. Approximately 10 L of sea water from the flow-through oil exposure apparatus are pumped through 3/8-in. columns containing XAD-2 macroreticular resin. The resin has been shown to efficiently adsorb the oil components from the sea water even at very low concentrations. Recovery of oil from a sea water suspension containing 115 ppb Prudhoe Bay crude oil was $92.4 \pm 1.7\%$ efficient, based on triplicate determinations. Analysis of the adsorbed oil is accomplished by eluting the column with carbon tetrachloride and determining the IR absorbance of the eluate. It has been found that the analytical columns can be regenerated at least 9 times (by washing with methanol, then water) before new resin must be employed.

The precision of the resin adsorption technique was investigated in two separate experiments. For these experiments, an oil-in-water suspension was simultaneously sampled using 3 resin columns, and the coefficient of variation of the triplicate analyses determined. In the first trial, seven such triplicate determinations gave an average CV of 9.5%; while in the second trial, eight triplicate determinations gave an average of 15%. Furthermore, these experiments have demonstrated that the flow-through apparatus is capable of delivering reasonably constant concentrations of oil in water over long periods of time. During the first trial, the oil concentration was determined to be 0.186 ± 0.070 ppm over a period of 23 days (CV = 37%).

In the first trial, the concentrations of volatile aromatics in the system were also determined using the helium extraction technique. The results obtained for concentrations of benzene through the xylenes are given in Table 3.1.

TABLE 3.1. Results of the First Trial (conc. in ppb)

Component	Run 1	Run 2
Benzene	32.6	32.6
Toluene	36.3	21.8
Ethylbenzene	4.6	4.0
m- and p-xylene	9.5	7.9
o-xylene	5.9	4.6
TOTAL	88.9	89.9

Considering that the average oil concentration in the apparatus had been determined to be about 190 ppb, it would appear that these five aromatic components constitute

almost one-half of the total hydrocarbon material in the flowing seawater suspension. Such is not the case, however. A study of the infrared absorbance characteristics of monocyclic aromatic hydrocarbons has shown that they absorb weakly in comparison to saturated hydrocarbons. In Table 3.2 the IR absorbances at the analytical wavelength employed for petroleum analysis of three pure aromatic hydrocarbons are compared with the absorbance of a saturate hydrocarbon.

TABLE 3.2. Comparison of Absorbances

Compound	Conc. in CCl ₄ (ppm)	Absorbance
Benzene	440	0.01
Toluene	434	0.09
o-xylene	440	0.16
n-Heptadecane	389	0.93

The IR method does not properly account for the light aromatic hydrocarbons; thus, they must be separately determined using helium extraction technique. A better estimate of total oil concentration is, therefore, 270 ppb rather than 190.

We propose to concentrate on obtaining detailed compositional information on the petroleum hydrocarbons adsorbed from oil-in-water suspensions onto the XAD-2 resin columns. Capillary gas chromatography and gas chromatography/mass spectrometry will be used for component identification and quantitation. In combination with the helium extraction method, application of these techniques should result in a comprehensive list of the major hydrocarbon components present in low-level flow-through exposure systems.

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- **LONG-TERM EFFECTS OF HYDROCARBONS ON SELECTED ECOSYSTEMS AND ASSOCIATED ORGANISMS**

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The basic objectives of this research project are to: 1) determine the effects of well-defined constant hydrocarbon contamination on the growth and reproduction of sensitive marine crustaceans (Anonyx: Amphipoda, and Neomysis: Mysidacea), 2) describe the extent of transfer by specific petroleum hydrocarbons from sediment to benthic organisms (sipunculids and bi-valves), and (3) define the rate of specific hydrocarbon depuration from oil-contaminated sediment in the field, as it relates to the recruitment of benthic organisms within this substrate.

Both Anonyx and Neomysis have been cultured in the laboratory for two or more generations, and the tolerance of the latter species to petroleum hydrocarbons has been determined. The extent of naphthalenes uptake and release by the sipunculide (Phascolosoma) exposed to oiled sediment has been reported. Depuration of saturate, di- and triaromatic compounds from oiled mud has been described, and it was found that several species of benthic organisms were recruited in both clean and oiled sediments placed in the intertidal zone.

Studies of the Fate of Prudhoe Bay Crude Oil in Marine Sediments

In order to more clearly define the potential for petroleum in marine sediments to exert significant long-term environmental effects, a study on the fate of crude oil in sediments is being conducted. The study is interdisciplinary, involving aspects of analytical chemistry, microbiology, and physical chemistry. Principal objectives of the study are to determine the rates and mechanisms of degradation of petroleum in the marine environment, to determine which component types are persistent, and to discover whether physical and chemical changes occurring with time render petroleum more or less biologically available. A laboratory study of the depuration of Prudhoe Bay Crude oil (PBC) from a fine-textured Sequim Bay sediment has been conducted to develop the analytical techniques appropriate to depuration studies.

The experiment was initiated early in 1976 by introducing into an aquarium the fine

sediment mixed with 1% fresh PBC. An additional aquarium, serving as a control, was charged with sediment free of PBC. Depth of sediment was approximately 4 in., and fresh seawater was allowed to continuously flow over the sediments in both aquaria. Triplicate grab samples were removed at intervals over 180 days and then frozen. The sample was mixed with anhydrous sodium sulfate and extracted twice with carbon tetrachloride. Over 97% of the oil was removed from the sediments. The values obtained for total oil content (mg oil/g sediment) as a function of time in flowing seawater are given in Figure 3.2. The values are based on analysis of three subsamplings from each of two sample replicates for each time period. The variance experienced among subsamples of the same sample jar (coeff. = 3.7%) was less than the total variance (coeff. = 5.8%). This finding, coupled with the scatter of the data around the linear regression (dashed line, $r^2 = 0.31$) indicates that the oil is not as homogeneous in the sediment as was expected.

FIGURE 3.2. Content of Total Oil and Aromatic Hydrocarbons in Sediment as a Function of Depuration Time

Hexane extracts of the sediment were also analyzed by high-pressure liquid chromatography (HPLC) utilizing three series - coupled 1/8 in. x 2 ft Durapak oxypropionitrile on proasil C columns. This column system was validated with model compounds to demonstrate separation of hydrocarbons into saturate monoaromatic; diaromatic and polyaromatic fractions. Hexane extracts from sediment contaminated with oil and analyzed under the same conditions produced a saturate fraction and a monoaromatic/diaromatic fraction which was approximately 80% resolved. UV absorbing components indicative of triaromatics and polyaromatics were also separated. The quantitative analysis of the aromatics in the sediment samples was performed with a calibration curve prepared from a PBC aromatics fraction using an ASTM method. The data from the aromatics analysis are shown in the same figure. The plot suggests slow depuration of these components, but at a rate somewhat faster than that of total oil. Correlation of the data with the linear regression (dashed line) was better ($r^2 = 0.44$) than that obtained from the total oil curve. Saturate, monoaromatic/diaromatic, and polyaromatic fractions separated by HPLC were collected. These fractions were analyzed by capillary column chromatography. The GC chromatograms from each fraction indicate good separation of saturates from aromatics and there appears to be good fractionation of the monoaromatics/diaromatics from the polyaromatics. This separation procedure only requires approximately 20 min, and is therefore a rapid means of deriving detailed quantitative and qualitative hydrocarbon data.

Analysis of hexane extracts of exposed sediments for normal paraffins and mono- and dimethylnaphthalenes by gas chromatography was also conducted (Table 3.3).

As in the case of the total oil analyses, these data are also scattered. However, there is a tendency for the concentration of methylnaphthalenes to be decreasing at a faster rate than the $C_{15}C_{23}$ saturates.

TABLE 3.3. Analysis of Contaminated Sediment for n-Alkanes and Methylnaphthalenes

Exposure Time (Days)	$C_{15}-C_{23}$ n-Alkanes (mg/g oil)	Mono- + Dimethylnaphthalenes (mg/g oil)
0	31.9	8.7
1	24.1	6.1
13	29.4	8.9
20	29.5	5.9
27	32.3	9.1
34	29.4	6.3
42	30.2	5.9
65	31.2	6.1
78	27.6	5.6
93	26.9	4.3
119	21.6	3.7

High-pressure liquid chromatography in conjunction with capillary gas chromatography (particularly when coupled with GC/MS technique) has been demonstrated to provide the analytical sensitivity and compositional detail required for this program. On the basis of the data generated thus far, the experiment demonstrated the potential persistence of crude oil in undisturbed fine-textured marine sediments. If the rate of decrease were to remain constant, it would require approximately 3 yr for complete release of contamination, but there is reason to believe that the rate would be modified by a number of environmental factors. Aeration and redistribution of the fine particles would enhance leaching and microbial degradation.

From qualitative inspection of the capillary chromatograms of saturate and aromatic oil fractions, extensive utilization of petroleum hydrocarbons by the bacteria indigenous to the sediments does not appear to have taken place within 120 days. However, it is apparent from odor and observation that some bacterial activity is present that could ultimately be a major mechanism of change in composition. A certain period of time (lag time) may be required for a build-up of microbial diversity and population size necessary to substantially alter the extent and composition of contamination in the sediment. Other microbial studies being conducted have already demonstrated that the presence of crude oil greatly increases the populations of bacteria which can utilize petroleum hydrocarbons. In this area, the initial studies have concentrated on enrichment of major aerobic and anaerobic bacteria isolated from Sequim Bay fine-sediment cores, using a synthetic seawater complex medium. This has laid the groundwork for quantitative studies now in progress which will measure the rate and extent of aerobic and anaerobic microbial attack by sediment

bacteria on petroleum hydrocarbons, and will determine the relative importance of direct metabolism vs cometabolism of hydrocarbons as a mechanism of oil utilization.

Exposure of Benthic Marine Organisms to Oil-Contaminated Sediment

It is evident from spill site surveys that levels of petroleum hydrocarbons (PH) in marine and estuarine sediments are substantially greater than those in the overlying water column. However, there is little known concerning the rate of hydrocarbon degradation or turnover in these sinks for contamination. Of considerable significance is the possibility that benthic feeding organisms may become contaminated by ingestion of these sediments after the event. While numerous studies have reported on aspects of hydrocarbon accumulation by organisms from solution and/or dispersion, only two studies have been concerned with the controlled exposure of benthic deposit-feeders to known concentrations of pH_5 on sediment and/or detritus (Rossi, 1976; Anderson et al., 1977). To further evaluate the significance of contaminated sediments serving as hydrocarbon sinks in the marine environment, we need to determine the rates and extent of uptake by organisms ingesting this material. The rate of transfer of a range of petroleum hydrocarbons should be determined from contaminated sediments to selected deposit feeders to derive the necessary information.

While some benthic organisms primarily utilize sediment for protection while feeding on suspended matter in the water column, those most likely to become contaminated from sediment-sorbed hydrocarbons are the deposit-feeders. We have, therefore, concentrated on determining the availability of sediment-sorbed hydrocarbons from Prudhoe Bay Crude oil to a deposit-feeding invertebrate. Our initial experiments were with the sipunculid worm, *Phascolosoma agassizii*. These burrowing organisms were exposed to sediments which had been contaminated with Prudhoe Bay Crude oil in two different ways. In addition to this research on accumulation of pH_5 , field studies have been conducted to determine the effects of contaminated sediment on the recruitment of benthic organisms in the natural environment.

The first system used to expose the sipunculid worms (*Phascolosoma*) to contaminated sediment involved the layering of the coarse sand substrate with oil. After oil had been in contact with this heterogeneous sediment for about 2 hr, simulated tidal flushes (2 per day) were begun and continued for 1 month. Animals were added to control and contaminated sediment trays after 2 weeks of tidal flushes and were therefore exposed to oiled sediment for the last 2-week period. Sediment cores taken over the 29-day period produced values

of hydrocarbon content which varied widely, probably as a result of changes in the proportion of fine to very coarse (pebbles) particles. Analyses of total hydrocarbons and total naphthalenes (naphthalene, N + methyl-naphthalenes, MN + dimethylnaphthalenes, DMN + trimethylnaphthalenes, TMN) showed a sharp decrease in concentration with depth. It is apparent that the top layer, which received about 4% (v/v) oil, still retained considerable contamination after 29 days. The animals exposed to these hydrocarbons, however, exhibited little or no contamination after 2 weeks of exposure (Table 3.4). As the sediment depth was only 8 cm, it was assumed that a reasonable portion of the animals spent sufficient time feeding and living in the highly contaminated top 2 cm. Because of variability, uneven distribution of contamination and animal activity, a second type of exposure system was utilized in a subsequent experiment.

TABLE 3.4. Levels of Naphthalenes in *Phascolosoma* Exposed to Surface Contaminated Sediment

Condition	Analytical Method	Concentration $\mu\text{g/g wet (ppm)}$			
		MNs	DMNs	TMNs	TNs ^a
Exposed for 2 weeks	UV	0.148	0.601		0.449
	UV	0.230	0.457		0.387
	HPLC-FS	1.00	0.90	0.74	2.64
	HPLC-FS	0.45	0.34	0.56	1.35
	UV	0.101	0.258		0.06
	UV	0.304	0.439		0.443
	UV	0.110	0.208		0.02
	UV	0.100	0.07	0.05	0.22
	HPLC-FS				0.04 (1) ^b
	UV				0.30 (5) ^c
Depurated for 6 days	HPLC-FS	0.15	0.36	0.27	0.78
	HPLC-FS				0.04 (5)
Depurated for 13 days	UV	0.334	0.430		0.464
	UV	0.166	0.311		0.177
	UV	0.218	0.278		0.196
	UV	0.223	0.526		0.449
	UV				0.300 (8)

^aTNs is total naphthalens, where UV analyses were capable of only determining methyl- and dimethyl-isomers, but the HPLC-FS method also produced values for the trimethylnaphthalenes.

^b() indicate the number of samples below the level of detection (0.04 for HPLC and 0.30 for UV).

^c0.30 was subtracted from all tissue samples analyzed by UV.

Sediment was first sieved through a 1.6 mm mesh screen and then mixed with a small volume of oil-water emulsion in a cement mixer. Uniformity of mixing was checked by liquid scintillation counting of 8 replicate samples for determination of C^{14} -2-methylnaphthalene which had been added to the oil. Additional evidence of quite uniform mixing was provided by analyses of

sediment conducted over the 2-week exposure period. Both total hydrocarbons (TH) and total naphthalenes (TN) in sediment samples remained quite constant (about 500-550 ppm TH and 3-5 ppm TN) from 16 hr to 2 weeks. Tissues of the sipunculids exposed to this oiled substrate reached maximum concentrations of naphthalenes after 40 hr, and showed only minor fluctuations during the first week (Figure 3.3). After 2 weeks, tissue content was less than that at earlier intervals, indicating a lack of accumulation and perhaps metabolic breakdown of naphthalenes. Depuration in clean sediment or water resulted in decreases from 63 to 100% over periods of 2 days and 2 weeks, respectively. Tissue analyses showed the expected variations between individuals, but rather close agreement occurred between the means derived from the ultraviolet spectrophotometric technique (Neff and Anderson, 1975) and gas chromatographic determinations.

The results of this study (Anderson et al., 1977) compare closely to those of Rossi (1976) and Rossi and Anderson (1977), in which *Neanthes arenaceodentata*, a marine polychaete, was exposed to sediment containing 9 ppm TN for 28 days, but was never found to contain levels above background (0.1 ppm TN). Using a contaminated artificial detritus (10 ppm ^{14}C -2-methylnaphthalene), Rossi showed that accumulated ^{14}C -activity was voided with the feces during a 24-hr period in clean water, even when feeding (^{14}C -food) was continued for 16 days.

FIGURE 3.3. Content of Methyl and Dimethylnaphthalenes in Sipunculids (*Phascolosoma*) Exposed to Oil-in-Sediments. Means, standard deviations, and the number of samples are shown for exposed (squares), depurated (triangles), and control (circles) tissues. Where standard deviations are absent, they were less than 10% of the mean. Solid symbols designate GC analyses, while all others were determined by UV. Depuration of the 40-hr exposed animals was for 48-hr, while that of the 2-week exposed groups was either 2 weeks (4 samples) or undetermined (5 samples)

As shown in numerous other studies with other species (reviewed in Anderson, 1975), sipunculid worms accumulate naphthalenes from sea water to a significant extent. The lack of similar magnification (ratio of tissue concentration to exposure concentration) when worms are exposed to sediment-sorbed naphthalenes (2-3 ppm) introduces some doubt as to the availability of petroleum hydrocarbons bound to sediment. A considerable amount of further research on the bioavailability of a range of hydrocarbons is required to provide a definitive answer to the question.

Because even the most carefully designed laboratory experiments cannot replicate conditions in the natural environment, observations should be validated in field experiments. Such procedures are particularly valuable when examining long-term effects. Field research on the recruitment of benthic organisms in oiled sediment provides data on the recovery potential of contaminated sediment and the relative sensitivities of various species, as well as some information necessary to evaluate bioavailability. In spring and summer 1976, sediment boxes were placed in the intertidal zone on a sand spit in Sequim Bay. Four sediment boxes in each installation received oil (4% v/v) by the layering-on-top exposure method, the remaining two serving as controls. A third series was set in place in the fall, but this sediment was treated as described above for mixed oil and sediment. Initial and subsequent sampling for hydrocarbon concentration and composition has taken place. Two collections have been made during spring and summer to determine the number of species and individuals present in control and contaminated sediments, and a third was just completed in the fall. Analyses of the samples, when completed, will provide valuable information on: 1) alterations in the composition of sorbed hydrocarbons subjected to natural leaching, microbial degradation, and photo-oxidation; 2) the effect of specific sediment-bound hydrocarbons on the settling and survival of larval or young benthic organisms; and, 3) the extent and compositional characteristics of tissue hydrocarbon contamination from sediment-sorbed compounds. This latter aspect of the research may require additional time, as the organisms must grow significantly to allow hydrocarbon analyses. While we await these data, similar results can be obtained by placing organisms of sufficient size in other oiled sediment boxes for various periods in the natural environment.

Extensive research is still required to produce the information needed to assess the effects of contaminated sediment on marine organisms, but the experimental approaches and analytical techniques developed are significantly contributing to this goal.

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4.0 NUCLEAR WASTES: FISSION

- **SUSPENDED PARTICLE INTERACTION AND UPTAKE IN TERRESTRIAL PLANTS**
- **TRANSURANIC COMPLEXATION IN SOIL AND UPTAKE BY PLANTS**
- **QUANTITATIVE ASPECTS OF PLUTONIUM FIELD STUDIES**
- **ECOLOGICAL DISTRIBUTION AND FATE OF PLUTONIUM AND AMERICIUM IN A PROCESSING WASTE POND ON THE HANFORD RESERVATION**
- **USE OF ANALOG ELEMENTS TO PREDICT THE EQUILIBRIUM AND BEHAVIOR OF TRANSURANIC ELEMENTS IN THE ENVIRONMENT**
- **WEATHERING AND AGING OF TRANSURANICS**
- **BIOGEOCHEMISTRY OF Pu AND Am IN THE MARINE ENVIRONMENT**
- **RADIOECOLOGY OF WASTE MANAGEMENT AREAS (ROWMA)**
- **RADIOECOLOGY OF ^{129}I AND ^{99}Tc**
- **INFLUENCE OF SOILS AND AQUATIC SEDIMENTS ON THE CHEMICAL BEHAVIOR, TRANSPORT, AND BIOAVAILABILITY OF POLLUTANTS**

One of the highest priorities associated with 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 inferred with confidence 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.

In terrestrial studies, investigations of transuranics in soils and plants have demonstrated the importance of valence state, complexation, competing elements, migration down the soil profile, and weathering cycles in governing transuranic, ^{129}I and ^{99}Tc availability to plants and, in the case of Pu, to the consuming animals. In the latter case, it was demonstrated, for the first time, that ingestion of plant tissues containing Pu may result in greater transfer across the gut compared to gavaging animals with inorganic Pu solutions, underscoring the importance of detailed studies of the soil, plant, and animal factors influencing uptake by the ingestion pathway. Further evidence of the importance of the ingestion pathway was provided in studies of foliar interception of airborne transuranic elements in which it was shown that Pu in particles in the respiratory size range were effectively intercepted and retained by plants, and significant quantities of intercepted Pu were transported to roots and seeds. Similar studies on the terrestrial ingestion pathway have been initiated with other actinides including, U, Am, Cm, and Np.

Radioecological field studies at PNL 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 and digestibility in cattle of several forage species. In studies on a pond ecosystem at the nuclear fuel reprocessing plant, Pu and Am uptake rates were studied for major biotic components including organic floc, algae, fish and ducks. The results indicated that assimilation of transuranics by the biota and export from the pond system were low compared to the total inventory.

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

We have more recently extended the approaches developed in terrestrial and freshwater studies to 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 mobility and availability to biota. Investigations have provided a basis for description of dispersion mechanisms on the sea floor of transuranics 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 Pu availability as a function of ocean depth.

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

• SUSPENDED PARTICLE INTERACTIONS AND UPTAKE IN TERRESTRIAL PLANTS

Principal Investigator: D. A. Cataldo

The purpose of these studies is to develop an understanding of the behavior and fate of airborne pollutants following interception by plant foliage. Our studies of PuO_2 particles intercepted by plant canopies indicate that small diameter particles ($<1 \mu\text{m}$) behave differently, with respect to retention time on foliage, than larger ($>10 \mu\text{m}$) particles characteristic of close-in fallout. Studies with simulated fallout particles ($1\text{--}200 \mu\text{m}$) demonstrate a much shorter retention half-time (e.g., 14 days), with both wind and rainfall being effective in their removal from foliar surfaces. Such data are employed in transport models to predict the impact of nuclear releases (both accidental and low level release from nuclear facilities) to the atmosphere. The present studies show submicronic particles (respiratory size range) to be effectively intercepted and retained on plant foliage. Even under rigorous leaching conditions, $>90\%$ of the deposited Pu is retained. The data also suggest that retention tends to increase with residence time on foliage. Transport studies show a significant fraction of the foliar deposits to be transported to roots and seed. The quantity transported to these tissues tends to increase following leaching treatments (simulated rainfall).

Investigations are continuing in an effort to understand the behavior of particulate forms of the transuranics in the environment. Areas of study include: 1) an evaluation of the retention characteristics of ^{241}Am , ^{244}Cm and ^{237}Np in addition to ^{238}Pu ; 2) the extent of uptake and translocation of the transuranics following foliar contamination; and 3) elucidation of the relationships between particle size and mechanisms associated with their retention and bioavailability.

Fate and Behavior of Particulate Plutonium Dioxide

The design and calibration of the wind-tunnel used to deposit the aerosols containing Pu has been described previously (Craig and Klepper, 1975). The exposure facility provides an effective and consistent method for the generation and deposition of particulates onto plant foliage. Particles employed in these studies are submicronic, ranging from 0.5 to $0.01 \mu\text{m}$, depending on the nature of the original suspension or fractionation performed on suspensions prior to generation. Mass loading of leaves is low and ranges between 10 to 50 ng per plant.

Suspension of $^{238}\text{PuO}_2$ employed is designated as fresh or water-aged. The fresh PuO_2 was prepared by suspending the particles in triple distilled water on the day of exposure; while water-aged PuO_2 had been suspended in water for 2 to 10 months prior to its use. Although both forms of oxide suspension are prepared from the same batch of $^{238}\text{PuO}_2$, aging of the particles in water results in a reduction in the median diameter of the particles present. This change in particle size distribution results from fragmentation of surface components of the original particles as a result of alpha recoil and hydrolysis.

Bushbean, sugarbeet and wheat were grown for 20, 42 and 40 days, respectively, and then exposed to aerosols containing Pu. All plants were held 28 days following exposure. During this time, plant foliage was subjected to various leaching regimes to evaluate the retention of both soluble and particulate Pu. The 28-day post-exposure period also provided time for the uptake and translocation of Pu to seed and roots of bushbean and wheat, and the tap root of sugarbeet, which were protected from aerosol contamination.

Retention of Foliar Deposits with Time Following Contamination

The retention behavior of submicronic particles of Pu intercepted by plant foliage differs substantially (Cataldo and Craig, 1976) from early concepts based on both natural and simulated fallout particles having diameters $> 10 \mu\text{m}$. Our initial investigation with submicronic particles of Pu indicated that the ability of Pu to be leached from foliar surfaces was dependent on both the solubility of the Pu (chemical form originally supplied) and particle size. In addition, the data showed that the availability of Pu for leaching decreased over the 21-day period following contamination. Subsequent studies have been concerned with the effect of leaf surface morphology on retention of Pu particles.

The fate of particles intercepted by plant foliage is dependent on the complex interaction between leaf surface characteristics and physical aspects of the individual particles. Leaf characteristics, important with respect to particle retention, include degree of surface relief (trichomes, structure and frequency), microtopography (cuticular arrangement and roughness) and wettability. Physical aspects of particles which can affect their retention include physical dimensions, density, charge and solubility. Current studies attempt to describe the interaction of particulate Pu with foliar surfaces of bushbean and sugarbeet which have different surface morphology.

To determine the influence of leaf surface morphology on particle retention, these two plant species were contaminated with poly-dispersed aerosols and the contaminated foliage subjected to various leaching regimes. Aerodynamic and physical data for the aerosols is given in Table 4.1. The count mode represents the particle diameter with the highest frequency and is employed as a first approximation of particle size in evaluating the behavior of particles following interception by plant foliage. Experiments A-3 and A-7 employed freshly prepared PuO_2 while A-4 and A-6 are similar suspension aged in water for 9 and 6 months, respectively.

TABLE 4.1. Aerodynamics and Physical Characteristics of Aerosolized Particles

Experiment Designation	Particle Characteristics					
	Aerodynamic			Physical		
	AMAD ^(a)	GSD ^(b)		MMD ^(c)	CMD ^(d)	CM ^(e)
A-3	1.27 · 0.05	1.63 · 0.03		0.366	0.178	0.140
A-7	1.59 · 0.13	1.76 · 0.09		0.462	0.179	0.130
A-4	0.73 · 0.11	2.16 · 0.09		0.201	0.033	0.018
A-6	0.75 · 0.05	1.84 · 0.05		0.209	0.069	0.048

(a) Activity mass median diameter (μm · sd)
(b) Geometric standard deviation (μm · sd)
(c) Mass median diameter
(d) Count median diameter
(e) Count mode diameter

The behavior of the fresh and water-aged PuO_2 particles on foliage of bushbean and sugarbeet is shown in Figure 4.1. The data indicate that only a small portion of the total Pu deposited onto foliage is readily leached under mild rainfall conditions. The behavior of these submicronic particles is contrary to early work with simulated fallout particles of greater than $10 \mu\text{m}$ (Witherspoon and Taylor, 1970; 1971) which were readily lost from foliage by both air currents and rainfall. However, Iranzo (1968) and Romney et al. (1975) have reported that Pu-containing material resuspended in contaminated field situations was difficult to remove from contaminated foliage, with as much as 50% being held on foliar surfaces. The present laboratory studies with submicronic particles tend to support these later observations. The leaching data (Figure 4.1) show a substantial reduction in the leachability of Pu particles with time following contamination. Although the mechanism responsible for this trend is difficult to assess, factors such as changes in wettability and surface roughness (wax deposition) may affect particle retention. In addition, changes in wettability and surface charge characterization of particles with residence time can influence their behavior.

A comparison of the behavior of particles on moderately rough and smooth surfaces indicates that Pu is more readily leached from smooth foliar surfaces (sugarbeet) than rough surfaces (bushbean). Again this observation can be accounted for by differences in surface roughness and wettability. The smaller particles, in the case of both types of leaf surfaces are more leachable, with the exception of the 1-day leach of bushbean. This behavior of smaller particles ($0.02\text{--}0.05 \mu\text{m}$) may result from the ease at which they can be

FIGURE 4.1. Leachability of Foliar Plutonium Contaminates as a Function of Time Following Contamination for Bushbean and Sugarbeet Leaves

suspended in water droplets and washed from the leaves, as compared with the fresh oxide particles (0.13-0.14 μm).

Differential Leaching of Foliar Deposited Plutonium

Apparent differences in particle retention for the two types of leaf surface were studied using continuous-leaching regimes. These employed a synthetic rainwater equivalent of 1.6 cm. The leaching rates for all treatments were linear when the log of the activity in the leachate was plotted vs. the leachate volume. Data on total Pu leached for each of these treatments are shown in Table 4.2. Particle retention appears dependent not only on the type of leaf surface, but also on particle size and nature of the leaching solution. In the case of bushbean, having a relatively rough surface texture, < 4% of the fresh and aged oxides are leachable with rainwater. However, acidified rainwater results in the removal of 28% of the water-aged oxide, with only a minimal

increase in the fresh oxide. In contrast, the hydrated oxide which contains > 50% of its activity in the soluble or colloidal size range was much more leachable with rainwater alone as compared with fresh and aged oxides. The observed behavior of sugarbeet leaves with respect to retention of particulates differed from that of bushbean. The fresh oxide appears to be more leachable than the aged oxide under both leaching regimes; acidified rainwater resulted in an increased removal of both oxide forms.

The present studies aid in our understanding of the interception and retention of submicronic Pu particles by plant foliage. It is the submicronic size range (respiratory size range) which will be of major concern as a result of reprocessing of nuclear fuel. Although the behavior and retention of submicronic particles is contrary to much of the early fallout work, the results obtained in the present study appear to be characteristic of insoluble submicronic particles (Carlson et al., 1976).

TABLE 4.2. Effect of Continuous Leaching on Retention of Foliarly Deposited Plutonium(a)

Plant	Plutonium Form	Leaching Regime % Leached	
		Synthetic Rainwater	Synthetic Rainwater + 0.1% HNO ₃
Bushbean (rough leaf surface)	Fresh Oxide (A-3)	3.8 ± 0.7	5.8 ± 0.3
	Water Aged Oxide (A-4)	3.8 ± 0.8	28.4
	Hydrated Oxide (b)	26.8 ± 2.9	34.2
Sugarbeet (smooth leaf surface)	Fresh Oxide (A-7)	18.0 ± 4.9	35.3 ± 9.8
	Water Aged Oxide (A-6)	4.3 ± 1.1	17.0 ± 3.5

(a) Plants leached 7 days following contamination.

(b) ²³⁸Pu(NO₃)₄ in 6N HNO₃ neutralized with 6N NaOH to pH 7.0, resultant suspension contained "soluble" or colloidal plutonium as evidenced by filtration through 0.01 µm volume filters. Day-7 leachates were 55% "soluble" based on filtration.

TABLE 4.3. Extent of Translocation of ²³⁸Pu from Contaminated Foliage in the Absence and Presence of a Solution Vector (in Simulated Rainfall)

Plant	Plutonium Form	Sink Tissue	Concentration Ratio (a)(b)	
			Absence of Solution Vector	Presence of Solution Vector
Bushbean	Hydrated Oxide	Seed	6.8 × 10 ⁻⁶ (2)	5.4 × 10 ⁻⁶ (10)
		Root	3.3 × 10 ⁻⁶ (2)	1.9 × 10 ⁻⁶ (10)
	Water Aged Oxide	Seed	1.1 × 10 ⁻⁵ (2)	3.4 × 10 ⁻⁵ (12)
		Root	4.8 × 10 ⁻⁶ (2)	2.7 × 10 ⁻⁶ (12)
Sugarbeet	Fresh Oxide	Seed	4.5 × 10 ⁻⁶ (2)	2.0 × 10 ⁻⁶ (12)
		Root	6.6 × 10 ⁻⁶ (2)	1.6 × 10 ⁻⁶ (12)
	Water Aged Oxide	Beet	(c)	(c)
		Beet	3.6 × 10 ⁻⁶ (2)	8.7 × 10 ⁻⁶ (8)
Wheat	Water Aged Oxide	Seed	5.2 × 10 ⁻⁶ (2)	2.5 × 10 ⁻⁶ (5)
		Root	2.0 × 10 ⁻⁶ (2)	2.5 × 10 ⁻⁶ (5)

(a) Concentration Ratio = pCi/g sink tissue/pCi/g contaminated leaf tissue.

(b) Number in brackets indicates number of samples.

(c) Currently being analyzed.

Uptake and Translocation of Foliar Deposited Plutonium

The foliage of bushbean, sugarbeet, and wheat plants was contaminated with various forms of ²³⁸Pu at 20, 40 and 42 days from planting, respectively. Experiments were terminated 28 days after contamination which provided time for the absorption and translocation of Pu from the leaf surface to seed and root.

Our early studies showed Pu to be relatively unavailable with respect to foliar uptake. These studies involved high mass loading rates (~180 ng/gm leaf), short uptake periods (7 days), and relatively dry culture conditions (Cataldo and Vaughan, 1974). Present studies employ low mass loading rates (10-30 ng/gm leaf), extended uptake period (28 days), and leaching regimes to evaluate the bioavailability of Pu under simulated environmental conditions such as rainfall. Leaf surface moisture has been shown to be an important parameter in foliar absorption; the presence of a solution vector aids in chemical modification and solubilization, and enables the diffusion of soluble species to the leaf interior. While it might seem unconventional to apply the term "concentration ratio" (CR) to seed concentration relative to leaf concentration, the idea of the leaf as a source for material moving to seed, or root, is well established on plant physiological grounds. It is of interest to note that leaf to seed CR values for plutonium reported in Table 4.3 are as high or higher than soil to seed CR values (Vaughan et al., 1976).

Table 4.3 presents summary data for the uptake and translocation of Pu from foliar surfaces to seed and root tissues. CR range

from 10⁻⁵ to 10⁻⁷ for the various treatments following dry deposition and in the absence of a solution vector (simulated rainfall). The application of a simulated rainfall over the first 21-day post-exposure period results in an increase in observed CR values. Each of the three plant species studied exhibit increased transport of Pu in the presence of a solution vector with the exception of bushbean root for the water-aged oxide treatment. Leaf surface morphology appears to have no marked effect of Pu absorption, especially following the application of a simulated rainfall. In studies with bushbean, the form of Pu supplied to foliage has little effect on availability in the absence of a solution vector; however, in the presence of a solution vector there is a substantial difference between the hydrated oxide (55% soluble at day 7), and the fresh and water-aged oxides (3.6 and 4.5% soluble on day 7). The effectiveness of leaf moisture in regulating the absorption of Pu would then appear to be related to the size of the soluble fraction.

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• TRANSURANIC COMPLEXATION IN SOIL AND UPTAKE BY PLANTS

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The principal objective of these studies is to define the role of soil microbial processes in influencing the long-term availability, distribution, and form of transuranic elements in plants.

In order to accomplish this objective, basic studies have been undertaken to determine:

1) the effects of transuranic elements on soil microbial populations and processes, 2) the potential for formation of transuranic-organic complexes in soil as a function of soil properties and soil microbial activity, and 3) the influence of soil microbial and plant processes on the kinetics, extent of uptake, sites of deposition, and form of transuranic elements in plants.

To the current year, progress has been summarized in BNWL-2000 and Wildung et al., 1976 (NAEG Symposium). Emphasis was placed on delineation of the relationships between soil chemical and microbial processes and the role of soil microorganisms in affecting solubilization and transformation of elements considered largely insoluble in soils strictly on the basis of their inorganic chemical characteristics.

Soluble, diffusible Pu in soils (usually less than 0.1% of total) appears to be largely present as particulates of hydrated oxide, but several lines of evidence suggest that microorganisms may influence the solubility of Pu and that the nonparticulate plant-available fraction is stabilized in solution by inorganic or organic ligands of limited concentration in soil. The role of soil microorganisms in influencing the solubility, form and plant-availability of the transuranics is discussed on the basis of the: 1) known chemistry of organic ligands in soils, 2) effects on the soil microflora, and 3) principal microbial transformation mechanisms, including direct alteration (valence state, alkylation), indirect alteration (metabolite interactions, influence on the physicochemical environment), and cycling processes (biological uptake and release on decomposition of tissues).

The toxicity of Pu to microorganisms depends on Pu solubility in soil. However, soil microorganisms are generally resistant to Pu, with toxicity apparently due to radiation rather than chemical effects. Highly resistant bacteria, fungi, and actinomycetes have been isolated from soil, and these organisms have been shown to be capable of transporting Pu into the cell and altering its form in the cell and in solution. The resulting soluble Pu complexes tend to be of higher molecular weight than simple complexes (Pu-DTPA) and negatively charged. The form of Pu, although not well-defined, is dependent upon organism type, carbon source, and time of Pu exposure during growth. These factors, in turn, are a function of Pu source, soil properties, and soil environmental conditions. Knowledge of the relative influence of these factors serves as a valuable basis for predicting the behavior of Pu and other transuranic elements in the soil.

During the current year, investigations have continued to measure 1) the solubility and plant availability of Pu as a function of soil properties, 2) the chemical speciation of Pu in soil extracts, microbial cultures, and plants, 3) microbial transformations of Pu in soil, and 4) availability to animals of Pu in plant tissues.

Studies to define the kinetics of Pu solubility and diffusibility in 28 well-characterized soils representing a range in physicochemical properties have continued. Solubility parameters will be related statistically to soil properties in order to establish a basis for prediction of Pu solubility and plant availability over a broad geographic region. This information, in conjunction with studies of transuranic speciation, microbial transformations, and plant and animal availability of Pu in different soils, should provide a broad predictive base to assess the long-term behavior and effects of Pu in the terrestrial environment.

Transuranic Valence States

Plutonium may exist simultaneously in four oxidation states in aqueous solutions. The behavior of Pu in the environment will be markedly influenced by valence state. Generally, Pu IV may be expected to hydrolyze, exhibiting low solubility in neutral, aqueous solutions characteristic of the environment. Other valence states, if stable, may be more soluble and consequently more mobile in the environment. Under normal environmental conditions, Pu III is expected to be easily oxidized. Pu V is not readily differentiated from Pu VI. Prime consideration has, therefore, been given to the differentiation of Pu IV from Pu V and VI.

Many ion complexes, soluble in organic solvents, are formed with TTA (thenyltrifluoroacetone), a β -diketone which chelates in its enol form through coordination with the carbonyl group and replacement of the enol hydrogen. Chelation is not favored by +1 and +2 cations, but improves greatly for +3 and +4 cations. The TTA, therefore, has potential for differentiation of Pu valence state in aqueous extracts of soils and plants provided they do not contain insoluble oxides, hydroxides, or polymeric forms. A study was undertaken to evaluate TTA extraction for determination of Pu form in these substrates.

Dependence on acidity and valence state is marked for Pu extraction with TTA. In the present studies, aqueous acidity was arbitrarily set at 1 M HNO_3 , the optimum acidity used for separation of U and Pu. Prepared Pu IV solutions stored in 2 M HNO_3 appeared to be stable with time toward valence change. However, extraction of aliquots of an approximately 6-month old stock solution containing 6 mCi/ml ^{238}Pu purified as Pu IV nitrate resulted in about 30% Pu not extractable. This fraction was TTA extractable following a redox cycle, but was unchanged after addition of an oxidant alone, indicating a sizable portion of Pu VI (or V)

in the solution. The Pu stock solution had been the source of Pu for many dilutions at low acidities (pH 1.5). Aliquots of these dilutions showed an unexpected 61-73% average extractability after several weeks despite the low acidity. Either Pu hydrolysis had not progressed, or the combination of acidification and TTA may reverse the hydrolysis during the course of the extraction.

The extractability of Pu IV and VI over a nitric acid concentration range of 1 M to 0.02 M is compared in Table 4.4. The Pu IV showed a minimum (but >50%) extractability at about 0.1 M HNO_3 with apparent quantitative extraction at acid concentration of 1 M and 0.02 M. The Pu VI exhibited low extractability, but extractability gradually increased with decreasing acidity over the same acidity range.

TABLE 4.4. Extractable Pu from Freshly Prepared Pu IV and Pu VI Solutions at Varying Acidities

Nitric Acid M ₃	% Pu IV Pu VI	
	Pu IV	Pu VI
1.0	99	< 1
0.5	99	< 1
0.2	75	5
0.1	74	3
0.05	56	7
0.03	77	9
0.02	97	13

In a preliminary study designed to evaluate pH effects over a range of acidities typical of environmental systems, three buffers at pH 4, 7, and 10 were utilized as the aqueous phase. The results indicated that TTA extractability was markedly dependent upon solution composition as well as pH. Extractability with TTA does not unequivocally identify Pu as ionic Pu IV. The TTA chelate itself has been shown to successfully compete for weaker metal complexes. Prior extraction with benzene in the absence of TTA could reveal a weak complex only if the latter is benzene-soluble. Acidification may alter the chemical distribution of TTA extractable Pu whereas unacidified solutions may not approach a stable equilibrium during the period of analysis and are difficult to maintain in a sterile condition.

As part of an overall program employing thin-layer chromatography and electrophoresis gel permeation chromatography, ultrafiltration,

and diffusion methods, the TTA extraction procedure should aid in the determination of Pu species in environmental systems. However, the application of the method for measurement of valence in these systems will require an awareness of its limitations and knowledge of the chemical and physical parameters influencing TTA extractability.

Microbial Transformations of Pu

Microorganisms, in intimate association with soil particles, are known to play an important role in affecting solubilization of elements considered insoluble in soils strictly on the basis of their inorganic chemistry. In-depth studies of the potential role of microorganisms in influencing the long-term fate and behavior of Pu are continuing. Current studies, using enrichment techniques, deal with the effect of carbon source on modification of soluble Pu by major soil microbial classes. In addition to studies with mixed microbial cultures of both fungi and bacteria isolated on the basis of carbon source (mixed sugars, organic acids, and aromatic acids), approximately 50 pure cultures of individual organisms have been isolated that are resistant to Pu. These cultures are being maintained in stock for use in tests of cross resistance to other metals and in the evaluation of common mechanisms for modification.

A large degree of metal cross-resistance in isolated cultures of soil microorganisms was observed. Biochemical characteristics suggested that, under certain conditions, cells selected by growth in the presence of high concentrations of metal, were capable of forming metal-containing materials distinct from those found when metals were allowed to interact with cultures grown in the absence of metal. If these materials were produced by the organisms as a detoxification mechanism in order to change the form of metal to one that does not interact with the cell, it is possible that the same materials are produced by cross-resistant organisms regardless of the nature of the metal to which the cultures were initially exposed. If this is the case, then identification of a limited number of Pu complexors may allow development of models for Pu complexation over a broad range of conditions.

Preliminary characterization, using gel permeation chromatography, of the mixed culture of fungi isolated from soil and grown in sugars indicated that Pu form was altered during fungal growth (Figure 4.2). The exocellular and intracellular soluble fraction obtained from organisms exposed to Pu in a single exposure and in continuous exposure exhibited a majority of material of molecular size greater than Pu-DTPA, which was used as the source of soluble Pu. Furthermore, there

FIGURE 4.2. Separation of Soluble Plutonium Complexes in Microbial Cultures Using Gel Permeation Chromatography

appeared to be a difference in Pu chemical form comparing Pu complexes formed on simple interaction of Pu with metabolites (single exposure) and Pu complexes formed on interaction after continuous Pu exposure of the culture. This suggests either that the culture grown in the continuous presence of Pu contained metabolites capable of interacting with Pu which were different chemically from those produced by the culture grown in the absence of Pu, or that the culture grown in the presence of Pu contained different organisms capable of adaptive response to the element leading to the synthesis of compounds relatively specific to detoxification of Pu.

Further chemical characterization using thin-layer chromatography and electrophoresis verified differences in Pu form. Several solvents of different polarities and pH values were employed to provide a range of chemical conditions for separation. Solvent systems included: A, butanol-pyridine, a system used in resolution of amino acids; D, pentanol-formic acid, a system used in separation of sugars and sugar acids, and G, water-acetic acid, a solvent utilized in resolving keto-acids and sugars. These systems were used to resolve Pu as Pu-DTPA, and Pu in the soluble exocellular and soluble

intracellular fractions of the above cultures. Thin-layer chromatography using solvent A indicated that the exocellular fraction contained one component of chromatographic mobility different than the added Pu-DTPA but the complex remained present in detectable quantities. The intracellular soluble fraction contained a component of lesser chromatographic mobility, but there was no evidence of Pu-DTPA. Solvent D did not mobilize Pu-DTPA or other possible complexes. Solvent G mobilized Pu-DTPA and indicated the presence of immobile Pu components in the exocellular and intracellular fractions, but these were not resolved.

Application of thin-layer electrophoresis (pH 6.6, pyridine-acetate buffer system; cellulose support) indicated the presence of a relatively large amount of material of greater negative charge than Pu-DTPA in the exocellular fraction along with Pu-DTPA. The Pu ligands in the intracellular fraction were either neutral in charge in this buffer system or were of a molecular size too large to migrate under the conditions of electrophoresis. Similar alterations of Pu form by a single Pu-resistant fungus exposed continuously to Pu during growth have also been reported (Robinson et al., 1975). Several phenomena may have been responsible for the observed changes in chemical form of Pu. Organisms may synthesize compounds which either bind Pu-DTPA or bind Pu more tightly than DTPA, thereby successfully competing for Pu in the presence of DTPA. Alternatively, the organism may degrade or modify the DTPA moiety allowing Pu transfer to ligands arising from microbial synthesis and degradation.

Plutonium Availability and Form in Plants and Animals

Plant studies have concentrated on determining the soil and plant factors governing the uptake and form of Pu in plants. Preliminary investigations, using soils varying widely in physical and chemical characteristics, have shown total Pu available for plant uptake to be related to soil type (BNWL-2000, Pt. 2). Although in short-term studies there is a marked discrimination in the plant uptake of Pu amended to soils (CR values of 10^{-2} to 10^{-7}), uncertainty exists as to the long-term potential for plant uptake of Pu and the impact of changes in Pu form on food chain transfer and ultimately on man. This results from a lack of information on potential chemical modification by soil microflora, decay of Pu-containing plant tissues, and subsequent availability of modified forms of Pu for plant uptake. Recent plant studies have been concerned with: 1) developing a basic understanding of the mechanisms involved in the root absorption of Pu in soils, 2) determining the chemical form of Pu in plant tissues following

uptake, 3) measuring the effect of cropping and root decomposition on the concentration of Pu in shoot tissues, and 4) determining the relative availability to animals of Pu contained in plant tissues as compared to inorganic Pu. The latter studies represent a cooperative study with the Biology Department at PNL.

Plants. Previous studies with soybean plants indicated that either a very small percentage of the Pu in soil was available for plant uptake or that the plant root itself was a barrier to Pu uptake and subsequent accumulation. To assist in separation of these effects and develop an understanding of the root uptake mechanism, hydroponically grown soybean plants were placed in $\mu\text{M } ^{238}\text{Pu}_2\text{DTPA}_3$ solutions and permitted to accumulate Pu for up to 48 hr. Concentration ratios for shoot tissues were found to be 6×10^{-3} and 0.3 after 1 and 24 hr, respectively. This would suggest that plants do possess the potential to effectively accumulate Pu with much of the apparent discrimination being a result of the soil environment and a reduction in the available fraction. In an effort to determine the root transport behavior of Pu, several plants were decapitated, xylem exudates collected at intervals up to 24 hr, and the samples subjected to TLE to resolve Pu-containing components. The electrophoretic mobilities of Pu in the medium, in the control exudate + $\text{Pu}(\text{NO}_3)_4$, and in the exudate collected from decapitated plants grown in $0.1 \mu\text{M}$ Pu-DTPA solution over a 24-hr period are illustrated in Figure 4.3. The presence of Pu-containing components in the spiked exudate indicated the presence of ligands capable of binding Pu and forming stable complexes. Similarly, several anionic and cationic Pu complexes were evident in exudates from treated plants. There was a component with an electrophoretic solubility similar to Pu DTPA which peaked in the second sampling and decreased in concentration in subsequent samples. These data suggest that both Pu^{+4} and Pu-DTPA can be absorbed by plant roots, with a large fraction of the absorbed Pu being complexed by the plant and transported as a metal-ligand to the plant shoots. These studies are continuing in an effort to resolve the uptake and transport behavior of Pu, and to determine the stability, fate, and potential effect of plant ligands on Pu transfer in the food web.

Animals. The accepted value for the gastrointestinal transfer ratio of Pu from food-to-man is 3×10^{-5} (WASH 1535, 1974). This value is based on the measured GI absorption of inorganic Pu IV in animals administered by gavage (Weeks et al., 1956) and the apparent assumption that food would contain primarily Pu IV in an inorganic chemical form since Weeks et al. (1956) had

FIGURE 4.3. Electrophoretic Separation of Pu Containing-Components Present in Xylem Exudate of Soybean Plants After Exposure to Pu-DTPA

shown that Pu VI and complexed forms of Pu IV gave GI absorption rates up to 600 times greater.

The ability to test these assumptions has only recently been possible, due to: 1) recent studies on this program which indicated that alfalfa plant tissues containing up to 400,000 d/m/gm could be obtained under a sequential harvesting regime, and 2) development of a sensitive analytical method to analyze Pu at levels of < 1 d/m per sample (Wessman, 1971).

In preliminary studies, two animals, rats (for comparison with previous gavage studies), and guinea pigs (digestion systems similar to ruminant), were used to compare gut transfer of Pu administered in inorganic forms and in alfalfa tissue. The results of the feeding study showed that in both rats and guinea pigs absorption through the gastrointestinal tract of Pu incorporated in alfalfa tissue was greater (7.7 to 53 times) than by gavaging inorganic Pu IV solutions. In these preliminary experiments, several variables, including animal species, duration of feeding, and types of plant tissues fed were evaluated,

but due to the small number of animals which could be utilized, the limited quantity of tissue and variability, further studies are required to evaluate the statistical significance of individual data sets.

Chemical characterization of the form of Pu present in the alfalfa tissue fed to animals, is in progress. Initial results indicated that the Pu is more soluble in stems than in leaves, perhaps by a factor of 2, and that > 90% of the Pu in either stems or leaves is not ionic Pu IV as determined from TTA extraction methods discussed earlier. The results of preliminary studies indicate that up to 45% (stems) of the soluble Pu is strongly complexed Pu IV with the remaining Pu in the Pu V or Pu VI valence state, or that all of the Pu is Pu V or Pu VI, with reduction occurring to Pu IV during chemical characterization. Further studies are underway to precisely determine the quantity of oxidized Pu species in plant tissues, however, based on the results of previous studies, it is likely that the presence of these Pu species accounts for the increased availability to animals of Pu.

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• QUANTITATIVE ASPECTS OF PLUTONIUM FIELD STUDIES

Principal Investigators: R. O. Gilbert, L. L. Eberhardt,
P. G. Doctor, A. R. Olsen, and J. A. Merrill

The purpose of this program is to provide quantitative and statistical support for environmental transuranic field studies with emphasis on nonarid regions. Our efforts during FY 1976 (the first year of funding) include: 1) visits to transuranic field study sites in various sections of the United States; 2) identification of statistical design and analysis problems needing attention; 3) preparation of reports and publications on appropriate statistical methods for transuranic field studies; 4) development of our capability to analyze the spatial "structure" of transuranic data to obtain more accurate estimates of average concentrations or total amounts; 5) identification of statistically valid operational procedures for deciding whether or not to clean up a land area contaminated to some extent by Pu; and, 6) research on iterative computer techniques for obtaining improved estimates of concentration contours of transuranics in soil.

Site Visits

Since July 1975 site visits have been made to Argonne National Laboratory (ANL) and the watershed of the Great Miami River in southwest Ohio (near Mound Laboratory), the Savannah River Ecology Laboratory Pu field study sites on the Savannah River Plant in South Carolina, the flood-plain study site at Oak Ridge National Laboratory (ORNL), the test reactor leaching ponds and radioactive waste management complex on the Idaho National Engineering Laboratory, and the canyons that receive treated radioactive liquid wastes from the Los Alamos Scientific Laboratory (LASL). These visits supplement our familiarity with transuranic field studies on the Nevada Test Site. We also assisted scientists of ANL in the design of a pond and watershed study to assess the influence of three land types (cornfield, pasture, forest) on the transport of fallout ^{239}Pu via erosion into small man-made ponds near Urbana, Ohio.

Ratios in Environmental Transuranic Research

Ratios are used extensively in transuranic field studies for studying the transfer of

transuranics from one environmental component to another. The ratio estimation problem is a subset of the more general problem of optimal estimation of the slope and intercept parameters of a simple linear model where both variables are subject to error, a common situation in transuranic studies. We have been reviewing the statistical literature in this area and are writing a computer program for evaluating different weighting schemes for estimating the parameters. This effort will be intensified after we complete our initial work on ratios. We also have an interest in identifying existing nonparametric statistical analyses and procedures that can be applied to transuranic field studies. Activities to date have centered on gathering appropriate references for use in writing a paper directed toward radioecologists engaged in Pu field studies.

It is necessary that the statistical properties of ratios be understood if they are to be used appropriately. We are reviewing the literature to identify methods of computing average ratios and their standard errors that seem particularly appropriate for

transuranic field studies and these will be reported in a later publication.

A ratio is used to express the relationship between two variables, say, Pu (Y) and Am (X) concentrations in the same aliquot of a soil sample. Calculating some form of an average ratio is particularly appropriate if we can assume that the ratio of, say, Y to X remains constant over the data set of interest. Expressed in geometric terms, this implies the relationship between Y and X is a straight line through the origin. In the absence of this condition the experimenter should carefully consider whether an average ratio is really very informative. It may be preferable, instead, to explain why the ratio is not constant from sample to sample.

There are several ways to calculate an average ratio if the data form a straight line through the origin. If the data come from a true experiment where the variable X is under the control of the experimenter and Y is normally distributed then there are several methods of calculating the average ratio and its confidence limit depending on the relationship between X and the variance of Y (Snedecor and Cochran (1967), pp. 166-171).

However, in the case of transuranic field studies, neither Y nor X is controlled by the experimenter. The estimate $\bar{Y}/\bar{X}$, where $\bar{Y}$ and $\bar{X}$ represent the mean of the Y and X observations, respectively, is an unbiased estimate of the average ratio. This is true whenever the data are a straight line through the origin, whether or not X is a random variable (Cochran, 1963, p. 161). Formulas for estimating the variance of $\bar{Y}/\bar{X}$ are generally approximations that are valid only for large samples. Also, if the data do not fit a straight line through the origin, the $\bar{Y}/\bar{X}$ is biased. This bias will be relatively unimportant if sample sizes are greater than 30 and large enough so that the coefficients of variation for $\bar{Y}$ and $\bar{X}$ (standard error/mean) are 10% or less.

One can also work with the individual ratios Y_i/X_i , $i = 1, 2, \dots, n$. If Y_i and X_i are distributed as bivariate lognormal variables, then the ratios Y_i/X_i are also lognormal. This implies the geometric mean of the ratios is a reasonable estimate of the "average" (median) ratio. There is one estimate that makes no distributional assumption; the median of the individual ratios. Another approach is simply to compute the mean of the individual ratio,

$$\bar{R} = \frac{1}{n} \sum_{i=1}^n Y_i/X_i$$

We compared several estimators and their confidence intervals for three data sets from transuranic field studies. In one case Y was $^{239-240}\text{Pu}$ and X was ^{241}Am in soil while in the others the average ratio of soil to vegetation Pu was of interest. For the one case where the relationship between Y and X was clearly a straight line through the origin, all 4 estimates were similar in magnitude, but their confidence intervals differed in length. The conclusion here was that if the data followed a straight line through the origin, any of the estimators investigated appeared to be satisfactory. In the cases where the data did not follow a straight line through the origin, the estimates did not vary significantly, except for $\bar{R}$. When several very large ratios were present, $\bar{R}$ was significantly larger than the other estimates since $\bar{R}$ is very sensitive to these large values.

As a result of our review of the literature, our past experience in the analysis of ratio data, and the examples discussed above, we recommend several approaches for estimating an average ratio. The first is to plot Y versus X to see if a straight line through the origin appears reasonable; if so, the ratio between Y and X may be assumed to be constant over the range of Y and X investigated, and it makes sense to calculate an average ratio. The second is to calculate the individual ratios; if there are any exceedingly large (or small) ratios, avoid the mean $\bar{R}$ of the individual ratios as an estimate of the average ratio.

Effective Methods of Summarizing and Reporting Pu from Environmental Studies

An evaluation of the present use of quantitative methods in environmental Pu studies has indicated that the present methods of reporting Pu data appear inadequate and improvements can be made if alternative methods of summarizing the data were available. Our objective is to evaluate present methods and to develop new methods when possible. We are writing a report on effective methods of summarizing and reporting Pu data. In general, because of the highly variable nature of Pu data and the problems associated with its collection over time and space, some of the commonly used methods of summarizing data do not adequately describe environmental Pu data sets. Our report will discuss these problems, indicate alternative methods and be the first of several reports which will deal with the analysis and description of environmental Pu data.

One of the first requirements in an environmental study is the preparation of the

data in a form suitable for publication and understanding. In the past, this has sometimes resulted in only reporting the sample sizes and describing the data's arithmetic mean and standard deviation. This is not adequate in most environmental Pu data sets because of the highly variable nature of the basic skewness of the data. Some researchers have used geometric means and geometric standard deviations, i.e., using log normality assumptions in an effort to more adequately present their data. That approach requires additional assumptions about the data that are not always justified. Our research indicates that alternative descriptive techniques can adequately describe the data's variability and structure. The techniques do not require distributional assumptions but are based on frequency histograms and order statistics.

Estimation of Spatial Pattern

An area of fundamental importance to Pu field studies is the optimal analysis of data collected over time or space. Traditional statistical analyses usually assume the data are independent (uncorrelated). In field situations, however, this is often not the case as evidenced, e.g., by systematic changes (trends) in concentrations from a point source of contamination. If the correlation structure over space or time is used in the analysis then improved estimates of average concentrations, total amounts (inventories), values at grid nodes, spatial pattern, etc., can be obtained. We have been conducting an extensive literature review on a technique called Kriging that may be particularly useful for these kinds of problems in transuranic field studies. We have established a working relationship with an expert on Kriging (Dr. Pierre Delfiner, Centre de Geostatistique, Fontainebleau, France) and expect soon to have access to his Kriging computer programs purchased by ERDA and installed on ERDA's computer in Las Vegas. This should be a valuable tool for exploring the potential of Kriging for transuranic field studies. Most of the initial work using this program will be in connection with our NAEG work on the Nevada Test Site. However, the experience gained there should be very useful for evaluating the applicability of Kriging in more moist climates.

One potential use of Kriging is to help identify those subareas within a transuranic contaminated area that require cleanup. The decision to clean up an area should be put on a probability basis since there is always some chance of missing a contaminated area during a cleanup operation or of cleaning up an area not really requiring cleanup. There are several statistical decision-making approaches that could be considered, some of

which could incorporate Kriging for obtaining improved estimates of average concentrations in soil over areas of specified size.

We have also been investigating the improvement in Pu concentration contours in soil that might be obtained by iterating on residuals from previous contour fits to the data. These efforts resulted in a paper presented at the American Statistical Association meetings in Boston, 23-26 August 1976. Our results indicate improved fits can be obtained. Future efforts in this area are expected to be directed toward Kriging since this technique allows for the estimation of error bars on the contours.

NAEG Transuranic Field Studies on the Nevada Test Site

The purpose of this effort is to provide statistical design and analysis support for environmental transuranic studies conducted by the Nevada Applied Ecology Group (NAEG) on the Nevada Test Site (NTS), Tonopah Test Range (TTR), and the Nellis Air Force Range. More specific activities over the period from July 1975 to 1 October 1976 are listed below.

We have assisted in the design of a field study to estimate the amount of Pu in blow-sand mounds at the Area 13 (Project 57) and Clean Slate 3 safety-shot sites. Previous estimates of Pu inventory at NAEG safety shot sites have been for the top 5 cm of soil. The amount of Pu below 5 cm within mounds are not included in these estimates. The present study is designed to fill that need. Entire mounds are being sampled as well as the 5 cm depth increment under the mounds.

We have prepared an initial synthesis of ²³⁹⁻²⁴⁰Pu data in soil, vegetation, small mammals, and cattle tissues for the Area 13 safety-shot site. These preliminary results were presented at the NAEG Information Conference in Las Vegas, 12-13 February 1976. The data for the above components as well as hypothetical Pu concentrations for lung, skeletal bone, and kidney of a "Standard Man" assumed to live in and obtain most of his food from the area were plotted on a single graph for visual comparison. These hypothetical values were obtained using the Pu transport and dose estimation model of Martin and Bloom (1976). This model was also used to obtain hypothetical Pu concentrations in muscle tissue of cattle assumed to graze Area 13 vegetation for the same periods of time that cattle were actually grazed. Martin and Bloom's model gave hypothetical concentrations in muscle that were from 2 to 4 times larger than obtained in muscle tissue of the cattle grazed on the area. This suggests their model may give conservative estimates for muscle of cattle. These results are quite

preliminary since they are based on tissue from only a few cattle (from 1 to 3). Data from several more cattle should become available within the next few months.

Considerable effort is being devoted to the design of transuranic studies on nuclear event sites on NTS. Initial effort has been directed toward the Little Feller I and II sites and the Palanquin, Cabriole test areas. Since the soils of these areas contain considerable amounts of rocks, care is being taken to minimize potential biases in average soil concentrations that might be introduced during the field soil sampling procedure. Study objectives at these sites are similar to those at safety-shot sites. These include estimating average concentrations or total amounts in soil, vegetation, and small mammals. A cattle grazing study is planned for the Palanquin-Cabriole site.

Experiments are continuing with automatic (computer) contouring methods for estimating spatial pattern of Pu concentrations in soil. Iterative techniques have been investigated using the Area 13 (Project 57) soil data. Also, we have recently begun using Kriging techniques on the NAEG safety-shot Pu soil data for estimating spatial pattern, average concentrations and total amounts (inventories). Kriging techniques appear at this time to be useful for Pu field studies. Our efforts during the next year using the Kriging BLUEPACK and GAMMA computer programs recently purchased by ERDA (Las Vegas) should be helpful in evaluating its potential.

We have also taken part in some of the planning for the upcoming cleanup of the Enewetak Atoll. This has involved attempting to obtain meaningful Pu soil concentration contours for the island of Janet (Enjebi) using data obtained during the 1972 Enewetak sample survey. This effort was not entirely successful due in part to the relatively wide spacing between soil sample locations. Delfiner applied Kriging techniques to these data and found a lack of structure in the data. This suggests the present soil data on Janet Island is not sufficient in itself for identifying areas needing cleanup. The use of in-situ measurements for ^{241}Am in conjunction with additional soil samples and Kriging is being considered for the upcoming cleanup effort.

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Statistical Design and Analysis for Terrestrial Ecology

We provide statistical design and analysis assistance to scientists engaged in terrestrial ecology programs. An example is the greater use being made of "double sampling" wherein nondestructive measurement of field biomass is combined with traditional harvesting methods. This technique has been effective in obtaining more reliable estimates of sagebrush biomass at reduced cost over traditional harvesting methods. A description of this application of double sampling will appear in the Journal of Range Management authored by D. W. Uresk, R. O. Gilbert, and W. H. Rickard.

An important aspect of our work is the processing of large amounts of vegetation biomass data from the ALE Reserve and other areas on the Hanford Reservation. These data are given a preliminary statistical analysis involving simple summary statistics and histograms on a routine basis. The data are then stored for possibly more sophisticated statistical treatment at a later time when several years' data are available for analysis.

We are also in the process of redesigning some of the forms used for recording data in the field. These new forms should result in fewer recording errors as well as reducing the time required to transfer data from field forms to data cards.

We have assisted in the design of revegetation studies of simulated strip mining areas. This study involves the construction of artificial earth mounds and the planting of various plant species between mounds. Assistance on statistical matters is also provided in the preparation of manuscripts for publication and in preparing manuscripts jointly with members of the Terrestrial Ecology Section.

● **ECOLOGICAL DISTRIBUTION AND FATE OF PLUTONIUM AND AMERICIUM
IN A PROCESSING WASTE POND ON THE HANFORD RESERVATION**

Principal Investigator: R. M. Emery

Other Investigator: D. C. Klopfer

U-Pond, located on the Hanford Reservation, has received low-level quantities of plutonium (Pu) and americium (Am) longer than any other aquatic environment in the world. Its ecological complexity and content of transuranics make it an ideal resource for information about the movement of these actinides within and out of an aquatic ecosystem.

U-Pond has been intensively inventoried for Pu and Am concentrations in the ecological compartments and characterized limnologically in terms of its physicochemical parameters, biological productivity and community structure. This work will provide a basis for evaluating the pond's performance in retaining waste transuranics. The quantitative estimation of export routes developed by this study is important in determining how effectively such ponds act as retainers for transuranic wastes. A more complete report has been prepared.

Transuranic Uptake Rates

Research during FY 1976 was directed toward measuring rates of transuranic uptake by 4 biological components in the pond. Organic floc, Cladophora, goldfish, and mallard ducks were selected for uptake studies on the basis of relative abundance in the pond or potential for exporting Pu and Am.

Because of the pond's high level of nutrient enrichment and resulting primary productivity, decomposing algae and plant material form a loosely compacted flocculent layer over the sediments. This floc contains a dense population of microorganisms and is also a major concentrator of Pu and Am. Cladophora, a filamentous green algae, grows luxuriantly in U-Pond and degrades into large quantities of floc each year. Both floc and Cladophora appear to accumulate most of their Pu and Am burden by capturing transuranic particles on their surfaces. Data are already available on amounts of transuranics accumulated by these components in the open pond (Emery et al., 1976) However, to determine the amount and rate of filterable Pu and Am accumulated by floc and Cladophora, in situ uptake experiments were performed by

enclosing them in dialysis membranes having a porosity of 0.1 μ m.

Results of these experiments show that accumulation of filterable Pu and Am is slow and that equilibrium concentrations are not established after 400 hr of exposure (Figure 4.4). Organic floc accumulated transuranics slower than Cladophora, and Am was accumulated at a slower rate than Pu by both components. Ratios of ^{238}Pu to $^{239,240}\text{Pu}$ accumulated by these materials were about the same as those occurring naturally in U-Pond. However, ^{241}Am was accumulated at a relatively slower rate than Pu, and the ^{241}Am to $^{239,240}\text{Pu}$ ratios were lower than those of the open pond. This likely occurred because a greater percentage of Am to Pu is present in U-Pond water as particulates. Nevertheless, the amount of filterable Pu and Am accumulated by floc in 400 hr represents less than 0.1% of the amounts concentrated in the open pond. Under open pond conditions floc accumulated on the average 1033 pCi of ^{238}Pu , 642 pCi of $^{239,240}\text{Pu}$, and 286 pCi of ^{241}Am /g dry wt. Cladophora accumulated a greater percentage of its mean total burden by way of filterable Pu and Am uptake, from 1 to 4% over a 440 hr period. In the open pond,

FIGURE 4.4. Rates of Uptake and Isotopic Ratios of Filterable Pu and Am Accumulated by Floc and Cladophora in U-Pond

Cladophora accumulated a greater percentage of its mean total burden by uptake of filterable Pu and Am, from 1 to 4% over a 400 hr period. In the open pond Cladophora accumulated averages of 46 pCi of ^{238}Pu , 34 pCi of $^{239,240}\text{Pu}$, and 36 pCi of ^{241}Am /g dry wt. In terms of environmental transport, the surface accumulation of particulate Pu and Am appears to be most significant.

Goldfish are the only fish in U-Pond and are fed upon by herons, coyotes and occasionally other mammals and waterfowl. To measure the uptake of Pu and Am, imported goldfish were exposed to U-Pond in cages that were set into the sediments, thus making floc available to them as food. Goldfish, analyzed whole and without gastrointestinal tracts, accumulated equilibrium concentrations within the initial 48 hr (Figure 4.5). The equilibrium concentration for whole goldfish, of about 11 pCi of Pu and Am/g dry wt, was almost identical to the concentrations of these isotopes in resident U-Pond goldfish. Approximately 95% of the whole body concentration is in the gut and the gut contents. Uptake rates and saturation levels were approximately the same for the three transuranic isotopes. Isotopic ratios for goldfish with and without guts were also similar to ratios found in native goldfish

samples. It is concluded that although goldfish feed heavily on floc, which is the pond's most active concentrator of Pu and Am, they assimilate and transport relatively minor quantities of these transuranics within and out of this ecosystem.

A short experiment measured the initial uptake rate of Pu by mallard ducks. These ducks were held along the shore in cages which extended into the pond to permit the ducks access to the water. Over the 5-day experimental period the ducks fed upon organic floc from the pond almost exclusively. Under natural conditions ducks would not likely remain on the pond feeding exclusively on organic floc for 5 continuous days. Thus, the conditions represent upper limits of continuous exposure which natural ducks would receive while contacting U-Pond during their period of occurrence in eastern Washington. Like goldfish, uptake of Pu in the first 2 days was rapid, approaching equilibrium in the gut and other tissues (Figure 4.6). This brief experiment also indicates that, again like goldfish, tissue assimilated concentrations of Pu are less than 5% of the whole body burden. This also suggests that a duck leaving U-Pond, having more than 95% of its Pu burden contained in its gut, would distribute about the same amount of Pu to

FIGURE 4.5. Rates of Uptake and Isotopic Ratios of Pu and Am Accumulated by Goldfish in U-Pond

adjacent environments as the gut contents are eliminated. If this duck were eaten by a human it is not likely that more than 5% of the duck's total Pu burden would be available for consumption via this route.

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FIGURE 4.6. Amounts of Total Pu Accumulated by Experimental Ducks after 2 and 5 Days of Continuous Contact with U-Pond, Compared with Minimum, Maximum and Mean Concentrations of Pu in Whole Waterfowl Occurring Naturally on U-Pond. Mean Pu concentrations in experimental ducks are shown by the location of the appropriate symbol within the ranges of data, depicted by the horizontal lines

- **USE OF ANALOG ELEMENTS TO PREDICT THE EQUILIBRIUM BEHAVIOR OF TRANSURANIC ELEMENTS IN THE ENVIRONMENT**

Principal Investigators: W. C. Weimer, J. C. Kutt, J. C. Laul
and J. L. Ryan

Several naturally occurring elements with chemical properties similar to those of the transuranic elements have been selected and are being examined as potential predictors of transuranic geochemical behavior. Such an approach might allow the estimation of the long-term behavior of transuranium elements in the environment by analyses of the steady-state behaviors of the analog elements.

Laboratory evaluations have been performed on the interactions of two transuranic elements and of a rare earth element with an aqueous suspension of an eastern Washington soil. These studies demonstrate the validity of using chemical analogs to evaluate transuranic element behaviors. The results have shown that the rare earth:transuranic elemental pairs interact with this terrestrial soil equivalently under the experimental conditions evaluated.

Concentrations of the native lanthanide elements in biological materials and many soil extracts are quite low and cannot be measured by standard instrumental neutron activation analysis techniques; therefore, a radiochemical group separation procedure has been developed to measure lanthanide elements in biological materials.

Rationale and Analytical Methods for Using Chemically Analogous Elements to Estimate the Environmental Behaviors of Transuranic Elements

Several naturally occurring elements with chemical properties similar to those of the transuranic elements have been selected and are being examined as potential predictors of transuranic geochemical behaviors. These elements would allow the estimation of the long-term behavior of transuranium elements in the environment by analyses of the steady-state behaviors of the analog elements.

Current theoretical and empirical data are inadequate to predict the effects of long-term biogeochemical weathering processes on the geochemical mobilization (or immobilization) and biological availabilities of the transuranium elements. The inability to predict these effects creates a serious problem in estimating the ultimate

consequences of the release of the transuranics into the environment. It is postulated that the long-term behavior of these transuranic elements can be modeled effectively by the behaviors of naturally-occurring analog elements which have been subjected to very long-term biogeochemical weathering processes and which have reached a steady-state equilibrium within the environment. Natural weathering processes should affect similar changes in analog elements and the corresponding transuranic elements due to sufficiently similar properties of the analog:transuranic pairs.

The chemical properties which are the most significant in the choice of analog:transuranic elemental pairs include: 1) the oxidation states likely to exist under normal Eh and pH conditions; 2) the ionic radii of these elements; 3) the hydrolysis constants; and 4) the solubility products of the principal species. Except for having a much wider

range of potentially stable oxidation states, the actinide elements are most similar to the lanthanide series elements. The known chemical behavior of the trivalent actinides is nearly identical to the behavior of the trivalent lanthanides except for a slightly greater effect of covalency in the bonding strength of the actinides. On these bases, the following pairs of transuranic and analog elements have been proposed: (Am^{+3} , Nd^{+3}); (Cm^{+3} , Nd^{+3}); (Pu^{+6} , U^{+6}); (Pu^{+4} , Th^{+4}); (Pu^{+3} , a suite of the heavier rare elements [Tb-Lu]). The proposed analog elements are all naturally occurring and are present in primary minerals, secondary minerals, and the reaction products of weathering processes which form terrestrial soils. The distributions of the analog elements between these geological compartments and soil solutions or other aqueous phases are governed by the steady-state processes that are the result of long environmental equilibration times and by their basic chemical properties. Once the analog elements are released from mineral phases, they equilibrate in the soil system and the same chemical, biological, and physical processes which affect them will affect transuranic elements which enter the environment. In an environmental steady-state condition, the transuranic elements would be expected to be distributed throughout the various phases of the soil system in the same manner as their analog element. Therefore, measurements of the concentration ratios for the biological incorporation of analog elements from soils to plants should permit an estimation of the concentration ratios for environmentally equilibrated transuranium elements.

Since the concentrations of the lanthanide elements in biological materials and many soil extracts are quite low and cannot be measured by standard instrumental neutron activation analysis techniques, a radiochemical group separation procedure has been developed to measure 12 lanthanide elements in biological materials.

Concentrations of this series of lanthanide elements in soils, soil extracts, and biological materials can be determined by neutron activation followed by a radiochemical group separation step and subsequent γ - or x-ray counting using a Ge(Li) diode or an intrinsic Ge diode. Samples and standards are irradiated at a neutron flux of approximately $6 \times 10^{12} \text{ n/cm}^2$ for 6 hr. After a 2-day decay time, the samples are transferred into nickel crucibles with a rare earth element mixed carrier already dried therein. The samples are then fused using a Na_2O_2 -NaOH mixture. The resulting fusion cake is decomposed with H_2O and neutralized with HCl. The rare earth elements are precipitated in a group as the hydroxides with NH_4OH . The rare earth hydroxides are dissolved in HCl and

precipitated as the fluorides with NH_4HF_2 . This fluoride and hydroxide precipitation carrier cycle is repeated three times to insure a radiochemically pure product. The analysis sequence is shown in Figure 4.7. The resulting group of rare earth elements is then counted on a Ge(Li) or intrinsic Ge system.

Table 4.5 lists the half life and γ - or x-ray energy selected for each rare earth isotope and Th and U, the decay interval prior to counting, and the approximate limit of detection for biological materials. After a decay period of 20 to 30 days, the samples are again counted, this time for 100-1000 min to optimize detection sensitivities for the longer-lived isotopes of Ce, Nd, Eu, Gd, Tb, and Tm. Chemical yields for the rare earth procedure are determined by the reactivation of the samples and enriched isotopes which are added to the samples prior to the rare earth group separation steps. These yields range from 50 to 80%.

Comparative Laboratory Behaviors of Am, Cm, and a Rare Earth Chemical Analog with Ritzville Silt Loam

Laboratory evaluations have been performed on the interactions of two transuranic elements and of a rare earth element added to an aqueous suspension of an eastern Washington soil. The results suggest that the rare earth:transuranic elemental pairs interact with this terrestrial soil equivalently, under the experimental conditions evaluated.

FIGURE 4.7. Flow Scheme Ab(U), Sr and Ree Elements

TABLE 4.5. Rare Earth and Other Analog Element Sensitivities

ELEMENT	T _{1/2}	γ-RAY keV	DETECTION* LIMIT (PPBI)	X-RAY keV	DETECTION* LIMIT (PPBI)
La	40.2 h	816	0.5	34.8	1.0
Ce	32.5 d	146	100	--	--
Pr	19.2 h	1576	3.0	--	--
Nd	11.1 d	531	50	38.7	13
Sm	46.8 h	103	0.3	41.6	0.06
Eu	12.7 y	122	0.3	40.1	0.03
Gd	242 d	98	30	--	--
Tb	72.1 d	299	1.0	--	--
Ho	26.8 h	81	0.1	49.2	0.02
Er	7.52 h	308	20	50.8	10
Tm	129 d	84	5.0	--	--
Yb	4.21 d	396	0.4	54.2	0.08
Lu	6.74 d	208	0.5	55.8	0.2
U	12.8 d	1596	10	--	--
Th (²³³ Pa)	27.0 d	312	100	--	--

*SAMPLE SIZE: 0.4 g
IRRADIATION TIME: 7 h
FLUX: 6×10^{12} n/cm²/sec
COUNTING TIME: 200 m
DIODE: 25% EFF. Ge(Li) OR PLANAR Ge(INTRINSIC)

If the rare earth Nd, which would be expected to behave similar to Am and Cm, can be shown to actually behave analogously in soil suspension, then its natural distribution might be used to predict the long-term soil behavior of Am and Cm. The verification of the similarities or differences in the long-term behaviors of rare earth:transuranic elemental pairs is not possible, but short-term laboratory comparisons using the same chemical forms of the analog and the transuranic elements are possible and have been performed. These laboratory evaluations have involved the determinations of the distribution coefficients (K_d) of ²⁴¹Am, ²⁴⁴Cm, and ¹⁴⁷Nd between a solution of 0.05 M Ca(NO₃)₂ and Ritzville silt loam as a function of pH, contact time, and soil pre-treatment conditions. The K_d values were determined for soil suspensions adjusted to pH values of 1.5 to 9.0 and equilibrated for time periods of 1, 4, 8, or 24 hr. The experimental conditions were duplicated for both unwashed Ritzville silt loam soil and soil which had been pre-washed by sequential treatments with 0.05 M Ca(NO₃)₂. Each of the radiotracer elements was added in the nitrate form to the soil suspension at a concentration level which was chosen to not exceed the solubility of the metal hydroxide species.

Representative data showing the relationship of the distribution coefficients for the

three elements to the pH of the soil:solution suspensions are presented in Figure 4.8 for the unwashed soil condition and for 1-hr and 4-hr contact times. The rare earth analog Nd when added to the soil behaved similarly to the transuranic elements Am and Cm under these experimental conditions, with the simple correlation coefficient ranging between 0.95 and 0.99 for all sets of experimental conditions (Table 4.6). These analogous behaviors lend support to the proposition of using naturally occurring Nd as an analog for evaluating certain soil geochemical processes which the transuranic elements may undergo.

FIGURE 4.8. Elemental Distribution Coefficients in a Soil-Ca(NO₃)₂ System

TABLE 4.6. Correlations of Analog: Transuranic K_d Values

SIMPLE CORRELATION COEFFICIENTS (r) FOR

ANALOG- TRANSURANIC PAIR	WASHED SOIL	UNWASHED SOIL	WASHED + UNWASHED SOILS
Nd-Am	0.95	> 0.99	0.99
Nd-Cm	0.95	0.98	0.98

• WEATHERING AND AGING OF TRANSURANICS

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The release of transuranium elements from the nuclear energy industry poses questions for which data are very limited. Information is needed to quantify the movement of radiocontaminants through food chains to assess the potential hazards to man. Quantitative data is required on the transfer of transuranics from soil to vegetation. Of special interest are the effects time (weathering, aging, and associated biological processes in soil) has on the availability of transuranium elements to be taken up by plants from soil. This study provides information on the effects of time on the uptake of transuranics from soil by range and crop plants.

Weathering and Aging Effects

Range plants, which provide forage for livestock and wildlife species, and crop plants, which are fundamental links in various food chains, are being grown under realistic field environments in transuranic contaminated soil contained in small weighing lysimeters. This allows the plants and soil to be exposed to annual climatic changes. The plants are then harvested yearly and radiochemically analyzed for transuranic content. Year-to-year variations will provide information to quantify the changes in phyto-availability of transuranium elements. Since these weathering studies are set up on an extensive scale and may extend beyond a decade, particular attention has been paid to isotopic purity and to the characterization of stock solutions by specific isotopic analysis. The lysimeter technic itself is somewhat unique and, in earlier studies, has been very carefully validated with respect to soil water and temperature profiles. (Hinds, 1973, 1975.)

At present 360 lysimeters have been prepared and placed in an enclosure located near the ERDA Arid Lands Ecology Reserve (ALE) headquarters. Isotopes of ^{238}Pu , ^{239}Pu , ^{244}Cm , ^{241}Am and ^{237}Np were individually placed in soil contained by the lysimeters. The water-tight lysimeters were constructed from polyvinyl chloride pipe (PVC), 13.2 cm

in diameter and 1 m long. The radioisotopes were mixed in either 3.4 kg or 100 g of soil. The 3.4 kg mixtures were placed in the lysimeters in a layer 20 cm thick, 10 cm below the soil surface. The 100 g mixtures were also placed 10 cm below the surface, but were only 0.5 cm thick. The number of lysimeters prepared at the present time in reference to the species planted (cheatgrass, peas, barley, and alfalfa) in each treatment is presented in Table 4.7. The lysimeters containing Ritzville soil were prepared and planted for the first time this past year. The Esquatzel soil lysimeters were planted and harvested for the third time this past summer.

The aboveground plant parts were harvested at maturity and divided into selected components. Cheatgrass (*Bromus tectorum*) and other plants which invaded the lysimeters were separated by species and the entire aboveground plant parts analyzed. The peas were divided into seeds, leaves, and stem/pod components. The seeds of the barley were analyzed separately from the stem/leaf parts. The entire alfalfa plant (3 separate harvests) were analyzed. The radiochemical analyses were done by LFE Environmental Analysis Laboratory in Richmond, CA. Sample analyses are at various stages of completion at this time. The significance of variations in plant uptake as affected by species or transuranic element differences will be assessed when the sample analyses are completed.

TABLE 4.7. Number of Lysimeters Prepared and Planted by Species and Treatment as of June 1976

Isotope (Nitrate Form)	mCi per Lysimeter	Number of Lysimeters Planted With			
		Cheatgrass	Peas	Barley	Alfalfa
<u>20 cm subsurface layer</u> (Ritzville soil):					
²³⁸ Pu	0.1	5	5	5	5
²³⁸ Pu	1.0	5	5	5	5
²³⁹ Pu	0.1	5	5	5	5
²³⁹ Pu	1.0	5	5	5	5
²⁴¹ Am	0.1	5	5	5	5
²⁴¹ Am	1.0	5	5	5	5
²⁴⁴ Cm	0.1	5	5	5	5
²⁴⁴ Cm	1.0	5	5	5	5
²³⁷ Np	0.1	5	5	5	5
<u>Thin subsurface layer</u> (Ritzville soil):					
²³⁹ Pu	0.1	10			
²⁴¹ Am	0.01				
<u>Control (Ritzville silt loam soil):</u>					
	0.0	30	10	10	10
<u>Thin subsurface layer</u> (Esquatzel soil):					
²³⁸ Pu	1.1	40	10	10	
<u>Control (Esquatzel silt loam soil):</u>					
	0.0	30	10	10	

Movement of ²³⁸Pu Through Soil

Soil cores removed from 4 lysimeters were used to investigate the vertical migration patterns of Pu in soil. After removal of the lysimeters from the enclosure, water was

added to bring the soil moisture to 10%. Horizontal core samples were then removed at distances of 5, 8, 9, 10, 11, 12, 20, 50, and 75 cm below the soil surface. At each depth the cores were sectioned into 3 horizontally adjacent samples, each 4.4 cm in length, resulting in a total of 27 soil samples per lysimeter. Cores were removed by first burning a 1.27 cm hole through the side of the lysimeter and then attaching a 1.27 cm OD (0.95 cm ID) aluminum guide tube to the wall. The samples were removed by inserting a 0.95 cm OD (0.70 cm ID) aluminum tube 4.4 cm into the lysimeter. The tube and first section were removed, another tube inserted 8.8 cm and the second section was removed. A third tube was inserted 13.2 cm through to the other side and the third section was removed. This was done to reduce chances of cross-contaminating the 3 horizontally adjacent sections. The soil void was then filled with a 0.95 cm OD aluminum tube and the hole sealed before proceeding to the next depth. Measured aliquots, approximately 1 g each, were sent to LFE Environmental Analyses Lab for Pu analysis.

Preliminary results are shown in Table 4.8. It would appear from this data that a significant migration of Pu occurred in both an upward and downward pattern. Completion of the data set and further analysis of the sampling technique will better substantiate the true significance of this phenomenon. Although processes controlling this movement are unknown, studies are being designed to investigate the relative importance of such processes as root movement, freezing, thawing, drying, wetting, and settling.

TABLE 4.8. Concentration of ²³⁸Pu (μCi/g DW) in Soil Cores. Initial concentration of the spiked layer was 11.6 μCi/g DW. Blanks indicate samples that analyses are not yet completed. Cores were only taken at the 8, 9, 10, 11, and 12 cm depths from the fourth lysimeter.

Lysimeter #:	1			2			3			4
Section #:	1	2	3	1	2	3	1	2	3	2
Depth (cm)	μCi ²³⁸ Pu/g DW									
5										---
8		0.00383	0.00248	0.000851	0.00219	0.00158	0.00640		0.00175	
9	1.13	3.70	5.99		1.82	0.0135	1.95		2.35	
10	1.76			2.26	2.12		2.10	1.48		
11		0.00444	0.00518	0.0249	0.0136	0.0109	0.0196		0.0152	
12			0.00341	0.00356	0.00315	0.00236	0.00333		0.00369	
20										---
50										---
70										---

Solubility studies have been initiated; however, radiochemical analyses are not yet available. The studies consisted of approximately 50 determinations of Pu solubility using soil samples obtained by the coring procedures as previously described. The method consisted of a timed mixing period of the undried soil with 0.01 M $\text{Ca}(\text{NO}_3)_2$ and removal of a 1 ml aliquot, filtration through a 0.01 micron membrane and evaporation of 500 λ on a stainless steel planchet, with subsequent alpha counting.

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• BIOGEOCHEMISTRY OF Pu AND Am IN THE MARINE ENVIRONMENT

Principal Investigators: W. C. Weimer, K. H. Abel, C. I. Gibson
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Geochemical and biochemical alterations may affect transuranic mobility in marine waters. Samples of sea water and sediment in Koa Crater in Enewetak Atoll were obtained for analysis of transuranic forms. The Pu distribution patterns show relatively large amounts associated with suspended sediments in the deeper waters and small amounts of microparticulate or colloidal Pu in mid-water and near-bottom samples. Additional data suggest that there is a substantial change in Pu availability as a function of depth within the sediment cores. Different geochemical or biochemical alteration may be affecting transuranic mobility at depth within the sediment or the near surface sediments may be leached of readily removable Pu. The crater sediments are a confirmed source of microparticulate + colloidal Pu as well as particulate Pu to the overlying waters.

Pu analyses of sea water and sediments collected off Greenland provided information to the dispersion mechanisms that spread Pu along the seafloor following the 1968 crash of a USAF B-52. This study complemented the monitoring survey conducted by the Danish Atomic Energy Commission by developing information of the physiocochemical speciation and mobilization of Pu in the marine environment. Results indicate that some mechanism, chemical, biological or a combination of both, converts a significant fraction of the subsurface Pu to a more easily leachable form that may be more biologically available to burrowing benthic animals. Pu appears to be resuspended from the bottom sediments and transported as extremely fine particles by the flushing of the tidal currents.

Characterization of Transuranic Elemental Forms in Enewetak Crater Waters

Samples from the water column in Koa Crater in Enewetak Atoll were obtained from three depths for analysis of transuranic forms. The Pu distribution patterns show approximately uniform soluble Pu concentrations throughout the entire water column, relatively large amounts of Pu associated with suspended sediments in the deeper waters, and small amounts of microparticulate or colloidal Pu in the mid-water and near-bottom samples.

The vertical profile of Pu distribution in the water column of Koa Crater was determined from samples taken with a large volume water sampler at surface, mid-water, and near-bottom depths. The sampler was fitted with

glass fiber filters followed by three Al_2O_3 beds to separate transuranic forms into particulate ($> 3\mu$), microparticulate + colloidal, and soluble species. This separation of physical and chemical forms is based upon: 1) filtering of the large particulate materials by the glass fiber filters; 2) consistent retention efficiencies for soluble Pu species by each of the three Al_2O_3 beds (laboratory investigations with well-defined Pu forms have demonstrated the validity of this sorption behavior in freshwater systems), and retention on the first Al_2O_3 bed of all non-soluble Pu species which pass through the glass fiber filters. Total concentrations of particulate, microparticulate + colloidal, and soluble Pu species are calculated based on these criteria.

The concentrations of these three Pu forms in the water samples are presented in Figure 4.9. The distribution of the soluble $^{239,240}\text{Pu}$ species was approximately uniform throughout the entire water column, ranging from 7-12 dpm/m³. In contrast, the concentration of both particulate and microparticulate + colloidal forms increased significantly with increasing water depths. The concentrations of particulate $^{239,240}\text{Pu}$ increased from 22 dpm/m³ at the air-sea interface to 1805 dpm/m³ in the near-bottom water sample. The microparticulate + colloidal $^{239,240}\text{Pu}$ concentrations increase from < 1 in the surface water to 4.6 dpm/m³ in the sample from near the crater sediments. These data indicate that the particulate Pu species (> 3 μ in dia) are associated with resuspended sediments from the bottom of the crater and that these sediments are transported through the water column to the surface waters. The microparticulate or colloidal Pu may result from resuspension processes. These microparticulate or colloidal Pu species are at concentrations in surface waters of the crater which are too low to quantify. These Pu forms possibly attach to larger particles in the water column or are completely solubilized in waters near the air-sea interface. The soluble Pu concentration levels in the water column are more than an order of magnitude greater than total Pu concentrations in mid-Pacific open-ocean samples.

FIGURE 4.9. Plutonium Distribution in Koa Crater Water Column

Characterization of Transuranic Elemental Forms Released from Enewetak Atoll Crater Sediments by Seawater Leaching

A 20-cm core of sediment from the Koa Crater in Enewetak Atoll was segmented into two 10-cm intervals and was subsequently leached on site with flowing sea water. A principal form of Pu released was a microparticulate or colloidal form which corresponded to a similar Pu form found in the overlying water column.

A sediment core from the Koa Crater was segmented into a 0-10 cm depth interval and a 10-20 cm depth interval. Each of these segments was placed in a container which formed a 1-cm thick x 30-cm dia bed and was leached by sea water from the Enewetak Lagoon. From 700-4600 l of water were passed through these sediment beds during leaching periods of 7-12 hr. Prior to passage of this surface water through the sediment, the Pu in the water was removed by passing it through a large volume water sampler. After passing through the sediment bed, the water was directed through another large volume sampler which differentiated between the soluble and the microparticulate + colloidal species that were released into the sea water.

The data for the Pu species released by this leach study are presented in Figure 4.10. Significant quantities of both soluble and microparticulate + colloidal forms of Pu were released into the sea water from each of these core segments. The concentrations of soluble Pu in the seawater leachate were 23 and 39 dpm/m³ for the 0-10 cm and 10-20 cm cores, respectively. These soluble Pu concentrations were 3 to 5 times greater than the concentrations in the Koa Crater water column and suggest that a steady-state natural leaching process could readily support the observed seawater concentration in Koa Crater. The concentration of microparticulate + colloidal Pu released from the 10-20 cm core segment was approximately 280 dpm/m³ of leaching solution and is more than 12 times greater than the 23 dpm/m³ released from the 0-10 cm core segment. These data for soluble and microparticulate + colloidal Pu suggest that there is a substantial change in Pu availability as a function of depth in the core. This may be due to different geochemical or biochemical alterations affecting transuranic mobility at depths or may be due to the fact that the near surface sediments had already been leached of readily removable Pu. These data confirm that the crater sediments are a source of soluble Pu and microparticulate + colloidal Pu, as well as particulate Pu, to the overlying waters.

FIGURE 4.10. Plutonium Released from Koa Crater Sediments by Seawater Leaching

Redistribution of Plutonium in the Greenland Marine Environment

Sea water and sediments collected offshore from Thule, Greenland, in August 1974, were analyzed to determine the important factors in mobilizing Pu contained in the sediments. Sediment cores were collected at 7 stations in Bylot Sound (See Figure 4.11) to determine the areal and depth distribution of Pu in the sediments. The depth distribution of Pu in 3 of these cores is shown in Figure 4.12. The Pu concentrations decrease by 3 orders of magnitude between the surface and a sediment depth of 14 to 17 cm, and over 90% of the Pu resides in the upper 6 cm of sediment (Figure 4.12). Plutonium concentrations in the surface sediments ranged from 302 pCi/gm at Station V (impact area) to 1.36 pCi/gm at Station H, approximately 5 km due north of the impact area. The Pu contamination on the seafloor spreads in a northeast by southwest direction from the impact area, and the Danish AEC has measured above-background Pu concentrations up to 30 km from the impact area.

FIGURE 4.11. Sediment Core and Seawater Sampling Locations in Bylot Sound, Thule, Greenland, August 1974

FIGURE 4.12. $^{239,240}\text{Pu}$ Concentrations in Bylot Sound Sediment Cores, Thule, Greenland, August 1974.

To determine if significant amounts of Pu are being redissolved from the sediments, particularly under anoxic conditions, the interstitial pore water was extracted from samples at Thule by pressure squeezing. The pore water was acidified and stored in polyethylene bottles and analyzed for Pu at PNL. The only pore water sample that contained detectable Pu (2.6×10^{-4} pCi/ml) was that extracted from the top 0-3 cm section of the sediment core from Station V. Since the sediment contained 2.8×10^2 pCi/gm Pu, this resulted in a distribution coefficient (K_d) of 9.2×10^{-7} for Pu distributed between the pore water and the sediment.

The squeezed sediments were frozen and leached to remove hydrous oxide coatings from the sediment particles. The leachate and residual sediments were analyzed for Pu. The objective of these analyses was to determine if Pu could be gradually dissolved from surface sediments by the leaching action of sea water and then eventually reattached to sediment away from the impact area by subsequent coprecipitation of hydrous iron and manganese oxide coatings or organic coatings on sediment particle surfaces. The results of this experiment (Figure 4.13A) show that very little (< 2%) of the Pu was leached from the surface sediments. However, for each sediment core the leachable Pu reached a maximum of 8-10% of the total Pu in the sediment sections at depths of 7 to 10 cm in the cores. This indicates that some mechanism, whether chemical, biological or a combination of both, converts a significant fraction of the subsurface Pu to a more easily leachable form that may be more biologically available to burrowing benthic animals.

Large volume seawater sampling was conducted at the surface and 5 to 10 m off the bottom at four stations at Thule to extract particulate and soluble forms of Pu from 420 to 2200 μ of sea water. The particulate forms were collected by filtration on 12-in. dia fiberglass filters, and the filtrate was passed through a

FIGURE 4.13A. Plutonium Leached from Thule Sediments by 0.1N HCl-H₂O₂

series of 1/4-in. thick by 12-in. dia activated aluminum oxide beds.

The Pu was leached from the filters and aluminum oxide by heating in strong HNO₃-HCl solutions, radiochemically separated, and measured by alpha energy analysis utilizing Si(Li) diode detectors. The results of analyses of samples collected at the impact area and 10 km to the north and south of the impact area, as well as a background seawater sample collected 20 km away (Station L') are shown in Figure 4.13B. Pu concentrations in all surface waters of Bylot Sound were typical of background fallout levels observed in the north Atlantic Ocean and the Pu was present predominantly in a soluble or at least nonfilterable form. However, in the near-bottom waters elevated Pu levels were observed and most of the Pu was present in a particulate form. This indicates that Pu is being resuspended from the bottom sediments and being transported as extremely fine particles by the

FIGURE 4.13B. Plutonium-239-240 Concentrations in Sea Water at Thule, Greenland, August, 1974

flushing action of the tidal currents in Bylot Sound.

The soluble Pu concentration in the bottom water at Station V (1.13 pCi/m³) was about 230 times lower than the Pu concentrations in the pore water extracted from surface sediments. Since the soluble Pu concentrations in the bottom waters were always higher than the soluble fractions in the surface waters at each station it is likely that some dissolution of Pu from the sediments and escape of Pu-enriched pore waters into the near-bottom sea water occurs in Bylot Sound.

Thus, Pu at Thule can apparently be mobilized by physical transport of resuspended very fine sediment particles, and by slow dissolution of Pu from the sediments by the sea water.

- **RADIOECOLOGY OF WASTE MANAGEMENT AREAS (ROWMA)**
- **RADIOECOLOGY OF URANIUM**

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Radioecology of nuclear wastes was pioneered in the western United States on the Hanford Reservation during the early 1940's. Initially, attention was paid to the Columbia River because it provided coolant water for production reactors. Ecological studies centered upon fish and waterfowl as vectors of radionuclides to people. With the abandonment of production reactors a few years ago, the radionuclide content of Columbia River water has declined and attention is now being directed towards radionuclides released into the environment as a result of chemical processing of irradiated fuel. Places that have received radioactive wastes are solid waste burial grounds, liquid effluent release belowground, surface ditches and ponds, and the terrestrial environment around chemical processing facilities that have received airborne deposits of radioactive debris from stacks.

ROWMA studies reported here are concerned with the degree of uptake of various radionuclides from soil, sediments, and water by terrestrial and aquatic biota, the potential of removal of radioactivity from waste management areas by biota, and the transfer of radionuclides between organisms and ecological trophic levels. The long-term objective of ROWMA effort is to delineate and quantify underlying ecological processes affecting transport of radioactivity. A portion of effort is allocated also to site specific problems caused by biotic vectors; i.e., birds, insects and wild animals that cause wider dissemination of radioelements. Important companion studies also are underway. They are funded through local intercontractor support agreements rather than through DBER and involve short-term assistance to Hanford plant personnel concerned with characterization of particular landscape subunits impacted by operation of the nuclear facilities. See Section 8, pp 8.1-8.44 for intercontractor studies.

First year studies on the ROWMA projects centered upon characterizing the abiotic environment and the biota of representative waste management areas on the Hanford Reservation. Environmental samples were collected to fill gaps in the field phases of the ^{129}I project and uptake of ^{129}I by plants from soil was continued in outdoor lysimeters. Studies were initiated to determine the availability of ^{129}I to cattle from ingested forage using fistulated steers.

First year studies using uranium isotopes centered around the growing of plants in uranium-contaminated soil.

Radioecology of Uranium (U)

The objectives of this portion of the Radioecology of Uranium program were to: 1) investigate the uptake of U from soil by plants utilizing ^{238}U as a tracer; 2) evaluate the applicability of a fluorometric technique for U analysis at environmental levels of U contamination; and 3) determine the toxicity level of U to plants grown in U amended soil.

Cheatgrass (*Bromus tectorum*) was planted in small weighing lysimeters (13 cm x 100 cm) containing ^{238}U (as uranyl nitrate) amended Ritzville silt loam soil. Amended concentrations of U ranged from 2 ppm to 20,000 ppm relative to the soil.

Control and U amended lysimeters were maintained in a field enclosure for 150 days. At maturity, the plants were harvested by clipping at the soil surface, separated into seeds and stem/leaf fragments, dried, weighed, and sent to LFE Environmental Analysis Laboratory in Richmond, CA, for analyses using a fluorometric technique.

The detection limits of the fluorometric technique used were insufficient at the lower concentration treatment resulting in a considerable portion of the data being at levels less than detectable. Future studies will necessitate either refinements in the present technique to optimize detection limits or utilization of another isotope of U as a tracer.

Table 4.9 shows concentration ratios obtained for cheatgrass from this study. Three replicates per treatment were sent for analyses. However, not all analyses have been completed. Therefore, numbers shown in Table 4.9 represent only maximum likelihood values and no statistical evaluation will be attempted until the data set is complete. It would appear that stems and leaves, in general, maintain a higher concentration of U than do seeds. Concentration ratios appear to be in the 10^{-1} to 10^{-2} range. Uranium concentrations in plant material also appear to be independent of soil concentration.

Perhaps the most significant phenomenon noted in this study is illustrated in Figure 4.14. Absolutely no plant growth was observed in the 20,000 ppm treatment and only minimal growth was apparent in the 2000 ppm treatment. Toxic effects can also be seen in the 200 ppm treatment. The critical range for noticeable toxic effects is between 20-200 ppm.

In conclusion, it would appear that U is very toxic at relatively low concentrations to at least one species of rangeland grass. Uptake of U from soil is in the 10^{-1} to 10^{-2}

TABLE 4.9. Concentration Ratios Observed for Plant Parts of Cheatgrass Grown in Uranium- ^{238}U Amended Ritzville Silt Loam Soil

TREATMENT	CR*		
	SEED STEM & LEAVES	STEM AND LEAVES / SOIL	SEED / SOIL
CONTROL	0.40	1.14	0.46
2 ppm	0.85	0.09	0.08
20	0.89	0.03	0.03
200	0.46	0.01	0.004
2000	0.22	0.10	0.02
20,000	**	**	**

$$*CR = \frac{\mu\text{g } ^{238}\text{U/g PLANT MATERIAL (DRY)}}{\mu\text{g } ^{238}\text{U/g SOIL (DRY) OR PLANT MATERIAL (DRY)}}$$

**NO PLANT MATERIAL AVAILABLE FROM THIS TREATMENT

FIGURE 4.14. Toxic Effects of Uranium on Cheatgrass

range. The fluorometric technique for U analysis is not satisfactory in its present state. Further studies are being initiated to better characterize uptake and toxicity of U not only in plant populations, but also in animal populations as well. Further studies will investigate the more potentially hazardous ^{232}U .

Small Mammal Populations in Shrub Communities

Small mammal populations have been intensively studied on the ALE Reserve (O'Farrell et. al., 1975) but little information has been previously obtained for sites elsewhere on the Hanford Reservation. Plots selected for study were shrub-dominated plant communities located near designated waste management areas. The purpose was to obtain quantitative information on small mammal populations associated with these kinds of communities.

Each study plot consisted of 100 Sherman live traps arranged in a 10 x 10 grid with 10 m spacing between traps. Captured animals were marked by toe clipping and released. In all, 4 plots were established in 4 different plant communities (Table 4.10). The preliminary data show the results of 2-day trap sessions conducted in May and August, 1976.

The pocket mouse, *Perognathus parvus*, was the most often trapped small mammal and the number of individuals trapped in each plot ranged between 32 and 63. Only adult mice were caught in May while the August catch was comprised mostly of young mice. Adult mice are believed to retire belowground in summer with only the young-of-the-year active aboveground in August. Our data indicate that pocket mice populations are widely distributed and that trappable populations in shrub-dominated communities are not very different.

TABLE 4.10. Small Mammals Trapped in Shrub Communities

Mammal Species	Plant Communities			
	Sagebrush hopsage	Sagebrush	Bitterbrush	Sagebrush bitterbrush
-----May-1976-----				
Pocket mouse	52	63	50	32
Deer mouse	0	6	4	3
Ground squirrel	1	0	0	0
-----August-1976-----				
Pocket mouse	42	52	52	38
Deer mouse	0	3	2	1
Ground squirrel	0	0	0	0

Literature Cited

O'Farrell, T. P., R. J. Olson, R. O. Gilbert, and J. D. Hedlund. A population of Great Basin pocket mice, *Perognathus parvus* in the shrub-steppe of southcentral Washington. *Ecol. Monogr.* 45:1-28, 1975.

Radiation Doses to Pocket Mice Living on a Retired Burial Ground

The site studied was the "WYE" burial ground. This facility was in service from 1962 to 1967 and terminated service in 1968. Records show that the buried waste contains <2000 Ci of β and γ emitters and less than 5 kg Pu covered with about 8 ft of backfill.

The most abundant small mammal living on the "Wye" burial ground is the Great Basin pocket mouse, *Perognathus parvus* (Table 4.11). This rodent is capable of burrowing deep into the soil to a maximum depth of 4-6 ft if soil conditions permit (Scheffer, 1938). Pocket mice spend most of their lives belowground. They normally spend the daylight hours belowground. During fall and winter months they remain in their burrows. The objective here was to determine if these mice were burrowing deep enough into the soil to become exposed to radiation emitted from the buried waste.

Live traps arranged in a grid pattern containing 100 traps spaced at 10 m intervals were placed on the burial ground. A control plot was set up nearby. When captured, mice were given an individual identification number by using a series of toe clips. Additional information recorded included species, sex, trap location, relative age, reproductive condition, and weight.

TABLE 4.11. Summary of Trapping Results After 2-Day Trap Sessions in May, July, August and October 1976

	-----WYE Burial Ground-----							
	TLD ^(a)		TLD		TLD		TLD	
	Apr	May Returns	July Returns	Aug Returns	Aug Returns	Oct Returns	Oct Returns	
<i>Perognathus parvus</i>	19	16	10	4	3	4	2	4
<i>Peromyscus maniculatus</i>	3	--	--	--	--	--	--	--
<i>Spermophilus townsendi</i>	--	2	--	--	--	--	--	--
-----Control Plot-----								
<i>Perognathus parvus</i>	8	14	3	7	5	9	1	5
<i>Peromyscus maniculatus</i>	--	--	--	--	--	--	--	--
<i>Spermophilus townsendi</i>	2	--	--	--	--	--	--	--

(a)TLD = Thermoluminescent Dosimeter

Small dosimeters were dorsally implanted beneath the loose neck skin of trapped animals. The dosimeters used were Harshaw TLD-700's® (Thermoluminescent dosimeters) encapsulated in a plastic film cover. The animals were anesthetized during the implanting operation and the small incision surgically sutured to prevent infection (Figure 4.15). After the TLD implantation and after the animals regained consciousness, they were returned to their exact capture location.

When an animal containing a dosimeter was recaptured, the old dosimeter was removed and replaced with a new one. The Wye burial ground has yielded 16 returned dosimeters over four trapping sessions and the control area has returned 10. All recovered dosimeters have shown that the mice are receiving only background levels of radiation exposure. The average mouse exposure on the burial ground was 47.91 mR per year and on the control plot it was 45.99 mR per year (Table 4.12).

From the data gathered to this date, it appears that the protective backfill of gravel and cobblestones is a sufficient shield that protects pocket mice from burrowing into the vicinity of the buried waste and receiving above normal levels of external radiation.

FIGURE 4.15. Implanting Dosimeter in Anesthetized Pocket Mouse

Terrestrial Invertebrate Populations

Insects and other invertebrates remain a poorly understood component of most ecosystems. This is particularly true in semi-arid regions of the western United States where usually their occurrence is noted only when their activities reach a level that seriously conflicts with man's goals or desires. Insects are the major consumer in many areas of the Hanford Reservation where domestic grazers

TABLE 4.12. Radiation Doses to Individual Pocket Mice Expressed in Milliroentgens (mR)

MONTH CAUGHT	mR/d	mR/wk	mR/year	
<u>WYE BURIAL GROUND</u>				
MAY 1976	0.15	1.05	54.7	
MAY 1976	0.11	0.77	40.1	
MAY 1976	0.11	0.77	40.1	
MAY 1976	0.15	1.05	54.7	
MAY 1976	0.11	0.77	40.1	
MAY 1976	0.11	0.77	40.1	
MAY 1976	0.11	0.77	40.1	
MAY 1976	0.13	0.91	47.4	
MAY 1976	0.15	1.05	54.7	
MAY 1976	0.11	0.77	40.1	AVG 45.2
JULY 1976	0.06	0.42	21.9	
JULY 1976	0.07	0.49	25.5	
JULY 1976	0.08	0.56	29.2	AVG 25.5
AUG 1976	0.25	1.75	91.2	
AUG 1976	0.25	1.75	91.2	AVG 91.2
OCT 1976	0.15	1.05	54.7	
<u>CONTROL</u>				
MAY 1976	0.11	0.77	40.1	
MAY 1976	0.13	0.91	47.4	
MAY 1976	0.13	0.91	47.4	AVG 44.9
JUL 1976	0.10	0.70	36.5	
JUL 1976	0.09	0.63	32.8	
JUL 1976	0.09	0.63	32.8	
JUL 1976	0.09	0.63	32.8	
JUL 1976	0.09	0.63	32.8	AVG 33.5
AUG 1976	0.24	1.68	87.6	
OCT 1976	0.19	1.33	69.3	

are excluded. Their abundance must be reckoned in schemes designed to evaluate energy and mineral cycling, translocation of radioactivity beyond burial sites, or, some kinds of food-chain transmission of radioactivity.

This study is designed to provide an initial evaluation of some potentially abundant insect groups. Darkling beetles (Tenebrionidae) are common on the Hanford Reservation and emphasis has been placed on understanding their population dynamics. Common shrub-inhabiting insects were sampled, as shrubs are particularly important since they are deep-rooted, inhabit radioactive waste burial sites, and some have been documented as having an affinity for radionuclide uptake.

Invertebrates inhabiting bitterbrush (*Purshia tridentata*) were sampled on two study sites (Table 4.13). The most abundant invertebrate group was the Homoptera (bugs). The Psyllidae (order Homoptera) comprised 75 and 77% of the total sampled in the two study areas (Table 4.13). The Psyllidae or jumping plant lice are small insects (ca. 2-5 mm) that resemble miniature cicadas. They feed on plant juices and are regarded as being specific feeders. They are frequently important pests of agricultural crops.

The abundance of invertebrates associated with big sagebrush (*Artemisia tridentata*) was also sampled (Table 4.14). The Homoptera again comprised the largest portion of total

TABLE 4.13. Invertebrate Abundance on Bitterbrush (*Purshia tridentata*) (a)

Taxa	Bitterbrush Site		Bitterbrush/Sagebrush Site	
Orthoptera (Grasshoppers)				
Acrididae	19	(2)	28	(3)
Tettigoniidae	3	(<1)	1	(<1)
Thysanoptera (Thrips)				
Phlaeothripidae	11	(1)	6	(1)
Hemiptera (True Bugs)				
Miridae	61	(5)	43	(4)
Nabidae	5	(<1)	4	(<1)
Reduviidae	2	(1)	2	(<1)
Pentatomidae	11	(1)	13	(1)
Unident. Nymph	1	(<1)	1	(<1)
Homoptera (Bugs)				
Cicadellidae	92	(7)	92	(8)
Delphacidae	4	(<1)	0	(0)
Issidae	2	(<1)	0	(0)
Psyllidae	917	(75)	851	(77)
Pseudococcidae	3	(<1)	0	(0)
Raphidoptera (Snakeflies)				
Raphidiidae	12	(1)	1	(<1)
Coleoptera (Beetles)				
Helodidae	1	(<1)	0	(0)
Buprestidae	1	(<1)	0	(0)
Cleridae	0	(0)	2	(<1)
Coccinellidae	0	(0)	1	(<1)
Curculionidae	1	(<1)	0	(0)
Chrysomelidae	1	(<1)	0	(0)
Lepidoptera (Butterflies and Moths)				
Geometridae	2	(<1)	0	(0)
Unident. Larvae	1	(<1)	8	(1)
Unident. Adult	1	(<1)	1	(<1)
Diptera (Flies)				
Chironomidae	1	(<1)	0	(0)
Sciaridae	1	(<1)	0	(0)
Chaemyiidae	1	(<1)	0	(0)
Agromyzidae	1	(<1)	0	(0)
Tachinidae	4	(<1)	0	(0)
Hymenoptera (Bees, Ants, Wasps)				
Braconidae	3	(<1)	2	(<1)
Ichneumonidae	0	(0)	2	(<1)
Eulophidae	5	(<1)	8	(1)
Eucyrtidae	6	(<1)	12	(1)
Perilampidae	1	(<1)	0	(0)
Pteromalidae	9	(1)	1	(<1)
Chalcididae	1	(<1)	0	(0)
Ceraphronidae	0	(0)	2	(<1)
Platygasteridae	2	(<1)	0	(0)
Formicidae	1	(<1)	5	(<1)
Unidentified Chalcid	0	(0)	2	(<1)
Aranidia (Spiders)				
Salticidae	5	(1)	12	(1)
Thomisidae	2	(1)	3	(1)
Others	34	(3)	4	(1)
Acarina (Mites)	2	(1)	0	(0)
TOTAL	1230	(100)	1107	(100)

(a) Values are the total number collected from 3 replicates of 50 sweeps, each using a standard sized sweepnet on June 23, 1976.

() = Relative percent of total.

TABLE 4.14. Invertebrate Abundance on Big Sagebrush (*Artemisia tridentata*) (a)

Taxa	Bitterbrush Site		Bitterbrush/Sagebrush Site	
Orthoptera (Grasshoppers)				
Acrididae	30	(12)	62	(17)
Tettigoniidae	1	(<1)	0	(0)
Thysanoptera (Thrips)				
Phlaeothripidae	0	(0)	2	(1)
Hemiptera (True Bugs)				
Anthocoridae	0	(0)	1	(<1)
Miridae	7	(3)	9	(2)
Nabidae	0	(0)	2	(1)
Pentatomidae	0	(0)	1	(<1)
Homoptera (Bugs)				
Cicadellidae	104	(41)	84	(23)
Delphacidae	0	(0)	1	(<1)
Psyllidae	1	(<1)	11	(3)
Aphididae	52	(21)	38	(10)
Orthozeidae	13	(5)	27	(7)
Pseudococcidae	0	(0)	1	(<1)
Neuroptera (Lace Winged Insects)				
Chrysopidae	2	(1)	2	(1)
Raphidoptera (Snakeflies)				
Raphidiidae	1	(<1)	0	(0)
Coleoptera (Beetles)				
Cleridae	0	(0)	2	(1)
Coccinellidae	4	(2)	6	(2)
Curculionidae	0	(0)	1	(<1)
Chrysomelidae	1	(<1)	0	(0)
Lepidoptera (Moths and Butterflies)				
Pyrilidae	0	(0)	3	(1)
Unident. Larvae	2	(1)	1	(<1)
Diptera (Flies)				
Chironomidae	2	(1)	0	(0)
Unident.	1	(<1)	0	(0)
Hymenoptera (Ants, Bees, Wasps)				
Braconidae	0	(0)	1	(<1)
Ichneumonidae	1	(<1)	0	(0)
Eulophidae	4	(2)	8	(2)
Encyrtidae	1	(<1)	1	(<1)
Perilampidae	0	(0)	4	(1)
Torymidae	2	(1)	2	(1)
Chalcididae	2	(1)	1	(<1)
Proctotrupidae	0	(0)	1	(<1)
Chrysididae	0	(0)	1	(<1)
Formicidae	10	(4)	56	(15)
Sphecidae	0	(0)	1	(<1)
Unident. Chalcid	2	(1)	0	(0)
Aranida (Spiders)				
Salticidae	7	(3)	17	(5)
Thomisidae	0	(0)	1	(<1)
Others	3	(1)	14	(4)
TOTAL	253	(100)	362	(100)

(a) Values are the total number collected from 3 replicates at 50 sweeps, each using a standard sized sweepnet on June 23, 1976.

() = Relative percent of total.

abundance, but the leafhoppers (Cicadellidae) and aphids (Aphidida) were the abundant families, not the Psyllids. The invertebrate populations associated with big sagebrush and bitterbrush were found to be distinctly different. In addition, far more individuals were associated with bitterbrush (1230 and 1107) than with sagebrush (253 and 362).

The abundance of ground-dwelling darkling beetles was sampled using a pitfall trapping technique. The abundance of darkling beetles in all study areas followed a bimodal pattern, being most numerous in the spring (May) and fall (September) months (Figure 4.16). Table 4.15 shows the percentage of each darkling beetle species in the total catch for each study site. *Eleodes hispilabris* was the most frequently captured species at all locations.

FIGURE 4.16. Spring Darkling Beetle Abundance

TABLE 4.15. Percentage of Darkling Beetle Species of Total Catch

SPECIES	STUDY AREAS			
	CONTROL	MIXED PLOT	B-C PLOT	BITTERBRUSH PLOT
Species	%			
<i>Eleodes hispilabris</i>	52	52	30	52
<i>Eleodes novoverrucula</i>	10	22	8	30
<i>Eusattus muricatus</i>	2	0	27	1
<i>Eleodes humeralis</i>	10	1	7	2
<i>Coniontis setosa</i>	9	6	11	5
<i>Eleodes nigrita</i>	9	1	1	2
<i>Eleodes granulata</i>	3	1	1	2
<i>Eleodes obscura</i>	0	0	0	5
<i>Philolithus densicollis</i>	5	19	15	1
	100	100	100	100

Eleodes novoverrucula was important in the catch at the bitterbrush and mixed study plots, but was less important at the control and B-C Cribs plots. *Eusattus muricatus* is a beetle species commonly associated with sandy substrates. It occurred in low numbers at the control and bitterbrush sites, was absent at the mixed plot, but abundant in the B-C Cribs areas. *Philolithus densicollis* was abundant in the mixed and B-C Cribs plots. Adults emerge about September 1 and remain active until killed by cold weather. Radical changes in abundance of *Philolithus* at specific locations has been documented at other study locations on the Hanford Reservation.

Densities of Large and Medium-sized Mammals on the Hanford Reservation

In order to understand the environmental aspects of management of radioactive wastes at Hanford the dynamics of the surrounding ecosystems must be considered. A definite part of the biota of any ecosystem involves the larger mammals, their food habits, behavior, and population densities. The purpose of this study was to estimate population densities of large- and medium-sized mammals on the Hanford Reservation.

For the purpose of this report the Hanford Reservation is considered as the 422 square miles south and west of the Columbia River. The Reservation was sub-divided into five habitat-types (Table 4.16). The sagebrush-bunchgrass (*Artemisia - Agropyron*) habitat type is the major habitat found on the Arid Lands Ecology Reserve. It is dominated by big sagebrush (*A. tridentata*), bluebunch wheatgrass (*A. spicatum*), and hawksbeard (*Crepis atrabarba*). Cusick bluegrass (*Poa cusickii*) thurber needle and thread (*Stipa thurberiana*). The sagebrush-cheatgrass (*Artemisia-Bromus*) is the most common habitat type found on the Reservation. It is dominated by big sagebrush in the overstory and cheatgrass (*B. tectorum*) in the understory. Long-leaved phlox (*Phlox longifolia*), and rabbitbrush (*Chrysothamnus nauseosus* and *C. viscidiflouis*) are common. Included in this habitat type are the homogeneous stands of cheatgrass characteristic of burned areas and badly disturbed soils in the Reservation. The bitterbrush-cheatgrass (*Purshia - Bromus*) habitat is dominated by bitterbrush and cheatgrass, big sagebrush, and rabbitbrush. Riparian habitat types are found along the Columbia River and in canyon areas of the ALE Reserve. The vegetation here consists primarily of willows (*Salix* sp.), juniper (*Juniperus scopulorum*), and poplar (*Populus*) trees, chokecherry (*Prunus virginiana*), sumac (*Rhus glabra*) and other introduced species.

An area-estimate method was employed to estimate densities of large mammals, which is a refinement of the strip census method

TABLE 4.16. Area Provided by Various Habitat Types on the Hanford Reservation

HABITAT TYPES	AREA, mi ²	% OF RESERVATION
HABITATS SOUTH OF COLUMBIA RIVER	423	74.2
SAGEBRUSH-BUNCHGRASS	96	16.8
SAGEBRUSH-CHEATGRASS	211	37.0
BITTERBRUSH-CHEATGRASS ^a	85	14.9
RIPARIAN-TREE SHRUB ^{a,b}	4	0.7
RIPARIAN + 1/2 MILE FROM COLUMBIA RIVER ^{a,b}	27	4.7
TOTAL RIPARIAN	31	5.4

^a MULE DEER HABITAT ON RESERVATION

^b PORCUPINE HABITAT ON RESERVATION

developed to study hares and rabbits on the shortgrass prairie of Colorado (Flinders and Hansen, 1971). Thirty, 1-mile by 80 yard transects were sampled at night with a hand-held 200,000 candle power airplane landing light, while driving at speeds ranging between 15-20 mph. Three consecutive nights were sampled every other month beginning in May 1976. The data were extrapolated to density per square mile and total numbers on the Hanford Reservation south of the Columbia River for all species sighted. Mammals encountered on the transects were black-tailed hares (*Lepus californicus*), Rocky Mountain mule deer (*Odocoileus hemionus*), coyotes (*Canis latrans*), porcupines (*Erethizon dorsatum*), and badgers (*Taxidea taxus*). For the purpose of analyzing these data, the Reservation was stratified into major habitat types. Population estimates for coyotes, and badgers were determined for the total area south of the Columbia River. Estimates of mule deer densities were based on the area of bitterbrush and riparian habitats since deer are generally confined to these areas. The population densities of porcupines were estimated to be within the two riparian habitat types. (Table 4.17).

TABLE 4.17. Average Number of Animals/mi² on the Hanford Reservation During 1976

SPECIES	MAY 6	JUN 22	SEP 9	AVERAGE
BLACKTAILED HARE	10.25	7.63	9.77	9.22
MULE DEER	1.43	2.15	3.10	2.23
COYOTE	0	1.20	0.24	0.48
PORCUPINE	0	0.48	0	0.16
BADGER	0	0	0.24	0.08

The population of blacktailed hares was estimated as approximately 3,015 or 9.22 per square mile. Densities of hares were determined on all habitat types south of the Columbia River except for sagebrush-bunchgrass which occurs on the ALE Reserve. Blacktailed hares were not observed in this habitat type. Highest densities occur in the areas immediately south of the 200 Area. During the three sampling dates from May to September no seasonal trends in population densities were observed ($P = 0.85$). No variation in densities occurred during nights ($P \geq 0.73$) during the three sampling dates.

Mule deer on the Reservation prefer certain habitats. Deer numbers on the Reservation are estimated to be 256 or 2.33/mi² within the riparian and bitterbrush habitats. Densities of deer increased from 1.43/mi² in May to 3.10/mi² in September. Most observations of deer on the transects indicate that they occur mostly in small groups; however, this may change with changing seasons.

Coyotes are wide ranging and are found in all habitat types. Total numbers of coyotes on the Reservation south of the Columbia River are estimated at 203. The data shown in Table 4.17 indicate that density estimates range from 0 in May to a high of 1.20/mi² in June. Average densities of coyotes on the Reservation are 0.48/mi² which agree with data from other similar areas in the western United States.

Badgers were also sighted and are assumed to have a wide distribution on the Reservation. From the data obtained, it is estimated that there are 34 badgers on the Reservation south of the river.

Porcupines generally occupy the riparian habitat types. Density estimates obtained show that there are approximately five porcupines on the Reservation. These animals are quite mobile within their habitat, the sighting of two during one sample date accounted for the variability in the data.

Long-term studies of this type will give useful estimates of populations and densities of the mammals present on the Hanford Reservation. Continued observations will yield trends in the population densities and movements over the years, and can be important to the radioactive waste management programs.

Literature Cited

Flinders, J. T. and R. M. Hansen. Abundance and dispersion of Leporids within a short-grass ecosystem. *J. Mam.* 54(1):287-291.

Reptiles of the Hanford Reservation

In April 1976 a study was initiated to investigate reptile distribution and densities on the Hanford Reservation. Four study plots were selected for intensive studies. They were located in: 1) a sagebrush-cheatgrass community on the ALE Reserve, 2) a sagebrush-cheatgrass community adjacent to the B-C Crib on the 200 Area plateau, 3) a sagebrush-bitterbrush-cheatgrass community near the WPPSS project #2 and 4) a bitterbrush community northeast of the WPPSS project #2 near the Columbia River. Permanent drift fence lines (Figure 4.17) were established to facilitate the capture of snakes and pitfall trap grids (Figure 4.18) were established to capture lizards.

FIGURE 4.17. Permanent Drift Fence Lines

FIGURE 4.18. Pitfall Trap Grids

The snakes captured at each study site are listed in Table 4.18. These data provide information on species composition and relative abundance of snakes in the various plant communities. Weight and reproductive condition of snakes were recorded and food habits were determined by palpating the GI tracts. Snakes were also individually marked by tattooing their ventral scutes. This will aid us in studying movements and weight changes over time.

TABLE 4.18. Snake Captures with Drift Fences

AREA	SPECIES CAPTURED	TOTAL NUMBER CAPTURED
SAGEBRUSH-CHEATGRASS COMMUNITY (ALE)	GREEN RACER (<i>Coluber constrictor</i>)	5
	BULL SNAKE (<i>Pituophis melanoleucus</i>)	4
SAGEBRUSH-CHEATGRASS COMMUNITY (B-C CRIB)	GREEN RACER (<i>Coluber constrictor</i>)	0
	BULL SNAKE (<i>Pituophis melanoleucus</i>)	1
SAGEBRUSH-ANTELOPE BITTERBRUSH COMMUNITY	GREEN RACER (<i>Coluber constrictor</i>)	4
	BULL SNAKE (<i>Pituophis melanoleucus</i>)	3
ANTELOPE BITTERBRUSH-CHEATGRASS SAND DUNE COMMUNITY	GREEN RACER (<i>Coluber constrictor</i>)	1
	BULL SNAKE (<i>Pituophis melanoleucus</i>)	2

Two lizard species were captured in pitfalls. The side-blotched lizard (*Uta stansburiana*) was captured on all but the bitterbrush-cheatgrass site while the fence lizard (*Sceloporus graciosus*) was captured only in the bitterbrush-cheatgrass area. Figure 4.19 presents a graph of the total number of lizards which were marked (toe amputations were used to mark individuals) on each study area for each trapping day. Adjustments have been made to allow for comparisons between the 73 trap grids and 25 trap grids. The graph simply shows the number of lizards which were captured over time and gives an indication of the population size. The initial rises on each graph level off as one comes closer to marking the total adult population and remains relatively constant until juveniles hatch and join the adult cohort.

Much of the data collected on lizards is currently being analyzed and will be reported later. Weights, reproductive conditions, territory size, and food habits are being studied.

FIGURE 4.19. Total Number of Lizard Marked on Each Study Area for Each Day

Reproductive Performance of Nesting Coots

During May and June 1976, we censused coot (*Fulica americana*) nests on 3 ponds to determine if there were any differences in the reproductive performance of coots nesting on a waste pond as compared to those nesting on control ponds. The waste pond, Gable Mountain Pond, is a 28 hectare impoundment, which was formed in 1957 to receive condenser coolant water from a chemical processing facility. Along with the coolant water, the pond has received low levels of radionuclides. The control ponds, Morgan Lake and Royal Slough, each are larger than Gable Mountain Pond and are located on the Columbia National Wildlife Refuge near Othello, Washington. All of the ponds support similar vegetation. Aquatic macrophytes occur along the pond margins and in the shallow water portions of the ponds. The important emergent aquatic plant species are broad-leaved cattail (*Typha latifolia*) and bulrush (*Scirpus acutus*).

Coot nests were located by searching emergent aquatic vegetation while standing in a slow-moving boat. We were often alerted to a nest location by carefully watching adults performing distraction displays.

Table 4.19 presents data on the number of nests found, clutch sizes, and hatching success of coot eggs found at the three ponds. There was no statistical difference in clutch sizes or hatching success between ponds. Apparently, the low-level radionuclide contamination present in Gable Mountain Pond is not having an appreciable effect on coot productivity. Further studies are needed

TABLE 4.19. Number of Observed Nests, Clutch Sizes, and Hatching Success of Coots of Radioactive Waste Pond and 2 Control Ponds in South-eastern Washington

LOCATION	NUMBER OF NESTS FOUND	AVERAGE CLUTCH SIZE	NEST HATCHING 100%	NEST WITH 1-3 INFERTILE EGGS
GABLE MOUNTAIN POND (WASTE POND)	12	6.00 ± 0.42	12	0
ROYAL SLOUGH (CONTROL)	18	6.53 ± 0.98	16	2
MORGAN LAKE (CONTROL)	12	6.16 ± 1.02	11	1

in order to determine if there are any behavioral differences in coot populations residing on the waste pond and the control areas.

Moss Taxa of Shrub Communities Near Waste Management Areas

Mosses are not a conspicuous part of the flora of shrub communities on the Hanford Reservation. Because of specialized taxonomy, they have received little research attention at Hanford or at other places in the dry interior region of Washington State.

Mosses along with attendant population of algae, lichens, and fungi can provide a crust on the soil that is resistant to wind and water erosion. The crust can be broken by walking across the soil, but is particularly vulnerable to trampling by grazing cattle and sheep.

Waste management areas have not been exposed to cattle or sheep grazing for at least 30 yr. These areas can provide information on the durability of soil crusts without interferences of livestock grazing.

Initial collections of mosses have been made with the following taxa identified: *Barbula brachyphylla*, *Bryum argenteum*, *Ceratodon purpureus*, *Ceratodon purpureus* var. *dimorphus* (only one other collection reported from the Pacific NW), *Pterygoneurum ovatum*, *Tortula brevipes* var. (a new variety--to be named), *Tortula intermedia*, *Tortula papillosissima*, and *Tortula princeps*.

Radioecology of Aquatic Systems

The Hanford Reservation has a number of waste ponds and ditches that have been exposed to a variety of radionuclides. This provides an excellent opportunity to study the distribution, fate, and effects of radionuclides in aquatic ecosystems. Such a study also gathers needed information about the effectiveness of these waste disposal systems in retaining radionuclides.

The radioecology of waste management areas study (ROWMA) was initiated in FY 1976 to answer questions pertaining to the distribution, fate, and possible effects of radwastes in environmental systems. Four ponds and four ditches were selected for comprehensive radioecological study on the basis of waste types currently being received, history of use, and ecological significance.

U-Pond, located in the 200-West Area, has been receiving wastes from plutonium reprocessing operations for over 30 yr (Figure 4.20). The Z-19 ditch (Figure 4.21), which has fed U-Pond for the past 5 yr, is the latest of a series of ditches carrying Pu reprocessing wastes to U-Pond. B-Pond, situated east of the 200-East Area (Figure 4.20), has received chemical sewer wastes and process cooling water from recovery operations since 1945. B-Pond is fed via the B-3 and the A-29 ditch (Figure 4.21). Gable Mountain Pond is located north of the 200-East Area and has received process cooling water since 1975 (Figure 4.20).

FIGURE 4.20. Designated ROWMA Study Pond with Associated Radionuclides

FIGURE 4.21. Designated ROWMA Study Ditches with Associated Radionuclides

The remaining 2 aquatic systems are considerably different in character, West Pond (Figure 4.20), which occurs naturally, is an expression of the groundwater table at the surface, and it has no surface water or waste water sources, although it did receive sanitary wastes at one time. It does, however, contain uranium which may be due to it being located near a natural uranium deposit. The 100-N trench is located at the N-reactor site near the Columbia River (Figure 4.21). It was created in 1973 and receives cooling water from the N-reactor crib.

Sufficient data are now available to tentatively describe annual ranges of physicochemical, biological and radiological parameters for the ROWMA study sites. Ranges

of physicochemical characteristics for ponds and ditches are shown in Table 4.20 and 4.21. Gable, B-, and U-Ponds differ widely in physical dimensions, retention times and sedimentation properties, but are generally similar in chemistry (Table 4.20). An exception to this is the unusually high concentrations of nitrogen in B-Pond. West Pond shows extreme variation from the other ponds due to the long-term accumulation of dissolved and suspended materials through evaporative concentration. This is readily evident from alkalinity and conductivity data (Table 4.20). Since West Pond received sanitary wastes during the early development of Hanford, nitrogen and phosphorus concentrations are also disproportionately high. All ROWMA study ditches are generally similar in

TABLE 4.20. Physical Description and Annual Ranges of Physicochemical Parameters for ROWMA Study Ponds

	GABLE POND	B-POND	U-POND	WEST POND
SURFACE AREA (m ²)	287,337	140,159	56,658	77,808
SURFACE AREA (ACRES)	71.0	36.9	14.0	19.2
VOLUME (m ³)	431,000	233,246	22,700	23,685
MEAN DEPTH (m)	1.5	1.6	0.4	0.4
RETENTION TIME (hr)	379	252	40	-
TEMPERATURE (°C)	0 - 25.3	0 - 25.0	0 - 28.4	0 - 25.5
INSOLATION RANGE (ANGLEY'S)	20 - 253	20 - 253	20 - 253	20 - 253
SESTON (mg/l)	0.59 - 60.15	0.29 - 214	2.13 - 76.5	2.61 - 27.5
SEDIMENTATION (mg/cm ² day)	1.43 - 6.76	0.21 - 2.76	0.34 - 1.01	5.12 - 26.8
pH	7.85 - 8.75	7.0 - 9.0	7.0 - 9.5	9.7 - 10.0
ALKALINITY (mg/l CaCO ₃)	48.9 - 73.0	48.4 - 71.4	74.0 - 132.0	6.764 - 13.177
DISSOLVED OXYGEN (mg/l)	7.9 - 12.7	8.1 - 13.8	9.0 - 13.0	8.0 - 13.8
HARDNESS (mg/l CaCO ₃)	50.49 - 80.59	51.46 - 85.48	39.0 - 62.14	112.6 - 145.7
CONDUCTIVITY (umhos/cm @ 25°C)	1118 - 1662	1189 - 1590	1000 - 2500	151,111 - 339,333
TOTAL NO ₃ -NO ₂ (mg/l)	0.035 - 0.330	0.062 - 6.10	< 0.100 - 0.295	-
TOTAL NH ₃ (mg/l)	0.143 - 0.640	< 0.100 - 5.30	< 0.100 - 0.360	1.95 - 3.50
ORTHO P (mg/l)	< 0.01	< 0.010 - 0.205	0.032 - 0.126	1.80 - 2.60
TOTAL P (mg/l)	0.025 - 0.075	0.010 - 0.081	0.088 - 0.156	1.85 - 2.70
TOTAL Si (mg/l)	0.520 - 1.53	0.480 - 2.45	0.390 - 0.840	< 0.100 - 1.10

TABLE 4.21. Physical Description and Annual Ranges of Physicochemical Parameters for ROWMA Study Ditches

	Z-19*	A-19	B-3	100-N**
LENGTH (m)	885	1325	1200	423
FLOW (m ³ /min.)	0.48 - 7.6	1.709 - 2.034	6.177 - 10.845	≈ 6.624
TEMPERATURE RANGE (°C)	10.5 - 24.6	13.4 - 27.0	10.0 - 25.5	18.0 - 37.0
INSOLATION RANGE (ANGLEY'S)	20 - 253	20 - 253	20 - 253	20 - 253
SESTON (mg/l)	0.04 - 7.60	0.21 - 0.684	0.11 - 3.92	-
pH	6.5 - 7.9	7.4 - 8.1	6.95 - 8.00	7.55 - 8.00
ALKALINITY (mg/l CaCO ₃)	55 - 87	45.91 - 69.26	48.94 - 65.46	29.26
DISSOLVED OXYGEN (mg/l)	7.8 - 11.2	7.8 - 10.5	8.0 - 12.1	≈ 8.0
HARDNESS (mg/l CaCO ₃)	-	54.38 - 70.88	58.26 - 80.59	36.41
CONDUCTIVITY (umhos/cm @ 25°C)	-	1151 - 1423	1040 - 2017	960
TOTAL NO ₃ -NO ₂ (mg/l)	0.004 - 0.450	0.029 - 0.910	1.20 - 6.40	-
TOTAL NH ₃ (mg/l)	≤ 0 - 100	< 0.100 - 4.40	< 0.100 - 4.40	-
ORTHO P (mg/l)	0.070 - 0.110	< 0.010 - 0.590	< 0.010 - 0.032	-
TOTAL P (mg/l)	0.080 - 0.120	0.022 - 0.720	0.024 - 0.084	-
TOTAL Si (mg/l)	1.80 - 8.40	0.600 - 3.15	1.30 - 2.90	-

* Z-19 DITCH HAS BEEN DRY SINCE 3-76, DUE TO AN ACCIDENTAL RELEASE SITUATION

** 100-N TRENCH WAS NOT ACCESSIBLE FOR STUDY UNTIL 9-76

physicochemical characteristics except B-3 ditch, which is relatively higher in nitrogen but lower in phosphorus concentrations (Table 4.22).

Biological characterization of the ponds and ditches are near completion. Primary productivity of periphyton appears to be highest in West and Gable Ponds (Tables 4.22 and 4.23). Secondary productivity is apparently low for all systems with B-Pond showing

the highest rates (Tables 4.22 and 4.23). In terms of the flora and fauna, all sites are fairly diverse in the taxa represented, and species present are not uncommon (Table 4.22 and 4.23). Because of its alkaline and saline water chemistry, West Pond appears different in its general biological profile than the other ponds, yet there are many similarities in algal and insect populations. Characterization of the 100-N trench is not as complete as the other systems. However, despite its relatively higher content of radioactive materials its biological profile appears to be similar to other systems.

Algae and other microbes, macrophytes, and insects appear to have dominant roles in the ecosystems structure of the ponds. There are a variety of transient insects present as larvae in these systems which emerge as adults and enter adjacent environments. Goldfish populations, which are present in the B-3 ditch, and in all ponds except West Pond are sufficiently large enough to be significant radionuclide export routes for via predation by mammals and birds. More attention will be given to these potential export routes in the future.

The relative radionuclide content of the study sites is presented in Table 4.24 in the form of gross α and β of the filterable and particulate water fractions. Data are from ARHCO's 222-S Analytical Laboratory Radiation Monitoring Reports (1975 and 1976) and from UNI's 1975 Environmental Release Report released March 9, 1976. The relatively high value for α activity in the filterable fraction for the West Pond samples may be due to the presence of the naturally-occurring uranium. Concentrations of radionuclides in 100-N trench are higher than the other 3 sites because it receives water directly from the 1301-N crib (Figure 4.21). Major radionuclides associated with ROWMA sites may be seen in Figure 4.20 and 4.21. Dosimetry at the sediment water interface in all ROWMA study sites is low except for 100-N trench (Table 4.25).

In FY 1977, following the completion of site characterization, we will begin to inventory each system for specific radionuclides. Combined with this, the rates of incoming and exported radionuclides will be monitored. Results of this phase of the ROWMA study are expected to provide an expression of material transport for these radionuclides through aquatic ecosystems.

TABLE 4.22. Biological Characteristics of ROWMA Study Ponds

	GABLE POND	B-POND	U-POND	WEST POND
PRIMARY PRODUCTIVITY (mg C / cm ² · day)	0.340 - 21.3	0.840 - 3.51	0.0330 - 1.16	1.99 - 6.96
SECONDARY PRODUCTIVITY (NUMBER OF ORGANISMS / cm ² · day)	0.023 - 0.112	0.009 - 0.547	0.005 - 0.180	0.002 - 0.084
CHLOROPHYLL <i>a</i> (µg / L)	-	-	0.5 - 2.0	
ALGAE (DOMINANT FORMS)	FILAMENTOUS GREENS DIATOMS	FILAMENTOUS GREENS DIATOMS	FILAMENTOUS GREENS NONFILAMENTOUS GREENS DIATOMS	DIATOMS
MACROPHYTES (DOMINANT FORMS)	PONDWEED WATER MILFOIL BULRUSH CATTAIL	PONDWEED BULRUSH CATTAIL	PONDWEED BULRUSH CATTAIL	BULRUSH CATTAIL
INVERTEBRATES (OTHER THAN INSECTS)	FLATWORMS CLADOCERANS AMPHIPODS SNAILS	FLATWORMS LEECHS CLADOCERANS AMPHIPODS SNAILS	FLATWORMS OLIGOCHAETES LEECHS OSTRACODS CLADOCERANS AMPHIPODS WATERMITES SNAILS	OLIGOCHAETES OLIGOCHAETES
RESIDENT INSECTS	WATERSTRIDERS WATERBEETLES GIANT WATER BUGS WATER BOATMAN BACK SWIMMERS WATER SCORPIONS	WATERSTRIDERS WATERBEETLES WATER BOATMEN BACK SWIMMERS	WATERSTRIDERS WATERBEETLES GIANT WATER BUGS WATER BOATMEN BACK SWIMMERS WATER SCORPIONS	WATERBEETLES
TRANSIENT INSECTS	MIDGE DAMSELFLY DRAGONFLY CADDIS FLY MAYFLY	MIDGE DAMSELFLY DRAGONFLY CADDIS FLY MAYFLY	MIDGE DAMSELFLY DRAGONFLY CADDIS FLY MAYFLY	MIDGE DAMSELFLY SHORE FLY HOUSE FLY
VERTEBRATES	GOLDFISH	GOLDFISH	GOLDFISH	

TABLE 4.23. Biological Characteristics of ROWMA Study Ditches

	Z-19	A-29	B-3	100-N
PRIMARY PRODUCTIVITY (mg C / cm ² · day)	--	2.10 - 14.0	1.58 - 9.53	--
SECONDARY PRODUCTIVITY (NUMBER OF ORGANISMS / cm ² · day)	--	0.0172 - 0.376	0.0134 - 0.386	--
ALGAE (DOMINANT FORMS)	FILAMENTOUS GREEN DIATOMS	FILAMENTOUS GREEN DIATOMS	DIATOMS	FILAMENTOUS GREEN DIATOMS
MACROPHYTES (DOMINANT FORMS)	BULRUSH CATTAIL SPEEDWELL	BULRUSH CATTAIL SPEEDWELL	BULRUSH SPEEDWELL	SPEEDWELL
INVERTEBRATES (OTHER THAN INSECTS)	--	--	SNAILS	SNAILS
RESIDENT INSECTS	WATERSTRIDERS WATERBEETLES WATER BOATMEN BACK SWIMMERS	WATERSTRIDERS	WATERSTRIDERS WATERBEETLES	WATERBEETLES
TRANSIENT INSECTS	DRAGONFLY	MIDGE DAMSELFLY MAYFLY	MIDGE DAMSELFLY MAYFLY	MIDGE DAMSELFLY DRAGONFLY
VERTEBRATES			GOLDFISH	

TABLE 4.24. Radiological Characteristics of
ROWMA Study Ponds and Ditches

	FILTERABLE α (pCi / L)	PARTICULATE α (pCi / L)	FILTERABLE β (pCi / L)	PARTICULATE β (pCi / L)	DOSIMETRY (mr / wk)* (SEDIMENT-WATER INTERFACE)
GABLE POND	< 4.0 - 170	< 2.0 - 145	36 - 140	< 1.7 - 1740	143.3 - 172.2
B-POND	< 1.1 - 322	< 2.1 - 4.3	< 30 - 190	7.8 - 111	6.5 - 8.3
U-POND	< 4.5 - 522	< 1.5 - 59	< 26 - 8100	< 17 - 5110	183.3 - 229.2
WEST POND	< 13 - 220	< 0.84 - 64	< 89 - 14,700	< 2.6 - 1740	$\leq$ bkg - 3.1
Z-19 DITCH	< 5.6 - 26,900	< 0.14 - 141,000	< 37 - 2310	1.9 - 3440	-
A-29 DITCH	< 1.1 - 100	< 2.6 - 189	< 26 - 773	< 16 - 662	$\leq$ bkg
B-3 DITCH	< 1.0 - 15	< 2.1 - 51	< 37 - 161	< 16 - 649	5.1
100-N TRENCH	22 - 860**		1900 - 5,700,000 ⁺		33,600 - 77,200 ⁺⁺

* BASED ON 1 MONTH MEASUREMENT
** TOTAL α MEASUREMENTS

⁺ TOTAL β MEASUREMENTS
⁺⁺ AT WATER SURFACE

- **RADIOECOLOGY OF ^{129}I AND ^{99}Tc**
- **INFLUENCE OF SOILS AND AQUATIC SEDIMENTS ON THE CHEMICAL BEHAVIOR, TRANSPORT, AND BIOAVAILABILITY OF POLLUTANTS**

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The terrestrial aspects of these investigations, involving close cooperation between soil and plant scientists, are directed toward identification of soil physicochemical factors and plant kinetic and metabolic factors governing the availability to plants of nuclear and non-nuclear wastes. Principal recent emphasis has been placed on ^{99}Tc and ^{129}I . An interpretive review of the literature on these elements is in preparation. In addition, laboratory studies have been initiated to define key processes governing their behavior.

In the case of radioiodine, its long-term environmental behavior and ultimate fate cannot be inferred with confidence from the often studied short-lived isotope ^{131}I . Technetium, it now appears, is not like iodine in its soil/plant behavior, as has been assumed in the past. Therefore, its long-term behavior and ultimate fate remains uncertain, also. The quantitative measurement of ^{129}I and ^{99}Tc in environmental samples is also technically complex and costly. ^{129}I can be measured in a variety of low-level environmental samples using neutron activation procedures developed at PNL. ^{99}Tc still presents difficulties, but a sensitive mass spectrometric approach has been under development for environmental application.

In aquatic sediment studies, investigations have been directed toward measurement of the physicochemical properties of suspended and bottom sediments of the lower Columbia River watershed and radionuclide (^{99}Tc , ^{129}I) and trace metal speciation, transport and biological availability as influenced by sediment properties and seasonal and man-induced changes in watershed and river conditions. Limited efforts were directed toward those studies in 1976. Principal emphasis was on development of methods to determine Hg speciation in waters to assist in interpretation of field studies conducted in previous years.

The soil and sediment studies closely interdigitate with several investigations dealing with the fate of radionuclides in other terrestrial and aquatic studies outlined in this report.

Uptake of ^{129}I by Forage Crops

Alfalfa (*Medicago sativa*) and orchard grass (*Dactylis glomerata*) were grown in lysimeters each with soil spiked with 2.5 μCi

of ^{129}I . The treatments and controls were replicated six times and irrigated throughout the growing season. The alfalfa was harvested three times and orchard grass twice. After each harvest, the tissues were dried,

weighed, and counted directly using a well-type NaI(TL) crystal low-energy photon counter.

Figure 4.22 illustrates the uptake of ^{129}I per unit during the 1975 and 1976 growing seasons. Alfalfa tissues contained 3 to 7 times more iodine than orchard grass but the difference tended to decrease with time. After the first harvest, plant uptake was reasonably steady. The concentration of ^{129}I from the terminal harvest in 1976 was slightly higher than earlier harvests of the same year.

The lysimeters will be maintained for several years to evaluate the effect of aging and variable weather conditions on the uptake of ^{129}I from soil by field crops.

FIGURE 4.22. Uptake of ^{129}I by Alfalfa and Orchard Grass from Field Lysimeters

Dry-Matter Digestibilities of Cattle Forage

The objective of this study was to determine the livestock-forage and environmental ^{129}I relationships on bluebunch wheatgrass pastures under field grazing conditions. This study reports the preliminary findings on forage digestibility while samples are in the process of being analyzed for ^{129}I .

Digestible dry matter (DDM) was determined for the 4 major plant species consumed by cattle. This is defined as the digestible portion of ingested plant parts that are available to the animal.

Individual plants of 4 species most palatable to cattle and known to be the most important food items were harvested throughout the grazing season by hand-clipping. All plant material was oven dried at 50°C for 48 hr and ground through a Wiley mill with a 1 mm screen.

A 4 g sample of plant material along with a small steel ball was placed into nylon bags (2 x 4 in.). All samples were replicated 5 times. Twenty nylon bags were tied to a nylon cord and inserted into the rumens of 2 free-ranging steers which were surgically fitted with rumen canula. Samples were allowed to remain in the rumen for 72 hr and removed. All bags were rinsed in distilled water until no distinguishable change in color of rinse water could be noticed. Oven-dried weights were obtained and digestibilities determined. Sampling was conducted during the grazing season of May and June.

Table 4.25 presents the dry-matter digestibilities for the major forage plants consumed by the steers. *Crepis atrabarba* was the most digestible forage plant followed by the grasses *Poa cusickii*, *Stipa thurberiana*, and *Agropyron spicatum*. Digestibilities of all plants except *Agropyron* did not change between mid-May and early June. These plants were in the reproductive stage of development during both sampling dates; therefore, little change is expected. *Agropyron* showed an increase of 7.4 percentage points, which may be due to differences between steers rather than forage.

Digestible dry-matter varied between the two steers ($P \leq 0.03$) for both dates. Individual variations in digestibilities may be contributed to the variation in microflora found within and between the rumens of the two animals.

In assessing the fate of ^{129}I ingested by the steers, the end result will be determined largely by the ability of the rumen microorganisms to utilize iodine and convert it to a form available to cell uptake. The end result will be measured in the tissues of the experimental animals.

TABLE 4.25. Dry Matter Digestibilities of Four Forage Plants Between Two Steers During the Grazing Season of 1976 on an *Artemisia/Agropyron* Pasture

SPECIES	HEREFORD		CHAROLAIS		AVERAGE
	N ^(a)	$\bar{X} \pm \text{S.E.}$	N	$\bar{X} \pm \text{S.E.}$	$\bar{X} \pm \text{S.E.}$
----- 19 MAY 76 -----					
<i>Crepis atrabarba</i>	3	84.0 $\pm$ 0.1	5	87.5 $\pm$ 0.1	85.9 $\pm$ 0.6
<i>Poa cusickii</i>	3	65.1 $\pm$ 0.7	4	75.8 $\pm$ 0.2	71.2 $\pm$ 2.1
<i>Stipa thurberiana</i>	3	56.0 $\pm$ 1.0	3	69.7 $\pm$ 0.1	62.9 $\pm$ 3.1
<i>Agropyron spicatum</i>	3	55.0 $\pm$ 0.6	2	62.7 $\pm$ 1.7	58.1 $\pm$ 1.9
----- 8 JUNE 76 -----					
<i>Crepis atrabarba</i>	5	84.0 $\pm$ 0.4	5	85.2 $\pm$ 0.2	84.6 $\pm$ 0.2
<i>Poa cusickii</i>	4	68.9 $\pm$ 1.0	5	75.7 $\pm$ 0.6	72.7 $\pm$ 1.2
<i>Stipa thurberiana</i>	5	61.6 $\pm$ 1.8	5	70.5 $\pm$ 0.9	66.1 $\pm$ 1.5
<i>Agropyron spicatum</i>	4	62.6 $\pm$ 1.7	4	68.4 $\pm$ 1.3	65.5 $\pm$ 1.1

(a) N = SAMPLE SIZE

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Technetium Behavior in Soils and Plants

The isotope ^{99}Tc is produced by the spontaneous fission of ^{235}U in nature and by the neutron activation of ^{99}Mo and the slow neutron fission of ^{235}U or ^{239}Pu in nuclear reactors (Campbell, 1961). It has a long half-life (2.15×10^5 yr) and an approximate fission yield of 6.1% which is equivalent to ^{137}C or ^{90}Sr . Accumulated quantities of Tc resulting from nuclear power generation in the U.S. are estimated to be 170,000 kg by the year 2000 (Burkholder et al., 1975). In addition to existing fallout levels, the potential exists for Tc entrance into the environment in emissions from nuclear reactors, nuclear fuel reprocessing plants, and other facilities such as hospitals that use Tc for commercial purposes. Technetium behaves somewhat like iodide in the human body, concentrating in the thyroid, and therefore its environmental behavior and routes to man are of considerable interest (Kotegov et al., 1968; Boyd, 1959). Recent evidence indicates, however, that technetium is unlike iodine at some points in its pathways from the nuclear fuel reprocessing plant to green plants and other organisms in the foodchain to man (Vaughan et al., 1976).

There are no published measurements of Tc in surface soils and few measurements in other segments of the environment (Kenna, 1962; Brown, 1967; Attrep et al., 1971; Moss et al., 1975; Sodd et al., 1975; Birks, 1975). Technetium has only rarely been measured in less complex materials such as natural waters because counting methods are much less sensitive than for other major isotopes. Complete chemical separation of ^{99}Tc from short half-life Ru isotopes has been difficult and the analytical developments in gamma or alpha spectroscopy, which have allowed more intensive study of other elements, such as ^{137}Cs and ^{239}Pu , in the environment, have not materially assisted in separation of ^{99}Tc , a beta emitter, from interferences. A sensitive neutron activation method for measurement of Tc at environmental levels has been published (Foti et al., 1972a, 1972b) but application of this technique to environmental measurements has not been reported. Currently, a mass spectrometric method for the analysis of ^{99}Tc in

environmental samples has been under development of PNL by mutual agreement with another DBER program. This method now appears feasible for analysis of sample concentrations in the range of 0.1 pCi (Kaye, 1977).

It is certain that ^{99}Tc is now present in soils as a result of world-wide nuclear testing, and the half-life and fission yield suggest it may be present at concentrations considerably higher than ^{129}I originating from fallout. In addition, Tc has been detected by counting methods in groundwater beneath deep sediments underlying nuclear waste storage facilities (Brown, 1967). The concentration in the groundwater was approximately equivalent to the concentration in the waste stream indicating that, in contrast to most of the radionuclides in the waste, it was highly soluble and mobile in soil.

Soluble ions in soil have the potential for competing with nutrient ions for uptake by plant roots. If the soluble ion serves as a nutrient analog, plant uptake may be accentuated, leading to accumulation in foodstuffs. Studies were therefore undertaken to assess: 1) the extent of Tc sorption in soils representing a range in physiochemical properties and 2) the accumulation, fate, and behavior of Tc in plants.

Behavior In Soils

The relatively high fission yield and apparent solubility of Tc in soil suggested that Tc availability to plants should be investigated. Under aerobic conditions, the most stable chemical species of Tc in aqueous solution is the highly soluble pertechnetate ion (TcO_4^-), and, over the range of slightly acid to basic pH values typical of most agricultural soils, the pertechnetate ion may be expected to be the predominant soluble species (Dezoudor and Pourbaix, 1966). In previous studies (Wildung et al., 1974), in which Tc was applied as pertechnetate to surface soils representing a range in physicochemical properties and equilibrated under aerobic conditions, Tc was highly soluble and was sorbed (Kd values of 0.007 to 2.8 mL/g) in significant quantities only in soils of high organic matter and low pH, perhaps through reduction to less soluble TcO_2 or TcS_2 .

Behavior In Plants

Phenotypic changes in response to Tc applied to soil as pertechnetate were observed within 5 days of plant emergence (Wildung et al., 1976). At the 0.001 and 0.01 $\mu\text{g/g}$ levels, soybean plants were similar in appearance to the controls. At the 0.1 $\mu\text{g/g}$ level, growth did not occur after cotyledon expansion. At the 5.0 $\mu\text{g/g}$ level, growth ceased 3 days after emergence. Toxicity symptoms in wheat

were also observed at the 0.1 $\mu\text{g/g}$ level. The plants were stunted and there was a necrosis of the blade tips and margins. Growth did not occur at the 1.0 and 5.0 $\mu\text{g/g}$ levels.

As suggested by the growth characteristics prior to harvest, Tc markedly reduced plant yields (Wildung et al., 1976) at soil concentration levels above 0.1 $\mu\text{g/g}$ (6 orders of magnitude above fallout levels as estimated from ^{137}Cs). The reason for Tc toxicity to the plant was apparent on analysis of the tissues. The Tc was readily taken up and marked increases in Tc concentration in the aerial portion of the plant occurred with increased soil concentration up to a high of 380 $\mu\text{g/g}$ in soybeans at a soil Tc level of 5.0 $\mu\text{g/g}$ (Wildung et al., 1976). The concentration gradient in the plants resulted from two phenomena. First, at the low concentration, where plants appeared healthy, the plants removed most of the Tc from soil, with 88 and 78% of the Tc applied to the soil present in the aerial tissues of soybeans and wheat, respectively, after 30 days of growth, limiting further uptake. Secondly, at the higher soil concentration levels, initial Tc uptake was sufficiently high to markedly reduce yields thereby limiting tissue dilution.

In related studies reported in Section 8, pp 8.43-8.44, native plants of the Hanford site showed plant/soil concentration ratios from 100-400, with up to 82% of the ^{99}Tc amendment removed from the soil. Concentrations were well below the toxicity threshold, e.g., 1 pg/g soil.

Recently, in an abstract of another study (Landa et al., 1975) dealing with ^{99}Tc uptake by plants, it was also reported that Tc may cross the root membrane; uptake by wheat of ^{99}Tc , applied as pertechnetate in irrigation water during the early stages of growth, was on the order of that reported in the present studies. It is clear that plant affinity for Tc applied to soil as pertechnetate is high and generally exceeds that reported for other non-nutrient nuclides arising from the nuclear fuel cycle (Menzel, 1965). However, from the studies to date, it is not possible to evaluate the full potential for Tc uptake by healthy plants in the field. In the field, plants would have access to a larger volume of soil containing Tc at lower levels and therefore the concentration factor ($\mu\text{Ci/g}$ dry tissue per $\mu\text{Ci/g}$ dry soil), often used as a measure of radioelement availability to plants, may be higher than would be calculated from these data. The principal factor controlling plant uptake in this case may be the concentration and long-term solubility of Tc in soil.

The Tc was mobile in the soybean plant with the highest concentrations in the cotyledon followed by the leaves, stem, and bud in tissues where these were formed. Wheat exhibited the highest Tc concentrations in the blade. Other studies (Wildung et al., 1974) with soybeans in a split-root system indicated that Tc may be translocated downward to root tissues, but concentrations in the roots were less than 1 $\mu\text{g/g}$. Dose rates calculated for individual aerial tissues of soybeans and wheat in which growth did not occur after cotyledon expansion did not exceed 20 rad. In contrast, the median external lethal dose rates for flowering plants generally range from 800-130,000 rad (Platt, 1973). The evidence, although presumptive, suggests that toxicity was due to biochemical rather than radiation effects.

The high affinity of plants for Tc and its potential toxicity necessitated further study of the mechanisms by which plants accumulate Tc and its fate and behavior in the plant. Although plants efficiently accumulate Tc from soils at 0.01 $\mu\text{g/g}$, the question arises as to whether plants have the active transport mechanisms for accumulating Tc at fallout levels. Soil levels of Tc resulting from fallout were calculated to be approximately 0.1 pg/g . To determine whether Tc was available for plant uptake at these levels, soybean plants were permitted to accumulate Tc at concentrations of 0.22 pM (0.02 pg/mL) pertechnetate for 2 hr. Of the 10.5 pg supplied, 6% (0.65 pg) was accumulated by the plant in 2 hr. The distribution of Tc between the shoot and root was 2 and 4%, respectively (Cataldo et al., 1976). Thus, the plant appeared to have a highly efficient mechanism for accumulating Tc, perhaps using mechanisms normally employed for a nutrient similar in ion size and charge to Tc.

The mobility and physical distribution of Tc within the plant was evaluated following a 24 hr exposure of 69-day old soybean plants to 0.1 μM Tc (Cataldo et al., 1976). At 69 days of growth, these plants contained pod and seed tissues, normally metabolic sinks for many metabolites and essential trace elements. Following exposure, one set of plants was harvested and analyzed immediately, while a second set was transferred to a nutrient solution without added Tc and permitted to continue growth for an additional 21 days. On transfer of the exposed plants to nutrient solution, desorption of Tc from the root was not observed. Immediately following the Tc exposure period, 85% of the accumulated Tc was localized in root and leaf tissue with only 3.7 and 0.03% transported to pod and seed tissues, respectively. From these studies, Tc appeared to persist in the xylem and was highly xylem mobile moving with the

transpirational stream. At 90 days of age, or 21 days following exposure to Tc, root and stem tissues showed a reduction in Tc content, while leaf, pod, and seed tissues increased. The increases were due to Tc movement to these tissues from root and stem tissue following the initial exposure period. Since the 90-day old plants were close to physiological maturity with seed filling near completion, significant phloem mobilization of Tc from vegetative tissues apparently did not occur. This was evident from the apparent lack of Tc mobilization from leaves on seed maturation and the minimal increases in Tc content of pod and seed tissues between 69 and 90 days.

A knowledge of the mobility patterns of ions in plants, namely xylem or phloem mobility, is important in anticipating the fate and distribution of specific ions during vegetative growth as well as at maturity. Although Tc did not appear to be phloem mobile, its xylem mobility would suggest that long-term, low-level accumulation of Tc could result in significant deposition of Tc in seed tissues. This becomes important in considering food chain transfer and ingestion hazards to man.

The mobility of Tc in plants may be expected to be highly dependent upon its form. The high xylem mobility of Tc suggested that Tc was present in a stable soluble form, perhaps at pertechnetate. Investigations were therefore undertaken to characterize the form of Tc in plant xylem exudates. Plant xylem exudates were collected, assayed for Tc content, and the chemical form of Tc characterized by electrophoresis (Figure 4.23). Migration of Tc in all controls was towards the cathode, indicating an anionic species. The electrophoretic mobility of Tc was similar for all solutions, indicating the form remained unchanged. The R_f for two exudate fractions was similar to the controls indicating that the xylem mobile form was pertechnetate. Further studies are underway to insure that Tc was not present in a reduced form which was rapidly oxidized to pertechnetate during electrophoresis. Compared to the original feeding solution, the concentration of Tc in the xylem exudate increased by a factor of 4 and 17 for the two samplings, that is, concentration of Tc occurred against a solution concentration gradient, indicative of an active process.

The high affinity of the plant for Tc, Tc toxicity to the plant, and Tc xylem mobility suggested that Tc may, in fact, behave as a nutrient analog, possibly an analog which behaved as an anion in the plant. Prior to conducting preliminary competition studies, its uptake behavior with respect to substrate concentration was determined.

FIGURE 4.23. Thin-layer Electrophoretic Behavior of Technetium in Plant Xylem Exudate and Controls

The rate of uptake of Tc by the intact plant was concentration-dependent (0.01 to 10 μM) and exhibited multiple isotherms (Cataldo et al., 1976). Multiphasic uptake behavior at low ion concentration is characteristic of a membrane, carrier-mediated process and indicates a degree of specificity for Tc. Multiphasic uptake responses obtained over a range of substrate concentrations are typical of those obtained for many cationic (Nissen, 1973b) and anionic (Nissen, 1973a; Vange et al., 1974) nutrient species.

To determine whether Tc as pertechnetate was functioning as a nutrient analog, several nutrient and non-nutrient ions were selected for preliminary competition studies. The concentrations of pertechnetate and potential competitor ions were chosen from the concentration-dependent uptake curves and represented a working concentration range for a single isotherm (0.2 μM to 0.7 μM). A number of nutrient and nonnutrient anions were screened to determine their effect on Tc uptake. In addition, several micronutrient cations were also investigated. These ions were selected on the basis of their similarity to the solution chemistry of Tc and/or their requirement in plant nutrition. Assuming a classical type of competition kinetics, an uptake rate equivalent to the control would indicate the presence of a noncompetitor, whereas a reduction in uptake rate, would suggest the presence of a competitor which interacts, either directly or indirectly, with Tc at some point in the uptake process. Of the ions studied, five reduced Tc uptake. These included Mn^{+2} ,

$H_2PO_4^{-2}$, MoO_4^{-2} , SO_4^{-2} , and SeO_4^{-2} . Detailed competition kinetic studies are currently underway to further elucidate the interaction between Tc and specific nitrilites. However, if Tc behaved as an analog of one or more of these species, many aspects of the fate and behavior of Tc in the plant could be explained. This would not only account for the high affinity of the plant root for uptake of Tc but would also provide a basis for the observed toxicity of Tc. If Tc acted as a nonfunctional analog of essential nitrilites, an impairment of critical physiological and metabolic processes could result from its substitution in functional proteins or as a cofactor in enzymatic reactions resulting in a reduction or inactivation of key biochemical processes. Furthermore, if Tc serves as a nutrient analog, discrimination at the plant root membrane may be minimal and there is a greater possibility that the observed high uptake may be general to a range of plant species, and perhaps animals, resulting in an avenue for introduction of Tc into food supplies. The ultimate concentration and behavior of Tc in the terrestrial environment will depend on the quantity and form which is emitted, its long-term concentration and solubility in soils, and its subsequent plant availability. The results of these investigations indicate that there is a need to develop this information.

Iodine Behavior in Soils and Plants

While there is an increasing body of knowledge regarding the behavior of I in the environment, little is known of Tc behavior. An interpretive review is underway to establish the state of knowledge and to compare the behavior of I and ^{99}Tc in the environment to determine the extent to which information on $^{129},^{131}I$ may be applied to prediction of the behavior of ^{99}Tc . The review encompasses source terms and physical, chemical, and biological processes responsible for the redistribution of these elements in the environment. A preliminary assessment indicates that significant differences in the cause and behavior of these elements precludes extensive use of I information for predictive purposes. Furthermore, it appears that major differences also occur in the environmental behavior of ^{129}I and stable iodine.

Behavior of Mercury in the Columbia River Watershed

Previous studies (Garland and Wildung, 1974) have indicated that the transport, deposition in sediments, and biological availability of Hg in the Columbia River watershed is markedly influenced by chemical and/or microbiological processes which alter the solubility of Hg in the river. Limited investigations were, therefore, undertaken to

determine the quantity and form of Hg dissolved in Columbia River water. A technique for adsorbing dissolved Hg on activated charcoal was investigated and subsequently applied to Hg analysis by flameless atomic absorption spectrometry (faas).

The procedure briefly consists of trapping dissolved Hg species on a small column of 30-40 mesh activated charcoal. The adsorbent column was attached to the bottom of a plexi-glass tube, and water samples placed in the tube were forced through the activated charcoal at 10 psig. An in-line filter placed immediately above the charcoal column removed suspended matter from the sample. The effectiveness of adsorbing dissolved Hg species on activated charcoal was evaluated by spiking filtered Columbia River water with standard aliquots of dissolved $HgCl_2$, CH_3HgCl , and C_6H_5HgCl . Results showed that all three Hg species were quantitatively recovered by the activated charcoal column.

Collected Hg was released by heating the activated charcoal column to 500°C in an inert gas stream. The effluent water and Hg vapors were directed into a second column containing gold-coated glass beads. This second column was simultaneously heated to 150°C and water vapor was quickly exhausted; Hg vapors were retained by amalgamation with the hot gold-coated beads. Hg retained by the gold-coated beads was liberated by increasing the column temperature to 500°C. The liberated elemental Hg was passed into a long-pathlength gas absorption cell where it was measured at the 254 nm line.

The developed procedure concentrates total dissolved Hg from water samples; minimum detectable concentration, as measured by faas, was 0.25 ng/l for a 2l sample. Preliminary use of the method for the analysis of laboratory distilled water and Columbia River water yielded mean dissolved Hg concentrations of 9.0 ng/l and 1.3 ng/l, respectively (Table 4.26).

TABLE 4.26. Total Dissolved Hg in Water

Source	Date	Concentration (ng/l)	Volume Sampled (l)
Columbia River (Richland, WA)	8/2/76	1.3 ^(a)	1.0
		0.9	1.0
		1.1	1.0
	10/29/76	1.2	0.5
		2.2	1.0
Laboratory Distilled Water	11/3/76	1.3	1.5
		Mean	
		1.3	
	11/3/76	8.3 ^(b)	0.2
		7.5	0.2
		110.4	
	Mean	100.4	
		8.7	0.6
		8.3	0.3

(a) estimated error = 20-40%
(b) estimated error = 15-25%

The application of this technique should be a significant aid to comprehensive studies of Hg in the Columbia River. Future refinements and adaptations to other adsorbent media should provide a simple, rapid, and inexpensive means of achieving Hg speciation analysis.

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5.0 NUCLEAR FUSION

- REVIEW OF BIOLOGICAL EFFECTS OF TRITIUM RELEASES FROM FUSION POWER REACTORS (DMFE TASK)
- REVIEW OF BIOLOGICAL EFFECTS OF ACTIVATION PRODUCTS AND OTHER CHEMICALS RELEASED FROM FUSION PLANTS (DMFE TASK)
- SUBLETHAL EFFECTS OF TRITIUM ON AQUATIC SYSTEMS
- EFFECTS OF LOW-LEVEL CHRONIC IRRADIATION ON EMBRYONIC DEVELOPMENT

Necessary information for defining the nature and magnitude of environmental effects was lacking during the preparation of the environmental analysis for the magnetic fusion energy program. This could result in impact estimates of questionable accuracy, so in response to this need, PNL undertook a study of two major areas of potential environmental impact. These are: 1) the biological effects of tritium releases, and 2) the biological effects of activation products and other chemical releases. Magnetic field effects, consequent on containment of the fusion plasma were considered at the time of this writing to be of biomedical interest, primarily, relating to in-plant occupational health (Mahlum, 1976), so are not dealt with in this report. Possible concerns over magnetic enhancement over natural background in the environment outside plant boundaries will be re-evaluated during the forthcoming year. Sponsorship for these efforts comes from both the Division of Biomedical and Environmental Research and the Division of Magnetic Fusion Energy, ERDA.

Current efforts have emphasized the effects of chronic exposure of early life stages of aquatic vertebrates and invertebrates to low level tritium irradiation. This includes: 1) investigation of the permanence of injury to the immune response in yearling rainbow trout, *Salmo gairdneri*, sublethally exposed to tritium during embryogenesis, and 2) investigation of the potential for uptake, release, and effects of tritium over the course of the life history of the crab, *Pinnixa occidentalis*.

Additionally, literature reviews on the fate and effects of tritium, activation products, and other chemical releases to the environment have been completed. The objective was to disclose weaknesses in the information base for preparation of an environmental analysis for the magnetic fusion energy program.

• REVIEWS FOR DIVISION OF MAGNETIC FUSION ENERGY

Principal Investigators: J. A. Strand, R. C. Thompson and
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As in either BNR or PWR designs, some tritium is anticipated to escape the fusion plant in both liquid and gaseous effluents. Tritium released as tritium oxide will behave like ordinary water throughout the hydrosphere and will be readily metabolized by all living organisms. Tritium released in elemental form is of much less concern than tritium oxide because it is not readily incorporated into living organisms. Extensive studies over the past 30 yr have failed to produce tritium effects data significantly different from those which might have been predicted from a general knowledge of the effects of ionizing radiation. If fusion reactors hold tritium releases within ICRP standards, it is unlikely that any significant impact to the environment from such releases will occur (Strand and Thompson, 1976).

Activation products which may escape fusion reactors include ^{26}Al , ^{49}V , ^{52}Cr , ^{54}Mn , ^{55}Fe , ^{58}Co , ^{60}Co , ^{93}Nb , and ^{94}Nb . The biological transport of many of these elements has not been studied in terms of atmospheric dispersal, deposition and accumulation in soil, hydrologic transport, and accumulation in plants and animals. Lithium releases to the environment either during the mining cycle, from power plant operation or accident, may be in the form of a number of compounds varying in solubility and affinity for biological organisms. Hence, toxicity would appear to vary according to solubility and possibly pH shift. The effects of a severe liquid metal fire or explosion involving Na or K will vary according to inherent abiotic and biotic features of the affected site. However, present research does not adequately deal with the environmental impact associated with such liquid metal disasters. Beryllium may be released to the environment during the mining cycle or under conditions of reactor accident. Beryllium toxicity has been demonstrated in a variety of organisms including plants, animals, and man. Adverse effects to the environment may also be expected from salt drift and such routine chemical releases of biocides, corrosion inhibitors, and dissolution products. (Strand and Poston, 1976).

Review of Biological Effects of Tritium Releases from Fusion Power Reactors

Tritium breeding in D-T burning fusion reactors is expected to occur at a high rate, with an estimated doubling time of months. Consequently, the inventories of tritium will be greater than for present fission designs. As in either the BWR or PWR designs, some

tritium is anticipated to escape the plant in both liquid and gaseous effluents. With this projected increase in release of tritium from fusion reactors, it is necessary to know how readily tritium is metabolized by biological organisms, and at what levels of radiation dose-adverse effects may be expected. However, relatively few studies of the effects and behavior of tritium have been undertaken.

A summary of the existing information pertinent to these needs and recommendations for future research follow.

Tritium released as tritium oxide behaves like ordinary water throughout the hydrosphere and is readily metabolized by all living organisms. Although most biochemical reactions discriminate against the incorporation of tritium, the possibility of some concentration should not be overlooked. A fraction of tritium in the body as tritiated water becomes organically bound; that is, exchanges with hydrogen bound in organic molecules. However, organic binding is not expected to result in significant bioaccumulation of tritium from tritiated water, because water is approximately as hydrogen rich (~11% by weight) as any compound of biological organisms (~10% by weight). Processes that allow accumulation and incorporation of tritium assist in its elimination. Tritium which is organically bound demonstrates a longer half-time in organisms, but it appears to constitute a small fraction of the total tritium label.

Tritium released as HT is of much less concern than HTO because it is not readily incorporated into living organisms.

Extensive studies over the past 30 yr have failed to produce tritium effects data significantly different from those which might have been predicted from a general knowledge of the effects of ionizing radiation. Although recent biological effects studies suggest uncertainty as to the appropriate RBE for tritium, this would likely be no greater than three or four. However, if fusion reactors hold tritium releases within ICRP standards, no significant impact to the environment from those releases is expected. However, some uncertainties still exist and should be eliminated by continuing research.

Future research needs include confirmation of the current estimates of concentration factors and turnover rates resulting from both acute and chronic exposures. Temporal and spatial variations must be followed and trophic relationships need additional study. Relatively few studies have been conducted on assessment of potential genetic effects, particularly under conditions of chronic exposure. More information on radiation effects on individuals, populations, and communities needs to be developed. Longer term or chronic studies should consider factors such as rates of growth, morphological abnormalities, onset of maturity, and reproductive capacity. Chromosomal studies under both laboratory and field conditions should be extended, and the somatic and genetic consequences of such changes on populations, communities, and ecosystems should be evaluated.

Other future research needs include better definition of source terms. The oxidation of released tritium and the degree to which it remains as elemental tritium should be determined. Other deficiencies in our knowledge lie in our lack of understanding of the basic relationships between low-level, low-dose-rate, total radiation exposure, and health effects. Properly designed largescale, multi-generation, tritium exposure studies in experimental animals are needed to extend present knowledge of these basic relationships.

Review of Biological Effects of Activation Products and Other Chemicals Released from Fusion Plants

Literature review indicates that existing information is incomplete, often contradictory, and of questionable value for the prediction and assessment of impacts of activation products and other chemicals released from fusion reactors. A summary of the pertinent existing information and recommendations for future research follow.

The spectrum of neutron-induced activation products in structural materials of fusion power plants may differ significantly from that of current light water reactors. It is still uncertain which structural materials will be used in the blanket and first wall of fusion power plants; however, niobium, vanadium, vanadium-chromium alloy, vanadium-titanium alloy, sintered aluminum product (SAP), and stainless steel have been suggested. The activation products of principal concern will be the longer-lived isotopes ^{26}Al , ^{49}V , ^{51}Cr , ^{54}Mn , ^{55}Fe , ^{58}Co , ^{60}Co , ^{93}Nb , and ^{94}Nb . These induced radionuclides may escape the reactor confines through neutron sputtering into or corrosion by liquid metal coolants. Activation products may also escape containment as aerosols under certain accident situations. Disposal of radioactive waste through burial may increase the potential for the environmental impact. The impact of fusion-associated activation products and other chemical releases on the terrestrial and aquatic ecosystem needs to be further determined. The biological transport of many of these elements has not been studied in terms of atmospheric dispersal, deposition and accumulation in soil, hydrologic transport, and accumulation in plants and animals.

Lithium releases to the environment either during the mining cycle, from power plant operation or accident, may be in the form of a number of compounds varying in solubility and affinity for biological organisms. Hence, toxicity would appear to vary according to solubility and possibly pH shift. Where comparative data are presented, animal forms are more susceptible than plant forms; the embryonic and juvenile life stages are

more sensitive than adult animals. From the literature, it is evident that the existing data base is of questionable value for the prediction and assessment of impact. The lack of source terms information, in particular, complicates this problem. Also, present research has not treated the potential for adverse impacts from long-term, low-level exposures of the metal. The potential for biomagnification has not been defined.

The effects of a severe liquid metal fire or explosion involving Na or K will vary according to inherent abiotic and biotic features of the affected site. Saline, saline-alkaline, and sodic soils of arid lands would be particularly susceptible to alkaline stress. Grasslands soils contain greater quantities of humus which increase buffering capacity of the soil, alleviating alkaline stress. The very acidic soil of forest regions would tend to neutralize alkaline insult. In marshlands, proximity to an aquatic biome would facilitate dilution; however, the ecology of wetlands is extremely vulnerable to contamination from a liquid metal spill. Wetlands or marshlands often provide special habitats required for rare and endangered species, and are among the most productive biomes. Lentic (still-water) environments represent the most vulnerable of aquatic biomes; estuarine or marine coastal sites the least sensitive due to the greater buffering capacity of more saline waters. Present research, however, does not adequately deal with the environmental impact associated with liquid metal disasters. Of particular significance are the dynamics involved with the hydrolysis of alkali metal oxides in ecosystems and the fate of the alkali and hydroxide derivatives.

Beryllium released to the environment during the mining cycle or reactor accident situation could be in the form of a number of compounds. Solubility and hence biological availability would vary with pH, the presence of certain inorganic and organic compounds, and the physiochemical forms of these compounds; i.e., ionic, colloidal, or particulate. Beryllium toxicity can be demonstrated in a variety of organisms including plants,

animals, and man. Animals are usually as susceptible as plants. Evidence suggests the beryllium concentrates in both plant and animal forms with potential for biomagnification in food chains leading to man. Research needs include study of both acute and chronic exposures. The potential for biomagnification also needs to be defined. Studies over longer time periods and at low levels need to be conducted to detect sublethal levels of stress.

Salt drift, although not unique to fusion plant, will contribute significantly to the accumulation of salinity in soils of arid regions, and to some degree in grasslands, with the potential for adverse impact on vegetation. Salt from cooling towers will not likely accumulate in forested regions. Cooling tower salt drift may contain trace quantities of arsenic, cadmium, lead, chromium, and mercury, and if threshold limits are exceeded, toxicity to both plants and animals will result. Future research needs include provisions to better characterize the heavy metal constituents and other compounds comprising salt drift, as well as assessing their relative toxicities to indigenous flora and fauna. Tests under site specific conditions and utilizing indigenous species need to be conducted before the significance of impact may be predicted.

Adverse effects to aquatic species from routine chemical releases (biocides, corrosion inhibitors, dissolution products) may occur in the discharge of both fission and fusion power plant designs. The potential impact is reduced abundance or total loss of one or more species, and sometimes the substitution of undesired species. Mortality may be direct or indirect when small quantities accumulate within the flesh of affected organisms. However, such routine chemical releases are well characterized, and if releases are controlled, their impact will be minimal. Future research needs are site specific and should include the determination of cooling and receiving water quality, the effect of water quality change on biota, and the potential for biological accumulation and biomagnification of discharged chemicals.

- **SUBLETHAL EFFECTS OF TRITIUM ON AQUATIC SYSTEMS**
- **EFFECTS OF LOW LEVEL CHRONIC IRRADIATION ON EMBRYONIC DEVELOPMENT OF MARINE FISH AND INVERTEBRATES**

Principal Investigators: J. A. Strand, M. P. Fujihara,
G. Roesijadi, and T. M. Poston

Freshwater laboratory investigations (funded by DBER) during the current year have continued to investigate the suppression of primary immune response in fish. Emphasis is on establishing how permanent this injury may be. Antibody synthesis in response to a secondary challenge from the bacterium, Flexibacter columnaris, was employed in these studies using yearling rainbow trout, Salmo gairdneri, sublethally exposed to tritium (0, 0.04, 0.4, 4.0, and 40 rads) during 20 days of embryogenesis. Results indicated that the immune response was significantly suppressed at dose levels as low as 0.4 rads. As such, it served to demonstrate the permanence of injury following irradiation of the embryonic life stage, as evidenced by secondary challenge in yearling rainbow trout.

The objective of our second project is to examine the potential for uptake and release of tritium over the course of the life history of a marine invertebrate species and to evaluate the possible effects of low-level chronic irradiation. Previous ecological investigation has concentrated on freshwater organisms. In the present studies, crabs, Pinnixa occidentalis, are being reared in the laboratory, and measurements of the HTO exchange of the fast compartment are being made during the course of development. Rates have been determined for the megalopa, 1st crab instar, 2nd crab instar, and 3rd crab instar. The results indicate that the exchange is extremely rapid and inversely related to the size of the animals.

Sublethal Effects of Tritium on Aquatic Systems

In continuing studies on enhanced susceptibility to infection in fish following tritium irradiation, antibody synthesis in response to a secondary challenge from the bacterium, Flexibacter columnaris, was employed to investigate the permanence of injury to the immune response in yearling rainbow trout, Salmo gairdneri, which had been sublethally exposed to tritium (0, 0.04, 0.4, 4.0, and 40.0 rads) during the first 20 days of embryogenesis. Previous experiments employing the same test fish, but at an earlier age (5 months), demonstrated that the immune

response of tritium irradiated fish was significantly suppressed at doses as low as 4.0 rads.

Test fish employed in the most recent series of experiments were maintained for an additional 12 months on dechlorinated sanitary water after receiving primary antigenic challenge. At an age of 17 months, control and tritium irradiated test fish were again administered intraperitoneally 0.1 cc of a heat inactivated antigen (1.8×10^8 cells/ml), F. columnaris, in 25% Freund's incomplete antigen. At 3 weeks and 1 week presecondary challenge, and at 2, 4, 7, and 10 weeks post secondary challenge, a standard tube

agglutination test for the specific antigen of antibody stimulation was performed on the serum of 20 fish from each of the control and irradiated test groups.

Table 5.1 shows the arrangement of tritium irradiated and control test groups in two ponds employed during the present series of experiments. Each test group was duplicated. The experiment was designed so that the data could be treated by analysis of variance.

TABLE 5.1. Arrangement of Tritium Irradiated and Control Test Groups in Two Pond Sampling Array^a

Compartment	Pond	
	I	II
1	A	E
2	B	D
3	C	C
4	D	B
5	E	A

^aThe letters A, B, C, D, and E represent control, 0.04, 0.4, 4.0, and 40.0 rad treatment levels, respectively.

Mean titer values (reciprocals) for specific agglutinins to *E. columnaris* for a selected experimental group over the 10-week sampling period following secondary challenge are graphically presented in Figure 5.1. Summary statistical information for both test groups are contained in Table 5.2.

FIGURE 5.1. Mean Titer Values in Pond I for Specific Agglutinins to *E. columnaris* Arranged According to Increasing Radiation Dose at Each Sampling Interval

TABLE 5.2. Summary Analysis of Variance for Agglutinin Titer ($\log_e$)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Ratio
Ponds	1	14.53	14.53	28.65 ^b
Treatments	4	24.53	6.13	12.09 ^b
Time	3	627.60	209.20	412.40 ^b
Ponds x Treatments	4	6.08	1.52	3.00 ^a
Ponds x Time	3	10.93	3.64	7.18 ^b
Treatment x Time	12	6.05	0.50	0.99 N.S.
Ponds x Treatment x Time	12	7.11	0.59	1.17 N.S.
Error	760	385.56	0.51	

^aSignificant F value at 0.05 level

^bSignificant F value at 0.01 level

N.S. Nonsignificant F value at 0.05 level

These data indicated that under the prescribed experimental conditions, a clearly observable and statistically significant change in mean titer values occurred during the 10-week sampling period following secondary challenge. As well, significant differences were detected in mean titer values among the five treatment means. There also was a significant pond effect, and significant pond X treatment and pond X time interactions.

From similar analyses performed for pond I at each sampling interval, it was found that on the 2nd, 7th, and 10th weeks, significant differences occurred among the five treatment means. In pond II, a significant difference in treatment means occurred only during the 7th week. Subsequent statistical analyses employing the Duncan Multiple Range Test, (Table 5.3) confirmed that specific agglutinins to *E. columnaris* in pond I increased rapidly in both control and tritium irradiated experimental fish following secondary antigenic challenge. These analyses also indicated that on the 2nd, 7th and 10th weeks in pond I, control and 0.04 irradiated test fish demonstrated significantly higher agglutinin levels, which may be interpreted as suppression of agglutinin synthesis in test fish exposed to higher tritium levels. Similar results were observed in pond II during the 7th week.

These results may mean that immune suppression as here measured, was dependent upon both agglutinin levels and time, and that opportunity for development of higher agglutinin titers beyond 10 weeks may have

TABLE 5.3. Duncan Multiple Range Test Comparisons of Mean Titer ($\log_e$) Values for Each Sampling Period Arranged According to Increasing Irradiation Dose,^a Pond I

Time	0 rad	0.04 rad	0.4 rad	4.0 rad	40.0 rad
2nd Week	4.93	4.79	4.03	4.07	4.02
4th Week	5.62	5.45	5.35	5.31	5.38
7th Week	6.42	6.35	6.01	5.97	5.83
10th Week	7.43	9.29	7.08	6.73	6.53

^aDifferences are significant at 0.05 level

resulted in significant immune suppression at the next dose level. That is, differences among treatment means would likely have become more pronounced as agglutinin levels in treatment groups continued to increase beyond 10 weeks. Nevertheless, it may be concluded that under the described experimental conditions, the immune response as evidenced by secondary challenge in yearling rainbow trout was significantly suppressed at dose levels as low as 0.4 rads, and, as such, served to demonstrate the permanence of injury following irradiation of the embryonic life stage.

Effects Of Low-Level Chronic Irradiation On Embryonic Development Of Marine Fish And Invertebrates

It is generally accepted that organisms possess several body compartments for tritium (Tucker and Harrison, 1974), and turnover rates for the different tritium compartments have been reported for numerous species. However, little is known about factors which affect tritium turnover. Such information will improve our general understanding of tritium metabolism and its movement in the marine coastal ecosystem. The rate of turnover of the rapidly exchangeable tritium compartment represents water turnover and, in the case of marine and aquatic organisms, is an estimate of water permeability. Although

it has been shown by Smith and Rudy (1972) and Hannan and Evans (1973) that relative HTO turnover rate is size-dependent in at least three marine species, studies with other organisms have failed to detect such a correlation (Hannan and Evans, 1973; Tucker and Harrison, 1974). The relationship between body size and turnover rate is not well understood.

The present investigation was undertaken to address this problem. Specimens of the marine crab *Pinnixa occidentalis* were collected as larvae and are being reared in the laboratory. Measurements of HTO turnover rates of the rapidly exchangeable compartment are being made as the crabs progress through their various stages of development.

This study commenced in May 1976, when zoeal stages of *Pinnixa occidentalis* were collected from the plankton of Sequim Bay, Washington, and transferred to the laboratory for rearing. Larvae were reared in culture dishes containing filtered seawater (0.45 μ) of 30 ‰ and approximately 10°C. The water was changed every other day, and the larvae were fed *Artemia* (San Francisco Bay) nauplii.

Larvae which molted to the megalopa stage were transferred to compartmentalized plastic boxes and reared individually in 30 ml filtered seawater. Measurements of HTO turnover were conducted with megalopa and the first, second, and third crab instars. Rearing is still ongoing, and measurements will be made on subsequent stages as the crabs develop.

To determine relative HTO turnover, crabs were first incubated in HTO for 20 hr, then transferred to clean sea water. Turnover rate was measured as the rate of appearance of tritium in the release medium as described by Roesijadi et al. (1976). Ten individuals were pooled for each determination, and 3 replicate measurements were conducted for each stage.

Rate constants (k) were calculated from the relationship

$$\ln \frac{C_{\infty} - C_t}{C_{\infty}} = -kt$$

where C_{∞} = counts as equilibrium, and C_t = counts at time t. Halftimes ($t_{1/2}$) were calculated as

$$t_{1/2} = \frac{\ln 2}{k}$$

Rate constants and halftimes for HTO exchange in the megalopa and first, second, and third crab instars of *Pinnixa occidentalis* are presented in Table 5.4.

TABLE 5.4. *Pinnixa occidentalis*. Rate constants (k) and half-times ($t_{1/2}$) for HTO exchange in the Megalopa and first, second, and third crab instars

Stage	Wet Weight (g) ^a	$k(h^{-1})^a$	$t_{1/2}$ (h)
Megalopa	0.0125 ± 0.0035	5.4 ± 2.7	0.13
1st Crab	0.014 ± 0.0017	4.23 ± 1.34	0.16
2nd Crab	0.018 ± 0.0015	3.4 ± 0.39	0.20
3rd Crab	0.034 ± 0.0009	1.5 ± 0.45	0.46

^aMean and 1 standard deviation

HTO exchange in *Pinnixa occidentalis* was extremely rapid, particularly in the first three stages. Rates were highest in the megalopa and decreased in subsequent stages. Values for the megalopa and first and second crab instars rank among the highest turnover rates reported for marine invertebrates (Maetz, 1974). Exchange rates for the third crab instars were more typical of rates reported in other marine animals.

The relative HTO turnover rate was highly dependent on body size and decreased with increasing body weight. Linear regression analysis of $\log_{10} k$ versus $\log_{10}$ wet weight resulted in a $b-1$ value of -1.18 ± 0.21 (S.E. of regression) which cannot be considered to be significantly different from -1 . Thus, b is essentially 0, suggesting that both small and larger crabs were exchanging similar

volumes of HTO per time over the size range considered. Only 3 other marine invertebrate species have been reported to possess relative HTO turnover rates which were affected by the size of the organism. Using the calculations of Kannan and Evans (1973), $b-1$ ranged from -0.11 to -0.27 for *Limulus polyphemus*, *Rithropanopeus harrisi*, and *Carcinus maenas*, values which are considerably different than that reported here. Size (weight) ranges for these species were not as low and did not include organisms as young as those in the present study. Differences in $b-1$ values in the present study and previous ones may be due to ontogenetic effects as well as strict size effects. It will be of interest to learn if the HTO turnover rate versus size relationship of *Pinnixa occidentalis* remains stable when stages older than third crab instar are examined.

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6.0 SOLAR HYDROELECTRIC GENERATION

• EFFECTS OF HYDROELECTRIC GENERATION ON RIVERINE ECOLOGY

This is a small ecological project designed to develop data for measuring aquatic environmental impact at hydroelectric and pumped storage sites. Dissolved gas supersaturation due to air entrainment, modification of ambient temperature regimes, fluctuation in river flow caused by the use of hydroelectric facilities, and loss of appropriate wildlife habitat caused by the impoundment of large quantities of water, all create major uncertainties in the further development of hydro-pumped storage. Nevertheless, in the Pacific Northwest, several hundred potential sites for pumped storage have been identified, and this hydro-development is the cheapest of all currently practicable means for nuclear, fossil, or solar (wind) energy storage.

Severe physical changes to impacted river systems result from development of hydropower. Species composition, productivity and energy flows in resulting reservoirs are much different than in the pre-impoundment stream or river. Effects of changes in water flow patterns, quantity and species composition of downstream drift, sediment load, dissolved gas concentrations, and temperature often extend to the river mouth and beyond.

Once dammed for the production of power, a river must be treated as a new and radically different system. To maximize production of useful fish species for commercial or sport harvest and to minimize the impacts downstream from the dam, sound management is an important obligation of agencies responsible for development. Evaluation of alternate design criteria and operating procedures should be based on a substantial body of ecological data. These data should relate to impacts of specific proposed plans and define their environmental significance.

• EFFECTS OF HYDROELECTRIC GENERATION ON RIVERINE ECOLOGY

Principal Investigators: D. H. Fickeisen and J. C. Montgomery

Currently, two tasks are underway in this project. Studies of gas bubble disease resulting from dissolved atmospheric gas supersaturation continued with emphasis on effects of temperature on tolerance of resident species. The second, a new task, was initiated to examine impacts of water level fluctuations resulting from regulating power generation to meet changes in consumer electrical demand. Several effects were identified qualitatively which await more rigorous analysis to assess their significance. Both tasks are of regional and general interest. In the Columbia River system, gas supersaturation and indirect effects caused by fluctuations in river flow play the predominant role in population dynamics of anadromous and resident fish, as well as other aquatic organisms.

Gas Bubble Disease

Laboratory studies on the effects of dissolved atmospheric gas supersaturation on aquatic organisms continued during the past year at a reduced level of effort. Modifications such as spillway deflectors to existing hydroelectric facilities on the Columbia River have reduced the degree of gas supersaturation which occurs primarily when air is entrained by water falling over dam spillways and carried to depths in the stilling basin. Hydrostatic pressure forces some entrained air to dissolve in the water and, as the water moves downstream and surfaces, the pressure is reduced and the water becomes supersaturated. Rates of re-equilibration of gases in water are slow and supersaturation caused by dams persists many miles downstream. River systems with many consecutive dams such as the Snake and Columbia Rivers in the Pacific Northwest become supersaturated for most of their length.

With engineering modifications at dams to reduce air supersaturation and the adoption of water quality criteria for dissolved gases, the urgency of gas bubble disease research has diminished, as it applies to tolerance of riverine organisms. However, a number of fundamental questions remain that

relate to sublethal effects, swimming depth distribution of migrating and resident fish, and the pathogenesis of gas bubble disease among fish populations.

Use of water for cooling in industrial processes, including thermal generating stations, is another source of gas supersaturation. Gas solubility in water decreases as temperature increases. In a typical condenser cooling system, gases do not re-equilibrate and the discharged water is supersaturated with air. This phenomenon has resulted in fish kills in reservoirs (e.g., Lake Norman, North Carolina) and in marine waters (e.g., Pilgrim and Millstone Nuclear Plants in New England) where fish have apparently been attracted to warmed discharge plumes.

When aquatic organisms are exposed to dissolved gas supersaturation, their tissues become supersaturated and gas-phase emboli form at nucleation sites. The emboli block blood circulation, resulting in hemostasis and tissue necrosis, hemorrhaging, or cardiac blockage. The resultant "gas bubble disease" has been reported in a variety of freshwater and marine fishes and aquatic invertebrates.

We have concentrated recent research efforts on completing bioassays designed to quantify interactions of temperature and supersaturation on tolerance of black bullhead (*Ictalurus melas*), pumpkinseed sunfish (*Lepomis gibbosus*), and rainbow trout (*Salmo gairdneri*) to gas bubble disease. The data apply to effects of cooling water discharges from nuclear and fossil fuel power stations. The protocol involves acclimating test fish to a specific test temperature at 4°C increments between 8° and 32°C (8° to 20°C for rainbow trout), and exposure to controlled combinations of temperature and dissolved gas in water during 96-hr bioassays. The fish are tested in shallow (25 cm) troughs to prevent their gaining significant benefits of hydrostatic pressure by sounding. Each test is conducted with 40 fish and at least 3 tests are required at each temperature to define the tolerance curve. We have completed our test series with rainbow trout and have nearly completed the extended series for the other two species (Table 6.1).

Tolerance to gas bubble disease is lower for rainbow trout than for other test species at the lower temperatures. There is a definite trend for tolerance to decrease as temperature increases for all three test species. Thus, fish exposed to the combined stress of temperature and gas supersaturation in a discharge plume will be less resistant than the same species exposed to saturated water below a dam where the temperature is not increased. We plan to complete this programmed series of bioassays and examine effects of thermal shock on tolerance to gas supersaturation next.

A mathematical model has been developed by the BNW Systems Department which describes the effect of temperature on the tolerance of fish to gas bubble disease. The model now fits experimental data for rainbow

trout fairly well and is being refined and expanded to describe the bullhead and pumpkinseed data. Our goal is to permit use of the model to generate a response surface for accurately predicting the tolerance of our test species at a given temperature and gas level.

Through cooperation with local sportsmen, we have collected data on incidence of gas bubble disease in wild resident fish. Twenty-five squawfish (*Ptychocheilus oregonensis*) and about 200 smallmouth bass (*Micropterus dolomieu*), representing over half of those sampled, were observed with emphysema of skin and fin membranes in the Snake and Columbia Rivers during the summer of 1976, indicating presence of gas bubble disease.

Proceedings of a workshop on gas bubble disease hosted in October of 1974 by Battelle-Northwest and the Atomic Energy Commission (now ERDA) were published and distributed early in the year (Fickeisen and Schneider, 1976). In addition, two manuscripts describing bioassays of fish in a deep tank and effects of gas bubble disease on aquatic insects have been prepared.

River Level Fluctuations

Changes in turbine loading at dams to meet variable power demands result in short-term fluctuations in discharge water flows. The resulting continuous variation in water level below the generating facility causes stranding, increased exposure to predators, entrapment, or desiccation of aquatic organisms depending on season, river stage, and topography. Future development of pumped storage facilities and even more extensive "management" of the Columbia River will compound these effects.

Water levels within the Columbia River adjacent to the Hanford Reservation vary on a daily and weekly cycle, and changes of over 1 m in a 24-hr period are common. The reach remains free-flowing and contains several sloughs used by spawning bass, extensive gravel areas used by spawning salmonids, and numerous sites of potential fish entrapment.

Casual observations of effects of water level fluctuations on fish incidental to other field work in past years led to our recent efforts specifically designed to identify the species impacted and the causes of mortalities. Our work has drawn the attention of several regional agencies with particular interest in our efforts to develop a quantitative basis for evaluating the impact.

TABLE 6.1. Tolerance of Fish to Gas Bubble Disease at Different Water Temperatures (96-hr TL₅₀'s)

Temperature (°C)	TL ₅₀ (Total Gas Supersaturation)		
	Rainbow Trout	Black Bullhead	Pumpkinseed Sunfish
8	120.5	126.9	129.6
12	119.4	125.1	126.1
16	119.6	123.8	127.6
20	117.8	124.4	123.8
24	--	122.9	115.1
28	--	119.8	114.3
32	--	120.2	115.1

An unusual runoff pattern this year reduced the degree of fluctuation, however, during an experimental flow period conducted for other purposes. Throughout the season, we were able to make limited observations at the study areas. Fish and invertebrates were affected by fluctuating water levels at all observed river stages, indicating the presence of entrapment ponds at a wide range of elevations.

Many fish species, as well as fish food organisms, are adversely affected by normal day-to-day water level fluctuations. Mortalities are caused by entrapment of adults and fry in small ponds that dry up or are excessively heated by solar radiation, by stranding of adult fish on rocky beaches as the water level drops and by enhanced predation from birds, mammals, and fish. In addition, smallmouth bass spawning is adversely affected by rising water levels, which flood the warmer sloughs with colder river water, and by falling water levels, which expose bass nests in shoreline areas or cause abandonment by guarding males.

Early work at Hanford discussed losses due to colder water influx to sloughs after egg development began (Henderson and Foster, 1957). We located five bass nests this year, three of which were flooded with cold water and the eggs apparently failed to hatch. The use of radiofrequency tags and subsequent telemetry monitoring of fish movement indicated that male bass normally guard the nest until the eggs hatch. The males then move from the sloughs, spend about a week near the slough outlet apparently foraging, and subsequently move into deeper parts of the river. One of the tagged male bass fish continued to guard a nest that was flooded with cold water well past the expected time of fry emergence.

Eggs from other bass nests were observed to hatch, but only about 35-50 fry emerged from each. A typical nest should have produced about 2000 fry (Scott and Crossman, 1973). Water level variations possibly are involved in the poor hatch.

Entrapment of adults and fry in intermittent ponds caused considerable loss of fish as the ponds dried. Nearly all fish species of commercial or sport importance were entrapped by declining water levels, as were most resident coarse fish. Many of the entrapped fish were dead or moribund. Aquatic insects were also entrapped in small ponds and many died, indicating a possible impact on fish food resources. Other aquatic organisms such as crayfish and annelids were subject to desiccation. Thousands of whitefish larvae and salmonid fry were entrapped and died from dewatering (Figure 6.1). Solar radiation heated some small ponds to temperatures near or above the upper lethal limit for salmonids.

Aerial photographs during the minimal river stage showed many potential entrapment areas. We were able to survey only a small fraction of these. Additional effort on sampling program design is needed to quantify the number and species of fish killed during low water stages in our study areas and to permit extrapolation of values for similar areas identified photographically. This is planned as part of our future research effort.

Predation, a factor of major importance, is enhanced by water level fluctuations. We observed predation by adult fish (particularly bass and squawfish) on fry entrapped in ponds, by coyotes on entrapped adult fish, and by raccoons on clams. In addition, scavenging by birds, rodents, amphibians,

FIGURE 6.1. Salmonid Fry Entrapped in Small Dewatered Pond

insects, and arachnids was apparent and reduced the numbers of dead fry observed.

Our objectives of initiating a qualitative investigation, defining the potential impacts of fluctuations in river level, and identifying future study needs have been accomplished. We plan to intensify field studies at selected sites with a goal of developing quantitative methods to define generally applicable impacts of variations in river flow.

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7.0 MULTITECHNOLOGY SUPPORT

- ANALYSIS OF NATURAL SYSTEMS
- QUANTITATIVE ECOLOGY OF IMPACT EVALUATION
- GEOCHEMICAL OCEAN SECTIONS STUDY (GEOSECS)
- CHEMISTRY OF OCEAN SOLUTIONS
- IN SITU POLLUTANT STUDY OF THE NEW YORK BIGHT
- EFFECTS OF WATER QUALITY ALTERATIONS ON FISH BEHAVIOR
- COMBINED EFFECTS OF THERMAL DISCHARGES ON AQUATIC BIOTA
- EFFECTS OF THERMAL DISCHARGES ON AQUATIC BIOTA
- POWER PLANT HEAT AND CHEMICAL EFFLUENTS EFFECTS ON SELECTED MARINE AND ESTUARINE COMMUNITIES
- IMMEDIATE EFFECTS OF ANTIFOULING BIOCIDES FROM COASTAL AND OFFSHORE POWER PLANTS
- EFFECTS OF THERMAL DISCHARGES TO COASTAL WATERS

Multitechnology Support includes research efforts that provide information applicable to several presently operating technologies as well as those being investigated for the future. In these technologies the nature of the environmental problem is equally applicable to any one technology; e.g., thermal and chemical pollution of water due to operation of steam electric plants, whether nuclear, fossil fuel, or gas fired; or, the statistical design needed for differentiating a general background of industrial pollution from the contributions, if any, arising from operation of an energy facility.

The two main groups of projects in this category include biomathematical effort represented by the first two projects indicated above and aquatic ecological studies represented by the remaining ten projects.

Biomathematical Projects: These represent important effort in applying statistical theory to real-world problems such that appropriate error limits may be set when quantitative descriptions of ecological relationships are attempted. Specifically four areas of investigation are underway: 1) the application of sampling theory for the reliable measurement of environmental contamination, 2) methods for the statistical estimation of wildlife populations, 3) the evaluation of simulation models, and 4) statistical approaches for supplying an overview of large environmental data sets (e.g., pre- and post-operational monitoring data from steam-electric plants). In addition to those projects covered in this section, an additional biomathematical project, described in Section 4, concerns the estimation of pollutant inventory at contaminated sites. The latter project concerns plutonium estimation at the NTS field site, but the approaches are applicable to other pollutants at heavily contaminated sites.

Aquatic Projects: These include both marine and freshwater studies and concern the bulk of Multitechnology Support effort. Generally, this research is directed at understanding the basic processes involved in the functioning of the aquatic ecosystems. Once these processes are understood, the task of determining the ultimate effects of man's use of the ecosystem will be easier and more complete.

One of the major questions being addressed by PNL scientists, in aquatic projects, is the biological availability and effects of effluents resulting from energy production and use. However, to do this, the entire system we are concerned with must be assessed to identify those factors and processes that have a significant impact on the behavior of a particular material in the ecosystem. We need to establish the baseline conditions, identify the impacts to the ecosystem, understand the processes that take place in the biogeochemical cycling of a material, and be able to accurately measure effluent bioavailability and effects so that the ultimate cost to the ecosystem can be estimated.

Presently, one of man's major concerns is how to best utilize the resources of the aquatic ecosystem. For the freshwater system, this means deciding just how much industrial and domestic waste materials our lakes and streams will absorb and still be desirable for other uses such as domestic water supplies, fisheries and recreation. The same is true for the marine environment, except that the coastal system is receiving pressure as a site for all types of industrial development, particularly, energy recovery and production. Indiscriminate disposal of waste products is not desirable for several reasons, but beyond a certain point, the removal or reduction of materials from an effluent stream requires costly energy. Thus, to conserve our available energy, it is important to determine and understand how to use the natural cycling capabilities of the aquatic systems to the benefit of man.

This section summarizes the research that PNL has undertaken to understand how the ecosystem functions and what impacts are caused by man's development and use of various energy technology. The state-of-the-art varies significantly between the energy areas that are involved in this multidisciplinary effort and, therefore, the studies are directed to establishing an understanding at the level necessary to assess the significance of the environmental impacts.

7.0 MULTITECHNOLOGY SUPPORT

- ANALYSIS OF NATURAL SYSTEMS
- QUANTITATIVE ECOLOGY OF IMPACT EVALUATION
- GEOCHEMICAL OCEAN SECTIONS STUDY (GEOSECS)
- CHEMISTRY OF OCEAN SOLUTIONS
- IN SITU POLLUTANT STUDY OF THE NEW YORK BIGHT
- EFFECTS OF WATER QUALITY ALTERATIONS ON FISH BEHAVIOR
- COMBINED EFFECTS OF THERMAL DISCHARGES ON AQUATIC BIOTA
- EFFECTS OF THERMAL DISCHARGES ON AQUATIC BIOTA
- POWER PLANT HEAT AND CHEMICAL EFFLUENTS EFFECTS ON SELECTED MARINE AND ESTUARINE COMMUNITIES
- IMMEDIATE EFFECTS OF ANTIFOULING BIOCIDES FROM COASTAL AND OFFSHORE POWER PLANTS
- EFFECTS OF THERMAL DISCHARGES TO COASTAL WATERS

Multitechnology Support includes research efforts that provide information applicable to several presently operating technologies as well as those being investigated for the future. In these technologies the nature of the environmental problem is equally applicable to any one technology; e.g., thermal and chemical pollution of water due to operation of steam electric plants, whether nuclear, fossil fuel, or gas fired; or, the statistical design needed for differentiating a general background of industrial pollution from the contributions, if any, arising from operation of an energy facility.

The two main groups of projects in this category include biomathematical effort represented by the first two projects indicated above and aquatic ecological studies represented by the remaining ten projects.

Biomathematical Projects: These represent important effort in applying statistical theory to real-world problems such that appropriate error limits may be set when quantitative descriptions of ecological relationships are attempted. Specifically four areas of investigation are underway: 1) the application of sampling theory for the reliable measurement of environmental contamination, 2) methods for the statistical estimation of wildlife populations, 3) the evaluation of simulation models, and 4) statistical approaches for supplying an overview of large environmental data sets (e.g., pre- and post-operational monitoring data from steam-electric plants). In addition to those projects covered in this section, an additional biomathematical project, described in Section 4, concerns the estimation of pollutant inventory at contaminated sites. The latter project concerns plutonium estimation at the NTS field site, but the approaches are applicable to other pollutants at heavily contaminated sites.

Aquatic Projects: These include both marine and freshwater studies and concern the bulk of Multitechnology Support effort. Generally, this research is directed at understanding the basic processes involved in the functioning of the aquatic ecosystems. Once these processes are understood, the task of determining the ultimate effects of man's use of the ecosystem will be easier and more complete.

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- **ANALYSIS OF NATURAL SYSTEMS**
- **QUANTITATIVE ECOLOGY OF IMPACT EVALUATION**

Principal Investigators: L. L. Eberhardt, J. M. Thomas,
M. I. Cochran, R. O. Gilbert and B. B. Vinson

Long-term efforts to understand and develop the quantitative aspects of ecology continue, with emphasis on both practical and theoretical considerations. With ERDA's broad research missions covering a wide array of energy technologies, all having evident environmental problems, it becomes increasingly important to have a firm quantitative basis for ongoing and future studies. In the past year, we have again emphasized sampling for contaminants in ecological systems, and have begun some investigations of statistical aspects of ecological effects of such substances. Several investigations of methods and techniques for animal population studies were carried out, and were augmented by practical experience in the field. We began an investigation of the difficult problems of animal population regulation, and cooperated in several studies of marine mammals. Our bibliography was published in "hard-back" form, and the Dairy Farm study was completed and the results published.

During the past year, also, we have begun to apply some of the methods suggested by our earlier work. This effort is covered in the Schedule-189, "Quantitative Ecology of Impact Evaluation." The work augments our applied research. Population aspects are principally considered in the Schedule-189, "Analysis of Natural System," but some methodological and conceptual studies were carried out under the second project. We intend to broaden the scope of the second project to consider other energy-related impacts, but necessary long-term (preimpact and operational) data sets are not yet available so some features of our current work may have to be "extrapolated." Some of our research on indices of population size has been submitted for publication. We organized and participated in a short course on environmental impacts at the Center for Quantitative Studies (University of Washington), presented two invited seminars, and have been invited to present some of our results at a symposium in early 1977.

Sampling for Contaminants

Our work on sampling for contaminants was continued in the past year with emphasis on a variety of topics. We have continued to stress specific aspects of methodology and particular applications (cf. Eberhardt, 1975) but have also attempted to consider broader aspects. An especially important issue continues to be the need to match a sampling plan to specific objectives.

Four rather different branches of sampling theory and methodology are considered: 1) descriptive sampling, 2) analytical sampling, 3) sampling for spatial pattern, and 4) sampling for modeling. Nearly all of the textbook results are subsumed under the heading of descriptive (survey) sampling, in which the main goals are those of accurately and efficiently estimating a total or a mean. Analytical sampling is concerned primarily with analysis--are there significant differences among survey units? As such, the main

distinctions from classical statistical analysis and experimental design seem to lie in the area of design and allocation of sampling effort over the population being surveyed.

Much of our recent research has been concerned with the other two fields. Sampling for spatial patterns has largely evolved in petroleum and mining exploration, with connections to the work of geographers and others concerned with patterns. We have been particularly impressed by the substantial differences in the manner in which samples should be allocated for these purposes, as contrasted to descriptive and analytical sampling (Eberhardt and Gilbert, 1976). We are currently gaining a good deal of practical experience in this area in connection with work reported elsewhere in this report (Quantitative Aspects of Environmental Plutonium Studies).

Sampling for modeling has its origins largely in industrial experimentation and can be exemplified by asking "what are the best sampling times for fitting a specific function to some process observed over time?" As a concrete example, one may consider fitting uptake and retention curves for radionuclides in situations where the analyses require sacrifice of individual animals and may thus be quite expensive. Hence, it would be highly desirable to specify a pattern of sampling times that yields a maximum of information for a given expenditure. It is, of course, necessary to specify a model and to know something of the relevant frequency distributions. These topics have been addressed in our earlier work (Eberhardt et al., 1976).

In April 1976 we participated in the second of a series of mineral cycling symposia sponsored by the Savannah River Ecology Laboratory, for which we organized a section on design and sampling, and presented a review of the topics discussed here. That paper includes details of methodology and some specific applications (Eberhardt, in press).

Literature Cited

Eberhardt, L. L. Some methodology for appraising contaminants in ecological systems. J. Fish. Res. Board Canada 32(10):1852-1859, 1975.

Eberhardt, L. L. Designing ecological studies of trace substances. In: Symposium on Mineral Cycling, D. C. Adriano and I. L. Brisbin (ed.).

Eberhardt, L. L. and R. O. Gilbert. Sampling the environs for contamination, pp. 187-196 In: Proceedings of the First ERDA Statistical Symposium. BNWL-1986, UC-32, 1976.

Eberhardt, L. L., R. O. Gilbert, H. L. Hollister, and J. M. Thomas. Sampling for contaminants in ecological systems. Environ. Science and Technol. 10(9):917-925, 1976.

Problems in Measuring Ecological Effects of Pollutants

Present-day concerns about environmental contamination are increasingly directed to the possible effects of chronic low-level pollutants. Such effects are extremely difficult to measure in nearly any situation, but the problems are particularly severe in ecological systems. In considering human health, one commonly thinks of the well-being and longevity of individuals. In contrast, the "health" of an ecosystem depends on the well-being of populations. We have, thus, adopted the point-of-view that the ecologically important responses to some stress are those that influence species populations and communities.

In reviewing the ecologically important issues in chronic low-level effects of pollutants, we have largely considered these topics: 1) problems in gathering statistically significant data, 2) problems in measuring effects from uncontrolled exposure, and 3) factors limiting the resolution of measurement uncertainties.

Details of the specific issues under each heading cannot be presented here, but the nature of these details is suggested in the following outline:

1. Problems in gathering statistically significant data
 - a) relevance of the traditional experimental approach
 - b) weaknesses of studies based on linear regression
 - c) a need for suitable mathematical models
 - d) difficulties in measuring population parameters
2. Measurement problems
 - a) compensatory responses of populations
 - b) indirect nature of measurements and effects
 - c) interactions between species and trophic levels
 - d) a multiplicity of response variables
3. Limiting factors
 - a) a suitable substitute for the experimental viewpoint

- b) removing present weaknesses in measurement technology
- c) inadequacies in present models
- d) lack of detailed understanding of regulatory mechanisms
- e) absence of reliable long-term data on most natural populations

We do not have suitable answers for the issues raised above, and believe they require continued and intensive research. A more detailed review of individual points was prepared in connection with some Congressional Hearings (Eberhardt, 1975).

Literature Cited

Eberhardt, L. L. Some problems in measuring ecological effects of chronic low-level pollutants, pp. 565-586. In: Hearings Before Subcommittee on Environment and the Atmosphere. Science and Technology Committee, U.S. House of Representatives, Ninety-fourth Congress. USGPO, Washington, D.C., 1975.

Methods and Sample Sizes for Population Studies

A perennial question in planning ecological studies is: "How large a sample size do we need?" We have been conducting research on this problem for some time and have recently begun to summarize the results, to extend our recent review of problems concerning the assessment of environmental impacts (Eberhardt, 1976).

One of the least-studied facets of animal population surveys has been the use of "indices" or relative measures of animal abundance. Thus, the major reference on estimation of animal population size (Seber, 1973) devotes several hundred pages to the capture-recapture or mark-and-recovery methods, but only a few pages to measures of relative abundance (cf. Eberhardt, 1975). Part of our study has been devoted to assessing indices, and to collecting data on the variability associated with a variety of indices. We have found that the coefficient of variation (standard deviation divided by the mean) provides a useful guide to variability of many index methods, and can be used in approximating sample sizes required for specified precision (confidence limits). A summary of an extensive collection of data on variability appears in Table 7.1.

In addition to reviewing the sample size problem and some related features, we have also been cooperating and assisting in a review of marine mammal census methods being conducted at the University of Washington,

TABLE 7.1. A Summary of Coefficients of Variation for Index Methods

Group of Organisms	Coefficient of Variation
<u>Aquatic biota</u>	
Plankton	0.70
Benthos	
Surber sampler, counts	0.60
Surber sampler, biomass, volume	0.80
Grabs, corers	0.40
Shellfish	0.40
Finfish	0.50-2.00
<u>Terrestrial biota</u>	
Roadside counts	0.80
Call counts	0.70
Transects (on foot)	0.50-2.00
Pellet counts	1.00

and have helped plan a number of specific surveys, including participation in an aerial and ground census of the California sea otter (*Enhydra lutris*).

Literature Cited

Eberhardt, L. L. The estimation of animal abundance (Book Review). J. Wildl. Manage. 39(1):224-228, 1975.

Eberhardt, L. L. Quantitative ecology and impact assessment. J. Environ. Manage. 4: 27-70, 1976.

Seber, G. A. F. The Estimation of Animal Abundance. Hafner, N.Y. 506pp. 1973.

Transect Methods for Population Studies

We have continued our research on transect methods. Our earlier work on line transects was broadened to include strip transects and the line-intercept method (1975 Annual Report). We continue to regard these methods as very important in field ecology because they are versatile and do not involve capture or marking.

Our efforts to appraise a nonparametric method for estimation (which we have called Cox's method) have continued, and the method has been tested on some field data. The estimates obtained seem to be "reasonable" in terms of values obtained through a parametric method. However, the main reason for

considering distribution-free methods is just to avoid the need for postulating a model in certain circumstances. Hence, satisfactory evidence for utility of Cox's method will have to come from other sources.

Approximate variance equation estimates for the nonparametric method suggest that it is substantially less efficient than a parametric model (the variance for Cox's method may be roughly double that based on the exponential model frequently assumed in line transect work). However, as we have remarked in previous reports, there are circumstances in which a nonparametric method seems essential. We are currently engaged in assisting the National Marine Fisheries Service (NMFS) in planning one such large-scale application of transect methodology. We also helped in designing and interpreting a similar study on a smaller scale in 1974. The survey will be directed towards porpoise (*Stenella spp.*) populations in the eastern tropical Pacific. Since these populations occupy a very large area, but are not locally numerous (2 or 3 schools per thousand square nautical miles may be a reasonable estimate), line transects from long-range aircraft seem to be one of the few available census methods. Probable scope of the survey may be indicated by noting that refueling in Hawaii and in Tahiti may be necessary.

Mechanisms for Animal Population Regulation

Much of the effort reported in several of the items covered in this and other sections of the Annual Report has to do with methods for censusing and otherwise determining relevant parameters of animal populations. Such information is of obvious importance in ecological studies, whether conducted for research or for environmental impact evaluations connected with any of the several energy technologies. Since most animal populations are able to "compensate" to some degree for insults (impacts) of one kind or another, the ability to measure population parameters may not provide all of the information needed. One should also have some notion of the factors regulating the population.

The matter of animal population regulation is complex and controversial. Arguments about density-dependence and density-independence have gone on for decades and continue today. Further, there are 6 or 7 present-day theories as to the mechanisms of population regulation. These range from limitations of food-supply or habitats through predator-prey interactions to genetic mechanisms. Although any worthwhile investigation should take into account these theories and the relevant evidence for each, we

have taken the point-of-view that an empirical approach is also needed.

The major empirical evidence for animal population regulation takes the form of the stock-recruitment curve of fisheries research and management. We have been attempting to review some of the information available in this area and have investigated some connections between the two major formulations of the stock-recruitment curve, that of Ricker and of Beverton and Holt (Eberhardt, 1976).

Although the stock recruitment (regulatory) mechanism is implicit in fisheries management as the "maximum sustainable yield" (MSY) principle, little such use has appeared for large mammals or in terrestrial animal management (other than in entomology). One exception concerns marine mammals, where a pioneering piece of legislation (The Marine Mammal Protection Act of 1972, Public Law 92-522) requires management be based on the principle of an "optimum sustainable yield" (OSY). The precise difference between OSY and MSY is still somewhat controversial. However, a working definition is emerging in consequence of the administrative and judicial requirements associated with the Marine Mammal Protection Act. We have recently explored some of the problems for marine mammals (Eberhardt and Siniff, in press).

We propose to continue the present effort on regulatory mechanisms, and to compile examples of data on terrestrial vertebrates, where possible. Some interesting aspects of regulation in an inhospitable environment (Antarctica) have turned up in a study there (Siniff et al., 1976) and may suggest some leads for further exploration in that system.

Literature Cited

Eberhardt, L. L. A relationship between two stock-recruitment curves (submitted), 1976.

Eberhardt, L. L. and D. B. Siniff. Population dynamics and marine mammal management policies. J. Fish. Res. Board Canada.

Siniff, D. G., L. L. Eberhardt, D. D. DeMaster and R. J. Hofman. An analysis of the dynamics of a Weddell seal population (submitted), 1976.

Marine Mammal Study

As noted in last year's report, and in the previous papers, we have been cooperating in various marine mammal studies. A number of species of marine mammals are variously

regarded as "threatened" by several energy development activities [notably Outer Continental Shelf (OCS) oil exploration, oil transport, and possibly some of the proposed "bioconversion" techniques]. It has thus seemed desirable that we become familiar with some of the problems, ongoing research, and census techniques. Among other things, the author participated in preparing a document for some recent United Nations (FAO) meetings in Bergen, Norway, on marine mammals and reviewed a number of issues concerning many of the U.S. coastal species and some of the cetaceans. Contacts with much of the U.S. research on these species have developed from the author's appointment to the Scientific Advisory Committee of the U.S. Marine Mammal Commission.

Dairy Farm Study

We have completed the final portions of this DBER-requested analysis with the publication of an extensive document (Thomas et al., 1976) in which we have recorded our detailed observations and a journal article (Hawthorne et al., 1976) where our modeling efforts are described. Some portions of the study not addressed in previous annual reports are presented below.

Soils

Representative soil samples collected in 1965 were composited to represent the soil profile in each of several fields. Mean ^{137}Cs and ^{90}Sr (determined by wet-chemistry) values were fit by nonlinear least squares (Figure 7.1) and indicate that the majority of change with depth can be associated with ^{137}Cs movement. The implications of this movement (a large portion of the radionuclide was deposited prior to 1962) for deep and shallow-rooted plants are probably different for each radionuclide, but for a deep rooted plant like alfalfa, ^{137}Cs would be more likely to be taken up by roots.

When we compared horizontal composites taken in 1962 with those mentioned above, we found ^{137}Cs values were higher in 1965 in both the lowest and uppermost portions of the soil profile (2.5 times at 0-1 in. and 2.9 times at 14-18 in.) whereas ^{90}Sr ratios were less than one below 2 in. Comparable ratios (1965/1962) calculated from vertical composites indicate that both radioisotopes increased slightly throughout the total soil profile (1.32 ± 0.21 for ^{137}Cs and 1.15 ± 0.28 for ^{90}Sr , respectively). ^{137}Cs to ^{90}Sr ratios for vertical soil composites ranged from 1.48 ± 0.36 (s.d.) in 1962 to 1.58 ± 0.24 (s.d.) in 1965. The small sample size

FIGURE 7.1. ^{137}Cs and ^{90}Sr Concentrations in the Soil Profile (1965 Data)

in 1962 ($n = 5$) and the size of the calculated standard deviations precluded any conclusions relative to changes over the 3-yr interval.

Growth Chamber Study

Alfalfa roots from the field in St. George, Utah, were transferred to the laboratory at U.C.L.A., grown in sand culture in a radioactively-clean, sealed environment and periodically harvested during the growing season. The results for four sampling times where adequate sample mass was available are in Table 7.2. Comparable field values were unavailable but alfalfa harvested at St. George in the previous spring exhibited values of 599 and 330 pCi/kg for ^{137}Cs and ^{90}Sr , respectively. Since remobilization of radionuclides may be different in the field than in sand cultures and because of the possibility of radioactive contamination "on" root-stocks, direct comparison of field and laboratory results may be unwise. However, inspection of the data in Table 7.2 suggests that a rather large fraction of the values observed at harvest could have been remobilized from stores in roots. A review of the literature relative to this point suggests that radionuclide storage in plant roots depends on a myriad of factors, thus any generalized conclusions are premature.

TABLE 7.2. Concentration of ^{137}Cs and ^{90}Sr in Alfalfa Shoots Grown in Sand Culture

Harvest Date	Radionuclide Concentrations (pCi/kg)	
	^{90}Sr	^{137}Cs
5/22	58	--
7/21	90	--
12/4	128	24
7/29	185	39
Mean	115	31

Literature Cited

Thomas, J. M., L. L. Eberhardt, H. A. Hawthorne, and S. D. Zellmer. Radionuclide Cycling in a Utah Dairy Farm. BNWL-1937. Battelle Northwest, Richland, WA. 221 pp, 1976.

Hawthorne, H. A., S. D. Zellmer, L. L. Eberhardt, and J. M. Thomas. $^{137}\text{Cesium}$ Cycling in a Utah Dairy Farm. Health Phys. 30:447-464, 1976.

Bibliography of Quantitative Ecology

As reported last year, the bibliography was substantially enlarged (roughly doubled). This year we were able to merge the computer tapes containing the data, complete checking and editing and prepare final copy for publication. The final volume has been published (A Bibliography on Quantitative Ecology, V. Schultz, L. L. Eberhardt, J. M. Thomas, and M. I. Cochran, Dowden Hutchinson and Ross, Inc., Stroudsburg, PA, 361 pp). We have also prepared a report documenting some of the computer procedures used to prepare the bibliography (An Application of Famulus: A Bibliography of Quantitative Ecology, M. I. Cochran and J. M. Thomas, BNWL-2130, UC-11,

Other Activities

As in past years, a substantial amount of effort on this project goes into consulting and technical service to other projects, and into various program and project reviews. Program and project reviews this year included several written reviews of proposals (ERDA), a site visit, participation in a major review of a NOAA laboratory (part of a National Academy of Sciences review of the agency as a whole). Our assistance was again required on

an ICRP committee, and extensive reviews of several drafts of an NAS document on "Biological Effects of Ionizing Radiation for the Environmental and Safety Program Office." We were again asked to review several epidemiological studies during the year and expect that our involvement may be more extensive if questions relevant to cancer deaths of Hanford employees become more numerous.

On the educational aspect, contacts with several universities continued, with participation on a Ph.D. Committee (Eberhardt) at Colorado State University, a continuing appointment as Affiliate Professor at the University of Washington (Eberhardt), and a request to serve as coordinator for Masters program in general biology jointly with Washington State University and the Joint Center for Graduate Studies in Richland (Thomas).

We have also continued to referee manuscripts for several journals, and two book reviews for journals were prepared and published.

Statistical Design and Analysis for Terrestrial Ecology

The purpose of this effort is to provide statistical design and analysis assistance to scientists engaged in terrestrial ecology programs. An example is the greater use being made of "double sampling" wherein nondestructive measurement of field biomass is combined with traditional harvesting methods. This technique has been shown to be effective in obtaining more reliable estimates of sagebrush biomass at reduced cost over traditional harvesting methods. A description of this application of double sampling will appear shortly (Uresk et al., 1977).

An important aspect of our work is the processing of large amounts of vegetation biomass data from the ALE Reserve and other areas on the Hanford Reservation. These data are given a preliminary statistical analysis involving simple summary statistics and histograms on a routine basis. The data are then stored for possibly more sophisticated statistical treatment at a later time when several years' data are available for analysis.

We are also in the process of redesigning some of the forms used for recording data in the field. These new forms should result in few recording errors as well as reducing the time required to transfer data from field forms to data cards.

Our activities have also involved assisting in the design of revegetation studies of

simulated strip mining areas. This study involves the construction of artificial earth mounds and the planting of various plant species between mounds. Assistance on statistical matters is also provided in the preparation of manuscripts for publication and in preparing manuscripts jointly with members of the Terrestrial Ecology Section.

Biostatistical Aspects of Impact Analyses

Our efforts during the past fiscal year have been devoted to: 1) assessing the use of quantitative methods for impact analyses using actual power plant data, 2) developing methods so we could obtain some preliminary sample size estimates useful for detection of environmental change, and 3) some preliminary work on assessing the statistical power of Goodall's Similarity Measure.

Under the aegis of this program we have investigated the usefulness of the analysis of variance (with suitable data transforms) as a tool to supply an overview of environmental data sets from operating nuclear reactors. We find that when more than one control and treated site are sampled frequently (at least once each nonwinter month) during operational and preoperational periods that such analyses will almost always be extremely unbalanced and very difficult conceptually and computationally to analyze. Even when analyses were done on a large computer, difficulties arose because of computer core size requirements, questions about the validity of F-tests, and decisions relative to mixed and fixed effects models which sometimes must be arbitrarily made. Practical problems arise because of the myriad of interactions present and the fact that some effects are "nested" or observed within another effect, making testing and interpretation the province of a professional statistician. Usually the interest is in detecting the statistical significance of an operational status by location interaction term for the measure of "change" (we must at present refrain from setting change = population impact). Almost all other statistically significant interactions or main effects, while interesting, usually would be expected (i.e., location, time, sites, subsites, operational status and all their interactions) by most field ecologists. Thus, considering all the complexities previously stated, we conclude that: 1) an analysis of variance overview of environmental impact data sets may be expensive, 2) the statistical model will be difficult to construct and implement on a computer, 3) the computer output must be professionally interpreted, and 4) care must be exercised not to lose sight of the datum of real interest, i.e., is there a change attributable to plant operation? As a

research tool, to explore all effects and interactions, this analysis may be more advantageous.

We have proposed (1975 Annual Report) that the ratio of impact measurements of concern in the "impacted" site relative to that of a control site can be a useful measure of change. The statistical analysis involves comparing pre-stress (site/control) to post-stress ratios to assess such change. We have encountered several problems in actual data sets where the "test" was used. First, no attempt (that we could discern) was made by power companies or their consultants to either physically or biologically match the characteristics of "treatment" and control sampling sites in any of three nuclear power station programs we evaluated in detail. Thus, it was very difficult to construct meaningful "after the fact" ratios. Further, in some cases only one control and two "treated" (or the converse) stations were sampled. In such a situation (where meaningful matches were not possible) an average of the two site or control stations may appear in the numerator or denominator of the ratio or two separate ratios may be calculated in which either the treated site(s) or control site(s) appear twice.

A second problem evolved when zero (sometimes unresolved blank) values were reported for the control station, resulting in infinite ratios. As a practical matter we discarded these ratios but this probably results in biased results because these counts are almost always small. Examination of counts from other stations on the same date usually indicated organisms present, so that the blank or zero sample value must have been either lost or mishandled in the laboratory. We have used the ratio procedure to assess some or all of phytoplankton, benthos, chlorophyll-a, and fish seining data from three nuclear plants but did not find statistically significant changes. In general, observed sample sizes ranged from 4 to 10 ratios per year, with either one or two operational and preoperational years observed. Thus, the statistical power of the test may not have been adequate to detect change. However, in the unusual case the observed mean preoperational to operational ratio change was usually so small that its meaning, insofar as future population changes are concerned, may be negligible.

Our efforts have also included several attempts to use nonparametric techniques (to avoid assumptions about the statistical distributions) to statistically analyze time series data from "matched" (in the sense discussed above) control and "treated" stations. We have principally used Wilcoxon's Signed Ranks Test because of our inability

to locate data sets with enough seasonal cycles to employ the methods of time series analysis. In the latter analysis, the assumption of no correlation between successive observations would be unnecessary. The principal drawback, so far, seems to be the lack of sensitivity of nonparametric tests to the size of differences. Our efforts have also included the use of regression analysis where station temperatures progressively decreased with distance from a discharge point. The principal disadvantage to this procedure is that few observations are available to decide if changes are linear or nonlinear in the logarithms of the dependent variable. In addition, the seasonal nature of most organisms results in new or changing relationships (models) for different periods of each year.

At this writing we have specifically tested the applicability of the methods of analysis of variance, ratio analysis ("pseudodesign"), regression and nonparametric analysis as tools to statistically assess data from the three nuclear power plants currently under study (actual data analysis was funded as a separate project by the Nuclear Regulatory Commission). While each quantitative procedure has some theoretical or practical drawbacks or is not applicable to all data sets from a specific power plant, they can be used as reasonable sets of quantitative procedures for evaluation of such data.

In other studies we have developed some preliminary quantitative methods to estimate sample size(s) necessary to detect specified ratio differences for benthic populations. For instance, we calculate that between 6 and 72 samples are necessary ($\alpha = 0.05$, $\beta = 0.20$) to detect a 2.5-fold difference depending on season and specific organisms. Similar calculations based on the analysis of variance suggests between 21 and 33 stations are necessary to detect such differences. We plan to continue some work along these lines in the next fiscal year, probably with emphasis on sample sizes for nonparametric methods. In a small study (conducted by Dr. J. Johnston of the Systems Department) we have calculated the power of Goodall's Similarity Measure as no better than 50%. This brings the use of such procedures as an analytical tool for impact assessment into serious question. Another aspect of our research has involved the use of "stem and leaf" and "hanging histograms" as tools for impact data display.

As part of our inter- and intra-department and Battelle involvement in the broad aspects of impact assessment, we helped organize and present a short course on "Quantitative Procedures in Impact Assessment" at the

Center for Quantitative Studies in Forestry and Fisheries at the University of Washington, April 5-9, 1976. The course was attended by a cross section of people actually involved with the quantitative aspects of impact assessment. In addition, we presented our results in invited seminars at the Savannah River Ecology Laboratory (May) and Washington State University (October). In our investigations of nonparametric methods useful for impact assessment, we became familiar with Scheffe's randomization test. While the procedure proved unusable in that context we were able to apply it to an experiment on the effects of PuO_2 on lung lipid biosynthesis and we have coauthored the resulting paper (Tombropoulos et al., 1976). Finally, we have been invited to extend the observations in Thomas and Eberhardt (1976) at the symposium on "Assessing the Effects of Power Plant Induced Mortality," early in 1977.

Literature Cited

Tombropoulos, E. G.; J. G. Hadley, J. M. Thomas, and D. K. Craig. Biochemical Effects of Inhaled $^{239}\text{PuO}_2$ on Lung Lipids. Health Physics.

Thomas, J. M. and L. L. Eberhardt. Ecological Impact Assessment, pp. 181-197. In: Conference on Computer Support of Environmental Science and Analysis, CONF-750706, Albuquerque, New Mexico, 1976.

Evaluating Simulation Models

We have continued the work outlined in last year's Annual Report and have evaluated the results of two additional simulation models (our own "dairy farm" model and a stream model reported in the literature). In both cases we found that the methods used by statisticians to examine the assumptions underlying both linear and nonlinear regression models (Draper and Smith, Chapter 3) to be extremely useful in visualizing the simulated results by using one or more of the following plots:

- 1) Simple residual (observed-simulated) plots against time.
- 2) Residuals plotted against simulated values.
- 3) Residuals plotted against common times within years or any other pattern suggested by the data.

Some of the relationships suggested by the above graphical depiction appeared to be nonlinear, so we attempted to fit several possible models to the data using a program

available on the newly acquired Biometrics Center PDP-11. The algorithm used did not require partial derivatives as do most such programs available to us. Furthermore, final parameter estimates were not in accord with those obtained by two computer programs we have used in the past. Thus, we have started a subproject to compare some of the commonly used computer algorithms used for nonlinear regression estimation with models used in ecological studies. We hope to be able to recommend particular situations where programs which do not require partials might be both easy to use and "accurate."

Quantitative Aspects of Field Inventory Estimation

This work is covered in Section 4 where it has been applied to plutonium concerns at

the NTS site. Topics covered include the following:

- Validating the use of averages of individual ratios
 $\bar{R}$ versus $\bar{Y} : \bar{X}$
- Improving the presentation of field data sets
 frequency histograms and order statistics $\left\{ \begin{array}{l} \text{versus} \\ \end{array} \right\} \bar{X} \pm \text{S.D.}$
- Estimating spatial pattern with error intervals
 contours weighted for correlation structure "Kriging" $\left\{ \begin{array}{l} \text{versus} \\ \end{array} \right\} \begin{array}{l} \text{contours through} \\ \text{"nearest neighbor"} \\ \text{data points} \end{array}$
- Streamlining the measure of biomass production
 double sampling (harvest and girth) $\left\{ \begin{array}{l} \text{versus} \\ \end{array} \right\} \begin{array}{l} \text{traditional harvesting} \\ \text{of total area} \end{array}$

- **GEOCHEMICAL OCEAN SECTIONS STUDY (GEOSECS)**
- **CHEMISTRY OF OCEAN SOLUTIONS**

Principal Investigators: D. E. Robertson, J. A. Young,
K. H. Abel, E. A. Crecelius, C. L. Wilkerson, W. C. Weimer,
and J. C. Langford

The GEOSECS project is a multi-institutional investigation of the geochemistry and mixing dynamics of the oceans. The objective of this study is to utilize the oceans' chemical and radionuclide constituents to characterize the biogeochemical and physicodynamic processes occurring in the oceans. Battelle-Northwest participation in this study has involved the characterization of a large group of trace elements in the oceans, and the measurement and utilization of cosmic-ray and fallout radionuclides as tracers of aerosol deposition and ocean mixing processes. The radionuclide studies at BNW have now been completed and the program has been directed towards measurement of the distributions of trace metals of geochemical and environmental interest in the oceans utilizing the valuable inventory of well-documented GEOSECS samples. The analyses of sea water from open ocean GEOSECS tracks are being supplemented by trace metal measurements of seawater samples collected on cruises extending from the coastlines of the U.S. to open ocean areas. These data will help define the natural and anthropogenic processes which create concentration gradients between the coastal and open ocean waters.

The second project is designed to determine the air-to-sea transfer of airborne trace metals to the coastal marine environment, the chemical species after dissolution, and subsequent chemical reactions which will affect bioavailability. The validity of ^{7}Be as a tracer for air-sea transfer of trace metals is being investigated presently at Quillayute, Washington. The distribution chemical/physical forms of plutonium in the Pacific Northwest coastal waters and sediments is also being studied to predict the future consequences of plutonium inputs to the coastal ecosystem.

Shipboard Mercury Measurements in Puget Sound and Washington Coastal Waters

Subtle mercury contamination problems encountered during storage of acidified seawater in polyethylene bottles have been recently identified. Because of these problems, many of the early mercury analyses of seawater reported in the literature

appear to be biased by an anomalously high baseline of contamination. We have discovered that these errors can be largely eliminated by conducting highly sensitive cold vapor atomic absorption mercury analyses at sea immediately after the seawater samples have been collected. The shipboard mercury analyses are beginning to produce data which show that the mercury levels in most areas of the

ocean are probably about 2 to 10 times lower than previous studies have indicated.

During the August 1976 R/V Cayuse cruise, in Puget Sound and off the Washington and Oregon coasts, shipboard mercury measurements were conducted immediately after sampling at six stations (Figure 7.2). The sampling and filtration procedures are described in the accompanying paper. The results of the mercury analyses are shown in Table 7.3.

FIGURE 7.2. Sampling Stations--R/V Cayuse, August 1976

TABLE 7.3. Mercury Concentrations in Puget Sound and Washington Coastal Waters

Station	Sampling Depth	Mercury Concentration (ng/l)	
		Unfiltered	Filtered
1	Surface	<4	25
	Near bottom	5	5
2	Surface	13	31
	Near bottom	<4	27
3	Surface	<4; <4	<4
4	Surface	--	<4; <4
	Near bottom	--	<4; 6
12	Surface	5	<4; <4; 6
	Midwater	7	<4; <4
	Near bottom	11	7; 5; <4
13	Surface	--	10; 10
	Midwater	--	<4
	Near bottom	--	7; 10

As shown, the mercury levels in these waters were extremely low. Despite careful precleaning of the filtering apparatus, it is clear that the seawater samples from the first two stations became contaminated during the filtering procedure. After these stations the filtering did not appear to contribute to any contamination. The new Go-Flo® sampling bottles used in collecting the seawater appeared to contribute negligible mercury contamination.

The observed extremely low mercury levels in these waters are of major significance, since this indicates that concentration factors for mercury by marine animals in these waters are at least an order of magnitude higher than original estimates. For example, the 0.1 ppm (wet weight) average mercury concentration in pink shrimp, dungeness crab and lemon sole muscle tissues represents a concentration factor for mercury from seawater of at least 2×10^4 . Thus, potential mercury pollution of these waters could have a greater impact than originally believed.

New Seawater Sampling Techniques for Trace Metal Analyses

Recent GEOSECS data on trace element distributions in the oceans have clearly shown that the actual concentrations of many trace elements in sea water are one to two orders of magnitude lower than previously reported. At the sub-ppb levels at which many trace elements are present in the oceans, one of the most formidable tasks in their measurement is the collection of representative, uncontaminated seawater samples. The collection of uncontaminated sub-surface samples is particularly difficult, because one is frequently faced with using a rusty metal hydrowire and some sort of sampling bottle which has not been designed for collecting seawater for trace metal analyses. Thus, faulty sampling technique is a common cause of unreliable trace metal data.

In August 1976, an oceanographic cruise aboard the R/V Cayuse was conducted in Puget Sound and off the Oregon-Washington Coasts to evaluate the use of three new sampling techniques which appear to greatly minimize the possibility of contamination during seawater sampling. The cruise track and sampling stations are shown in Figure 7.2.

First, we have employed the use of an all plastic hydrowire made from synthetic Kevlar® aramid fibers. The Kevlar fibers are sheathed with a braided nylon jacket to enhance ruggedness. The 0.21 in. diameter Kevlar cable used in this work has a breaking strength of 4500 lb and weighs one-sixth the weight of steel cables of equal breaking strength. One thousand meters of cable was

coiled on the main hydrowinch of the R/V Cayuse after wrapping the drum with plastic sheeting and carefully degreasing and cleaning all points of contact with the cable. The Kevlar cable was then used for all seawater sampling at depth.

Secondly, we used new Go-Flo[®] water sampling bottles which have been designed specifically for collecting seawater for trace metal analyses. These 5 l sampling bottles of all plastic (PVC, nylon and teflon) construction, have no inner springs or closure mechanisms which come in contact with the seawater. All inside surfaces of the bottle which are exposed to the seawater have been teflon coated. The closing mechanism utilizes PVC ball valves on each end of the bottle which are in a closed position when the sampler is lowered through the sea surface. At a depth of approximately 20 m the ball valves are automatically pressure-activated open, and the bottle can then be lowered in a free-flushing configuration to the desired depth. The sampler is then triggered shut when actuated by a messenger which is sent down the cable. The closure device for keeping the bottle closed while lowering it through the sea surface layer prevents contamination from surface slicks or ship effluents from entering the bottle.

The third technique employed for optimum sampling utilized in-line pressure filtration for separation and collection of the particulate matter. After bringing the Go-Flo[®] sampling bottle aboard ship, a polyethylene hose was connected to the outlet spigot and the other end was attached to the inlet of an all-plastic (PVC and polyethylene) custom made 140 mm diameter in-line filtration assembly. The Go-Flo[®] bottle was pressurized with filtered nitrogen gas to force the seawater through 140 mm diameter 0.3 μ pore size nucleopore membrane filters. Thus, the seawater remained in a closed system and was never exposed to the contaminating atmosphere of the ship. The filtered seawater was contained in pre-cleaned polyethylene bottles and was acidified to pH 1.5 with Ultrex[®] hydrochloric acid.

Seawater samples collected 2 m below the surface and at several depths to 800 m were obtained at 30 stations in Puget Sound and off the Oregon-Washington coasts to provide a continuity of trace element distributions between the open ocean GEOSECS stations and the coastal environment in this area. The employment of these new seawater sampling techniques will provide uncontaminated representative samples for obtaining intercomparable data.

Cobalt, Zinc, Silver and Arsenic Distributions in the Atlantic Ocean

The expansion of energy facilities in the U.S., especially along the coastlines, will require research and monitoring programs to describe and predict the environmental quality of the oceans. Several papers have recently appeared in the literature which document or suggest the alteration of trace metal distributions in coastal waters by anthropogenic additions to the ocean.

If man is causing a measureable trace metal impact upon the oceans it is essential that we understand the natural levels of trace metals in the oceans and the natural sources and biogeochemical processes which affect the trace metal distributions and economy in the oceans. Only when quality data of this type are available can we adequately assess the impact of anthropogenically derived trace metals in the marine environment. The GEOSECS program is helping to provide this important information and some of the new data are startling when compared with the present literature.

Figures 7.3 and 7.4 show the geographical and depth distributions of four trace metals of diverse marine biogeochemistries: cobalt, zinc, silver and arsenic. The data plotted in the section of the western Atlantic Ocean shown in Figures 7.3 and 7.4 represents the neutron activation analyses of seawater samples collected during the July to December, 1972 Atlantic GEOSECS expedition in the western basins of the north and south Atlantic Oceans. Because of the very large number of measurements performed, a convenient method for graphically illustrating the data obtained is to represent various concentration ranges by different sized dots.

The cobalt distribution is characterized by rather well-defined concentration maxima at various depths at most stations. There appear to be no systematic depth variations extending from station to station as is seen for the nutrient elements SiO_2 , PO_4 , NO_3 and O_2 . Cobalt concentrations in waters near the Antarctic (Station 74) contained extremely low cobalt levels (5-9 ng/l). The cobalt distribution is not systematically correlated with the nutrient distributions. It is obvious that the biological and geochemical processes which control the distribution of nutrient elements in the oceans do not create similar distributions for cobalt.

The geographical and vertical distribution of zinc in the western Atlantic Ocean is, in many respects, quite similar to that of

FIGURE 7.3. Cobalt Distribution (top figure) and Zinc Distribution (bottom figure) in the Western Basin of the Atlantic Ocean

cobalt. Many of the zinc maxima at various depths correlate with cobalt, but this correlation is not ubiquitous. No systematic geographical or vertical variations extending from station to station were observed.

The silver concentrations in the oceans are extremely low, averaging about 5 ng/l and ranging from 0.4 to 20 ng/l. The geographical and vertical distributions in the western Atlantic appear to be uncorrelated with the nutrient data and the cobalt and zinc distributions. Except for several relatively high concentrations of silver at different depths at Stations 3, 11 and 17

there does not appear to be much variability over much of the ocean section.

The arsenic distribution in the oceans is very homogenous at an average concentration of about 1.6 $\mu\text{g/l}$. This is contrary to earlier reported data which showed up to 15-fold variations in the arsenic concentrations in the Atlantic Ocean. This early work was done colorimetrically and could have reflected interferences from phosphate whose chemistry is reported to be similar to arsenate, the dominant chemical form of arsenic in the oceans. Because of the close similarity between arsenate and phosphate and the fact

FIGURE 7.4. Silver Distribution (top figure) and Arsenic Distribution (bottom figure) in the Western Basin of the Atlantic Ocean

that arsenic is greatly accumulated by many marine organisms, it was thought that the arsenic distribution might be similar to that observed for phosphate. However, this was not the case since the large decreases in phosphate in going from surface waters to deep waters were not accompanied by similar decreases in arsenic.

The trace element distributions of these four metals at GEOSECS stations have greatly increased our knowledge of the ambient levels present in the oceans and have given us a better understanding of their marine biogeochemistries. Data of this type being

generated in the GEOSECS program will be extremely valuable in evaluating the environmental quality of the oceans.

Mercury Distributions at Pacific Ocean GEOSECS Stations

Because of the extreme toxicity of mercury to marine organisms and its ability to accumulate as methyl mercury in certain fish of high commercial value, there is an intense interest in understanding its occurrence, behavior and fate in the oceans. The GEOSECS expeditions have provided an unprecedented opportunity to examine the geographical and

vertical distributions of mercury over large areas of the world's oceans. During the past year we have completed the mercury analyses of all of the Pacific Ocean GEOSECS stations shown in Figure 7.5. The results of these analyses show that most of the Pacific Ocean is characterized by extremely low (5 to 40 ng/l) mercury concentrations. However, as also observed in the western Atlantic Ocean, we have discovered areas in the Pacific Ocean which exhibit extremely elevated mercury levels. The accompanying three figures show mercury distributions along major cross-sections of the Pacific Ocean where unusually high mercury concentrations have been observed at various depths. Figure 7.6 shows the observed mercury distribution along Stations 212 to 224 which transect an east to west ocean section at 30° to 35° north latitude. Most of these waters contained very low mercury levels, but two well-defined mercury maxima were observed at Station 213. The maximum at a depth of about 800 m at Station 213 was observed to extend to Station 212 where it

was found at depths between about 400 to 600 m. The deep mercury maximum at Station 213 peaked out between depths of 3100 to 4500 m with the highest mercury concentration of 3600 ng/l found at a depth of 4250 m.

Figure 7.7 shows the mercury distribution between Stations 241 to 251 which transect a north-south section of the south Pacific Ocean along 179° east longitude. At Station 241 three mercury maxima occurring at depths of about 1600 m, 2500 to 2800 m and from 4000 m to the bottom were observed. The highest mercury concentration (3115 ng/l) was found at a depth of 4137 m. At Station 251 less pronounced mercury maxima were observed at depths of 2530, 2990 and 4330 m.

In Figure 7.8 the mercury distribution between Stations 322 and 343 is presented. This section runs south to north between 122° to 130° west longitude and represents gradually "aging" deep water which flows from south to north. The lowest mercury concentrations in this section were observed

FIGURE 7.5. Pacific Ocean GEOSECS Stations

in the "oldest" deep water between Stations 331 to 343. Mercury maxima were observed in the "younger" waters of Stations 322 and 326 at depths between 1500 to 3200. At Station 320 a well-defined mercury maxima occurred at a depth between 2200 to 2400 m. This maxima extended to Station 317 where the highest mercury concentration occurred at a depth of 2200 m.

An opportunity was afforded at Stations 337 and 343 to compare mercury concentrations in seawater stored in carefully precleaned

FIGURE 7.6. Mercury Distribution in the North Central Pacific Ocean Along 30°-35°N

FIGURE 7.7. Mercury Distribution in the Central Pacific Ocean Along 179°E

FIGURE 7.8. Mercury Distribution in the Eastern Pacific Ocean Along 122°-130°W

glass bottles with the normal GEOSECS storage procedure utilizing acid-leached polyethylene bottles. For mercury measurements in seawater we have now found that it is preferable to use glass bottles for storing the seawater. The mercury concentrations in the seawater stored in the glass bottles usually ranged from <1 to 10 ng/l, compared to mercury levels which ranged from about 10 to 50 ng/l in seawater stored in the polyethylene bottles. If the mercury concentrations in the seawater stored in the glass bottles are accurate, it would imply that a rather consistent baseline of mercury contamination averaging about 20 ng/l and ranging from about 10 to 50 ng/l for the seawater stored in the normal GEOSECS polyethylene bottles had occurred. This low level of mercury contamination would not, however, appear to obviate the observed systematic high mercury maxima observed at many of the GEOSECS stations.

Since the observed mercury maxima are always found at mid-depths or in near bottom waters, it is obvious that manmade pollution is not the source of the elevated mercury levels. The only conceivable mechanism for injecting such large amounts of mercury into the deep waters is submarine volcanism and/or tectonic activity on the sea floor. Recent studies by investigators in Iceland have confirmed that volcanic fallout can increase the mercury levels in ambient seawater receiving this material by two orders of magnitude, and this does not even take into account the volatile mercury released during volcanism that remains in the atmosphere. Recent studies at our laboratory of geothermal gases and waters have shown that these fluids contain many orders of magnitude more mercury than ambient air or "uncontaminated" natural waters.

Development of a Rapid Neutron Activation Procedure for Vanadium in Seawater

There is a growing interest in gaining a better understanding of the marine geochemistry of vanadium. The present interest stems mainly from the fact that vanadium is present in relatively high concentrations in crude oil (1 to 6000 ppm), and the effects of vanadium on marine ecosystems from potential oil spills during shipping and off-shore drilling are largely unknown. Certain marine ascidians and tunicates have an organo-vanadium compound as a respiratory pigment, and the effects on these intertidal and benthic animals from accumulated organo-vanadium compounds present in crude oils could be detrimental. It is, therefore, important to establish natural, baseline levels and distributions of vanadium in the marine environment.

Several determinations of vanadium in seawater have been reported. However, the data are too sparse to determine whether the reported variations in concentrations are real or brought about by biological or geochemical processes, or whether, as seems more probable, they are the result of inaccurate analytical methods. Since most analytical techniques presently available for determining vanadium in seawater were time consuming and had sensitivities near the detection limits it was desirable to develop a procedure which would be rapid, sensitive, accurate and precise.

Neutron activation analysis possesses more than adequate sensitivity and was selected as the method of choice. Vanadium-48 ($t_{1/2} = 16$ days) tracer was produced by proton bombardment of a titanium foil in the University of Washington cyclotron, and carrier-free ^{48}V was radiochemically separated and used as a tracer in the methods development. Because of the large amounts of Na, Cl, Br, Mg, and Ca present in seawater it was necessary to separate and concentrate the vanadium from these interferences prior to the neutron activation. The following procedure was developed:

To 100 ml aliquots of seawater in precleaned polystyrene beakers was added 5 mg of high purity iron solution and 250 μl of phenol red indicator (0.1 gm per 250 ml of 0.001 M NaOH). High purity 1.5 M NH_4OH was then added dropwise while stirring until the first permanent color change from yellow to red occurred at a pH of about 7.8 ± 0.3 . The $\text{Fe}(\text{OH})_3$ precipitates were allowed to settle for about 20 min and the precipitates were then centrifuged and washed 3 times with 0.5 M ammonium acetate containing 1 g/l of Magnifloc.

The precipitates were then transferred to 2/5 dram precleaned polyethylene snap-top vials and were dried slowly under a heat lamp. The vials were then sealed in polyethylene bags and encapsulated in 2 dram polyethylene vials for neutron activation. Vanadium standards (14.2 μg) were prepared by pipetting 10 μl of standard solution onto discs of high purity IPC filter media, drying the discs and encapsulating them in the same manner as the $\text{Fe}(\text{OH})_3$ precipitates. The samples and standards were neutron irradiated at the Washington State University TRIGA reactor, one at a time, standards interspersed between samples, for 2.0 min each. Following the irradiation, the samples and standards were allowed to decay for 2.0 min and were then counted directly on a Ge(Li) diode detector for 10 min to measure the 1434 keV γ -ray of ^{52}V (3.8 min).

The chemical recovery of vanadium for this procedure was $95.6 \pm 1.4\%$, and the precision and accuracy were estimated to be less than 10%. This procedure was used to measure vanadium in 58 seawater samples collected at the surface and near the bottom at 36 stations in the Bering Sea and Gulf of Alaska. The vanadium concentrations in these samples averaged $1.5 \pm 0.2 \mu\text{g/l}$, and ranged from 1.0 to 1.7 $\mu\text{g/l}$. The average procedural blank amounted to $0.11 \pm 0.03 \mu\text{g/l}$. The observed range in concentration in these samples is much less than that reported in the literature (0.2 to 4 $\mu\text{g/l}$). It appears that this neutron activation procedure possesses that required sensitivity, accuracy and precision to show that vanadium is rather homogeneously distributed in the oceans at an average concentration of about 1.5 $\mu\text{g/l}$ --an important conclusion for establishing reliable baseline data for this trace element in the oceans.

Iron-55 and Stable Iron Forms in Oceanic Aerosols

The preferential uptake of ^{55}Fe over stable Fe by many marine organisms and the transfer of this higher $^{55}\text{Fe}/\text{Fe}$ specific activity up the food chain to, and including, man has been well-documented. Since the sole transport vector for the input of ^{55}Fe into the ocean is the atmospheric aerosol, we sought to characterize the chemical forms of both ^{55}Fe and stable Fe on clean atmospheric aerosols and to simulate in the laboratory the release of ^{55}Fe and stable Fe from atmospheric aerosol particles upon contact with sea water. It was demonstrated that the ^{55}Fe in these aerosol samples is present as an amorphous Fe oxide or as very small particles on the surfaces of the bulk atmospheric aerosol particles. These ^{55}Fe forms are more readily solubilized by seawater or chemical solutions than are the stable Fe forms.

A two-step leaching procedure was used to estimate the potential initial release of Fe into sea water and the longer term removal of amorphous Fe oxides and other surface-associated Fe species from the aerosol particles. Aerosol samples were contacted under quiescent conditions with fresh sea water for a period of 24 hr followed by a 4-hr treatment with 0.5% $\text{NH}_2\text{OH}\cdot\text{HCl}$ or 10^{-3} N $\text{H}_2\text{SO}_4\text{-HNO}_3$. These treatments were demonstrated to have little effect on the dissolution of crystalline Fe minerals such as terrestrial dusts.

Atmospheric aerosol particulate materials were collected on glass fiber filters through the use of a high volume pumping system at Quillayute, Washington, a location on the western side of the Olympic Mountains which, under proper meteorological conditions, receives very clean air moving inland from the Pacific Ocean. The material collected during these clean periods contains aerosols which represent essentially atmospheric baseline conditions at 46° north latitude. Large volume "clean" aerosol samples for baseline measurements were also obtained from 71° north latitude at Point Barrow, Alaska. The aerosol samples were treated with the two-step leaching procedure. The ^{55}Fe and stable Fe concentrations were determined in the seawater leach, the chemical solution leach, and the residual total filter sample.

The data for the specific activities in the two leaching fractions and the original total filter sample (sum of the leaching solutions plus the residual filter) of five Quillayute aerosol samples are presented in Figure 7.9. The ^{55}Fe in these aerosol samples was much more chemically available than was the stable Fe, as can be seen from the ^{55}Fe specific activity in the seawater leach and in the chemical leachant data in comparison to the specific activities of the unleached total filter samples. Up to 30% of the total ^{55}Fe present on the Quillayute aerosol samples was released into the seawater and chemical leaching solutions. This compares to a maximum of 5.7% of the total stable Fe released by these treatments. Thus, one would predict that a much larger proportion of ^{55}Fe than stable Fe would be released shortly after contact of the atmospheric aerosols with surface ocean water.

The effects of the seawater and chemical leaching solutions on the two air filters from Point Barrow, Alaska, 71° North, are shown in Figure 7.10. There was a significant release of ^{55}Fe into the seawater leaching solution from both of the Point Barrow filters tested. These solutions contained very high specific activities of ^{55}Fe , 4-19 times greater than the specific activities of the total filters. From 67-97% of the total

FIGURE 7.9. ^{55}Fe Specific Activities in Quillayute Aerosols and Aerosol Leachates

FIGURE 7.10. ^{55}Fe Specific Activities in Point Barrow Aerosols and Aerosol Leachates

^{55}Fe content of these filters was solubilized during the 24-hr contact with sea water; there was little additional ^{55}Fe release into the chemical leachant. This behavior indicates that a rapid release of ^{55}Fe is likely when these atmospheric aerosols contact sea water in nature.

The ^{55}Fe associated with the aerosols from Quillayute, Washington, and Point Barrow, Alaska, was much more readily solubilized than the stable Fe in these aerosols. This high solubility of ^{55}Fe suggests that a large proportion of it is associated with the surface of the aerosol particles. The physical/chemical forms of this ^{55}Fe are likely amorphous Fe oxides or very small particulate species attached to the surfaces of the larger aerosol particles. These are easily dissolved by the $\text{NH}_2\text{OH}\cdot\text{HCl}$ solution or the mixture of dilute acids, while more crystalline Fe forms are not.

These differences in the physical/chemical forms of stable Fe and ^{55}Fe assist in clarifying the phenomenon of preferential marine organism uptake of ^{55}Fe relative to stable Fe. Since marine organisms incorporate soluble forms of elements and since ^{55}Fe is more readily solubilized than the stable Fe in atmospheric aerosols, this soluble ^{55}Fe is more bioavailable than the non-solubilized stable Fe on the aerosols. Much of the stable Fe already present in marine waters exists in a particulate form or an unavailable soluble form; thus, this stable Fe already in the oceans does not effectively dilute the input of available ^{55}Fe . The difference in ^{55}Fe and stable Fe availability underscores the necessity of knowing the chemical and physical forms of all materials in both the source terms and the receiving environments in order to predict potential environmental impacts.

Iron-55 Specific Activities of Surface Water Samples from the Gulf of Alaska

Observations of the decrease in ^{55}Fe with time in salmon have previously suggested that ^{55}Fe is present and biologically available for a short time in the surface waters of the ocean. Measurements of ^{55}Fe in surface waters from the Gulf of Alaska have verified the very short residence time of Fe in these waters.

Surface water samples were collected in the Northeast Pacific Ocean along the coast of British Columbia and into the Gulf of Alaska. The purpose of this collection was to compare the ^{55}Fe specific activities in the seawater samples in early and late summer with the specific activities of atmospheric aerosol samples during the annual early summer fallout maximum. The specific activities of the water samples collected during the spring fallout peak were approximately equal to the specific activities of atmospheric aerosols collected at the same time. The seawater specific activities decreased by more than an order of magnitude by late summer. These data support the theory that the rate of flux of ^{55}Fe through the photic zone of the ocean is quite rapid.

Samples for measurement of stable Fe in sea water were carefully collected with a plastic bucket in a manner to avoid contamination by the ship, acidified to pH 1-2, and stored in polyethylene bottles. Samples for the measurement of ^{55}Fe were collected by sorption on the Al_2O_3 beds by a Battelle large volume water sampler. After transport to the laboratory, the clean seawater samples were analyzed directly for the total Fe which was soluble under the acid conditions. Filters and Al_2O_3 beds from the large volume sampler were analyzed separately for ^{55}Fe .

The filters were ashed with a mixture of HNO_3 and HClO_4 . After these digestions and leachings, the solutions were prepared for radio-Fe analysis.

The ^{55}Fe specific activities for seawater samples collected during May and August 1974, are given in Figure 7.11. The late May sampling corresponds approximately to the time of the annual maximum in atmospheric fallout. The seawater specific activity at this time ranged from 38-70 dpm $^{55}\text{Fe}/\text{mg Fe}$ and averaged approximately 49 dpm $^{55}\text{Fe}/\text{mg Fe}$. This average value compares favorably to the average specific activity of atmospheric aerosols from Quillayute, Washington (approximately 22 dpm $^{55}\text{Fe}/\text{mg Fe}$) during May 1974. These data indicate that atmospheric aerosol input is the major source of Fe to these North Pacific waters.

FIGURE 7.11. ^{55}Fe Specific Activities in Surface Water Samples-1974

The values of the specific activities of the surface water samples collected during late August 1974, are also given in Figure 7.11. These values ranged from 0.7-9.9 dpm $^{55}\text{Fe}/\text{mg Fe}$ and averaged 3.9 dpm $^{55}\text{Fe}/\text{mg Fe}$. (The stable Fe concentrations were approximately constant in all water samples.) Thus, the surface water specific activities decreased by more than an order of magnitude between these two samplings. This decrease corresponds well with the concentrations of radioactive fallout, which decreased by a factor of five during this time.

In this relatively isolated Pacific Ocean water mass, the surface waters appear to reflect the inputs of the atmospheric aerosols very strongly. Since this portion

of the Pacific Ocean is not subject to rapid and massive exchanges of water, the difference in surface water specific activities between these two sampling times also reflects the relatively rapid removal of ^{55}Fe from the surface waters subsequent to its deposition on the atmospheric aerosol. This rapid removal from the surface waters is consistent with the short-term biological availability of ^{55}Fe in sea water previously estimated from: 1) the ^{55}Fe half-time in salmon species and 2) the high concentrations of ^{55}Fe found predominantly in surface-associated organisms. These data provide confirmatory evidence that the ^{55}Fe flux through the photic zone of the ocean is quite rapid and that ^{55}Fe is in a physical/chemical form available for biological incorporation for only a short time after its deposition.

Dissolution Rate in Seawater of Trace Metals from Anthropogenic Dust

Atmospheric fallout of anthropogenic dust is a major source of trace metals to coastal marine waters. The limited data that is now available indicates that this process can be the most important input of trace metals to the coastal environment. A sampling program is now underway to determine the rate of deposition of trace metals near the Washington Coast and to establish the validity of ^7Be as a tracer for this deposition. Of particular interest has been the results on the rates of dissolution of airborne dust collected at several coastal air sampling sites.

Dust collected on air filters and flyash from coal-fired power plants were added to fresh sea water. The rates of dissolution of Cu^{++} , Pb^{++} , Cd^{++} , As^{+3} , and As^{+5} from air filters were rapid. The majority of these trace metals dissolved within 4 hr and entered sea water in, presumably, a biologically available form. The dissolution rates of As, Sb, Se, Co, Cr, Na, Br, La, Eu, and Sr in coal flyash showed great difference between elements. The elements that showed significant solubilities within a day included: As, Sb, Se, Cr, Br, and Sr. The other elements studied (Co, Na, La, and Eu) showed less than 5% dissolution after 1 day of contact with sea water. Flyash samples from different power plants that burned different coals had different solubilities. The elements that dissolve will be available for both organic and inorganic reactions. The elements in flyash that are insoluble will probably be accumulated in coastal sediments.

Aerosol Deposition Velocities on the Pacific and Atlantic Oceans Calculated from ^7Be Measurements

The concentrations of ^7Be in the top 100 or 200 m of the Atlantic and Pacific Oceans

have been measured for the past several years to determine the deposition velocity of aerosol particles on the ocean surface, where the deposition velocity is defined as the flux across ocean surface divided by the atmospheric concentration. Beryllium-7 is produced at a relatively constant rate in the atmosphere by spallation reactions of cosmic rays with atmospheric nitrogen and oxygen. Immediately after formation, ^7Be becomes attached to aerosol particles, and therefore can serve as a tracer of the subsequent behavior of these particles. If the deposition velocity of the aerosol particles can be determined, then the flux across the ocean surface of any material contained in these particles can be calculated from the measured atmospheric concentrations of the material. The fluxes of most materials across the ocean surface are difficult, if not impossible, to measure directly.

Beryllium-7 is an ideal tracer for the determination of aerosol deposition velocities for two reasons. First, it is attached primarily to submicron size particles. At Richland, Washington, 88% of the ^7Be was found to be attached to particles less than 1.1μ in diameter, and less than 1% on particles greater than 7μ in diameter (Young, 1974). Submicron particles are transported to the ocean surface primarily by precipitation and turbulent diffusion rather than by gravitational settling. The rate of deposition of a particulate material whose size distribution contains an appreciable large particle fraction could probably be calculated by adding the gravitational settling velocity to the deposition velocity determined from ^7Be measurements. In the period immediately following a nuclear test, the debris may contain a large fraction of larger particles, especially in the case of a ground level test which ingests considerable material into the fireball. However, gravitational settling removes the large particles and eventually the remaining debris is present primarily on submicron particles which should have the same deposition velocity as ^7Be . Many other anthropogenic pollutants are present in the atmosphere on submicron particles. For example, in St. Louis in 1973, 67% of the lead, 75% of the bromine, 62% of the copper, 69% of the arsenic, and 81% of the sulfates were on particles smaller than 0.3μ (Young et al., 1975).

The second reason that ^7Be is ideal for the determination of particulate deposition rates is that its deposition velocity is relatively easy to measure. Atmospheric and oceanic ^7Be concentrations can be measured without much difficulty. The relatively short 53-day half-life of ^7Be insures that it is confined to the top 100 or 200 m of the ocean and also that the measured ^7Be in the ocean was deposited on the ocean surface within a few months prior to its measurement. Therefore, the ^7Be inventory

Experimental

Results and Discussion

The surface water ^7Be concentrations generally did not change very greatly with time of year. The concentrations at high latitudes were somewhat higher from May through October than from November through April, presumably because of the higher atmospheric ^7Be concentrations and lower rates of vertical diffusion in the ocean from May through October. There are two concentration maximums in the data for May through October which were absent in the November through April data. The concentrations measured close to the west coast of Central America in June 1968 and October 1974 were more than double the concentrations measured at about the same location in March 1973 and February 1974. Also the concentrations

7.21

FIGURE 7.13. Mean Annual Distribution of Precipitation Over the Ocean in cm Per Year (after Drozdov 1953)

measured off the East Coast of the United States at around 30°N from May through October were more than double the concentrations measured in March 1973 and 1974.

Beryllium-7 Inventories. Inventories of ^7Be have been calculated from vertical profiles that have been measured at several locations in the Pacific and Atlantic Oceans during the past several years. However, the number of surface ^7Be concentrations that have been measured is far greater than the number of vertical profiles that have been measured. Since the ^7Be inventory is necessary for the calculation of the deposition velocity, it would be desirable to be able to estimate the inventory from the measured surface concentrations. Therefore, the ratio of the surface concentration to the inventory for the vertical profiles was plotted versus latitude to determine whether the ratio was constant enough to use to calculate inventories from surface concentrations (Figure 7.14). It was found that the ratio was fairly constant for a given latitude and time of year, but that the ratio increased with latitude. Also the ratio was considerably higher during August, September, and October than during the rest of the year, especially at high latitudes. This indicated

that a larger fraction of the total ^7Be in the water column was at the surface during these 3 months, presumably because of the lower rates of vertical diffusion in the ocean.

The ratios of surface concentration to inventory given in Figure 7.14 were used to calculate the inventory for every measured surface concentration and the isopleths of inventory and therefore isopleths of average flux across the ocean surface for the Atlantic and Pacific Oceans plotted in Figure 7.15. The isopleths of inventory are very similar to those of ^7Be surface concentration. The inventory increase with latitude is slightly less than the surface concentration increase.

The inventories shown in Figure 7.15 were integrated over 10 degree latitude bands to obtain average values of the flux across the ocean surface. The ^7Be flux increased from 0.018 atoms $\text{cm}^{-2}\text{sec}^{-1}$ at 0 to 10°S to 0.034 atoms $\text{cm}^{-2}\text{sec}^{-1}$ at 40 to 50°N. The average flux for the Northern hemisphere was 0.027 atoms $\text{cm}^{-2}\text{sec}^{-1}$. This number is probably not significantly greater than the average worldwide rate of deposition on land and ocean surface of 0.022 atoms $\text{cm}^{-2}\text{sec}^{-1}$ estimated by Lal and Peters (1967) from ^7Be production rates.

FIGURE 7.14. Ratio of Surface ^7Be Concentration to the Inventory in the Water Column

FIGURE 7.15. ^7Be Inventory in Disintegrations Per Minute Per cm^2

FIGURE 7.16. Deposition Velocity in cm sec^{-1} for November Through April

Atmospheric ^7Be Concentrations. Atmospheric ^7Be concentrations are necessary for the calculation of ^7Be deposition velocities. Unfortunately, atmospheric concentrations have not been measured at oceanic locations over the extended periods of time necessary to determine seasonal variations and to average the short-term fluctuations resulting from meteorological processes such as precipitation scavenging. However, several investigators have measured ^7Be at continental locations. Health and Safety Laboratory (HASL) has reported ^7Be concentrations from 1970 through 1975 at 28 stations from 82°N to 90°S on its 80th meridian network (HASL-302). Battelle-Northwest has measured ^7Be at Richland, Washington (46°N) and Point Barrow, Alaska (71°N) since 1972 (Thomas 1973). Two-month averages of ^7Be concentrations reported by HASL and Battelle were then plotted as a function of latitude for use in calculating ^7Be deposition velocities.

In order to assess how well the ^7Be concentrations, measured at continental locations, corresponded to the concentrations over the oceans, the ^7Be concentrations measured at ocean stations were compared with the concentrations obtained from the above plots of ^7Be concentration versus latitude and time. Beryllium-7 concentrations were measured by PNL researchers in several hundred air samples collected on a daily basis aboard ships stationed from 7°N to 18°N east of Barbados, British West Indies, during the Barbados Oceanographic and Meteorological Experiment (BOMEX) in 1969. The ^7Be concentrations averaged 90% of the concentrations expected

from the continental measurements. During the GEOSECS experiment 45 air samples were collected at sea on a cruise from 45°N , 177°W to 66°S , 173°W during the period from 23 August 1970 to 19 June 1971. The concentrations in these samples averaged 70% of the concentrations predicted from the continental measurements. The ^7Be concentrations measured in air filters collected from 49°N , 129°W to 10°S , 170°W on another cruise in March 1971 also averaged 70% of the concentrations measured at the continental locations. These measurements suggest that ^7Be concentrations at continental locations average 10 to 30% higher than at oceanic locations, possibly because of vertical mixing caused by the passage of air over the continents. Beryllium-7 concentrations at Richland, Washington have averaged three times those at Quillayute, Washington on the coast of Washington on the other (upwind) side of the Cascade Mountains.

Beryllium-7 Deposition Velocity. The ^7Be deposition velocity was calculated for each oceanic inventory from the atmospheric concentration obtained for that latitude from the plots of atmospheric concentrations versus time of year and latitude derived from the HASL and PNL measurements at continental stations. The average atmospheric concentration for the month preceding and the month during which each seawater sample was collected was used. Isopleths of ^7Be deposition velocity are shown in Figure 7.16. The contours are much the same as the contours of surface concentration and inventory. The high atmospheric ^7Be concentrations at

mid-latitudes tend to depress the mid-latitude deposition velocities. The deposition velocity varied from a low of 0.2 cm sec^{-1} off the west coast of Africa to a high of 2 cm sec^{-1} off the west coast of South America. The average deposition velocity for the northern hemisphere was calculated to be 0.8 cm sec^{-1} . If the ^7Be concentrations measured at the continental locations are 10 to 30% higher than the concentrations over the ocean, then the deposition velocities reported in Figure 7.16 are 10 to 30% too low.

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• IN SITU POLLUTANT STUDY OF THE NEW YORK BIGHT

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This program is for feasibility evaluation, development and application demonstration of instrumental technology for the in situ analysis of the wide spectrum of inorganic, organic, and radionuclide species in oceans and freshwater sediments.

In Situ Parts per Million Analysis of Marine Sediments by X-Ray Fluorescence Techniques

A prototype energy dispersive x-ray fluorescence analyzer has been applied to the in situ elemental analysis of Puget Sound, Washington, marine sediments and Lake Washington-Lake Union, Washington, freshwater sediments. This technique allowed 20 elements (Mg, Fe, Co, Ni, Cu, Hg, Pb, As, Br, Rb, Sr, Y, Zr, Nb, Mo, Ti, C, Cr, Ba) to be quantitatively measured to the 10 ppm level in water depths of 50 ft.

An in situ x-ray fluorescence system was utilized in April 1976, in a study of concentrations of some 20 elements in Lake Washington and Lake Union, (Figures 7.17A and 7.17B) and a connecting ship canal. The analyzer system consists of a solid cryogen-cooled Si(Li) x-ray detector with a 20 mil Be window, a 50 mCi ^{109}Ce excitation source, and an analyzer/ computer system for field data storage and manipulation. This real time data capability allowed variations in elemental concentrations to be pursued by permitting sampling decisions while the data were being obtained. In this study, emphasis was placed on the measurement of Pb concentrations in the freshwater areas and on the measurements of Pb and As concentrations in the marine sampling program (Figure 7.18). The marine sampling area was near a smelter in Puget Sound with the specific pollutant elements of interest being Hg, Pb, As, Nb and Mo. Lead concentrations averaged 70 ppm. Lead concentrations in the harbor varied from 20 to 350 ppm with As varying from less than 13 to 270 ppm. It was interesting to note that a 280 ppm Br concentration was measured,

FIGURE 7.17A. In Field Operation, the Underwater X-ray Fluorescence System was Placed Aboard a Vessel Containing its Portable Electronics. An Analyzer recorded the spectral response of the detector for 5 min after which the sample position was changed by lifting the X-ray fluorescence analyzer to the surface, moving the boat to a new position, and repositioning the analyzer on the sediment surface.

which is above the 65 ppm normally seen in sea water. The system was analyzing colloidal or soluble material that was locked in the harbor built with a slag pile on one side and normal sedimentary material on the other. Elevated concentrations were also determined for Sr and Y. The relationship of other

FIGURE 7.17B. Exploration of the Ocean and Ocean Floor by in Situ X-ray Fluorescence

FIGURE 7.18. In Situ X-ray Fluorescence Spectra of Puget Sound, Washington Sediments

measured elements, such as Mg, Co, Ni, Zn, Br, Rb and Sr, Zr and Fe, provided a fingerprint as to the type of sedimentary material, as well as the quantity of water being viewed in the interstitial matrix.

In West Lake, Washington the in situ collected data show the Pb concentration to be relatively constant at 80 ppm. In the ship canal, concentrations of Pb were obtained which were quite variable ranging from 30 to 370 ppm. Beneath Interstate-5 in the ship canal the Pb concentration was 110 ppm. In the Lake Union area, the Pb concentrations rose from a few hundred to 720 ppm Pb. In all of the sites studied, variations in Fe, Co, Ni, Zn, Tb, Sr, Y and Zr concentrations were obtained indicating that a varying sedimentary matrix was being studied.

Historically, bulk sediment samples are collected to allow determination of pollutant concentrations in the sediments. Grab samples are thus obtained at each marine and freshwater sampling site for later laboratory analysis and thus for comparison to the in situ data. There was consistency between the in situ and laboratory analyses on the samples. The conservative elements such as Br, Rb, Y, and Zr agreed to within twofold. Concentrations of Fe varied up to tenfold; Pb concentrations varied from six- to tenfold to within 25%. In general, it seemed that the surficial sediment contained a different Pb-As concentration than did the bulk material. It should be recognized that the in situ system always analyzes surficial sedimentary material and not bulk matrix which is grab sampled for laboratory analysis.

This study demonstrated the feasibility and practicality of in situ x-ray fluorescence analysis. The system measured major pollutants and elements at levels of economic interest in the top few millimeters of the surface of undisturbed sediment at concentrations down to 10 ppm allowing real time measurements within a freshwater and marine ecosystem. The technology illustrates that sediment analysis is important in mapping the extent of pollution and determining the actual concentrations at the sediment-seawater interface. The technique illustrated that concentration changes vary within a very few feet as transverse segments were observed across the specific sediment area. It also illustrated that the probe monitors different chemical composition not detected by bulk analysis which heretofore have been the only measurement capability utilized in studies of our environment.

Quantification of In Situ X-ray Fluorescence Analysis of Sediments

A method has been developed to measure the total sample mass viewed by our in situ x-ray fluorescence analyzer, from which an estimate of the water content of the sample can be made. This development thus permits a continuous calibration for concentrations in mixtures varying from pure water to highly compacted sediments.

In situ analysis of ocean and lake sediments has recently been demonstrated using a submersible x-ray fluorescence analyzer. Although quantities of some twenty elements were measured, their interpretation was complicated by the variable sample mass viewed by the analyzer (due to x-ray attenuation) and by the variable high water content of the surficial sediment-water mixtures. The estimation of water content will also permit concentrations to be expressed as concentration in the solid matter, thus simplifying interpretations in terms of conventional analytical data.

The continuous calibration method utilizes the coherent and incoherent backscattered x-ray intensities from the ^{109}Cd exciting source to estimate both the mass and bulk composition of the mixture being analyzed. A computer program performs the calibration for each sample by iteratively correcting the fluorescent and scattered x-ray peaks for absorption and correcting the scatter peaks for contributions from the observed element masses. The total sample mass is then determined from the masses of the measured elements plus those of selected representative light elements. The light elements are selected from the corrected coherent/incoherent scatter intensity ratio and their masses are determined from the corrected scatter peak intensities.

The laboratory computer program was modified to utilize a thick sample absorption function and to use the scatter-determined sample mass with measured elemental masses for the part-per-million concentration calculations. The data in Figure 7.19 illustrate the accuracies obtained by this method in several analyses of standard reference materials. All dry sediment or aqueous solution standards were analyzed to within $\pm 15\%$ even without any knowledge of the matrix. Application of the method to 25 in situ sediment analyses yielded estimates of water content ranging from 10-100%, depending on the degree of sediment compaction and the physical placement of the x-ray fluorescence analyzer. The precision of these estimates and interpretation of the data remains to be studied.

*DEFINED AS THE DIFFERENCE BETWEEN THE MEASURED AND STANDARD VALUES DIVIDED BY THE STANDARD VALUE.

FIGURE 7.19. Analysis of Standard Reference Materials as Dry Sediment or Aqueous Solution by the Backscatter Calibration Method

• EFFECTS OF WATER QUALITY ALTERATIONS ON FISH BEHAVIOR

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Objectives of this project are to study behavioral patterns of ecologically or economically valuable fish. Information on sensory--avoidance behavior, or preferential foraging habits, if definitively established by systematic observation can be constructively used in both outfall and water intake design to ameliorate potentially noxious disturbances caused by these structures. The work is applicable to both nuclear and fossil fuel-fired steam electric plants.

In salmon, pressure sensitive radiofrequency transmitters were developed, tested and used to monitor behavior of the returning adult chinook in relation to gas supersaturated water on the lower Snake River. During spring 1976, 60 chinook salmon were monitored between the river mouth and the fourth upstream dam. Results indicate fish spend more than 85% of their time below the critical zone of gas supersaturation.

Twenty-nine white sturgeon also were tagged with radio transmitters to study location, seasonal movement, and behavior of a valued species in a free-flowing stretch of the Columbia River influenced by two hydroelectric dams and thermal loading from a nuclear power plant. Preliminary results suggest all size classes prefer free-flowing areas of the river and are relatively inactive in winter. Most movement occurs in summer.

The instantaneous response of juvenile chinook salmon encountering a simulated river thermal plume interface was also evaluated in a model raceway. Tests indicate that juvenile chinook salmon perceive and avoid discharge temperatures greater than 9-11°C above ambient, regardless of acclimation temperature.

Chlorine is a major chemical compound to reduce biofouling in steam electric power plants. Chlorination of large volumes of cooling waters poses the problem of the formation of chlorination by-products discharged to natural water systems. Long-term bioassays, both fresh and salt water, are underway with indepth analytical chemistry to determine the magnitude of the chlorination by-product problem.

Depth Distribution of Chinook Salmon in Relation to Gas Supersaturated Water

Depth telemetering radiofrequency transmitters and receiver gear developed at the Bioelectronics Laboratory, University of Minnesota (Tester and Siniff, 1976) were used to monitor behavior of returning adult chinook salmon (*Oncorhynchus tshawytscha*) in relation to gas super-saturated water in the lower Snake River, southeastern Washington

(Figure 7.20). Supersaturation of the Columbia and Snake Rivers in the Pacific Northwest routinely occurs during spring runoff when water spilling over hydroelectric dams entrains large volumes of air.

Salmonids exposed to supersaturated water may suffer from gas bubble disease when excess dissolved gases leave solution and form bubbles in fish tissues. The disease can be lethal, usually from vascular blockage

FIGURE 7.20. Columbia and Lower Snake River Study Areas

or hemorrhaging caused by emboli and has been responsible for severe fish losses. Swimming depth is critical for fish in super-saturated water. Gas bubbles form when dissolved gas tension in fish exceeds partial pressure. However, as hydrostatic pressure increases with depth, gas phase partial pressure increases and maintains gases in solution. Each meter of depth affords about 10% compensation. This compensatory phenomenon led to formulation of the critical zone concept, defined as "the portion of upper water where saturation exceeds the tolerance of a particular species." Saturation levels above 115% generally produce gas bubble disease in juvenile and adult salmonids. As long as fish remain below or spend minimal time in the critical zone, gas bubble disease will not occur. However, fish may alter swimming depth and enter the critical zone to move up fish ladders and pass over dams.

Pressure sensitive radio transmitters placed externally or internally permitted in situ monitoring of fish in relation to areas of differing gas saturation. Particular attention was given 24-hr depth distributions, frequency of vertical movements, maximum, minimum and mean swimming depths, and the amount of time spent below the critical zone. Gas saturation levels were measured with a satumeter and compared to similar data collected by other agencies.

During spring 1976, 60 chinook salmon were trapped and anesthetized at Little Goose Dam (Figure 7.20) in cooperation with the

National Marine Fisheries Service. Thirty fish were tagged with dart tags and radio transmitters of separately identifiable frequencies. Thirty control fish were dart-tagged only to evaluate radio tag effects. Of the 30 fish with radio tags, 15 carried external transmitters and 15 had internal transmitters. Fish were measured, weighed and transported 4 miles downriver and released after tagging at Texas Rapids (Figure 7.20). Fish movement in the lower Snake River from the point of release to a fish trap at Lower Granite, the fourth upstream dam, was monitored using truck and boat-mounted receiver gear. Additionally, swimming depth of fish below Little Goose Dam was continuously monitored by an automatic multichannel scanning receiver mounted on the dam. Radiotagged and control fish passing through the fish ladder at Little Goose and Lower Granite Dams were diverted into a fish trap by a device triggered by a magnetometer. This allowed fish recapture, retrieval and recalibration of some radiotags, and provided data on travel time and percent return to each dam for radiotagged versus control fish.

Comparison of fish travel times indicated external radiotags had no effect on upstream movement when fish retained the transmitter. However, chinook salmon initially tagged but which subsequently lost the transmitter moved upstream slower than controls (Table 7.4). The percent external radio-tagged and control fish passing Little Goose or trapped at Lower Granite Dams was similar (Table 7.5). Two external radiotags, one from a fish not

TABLE 7.4. Number and Travel Time of Radiotagged and Control Chinook Salmon Released at Texas Rapids and Trapped at Little Goose Dam and Lower Granite Dam^a

Radiotag Attachment	Number Fish Tagged	Little Goose Dam			Lower Granite Dam		
		Number Fish Trapped	Travel Time (hr) Range	Mean	Number Fish Trapped	Travel Time (hr) Range	Mean
External	15						
Fish retained tag		6	92-553	266	2	102-245	174
Fish lost tag		4	501-1556	933	3	337-651	520
Total		10	92-1556	533	5	102-651	382
Internal	15	0			0		
None (control)	30	19	44-1131	256	10	93-642	254

^aLittle Goose and Lower Granite Dams are 4 and 41 miles, respectively, from Texas Rapids.

TABLE 7.5. Number and Percent Radiotagged and Control Chinook Salmon Released at Texas Rapids and Crossing Little Goose Dam or Trapped at Lower Granite Dam

Radiotag Attachment	Number Fish Tagged	Little Goose Dam		Lower Granite Dam	
		Number Fish Crossing ^a	Percent of Tagged Fish	Number Fish Trapped	Percent of Tagged Fish
External	15				
Fish Retained Tag		7 ^b	47	2 ^c	13
Fish Lost Tag		6	40	3	20
Total		13 ^b	87	5 ^c	33
Internal	15	0	0	0	0
None (control)	30	26	87	10	33

^aSome fish were missed at the Little Goose Dam trap, but trapped at Lower Granite Dam indicating they crossed Little Goose Dam.

^bOne additional tag from a fish not monitored over Little Goose Dam was returned by a fisherman from the Grande Rhonde River, Oregon.

^cTwo additional tags from fish not monitored over Lower Granite Dam were returned by fishermen from the Little Salmon River, Idaho, and the Grande Rhonde River, Oregon.

monitored over Little Goose or Lower Granite Dams and one from a fish not monitored over Lower Granite Dam were returned by fishermen from the Little Salmon River, Idaho, and the Grand Rhonde River, Oregon. Therefore, 7 of 15 fish (47%) initially tagged with external radio transmitters reached Lower Granite Dam compared to 10 of 30 (33%) controls. However, the control tag was smaller and less obvious than the radiotag, and a reward was not offered for returns. Thus, fishermen

recovery was not as likely for control fish. The reduced travel times and lower fish recovery at Lower Granite Dam compared to Little Goose Dam (Tables 7.4 and 7.5) were due to problems at the Lower Granite Dam trapping facility early in the study which precluded recovery of slower fish.

Internally-tagged fish never crossed Little Goose Dam indicating serious retardation of upstream movement. Because of delay

due to tagging, radio transmitter design was modified and tags used during fall 1976 were half the volume and weight of those used previously (Figure 7.21). Tagging and tracking fall run chinook will provide comparable information on fish movements in the absence of gas supersaturation. Fall tracking data are currently being evaluated.

Data on 24-hr depth distribution of spring run chinook salmon was similar for externally

and internally tagged fish (Table 7.6), and indicated fish spent over 85% of their time below 2 m. Based on gas saturation levels (<130%) in the Snake River below Little Goose Dam during spring 1976, fish deeper than 1.5-2 m were below the critical zone most of the time. This information allows a more realistic assessment of the gas supersaturation problem as a challenge to upstream migrant salmonids.

FIGURE 7.21. Radiotransmitters: A) external and B) internal pressure tag used during spring 1976 on adult chinook salmon (*Oncorhynchus tshawytscha*) in the Snake River, C) reduced internal pressure tag used during fall 1976 on adult chinook salmon in the Snake River, and D) external location tag in use on white sturgeon (*Acipenser transmontanus*) in the Columbia River

TABLE 7.6. Depth Distribution of Chinook Salmon Tagged Externally or Internally with Pressure Sensitive Radio-transmitters in the Snake River

Depth	Percent Time at Depth	
	External Tags	Internal Tags
0-1	2.9	2.4
1-2	7.4	9.4
2-3	12.0	17.1
3-4	7.1	12.3
4-5	21.2	10.8
5-6	10.2	11.3
6-7	6.5	4.3
7-8	4.9	4.2
8-9	9.5	7.6
9-10	7.1	2.3
10-11	5.1	2.8
11-12	4.3	4.6
12-13	0.6	8.9
>13	1.1	1.9
Number of Fish	12	14
Number of Depth Recordings	1444	1246

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Tester, J. R. and D. B. Siniff. Vertebrate Behavior and Ecology Progress Report for Period July 1, 1975 - June 30, 1976. C00-1332-123. Prepared for U.S. Energy Research and Development Administration, Contract No. E(11-1)-1332 by University of Minnesota, Minneapolis, MN, 1976.

Seasonal Location, Distribution, and Movement of White Sturgeon in the Hanford Reach of the Columbia River

As part of the dissertation research of a NORCUS doctoral student supported by ERDA, studies began in FY 1976 to determine the seasonal location, distribution, and movement of white sturgeon (*Acipenser transmontanus*), in the Hanford reach of the Columbia River (Figure 7.20). No information exists on white sturgeon movements in the middle Columbia and lower Snake Rivers. The area extending downriver to McNary Dam offers several unique advantages for assessing white sturgeon movements in relation to hydroelectric and thermoelectric power generation. This segment of the Columbia River survives as the last free-flowing stretch above Bonneville Dam. One operating nuclear reactor and a steam generating plant now discharge warm water to the river. Three additional nuclear power plants are under construction or proposed for the future. Baseline data on fish behavior in the area, particularly the more valued commercial and sport species, are essential to provide protection in the face of continued environmental change. White sturgeon may have been anadromous but are now landlocked by a consecutive series of hydroelectric dams in the Columbia River system. The effect of habitat alterations from electric power generation may eventually eliminate the species from the middle Columbia River.

During the past year, 29 white sturgeon were collected near White Bluffs (Figure 7.20) and tagged with different frequency radio transmitters (Figure 7.21) to obtain information on location, distribution, and movement. Nine fish were tagged in the zero to 20 lb size class, 8 in 20-50 lb class, 4 in the 50-100 lb class, and 8 in the 100+ class. Twenty-four of the attached radio transmitters remain functional and are providing valuable information on location and movements of tagged individuals.

Although 62% of all tagged fish remained in the White Bluffs area after release, smaller sturgeon tended to move downriver, larger fish moved upriver, and intermediate-sized fish remained in the White Bluffs area. Preliminary indications are that all size classes prefer free-flowing areas of the river and are relatively inactive in winter. Most sturgeon movement occurs in summer. We hope to locate spawning areas with subsequent monitoring, determine if spawning requires current or slack water, and assess potential impact of continued nuclear power plant siting in the Hanford reach of the Columbia River on long-term maintenance of white sturgeon populations.

Initial Response of Juvenile Chinook Salmon to a Simulated River Thermal Plume Interface

Knowledge of behavioral responses of aquatic organisms in relation to thermal discharges is essential to quantify thermal exposure and understand the effect thermal discharges have on species survival. A model raceway (Figure 7.22) supplied with untreated Columbia River water was used to assess the instantaneous response of juvenile chinook salmon (*Oncorhynchus tshawytscha*) encountering a simulated river, thermal plume interface. Hatchery-reared fish were used for initial tests in January. During May and June, juvenile chinook salmon were collected by beach seine from the Columbia River at Hanford, transported in oxygenated containers to our laboratory and held 1-2 days in flow-through fishways at ambient river temperatures until testing. To initiate a test, groups of 10-15 fish were placed in the upper quarter of the raceway 2 m above the discharge, and retained by a screen (Figure 7.22). The screen was then raised by remote control and the fish were allowed to swim or drift towards the lower end of the raceway. Fish reactions were recorded on videotape for subsequent analysis as they passed over the discharge. Tests were conducted with no discharge (control), ambient temperature discharge (control of effects of current only) and heated discharge. Heated discharge tests included up to 6 different temperature conditions measured with small diameter thermal probes located in various parts of the plume (Figure 7.22).

Four series of tests, 1 with hatchery and 3 with wild fish, were conducted at different ambient raceway temperatures (Table 7.7). After all tests in a series were completed, they were grouped by thermal regime.

Fish response was evaluated from several viewings of the taped records. Six general responses were noted (Table 7.7): 1) fish passed through the plume with no apparent reaction, 2) fish held position in the plume downstream of the discharge orifice for a period of time, 3) fish darted or increased speed, 4) fish twitched, or exhibited a spasmodic movement while passing the discharge orifice, or either 5) some or 6) all fish darted upstream on encountering the plume. Category 5 also includes tests in which fish voluntarily held position in the plume for several minutes before darting upstream.

Although the plume temperature resulting in avoidance increased with acclimation (ambient temperature, the mean Δt of avoidance remained relatively constant at 9-11°C (Table 7.7). In the series of tests at ambient temperatures of 13°C some avoidance

FIGURE 7.22. Schematic of Raceway Showing Discharge Orifice, Video Cameras, Retaining Screen (upper left) and Position of Temperature Probes (lower right)

TABLE 7.6. Behavior of Juvenile Chinook Salmon Drifted Through Simulated Thermal Discharges

Water Temperature (C)					Fish Responses						
Ambient	Point of Discharge	Plume		Mean Δt	Number Tests	Pass Through	Hold in Plume	Dart	Twitch	Some Avoid	All Avoid
		Mean	Range								
Series 1: Hatchery Fish											
5	-	11	10-13	6	1	X	X				
6	-	14	10-17	8	2	X	X				
5	-	16	12-20	11	4	X	X			X	
6	-	19	12-24	13	5		X			X	X
5	-	21	15-25	16	1						X
Series 2: Wild Fish											
12		(No Plume)			2	X					
12	12	(Ambient Plume)			2	X	X				
12	23	16	14-16	4	1	X					
12	29	17	15-19	5	3	X					
12	29	18	16-21	6	10	X			X		
12	27	22	20-23	10	1	X				X	
12	35	24	24-25	12	2				X		X
12	37	26	25-27	14	1				X		X
Series 3: Wild Fish											
13		(No Plume)			2	X					
13	13	(Ambient Plume)			2	X	X				
12	16	13	13-16	1	1	X	X	X			
13	23	15	13-18	2	2	X	X	X			
13	27	16	13-20	3	2	X	X	X	X		
13	25	17	14-21	4	2	X	X		X	X	
13	26	19	16-22	6	2	X			X		
Series 4: Wild Fish											
15		(No Plume)			2	X					
15		(Ambient Plume)			1	X	X				
16	23	21	19-21	5	2	X	X				
15	28	23	23-25	8	2	X	X		X		
	31	25	24-26	9	2						X
	34	27	26-28	11	2						X

was observed at mean Δt 4 but not at 6°C. However, fish that exhibited avoidance appeared to move along the raceway bottom, pass directly over the discharge orifice and intersect the warmest discharge temperature (25°C).

With no discharge, fish passed to the downstream end of the raceway and then moved irregularly up and down along its length. At ambient or low plume temperatures, fish tended to hold for periods in the plume, swam up and down the raceway, but showed minimal thermal response. Fish did not go to the downstream end of the raceway at Δt 's above 9-11°C.

Each separate test utilized native fish. However, fish in each test were allowed several exposures to each thermal regime. After repeated trials at higher plume temperatures, fish would not move downstream or enter the plume, indicating some "learning" had occurred. At some of the highest discharge temperatures (25-37°C), twitch responses or spasmodic contractions of the fish were recorded.

We found no evidence of thermal attraction. Fish behaved similarly and displayed positive rheotaxis or held position in the discharge current with both ambient and low temperature discharges. This suggests juvenile chinook salmon encountering low temperature discharge plumes in nature may orient or hold in discharge currents. Nuclear power plants that discharge water at ambient, or slightly above ambient temperatures, may not be protective if other effluent components such as chemicals fail to enhance avoidance and act in combination to cause mortalities. Discharge of higher temperature effluents may be necessary to cause fish avoidance of lethal conditions.

The Synthesis, Pathways, Effects and Fate of Chlorination By-Products in Freshwater, Estuarine and Marine Environments

This program, funded through the Nuclear Regulatory Commission in September 1976, is designed to further define an information base for the generation and modification of regulations of chlorine as a biocide with regard to potential impacts of chlorination and dechlorination by-products. The study is divided into water chemistry and biological divisions with freshwater and marine or estuarine subdivisions. The water chemistry

division provides analytical support for the freshwater and marine biological studies.

Initial studies of the water chemistry task are to determine differences in organic composition between chlorinated and chlorine-free waters. Subsequently, specific compounds resulting from halogenation of water will be identified. Methodology will be developed for analysis of important halogenated compounds and their metabolites in tissues of exposed organisms. Major emphasis will be placed on saline water systems at first, since the extent of knowledge in this area is limited.

Fresh and saline water samples will be collected from several regions across the United States and chlorinated to provide more information of chlorination by-product formation nationwide and to enhance the comparability of the results.

As information is developed concerning the compounds formed by chlorination, specific experiments will be designed to study the rate and mechanism of formation of the important toxic components and their ultimate fate. Routine analytical methods for chlorination by-product will be developed to support the biological testing programs.

The marine biological task includes chronic and acute exposures of the native littleneck clam, *Protothaca staminea*, to sea water chlorination by-products. This species was chosen as being indicative of invertebrates which cannot physically move out of the region of a plume. Experiments will be conducted involving continuous and intermittent chlorination. Lethal and sublethal effects will be investigated so that in addition to identifying lethal concentrations, such parameters as growth efficiency, tissue and organ changes, structural deformities, toxicant bioaccumulation and biotransformation, will be examined.

Objectives of the freshwater biological studies are to investigate immediate and relatively long-term toxicity and fate of several chlorination by-products and to follow their pathways of action in freshwater fishes. This should elucidate the mechanisms of toxicity and the fate of the chlorination by-products identified. During the latter portion of this year, an investigation will be initiated of the effects of chlorination-dechlorination on the short- and long-term toxicity and the fate of associated by-products.

- **EFFECTS OF THERMAL DISCHARGES ON AQUATIC BIOTA**
- **COMBINED EFFECTS OF WASTE HEAT AND ENVIRONMENTAL FACTORS ACTING IN CONCERT**

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The two projects reported here are closely related. The first was designed to measure thermal injury, latent and manifest, in fish and other organisms important in aquatic food chains. The second makes use of such thermal data--always a variable in any real-world situation--to determine when and how multiple pollutants, including thermal increments, affect fish. A key concept emerging from these studies is that of multiple stressors; i.e., where several pollutants each in itself at "sublethal" concentration act together to cause significant abnormality or death. This is probably the more typical situation where environmental impact becomes manifest.

In current investigations on the thermal effects project, two of the most important environmental factors influencing the metabolic activity of fish are temperature and exercise. Fish respond to environmental stress by exhibiting an elevation of the blood sugar level. The tolerance of the fish to thermal stress is evaluated by measuring the blood glucose levels. The metabolic effects of exercise are determined by measuring the level of lactic acid in the circulating blood. Findings thus far indicate that, within specific limits, rainbow trout are physiologically less responsive to thermal change than might be expected from the literature. This information is important to the designer and operator of thermal power plants. Assessment of cold shock on aquatic organisms was also completed in early 1976, and publication efforts are reported here. Critical thermal maxima (CTM) have also been found useful as a criteria of thermal tolerance. They provide a rapid, relatively sensitive index of response to thermal stress. Reported here also are the results of CTM tests performed on fish which had survived bioassay exposure to chlorine and nickel.

In the second project, the studies of combined effects of multiple chemical and thermal parameters, in a single bioassay represent a relatively new area of research. One of the very interesting findings in this research is a heightened toxicity of Ni caused by rather low concentrations of chlorine. Work is based on flow-through bioassays, using multiple temperatures and chemical species. Rainbow trout, Salmo gairdneri, and coho salmon, Oncorhynchus kisutch were assayed. Sublethal parameters such as growth, bioaccumulation, tissue destruction and changes in critical thermal maxima have been measured in response to the low level, sublethal toxicant dosages.

The objective of these investigations will be to produce information on the interactions of several chemical species, typical of cooling water discharges, and elevated water temperatures in economically and ecologically important aquatic organisms.

The Combined Effects of Nickel, Chlorine and Temperature in Rainbow Trout and Coho Salmon

The utilization of natural waters for cooling purposes has been increasing in recent years. While many industries use water from rivers, lakes and estuaries for cooling, the power generation industry probably uses the largest volume on a site-to-site basis. Continual passage of cooling water through piping, condensers, or cooling towers, often results in an eventual buildup of biological growths, e.g., periphyton, and fouling organisms. If uncontrolled, this fouling can interfere with the efficiency of cooling which is directly related to power output. Common practice to control fouling is to inject chlorine into the cooling water as it is drawn into the plant. The chlorine is then discharged to the environment in the receiving waters. The effects that chlorine may have on resident biota must be evaluated. An assessment must also be made on heat and other chemical additives characteristic of such discharges.

The Combined Effects Program is designed to quantify the combined action of thermal stress and two chemical pollutants, nickel and chlorine, on the physiology of rainbow trout, *Salmo gairdneri*, and coho salmon *Oncorhynchus kisutch*. These chemicals were selected because of chlorine's use as a biocide and the use of nickel in a large percentage of the types of condenser tubing in steam electric stations. The effect of temperature on the toxicity of these two components is being studied due to the thermal component normally associated with effluents from steam electric stations.

The use of the combined effects approach in estimating the toxicity of multiple chemical and thermal parameters is a more realistic approach than a single factor approach in estimating the actual toxicity of effluents which contain multiple toxicants. The objective of the program is to study the interactions and mechanism of toxic action of nickel and chlorine at several water temperatures on coho salmon and rainbow trout.

In addition to the basic nickel chlorine bioassays, the experimental apparatus has been designed and constructed to investigate the difference in toxicity to salmonids when exposed in round aquaria with constant moderate swimming to improve respiration efficiency versus that in standard boxlike aquaria. Toxicity tests will be conducted to compare the mortality in replicate concentrations of

toxicant of fish swimming in round aquaria against a moderate current versus the mortality in standard boxlike aquaria.

Both acute and chronic flow-through bioassays are underway in a modified Mount-Brungs proportional diluter. Sublethal parameters of growth, bioaccumulation and tissue destruction are being measured in response to low-level toxicant dosage. A factorial experimental design including three levels of chlorine and three levels of nickel at different temperatures is being used. This enables statistical evaluation of each effect and the interaction of the combined effect of the toxicants and temperature. Both 96-hr (4 day) and 336-hr (14 day) bioassays are being conducted. Samples of gill, liver, and muscle tissues are being taken to monitor bioaccumulation of nickel tissue destruction.

The initial studies of nickel toxicity indicated a high sensitivity of rainbow trout to nickel. Further tests indicated that this sensitivity was related to the diet of the fish. A change in diet from "New Age"® feed to "Silver Cup" feed paralleled an improvement in overall condition and decreased the sensitivity to nickel. Preliminary physiological indications are that the fish are healthier on the latter diet and thus better able to cope with a chemical stress. The diet studies were not continued to determine the specific elements of the diet which had been lacking beyond monitoring a major change in health and physiological condition.

Ten bioassays have been conducted using rainbow trout or coho salmon. These have been conducted at temperatures ranging from 5°C to 15°C and from 4 days to 2 weeks with the most recent bioassays of a 2-week duration. Statistical evaluation of the bioassays and comparison of differences between bioassays is underway but not completed in light of the recent findings concerning the importance of the bioassay length to the LC₅₀ determination. Studies with both rainbow trout and coho salmon indicate that a 96-hr bioassay does not give a realistic estimate of nickel toxicity. A comparison of results using rainbow trout of a 96-hr (4 day) and a 264-hr (11 day) bioassays (Figures 7.23, 7.24, and 7.25) indicate that in the same range of nickel concentrations a greater percent mortality and thus a lower LC₅₀ occurred when the bioassay is carried beyond

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the standard 96-hr bioassay. Preliminary studies indicate a similar range in sensitivity, and effect of length of the bioassay, with nickel using both rainbow trout and coho juveniles.

FIGURE 7.23. The Percent Mortality of Rainbow Trout in a 96-hr (4 day) Nickel Bioassay with 25 Fish Per Aquarium and 0% Mortality of the Control Fish

FIGURE 7.24. The Percent Mortality of Rainbow Trout in a 264-hr (11 day) Nickel Bioassay with 25 Fish Per Aquarium and 0% Mortality of the Control Fish

FIGURE 7.25. The Time to 100% Mortality at Each Concentration in the 264-hr (11 day) Nickel Bioassay with 25 Fish Per Aquarium and 0% Mortality of the Control Fish

Effect of Sublethal Toxicant Exposures on Thermal Tolerance

Routine bioassays with aquatic organisms and chemical toxicants normally use death as the sole criterion of effect in relation to toxicant concentration and the duration of exposure. Only recently has the existence of possible sublethal stresses been considered among survivors, which would detract from the inherent ability of the organism to cope with other environmental changes.

Sublethal effects can be measured by a variety of physiological responses. However, in relation to development of the nation's energy resources, the effects of a toxicant and temperature acting in concert hold importance. Such interrelations are being examined in bioassays by changing the two variables, toxicant and temperature, to reflect various possible exposure combinations and reveal synergistic actions.

One vital question remains unexplored: "Do sublethal exposures to a potentially lethal toxicant significantly alter the thermal resistance of aquatic organisms, particularly fish?" Consequently, we have adopted the procedure of quantifying the upper thermal tolerance limit of at least some survivors from each toxicant bioassay as a logical extension of our research program.

Results are limited to date, although the basic method has been refined and applied. Our method is a modification of the Critical Thermal Maximum concept where the temperature is gradually, but steadily, increased from the acclimation and/or bioassay level at 6°C/hr. Time and temperature representing loss of equilibrium (LE) and death (D) are recorded, and mean values of control and exposed (toxicant survivors) fish are compared to determine likelihood of an altered upper thermal tolerance limit. "Follow-up" thermal tolerance tests are run 24-48 hr after termination of a toxicant bioassay, and usually involve only 5 control and 5 exposed fish so that LE and D data can be obtained accurately from each test organism by a pair of observers.

Thermal tolerance data obtained from survivors of a 14-day bioassay involving fingerling coho salmon (*Oncorhynchus kisutch*) and nickel at 15°C are given in Table 7.8. At all levels tested (Ni at 4.5, 11.0, 11.2 and 16.0 ppm), resistance to upper lethal temperatures was lower in exposed fish than in unexposed controls. The difference was greatest among the few survivors exposed to 16.0 ppm Ni, where LE and D temperatures were about 3°C below values for control fish. Differences were evident, but probably not statistically significant, between control fish and those previously exposed to Ni at 4.5 ppm.

TABLE 7.8. Thermal Tolerance of Surviving Coho Salmon Fingerlings Exposed for 14 Days to Various Concentrations of Ni, Based on 6°C/hr Temperature Increase from 15°C Acclimation Level (n=5)

Nickel Bioassay			Thermal Tolerance			
Ni Exposure (ppm)	Group	Mortality	Loss of Equilibrium Time (min)	Temp. (°C)	Death Time (min)	Temp. (°C)
4.5 ppm	Test	5.0	136.4	28.6	143.1	29.4
	Control	0.0	140.0	28.9	145.3	29.5
11.0 ppm	Test	25.0	124.2	27.4	132.7	28.3
	Control	0.0	138.7	28.9	145.4	29.6
11.2 ppm	Test	50.0	129.5	27.8	133.6	28.3
	Control	0.0	137.0	28.5	141.6	29.1
16.0 ppm	Test	70.0	104.0	25.4	113.4	26.3
	Control	0.0	134.1	28.4	141.9	29.1

Similar tests should be conducted to detect possible changes in upper thermal tolerance limits of organisms surviving exposure to other toxicants. The effects of sublethal resistance of aquatic organisms is important ecologically. The sum of all environmental factors must be considered in impact assessment.

Factorial Experiments to Test Synergistic Effects

Water concentrations of total residual chlorine (TRCl), at 0.05 ppm and less, ordinarily result in no more than 5% mortality in fish for the temperature, acclimation and other conditions indicated in Table 7.9. Nickel concentrations up to 8.5 ppm are non-toxic under similar observation and holding conditions, but in the presence of TRCl concentrations as low as 0.05 ppm, mortality reaches 95%. This finding might have been anticipated, but it represents a somewhat surprising potentiation of the response of fish to nickel caused by chlorine residuals. The reporting format of the bioassay shows three levels of chlorine alone, at the left, and three levels of nickel alone, across the top. The experiment was factorially designed and involved four combinations of these pollutants, in the lower right cells of the matrix, in addition to the controls. Future studies of how these responses change with different past exposure time intervals and different temperatures.

TABLE 7.9. Mortality in a 96-hr Ni/Cl Bioassay with 20 Rainbow Trout Per Concentration at 8.0°C

		Nickel (ppm)		
		0	4.0	8.5
TRCl (ppm)	<0.001	0%	0%	0%
	0.054	5%	95%	100%
	0.10	100%	100%	100%

TRCl = Total Residual Chlorine

Effects of Water Temperature and Exercise on Blood Glucose and Lactate of Rainbow Trout

A variety of environmental factors are known to influence the metabolic activity of fish and, therefore, their response to stress. Two of the more important factors are water temperature and the level of fish activity. The purpose of this study is to investigate the interrelationships of fatigue and water temperature on fish tolerance to stress. This project is assessing the combined effect of induced swimming and exposure to thermal stress on the performance ability of fish and physiological parameters, blood lactate and glucose. In the 1975 Annual Report, a detailed

description of the experimental design was given. Essentially, it is a multifactorial design using three acclimation temperatures, four test temperatures, and three levels of exercise. All cells of the matrix were completed in 1975 but the data analysis was incomplete. Analyses are completed and the major effort in 1976 was on data interpretation and publications of results.

A paper entitled "A Device for Serial, Nontraumatic, Sampling of Fish Stocks in Physiological Studies" has been completed for publication. This article describes a device which provided the solution to a problem which has interfered with many physiological studies in the past. The parameters measured in these studies are often responsive to a variety of stimuli and special precaution is required to avoid influencing the results by the handling technique alone. The "trap tank" solved this problem and its design and operation are discussed later in this paper.

Rainbow trout were divided into three temperature acclimation groups. The data from the 12°C group has been analyzed and the results will be published soon under the title, "The Effect of Water Temperature and Exercise on Fish Energy Physiology." A third publication discussing the effect of acclimation temperature on fish blood lactate and glucose is in the initial drafting stage.

Changes in the lactate and glucose levels in response to temperature and exercise have revealed some interesting trends. These trends are of special significance when related to thermal discharges. The results have been discussed in the two previous annual reports and will be discussed in detail in the forthcoming publications. Briefly, both blood parameters display an effect described by Prosser as "instantaneous temperature compensation." That is, there was little evidence of a temperature effect over the first 10 degrees above acclimation temperature. One might expect that an increase in temperature would have resulted in an increase in blood glucose and lactate. However, there is no change in the blood levels until the temperature is raised to 15°C above the acclimation state. This is a significant finding. Classical poikilothermic physiology would suggest that any temperature alteration would be reflected in rate functions and other physiological indicators. Our findings reveal no evidence of this, at least within the limits of a 10°C ΔT .

The second trend is that there is a combined effect of temperature and exercise on fish performance. Fish from the 12 and 16°C acclimation groups failed to complete the assigned exercise tasks at the test temperatures $\Delta T = 15^\circ\text{C}$. In the case of the 12°C acclimated group, a combined effect was also noted in the blood glucose.

At the outset of this study, we anticipated that the combined effects of exercise and elevated temperature would be classical muscular fatigue, i.e., exceptionally high blood lactate and associated exhaustion. We did find stress/exhaustion as a combined effect of temperature and effort as measured by blood glucose change and the inability of certain test groups to complete assigned tasks. However, the exhaustion is not muscular fatigue because the blood lactate is the same regardless of the effort and, therefore, is a symptom of the fatigue, although probably not the cause. The literature has been reviewed as a basis for data interpretation, and we now hypothesize that the cause of the fatigue is hypoxia, due to increased metabolic demand from elevated temperature and effort. Both heat and activity increase oxygen consumption, cardiac rate, and opercular rate until the temperature reaches a critical level at which cardiovascular and ventilatory efficiency is reduced, producing hypoxia.

The development of this hypothesis is a significant point of progress, because if proven correct, it will explain the physiological basis for failure of fish stamina under combined stress of swimming and elevated temperature. This information would provide the necessary data base to substantiate engineering decisions regarding allowable discharge temperatures in flowing waters. It is the testing of this hypothesis that we will next address. Should the hypothesis prove correct, it will be useful to the understanding of fish performance capability under physiological stress, particularly stressors other than temperature and exercise which interfere with oxygen uptake and transfer, e.g., chemical toxicants.

To test the hypothesis the next phase of the study will determine the "critical swim speed" of fish from a variety of acclimation temperatures and at various test temperatures. This will require a different exercise system. The annular ring described in previous annual reports is not capable of producing the higher swim velocities necessary for this next phase. In the last year, considerable effort was devoted to the construction of an exercise system. In order to develop data on critical swim speeds, the system must provide accurate control of swim velocities over a wide range, nonturbulent flow and a wide range of temperatures. Several different designs were tested with the final result seen in Figure 7.26. The exerciser is made of 10 in. PVC pipe. It is approximately 5 ft high, 4 ft wide and 11 ft long. It has an 8 ft long transparent PVC section that is the test area for the exercising fish. A control fish will be confined to a 30 in. pond designed to give the same temperature exposure as the experimental fish but not subject it to flow velocity sufficiently strong to stimulate rheotactic response. The circulation of the water in the

FIGURE 7.26. Exercise System to Measure Critical Swim Speeds

loop is by a special pump chosen for its low head, high volume capacity, and the nontoxic materials used in its construction. The pump used a jet principle that delivers large volumes with minimal turbulence. It is powered by an electric motor and moderated with an inline hydraulic transmission enabling control of the pump.

Much attention has been given in the design and construction to minimize turbulence in the test section of the loop. To accomplish this, flow straighteners, in the form of a bundle of stainless steel tubing, have been placed just upstream at the test station.

Effects of Cold Shock on Aquatic Organisms

Assessment of cold shock impact on aquatic organisms were reported in the 1974 and 1975 Annual Reports. This basic work was terminated in early 1976, except for final analysis of data and preparation of material for open literature publication.

One completed phase outlined methods for predicting effects of cold shock on aquatic organisms, based on bioassay data that quantifies the resistance of acclimated test species to gradual declining, or abrupt falling, temperatures. This phase also developed a simplified analytical model for estimating temperature declines in terminated thermal plumes that originate from offshore submerged discharges where current and boundary effects are minimal. The practical application of these data is to assess biotic impact following rapid shutdown of fossil or nuclear-fueled power plants using once-through cooling. The manuscript resulting from this work is entitled, "Predicting Effects of Cold Shock: Modeling the Decline of a Thermal Plume."

The second completed phase was an extensive series of bioassays where resistance to abrupt and gradual temperature decline (cold shock) was determined with pumpkinseed sunfish (Lepomis macrochirus), rainbow trout (Salmo

gairdneri), and crayfish (Pacifastacus leniusculus). Cold resistance depended on original acclimation temperature and varied among species in this order: pumpkinseed < rainbow < crayfish.

The lower median tolerance limits (TLm) were as follows: pumpkinseed, 12.4, 9.5, 4.6, and 2.8°C at acclimation temperatures of 30, 25, 20 and 15°C, respectively; rainbow trout, 3.5, 1.4, and 0.5°C at acclimations of 20, 15 and 10°C respectively; crayfish, 2.5, 0.4, and 0.0°C at acclimations of 25, 20 and 15°C, respectively. TLm values were slightly above the 50% loss of equilibrium values for both fish species, but well below for crayfish. The manuscript resulting from this work is entitled, "Comparative Cold Resistance of Three Columbia River Organisms."

Evaluation of the "Critical Thermal Maximum"

The objective of this program is to critically evaluate the effects of increasing water temperature at different rates on Critical Thermal Maximum (CTM) determinations by using a representative warmwater and coldwater species of fish.

Thermal resistance to higher (or lower) temperatures in fish is usually determined with acclimated test organisms by either abrupt or gradual exposures. Either method is amenable to statistical analysis, and each has its purpose and advocates. However, changing the temperature at a constant rate until loss of equilibrium (LE) and death (D) occurs is more efficient and economical for many purposes. The upper lethal limit determined in this manner is the CTM: "the point where locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that promptly lead to its death."

The CTM is derived from two variables, time and temperature, which operate simultaneously. If different rates of heating are applied to organisms first acclimated to a constant

temperature, CTM values will probably vary. Unfortunately, various investigators have used different rates of heating for CTM determinations. Thus, the results are not strictly comparable.

We are examining the effects of various chemical toxicants, and the combined effects of temperature and these toxicants, on fish and other aquatic organisms in controlled bioassays. Such bioassays, in fact, have become a major part of our overall research program. One practical extension of the bioassay is the use of CTM tests to determine if sublethal exposures to a toxicant significantly alters the thermal resistance of the test species.

However, certain features of the time-temperature relationship of the CTM must first be critically examined. Basic questions to be answered include: 1) What is the most practical rate of temperature increase for determining CTM values? 2) At what lower increase rate does partial compensating acclimation to temperature take place? 3) At what higher increase rates are LE and D values unnecessarily inflated by time? 4) Do warmwater and coldwater fish species exhibit the same response to temperatures increasing at a given or different rate? 5) What is the effect of acclimation level (low or high) on CTM values at different rates of temperature increase? These questions cannot be adequately answered from available literature.

In FY 1977, we initiated short-term CTM studies with a warmwater fish species, the

pumpkinseed sunfish (*Lepomis gibbosus*). Acclimation levels were 10 and 20°C, and temperatures were increased at 1, 6, 18, 30 and 60°C/hr. To accurately monitor LE and D, only four fish were used per test. The results, shown in Figure 7.27, represent responses of 16 fish for each combination of acclimation level and temperature rise.

Preliminary assessment of data leads to the following conclusions. First, increases of 6°C/hr and 18°C/hr are the most reliable for determining CTM. Second, partial acclimation is manifest only at the slowest increase rate of 1°C/hr from a 10°C acclimation level. Third, LE and D values are abnormally inflated by the more rapid increase rates of 30 and 60°C/hr because responses lag behind the rapidly rising temperature. As might be expected, CTM values are higher when derived from 20°C than from 10°C acclimation levels. Also, the span between LE and D value in terms of temperature and time is extended at increase rates of 18°C/hr or more.

The above observations apply only to the pumpkinseed sunfish, a representative warmwater species. Subsequent tests are planned with a representative coldwater fish species to determine any significant differences in thermal response under similar test conditions.

Once temperature-time relationships of CTM determinations are properly assessed, we believe the method will have broad application in quantifying any change in thermal resistance of aquatic organisms previously exposed to sublethal concentrations of various chemical stressors.

FIGURE 7.27. Mean Loss of Equilibrium and Death Temperatures of Pumpkinseed Sunfish Acclimated to 10 and 20°C When Subject to Different Rates of Temperature Increase. ▲, ● = Loss of Equilibrium; △, ○ = Death; Range Shown by Vertical Lines

- **POWER PLANT HEAT AND CHEMICAL EFFLUENT EFFECTS ON SELECTED MARINE AND ESTUARINE COMMUNITIES**
- **IMMEDIATE EFFECTS OF ANTI-FOULING BIOCIDES FROM COASTAL AND OFFSHORE POWER PLANTS**
- **EFFECTS OF THERMAL DISCHARGES TO COASTAL WATERS**

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This section of the report covers research on the effects of energy-related effluents on marine coastal organisms and communities. The work from the listed 189s is combined under one title because of the interrelationships and dependence of the programs. The goal of this research on the effects of energy effluents on marine coastal organisms and communities is to: 1) assess the immediate impact of an energy technology; 2) make predictions about the long-term impacts and ecological consequences; and, 3) provide background on the biological processes needed to understand biological availability and fate of energy effluent materials.

The information resulting from this research is needed for the design and siting of existing energy technologies, i.e., nuclear and fossil fuel steam electric generation stations, gas liquefaction plants, and for new technology such as the Ocean Thermal Energy Conversion concept.

During the past year, research has been continued on the effects of temperature primarily as a cofactor with other effluent materials, copper and biocides. The effects of these materials have been studied alone and in combination for periods of time ranging from 96 hr to 6 months.

The Effects of Cooling System Effluents on Marine Coastal Organisms

One of the basic concerns in the development and use of an energy technology is its effect on the environment. This concern has caused considerable controversy for those technologies that use cooling systems for the disposal of waste heat in the aquatic ecosystem.

For the coastal zone, energy-related activities have been a relatively recent phenomenon. Until the early 1960s, there were very few thermal electric stations using

marine waters for cooling purposes; oil and gas movement was limited and consisted, primarily, of piping offshore oil in the Gulf region; and other energy-related technologies were not being emphasized. Since that time, increased energy requirements have necessitated the use of the coastal zone for the siting of an increasing proportion of these activities, including steam electric generating stations, gas liquefaction plants, and oil recovery, transport and transfer facilities. Ocean Thermal Energy Conversion (OTEC) will be developed and sited in the open ocean, but the current research on heat exchangers will be useful in its development.

Unfortunately, research to date has not provided the information needed to understand coastal and nearshore processes, much less the effects of a particular effluent. However, in order to forecast the ecological consequences of a technology, there must be an understanding of: 1) the biogeochemical processes in the natural system, 2) the manner in which technology affects these processes; and, 3) how the processes affect the system as a whole.

The research discussed under this section deals with short- and long-term effects of heat, copper and chlorine. Our research effort on the basic coastal processes and bioavailability of energy-related effluents is discussed in the section entitled "Biological Availability of Energy Related Effluent Material in Coastal Ecosystems."

Waste heat, the major effluent component, has lost some importance as a potentially serious detriment to the environment, because research has provided an understanding of its biological effects, and engineering technology has reduced these effects. However, heat is still a potential compounding factor in the effects from other effluent materials (metals, biocides, residual biocide by-products) and other factors (pressure, turbulence) associated with the operation of a cooling system. In this program, heat is being studied primarily in combination with other effluent materials; however, tolerance work has been required to provide the data needed to initiate the combined effects studies. A manuscript is being prepared on the thermal tolerance work that has been completed.

A study was completed on the interaction of temperature and salinity on the hatching success of eggs of the sand sole, Psettichthys melanostictus, a commercially important flatfish. The thermal conditions of the test were similar to those an egg would experience upon being pumped through a cooling system. The results indicate that there was little or no effect on hatching success at temperature increases from ambient (8.5°C) to 25°C, at salinities from 24 to 30 ppt. However, at salinities of 12 and 18 ppt, there was significant mortality at temperature increases from 8.5°C to 22 and 25°C.

In marine-sited cooling systems, 90-10 copper-nickel alloys have been the primary material used for heat exchangers. This alloy is chosen because of its resistance to corrosion in the marine environment; however, it does erode or dissolve slowly, and the heat exchanger tubes have to be replaced periodically. There is considerable concern about the effects and fate of the copper and nickel released by this process. Because copper is the most abundant metal in the alloy, its occurrence in large quantities (naturally in

marine sediments), its demonstrated uptake by oysters, and its known toxicity at high concentrations, research has been undertaken to determine the effects of long-term exposure to copper of selected marine coastal organisms. These studies are an integral part of the bioavailability studies and chemical programs discussed elsewhere.

The coon stripe shrimp, Pandalus danae, and the sabellid polychaete, Eudistylia sp. are two animals currently being studied. Pandalus contains hemocyanin, a copper based, oxygen transporting respiratory pigment, in its plasma and is thought to be a copper regulator. Eudistylia, on the other hand, uses chlorocruorin, an iron-containing pigment structurally similar to hemoglobin, for oxygen transport and is considered a copper conformer.

Pandalus is being used for a detailed study of the effects of short and long-term exposures to copper at the ppb level. The concentrations are similar to those found in the waters near existing industrial/metropolitan areas. To date, we have established: 1) excess copper is deposited in the hepatopancreas, gills, exoskeleton, and other parts of the shrimp, but not in the skeletal muscle; 2) copper is regulated primarily through the moult, but also through the gut; 3) all tissues except the hepatopancreas return to baseline levels during recovery from copper exposure. This indicates that copper accumulated by the hepatopancreas is eliminated slowly or, possibly, that accumulation is nonreversible; 4) excess copper accumulation causes tissue necrosis in the gills at levels down to 20 parts per billion (ppb); 5) the 96-hr LC₅₀ for Cu⁺⁺ is about 70 ppb; and 6) primary sites of copper absorption are the gills and the gut.

One problem in determining the accumulation rate and threshold concentration for copper has been the variability of tissue copper concentrations found in animals from the same exposure units. It is felt that this variability is the result of copper being eliminated through the moult and, thus, the content of the various tissues is dependent on where, in the ecdysis cycle, a shrimp is. To eliminate this variance, a system is now being used whereby the moult cycle of each shrimp exposed to copper can be followed.

Eudistylia, the first to be tested in an array of species that would be present in a fouling community, is being studied for its ability to regulate copper, the threshold concentration above which it begins to accumulate copper, its rate of copper uptake, and any behavioral or toxicological responses that occur. This research is a prelude to examining the combined effects of cooling system effluent chemicals and heat on fouling communities.

To date, we have established the following:

- 1) 4 days exposure to 400-500 ppb copper is lethal to worms that have been extracted from their parchment-like tubes, but not necessarily to those that remain in their tubes, (death was difficult to define, though severe tissue necrosis of gills and body is evident);
- 2) concentrations down to 80 ppb will cause gill necrosis within 1 week;
- 3) Eudistylia will sense and avoid copper at levels below 10 ppb by more frequently withdrawing into its tube;
- 5) there is no relationship between the size of an animal and the amount of copper it accumulates in a given concentration over a given duration of exposure; and,
- 6) copper accumulation is greater but more variable in the gills than in the body. We are interested next in finding if copper is accumulated at levels between 1 and 10 ppb, and whether Eudistylia has the ability to regulate copper.

There is the problem of preventing growths of fouling organisms in cooling water systems (e.g., intake structures, heat exchanger tubing, cooling towers, and effluent piping). Bacterial slimes, periphyton assemblages, and macroattached organisms such as mussels and barnacles attack the surfaces and create water flow and heat transfer problems. These problems are significant but controllable in coastal electrical generating stations by use of appropriate chemical or mechanical cleaning methods. Presently, the use of the chemical biocide, chlorine, is the most widely used method. However, this means is becoming more costly, and the effects of chlorination have potential environmental consequences that need to be defined. One problem in developing an understanding of the effects of chlorination on nontarget organisms is that the time-dose patterns of its usage are by no means standardized from site to site. They range from continuous chlorination at low concentrations to brief, relatively high concentrations introduced periodically.

The acute biocidal properties of chlorine dissipate relatively soon after entering the receiving water body through physical, chemical and biological processes which result in the formation of chlorides and, potentially, numerous other halogenated (and nonhalogenated) compounds. Neither the synthesis, pathways, effect or fate of these chlorination by-products, nor the effects of other effluent components upon them have been identified. In fact, only since the recent revelation of similar problems in freshwater systems, has the instrumentation required to attack these complex problems been allocated to investigating their probable impact in seawater ecosystems.

This past year's investigations have been concentrated on acute and chronic effects of chlorinated seawater on coastal species and an

understanding of the mechanisms involved in producing these effects.

Results of acute lethality tests (96-hr LC_{50}) with 20 species of juvenile seawater fishes, crustaceans and clams indicate that coho, chinook and pink salmon, herring, English sole, shiner perch, sand lance, and a shrimp (Pandalus goniurus) are the most sensitive group. All have a 96-hr LC_{50} (derived from 4-6 experiments per species) of less than 0.1 mg/l total residual oxidant (TRO). An intermediate sensitivity group (96-hr LC_{50} from 0.1 to 0.2 mg/l) included a shrimp (Crago sp.), an amphipod (Anonyx nugax), a mysid (Neomysis sp.), threespine stickleback, and coon stripe shrimp. Two species were relatively resistant with 96-hr LC_{50} values greater than 0.5 mg/l: an amphipod (Pontogenia sp.), and a crab (Hemigrapsis sp.). Clams could not be tested by standard 96-hr procedures because of their ability to close their valves and stop pumping for significant periods of time; however, mortality resulting from extended periods of exposure suggested that the order of sensitivity, from most sensitive to most resistant, was: gaper (Tresus capox), bentnose (Macoma nasuta), cockle (Clinocardium nuttallii), butter (Saxidomus giganteus), and littleneck (Protothaca staminea).

By using chronic exposures, we have found that shiner perch juveniles, released by females during exposure to chlorinated seawater: 1) appeared to be more resistant than the young-carrying females, 2) exhibited 100% mortality after 10-12 weeks at 0.08 mg/l TRO and above, 3) grew as well at 0.4 mg/l TRO as the controls during the 5-month exposure, but 4) were characterized by a malformation of the mouth, eye and opercular cover on one side of the head at all levels of chlorination (the lowest being 0.02 mg/l TRO), which obviously affected feeding efficiency. This defect was also observed in all individuals which died at higher chlorination concentration, but in none of the controls.

Up to 30-day exposures of coon stripe shrimp indicate that the growth-effect concentration was 0.08 mg/l TRO. Another series of experiments identified a delay in the hatching process of near full-term eggs, and at 0.16 mg/l TRO, eggs did not hatch until transferred to unchlorinated water.

In the study of the effects of chlorination of seawater on marine organisms, a number of questions have arisen about the chemistry of chlorine in seawater. Because of the complex reaction between chlorine and the various components of seawater such as iodine, bromine, ammonia and dissolved organics, it is not possible to predict the chemical makeup of the solution to which an organism is being

exposed. To further complicate the matter, the concentration of two compounds that are reactive with chlorine (ammonia and organics), vary from area to area, depending on natural and man-influenced conditions. Thus, in order to relate the research conducted on the effects of chlorine to real-world problems, it will first be necessary to determine how the composition of sea water affects the reaction of chlorine, and what the residuals are under defined conditions, i.e., salinity, temperature, pH, ammonia concentration, dissolved oxygen, and total dissolved organics. Studies to characterize the reactions which determine the components of the total residual oxidant category, and resulting halogenated products were initiated late this year. These latter studies are directed at determining the halogenated organics formed by extraction directly from the water through the use of column filtration and by analysis of bivalves that are known to concentrate halogenated organics and have been exposed to chlorinated seawater for long periods of time (3 to 6 months). Work on the inorganic reactions by the use of radiotracers is being initiated to determine if it is chlorine, bromine, iodine, or a combination that is causing the biological effects.

The determination of toxicity of exposure to a single contaminant will not allow direct relation to the natural environment where an organism will be experiencing exposure to multiple contaminants. Therefore, after the

necessary research has been conducted to determine the toxicity of particular contaminants, it is necessary to test them in combination to determine if their actions will be additive, antagonistic or synergistic. Additive means the toxicity is equal to the sum of the effects observed when the stressor components were studied individually. Synergistic means the observed effects are greater than would be predicted if the toxicants were acting in an additive manner, and antagonistic effects would be of a lesser degree than additive.

In one series of 96-hr continuous-flow experiments in which coon stripe shrimp were exposed to chlorine and thermal stress, the shrimp, previously acclimated at 8°C, were subjected to greater ΔT increases, their tolerance of chlorinated seawater determined by comparing LC_{50} values, decreased.

Another series of short-term lethality exposures including combinations of copper, chlorinated seawater and altered temperature has been initiated, and the preliminary exposures revealed an interaction between copper and chlorine toxicity. The extent and form of this interaction will be investigated through a matrix of 27 combinations of the three variables. Following these short-term tests, the most critical combinations of the three stressors will be employed at sublethal levels in chronic exposures.

• THE BIOLOGICAL AVAILABILITY OF ENERGY RELATED EFFLUENT MATERIAL IN THE COASTAL ECOSYSTEM

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In order to make the predictions necessary to forecast the ecological consequences of an energy-related technology, there must be an understanding of: 1) the biogeochemical processes involved in the natural system, 2) the manner in which an energy technology affects these processes and how, in turn, this affects the ecosystem as a whole.

Direct biological effects such as lethality, behavioral changes, and physiological changes, are being studied under the program previously discussed. The biological availability and impact studies are investigating: 1) the chemical, physical, and biological processes that occur in the "natural" marine ecosystem; 2) how energy effluents affect these processes, and 3) the factors involved in regulating the bioavailability of effluent material. This past year's effort has centered on defining the quantities and forms of metals and radioisotopes in nuclear power plant effluent streams, the chemical forms present in bioassay systems, the chemical and microbial processes controlling the forms of metals available from the sediments, and the uptake and control of copper in shrimp. In addition, several sites in Sequim Bay have been monitored for potential use in field verification studies.

1976 Studies

The continued increase in demand for energy by our country and the developing world will require the siting of energy facilities, i.e., nuclear power plants, gas liquefaction plants, coal gasification plants and fossil fuel power plants in the coastal zone where sufficient water is available for the efficient and safe disposal of waste heat and chemical effluents. Most sites will have facilities designed with an effective life of at least 30 to 40 yr with new facilities added periodically, so that in effect, any one site can be expected to be in use for 100 yr or more.

This long-term use of a single site will result in sections of the coastal ecosystem receiving a continuous input of effluents from these and other industrial activities. Present regulatory guidelines limit effluent

material to quantities not considered harmful in the short term. However, the impact of the components of these effluents, whether as thermal increments, trace metals, biocides or artificial radionuclides, over the long-term have not been clearly defined. The sources, inventories, and distributions of these materials are incomplete, and many of the physiochemical-biological processes that control their movement, distribution, biological availability, and toxicity are poorly understood.

Our research program is directed at understanding the physical, chemical and biological behavior of the pollutants and their potential effect on the marine coastal ecosystem. The program has three major objectives: 1) increase our understanding of abiotic and biotic factors which influence physical, chemical and biological movement and accumulation of coastal and offshore energy-related effluents, 2) develop

an insight as to how the effluents may affect the coastal marine community, and, 3) develop realistic environmental criteria for marine-based, energy-related facility discharges.

The research effort is a multidisciplinary one that includes scientists trained in the physical, chemical and biological aspects of the marine environment. Their studies are centered at the Marine Research Laboratory in Sequim, Washington. Staff and resources from the Battelle-Northwest Laboratories, Richland, Washington are included in many aspects of the investigation. The research conducted during the past year has been concerned with the four major segments of the overall picture that we identified earlier. These are the identification and quantification of the effluent materials as they enter the marine coastal ecosystem, the physical transport and dispersion of the material from its source, the chemical interactions between the water and sediment compartments in the coastal ecosystem, and the biological availability of the contaminants to coastal organisms and communities. The latter segment is closely related to the Effects studies that are discussed in the preceding section.

Source Term Identification

Analytical developments and regulatory actions over the past decade have allowed us to develop better estimates of the total amounts of effluent material that are entering the coastal system from a given source. Unfortunately, many of the estimates are based on limited samples or calculations made from laboratory tests. Actual measurements where the material enters the coastal ecosystem are few, and form of the contaminant is generally not known. This latter factor is very important because of the demonstrated influence of chemical form on bioavailability. An example of this is the greater toxicity of hexavalent chromium as compared to the trivalent form.

During the past year, part of our research effort has been to: 1) define the quantities and chemical forms of metals and radioisotopes in the effluent stream of a coastal-sited nuclear fueled stream electric generating station; 2) define the chemical behavior of metals in laboratory test systems intended to simulate the real world; 3) obtain a clearer definition of the ambient concentrations and forms of metals in sea water used in our biological experiments; and 4) determine the role of mixing in the fate of sediment contained pollutants.

The work at the coastal steam electric generating stations has provided us with information on the quantities and form of materials to be expected in the coastal

environment as the result of future development. These data will be used to design experiments on the biological availability of these materials and determine their ultimate fate and consequences to the coastal ecosystem. Results of sampling at four different times in the nuclear fuel cycle show that the radioisotopes being released are at extremely low levels ($<1\gamma$ dpm/l after dilution of radioactive waste by tertiary coolant), that several different physicochemical forms are present at the point of release (particulates, cations, anions and nonionic species), and that composition of the released radioactive wastes varies significantly. Detailed examination of particulate species has shown significant variability with time in the radioactivity in four distinct sizes, ranging from $>8\mu$ to 0.2μ . Further work with radioactive waste particulates ($>0.4\mu$) shows that there is a slow release of the radionuclides with time after seawater contact is initiated.

Work in the next year will examine waste materials generated during decontamination operations in the reactor. Because of the presence of complexing agents such as detergents, these wastes are not treated by the radioactive waste system, but released directly into tertiary coolant at very low levels. The fact that radionuclides are presented to the environment in a complexed soluble form different from other waste may alter environmental pathways.

In the past, laboratory experiments on the toxicity of contaminants have not taken into account the chemical form to which an organism is exposed. Classically, the contaminant is added stoichiometrically, and toxicity is based on calculated concentration, or a measure is made of total contaminant in the test system. Recent work has shown that the chemical form is important, and that certain elements begin changing form as soon as they enter seawater and continue to change as the physical, chemical and biological conditions change. Because of this, we have been developing methods that will allow us to obtain real time data on the form and quantities of a toxicant in our test systems. Our primary efforts have been with copper. As a result of this work, we routinely measure the more toxic form of copper (Cu^{++}) in our tests by specific ion electrodes when working at exposure levels greater than 50 ppb, and by anodic stripping voltammetry (ASV) for those in the 50 to 10 ppb range. For concentrations below 10 ppb, we use an ASV system but with a glassy carbon electrode that enables us to measure ionic copper down to the ng/l level in sea water.

With this latter system, we have been able to determine that the copper levels in our ambient seawater are in the 40 to 60 ng/l range, and that copper bound to oxidizable

materials (UV and ozone oxidation) is in the 100 to 200 ng/l concentration range. These levels are about 2 orders of magnitude below the level where we get effects in 30 days. This information provides valuable data in our effort to define all of the factors affecting a particular experiment.

Sediment-Water Interactions

A large portion of the contaminants introduced to the aquatic system ultimately becomes incorporated into the sediments. Because sediments may potentially serve as either a source or a sink for man-produced contaminants, the chemical interactions between the water, biota and sediments are being investigated.

The biogeochemistry of copper in coastal sediments is being investigated initially because of its potential as a pollutant and its relatively high natural occurrence in marine sediments. A literature review of the chemistry of copper in the marine environment was conducted to provide a perspective of current knowledge and to identify areas requiring further study. This review is in final draft and will be published during FY 1977. Research to establish the baseline conditions in Sequim Bay is being undertaken to provide data for a well-characterized system for future verification of laboratory research.

The Cu concentration in the Sequim Bay sediments is about 48 µg/g; while soils from the watershed of the bay contain about 36 µg/g. In areas of Sequim Bay having fine sediment with high organic carbon (C) content, Cu levels in the top 2 cm are about 4 times greater than in sediment from 2-30 cm below the surface. The accumulation of Cu at the sediment:water interface may be attributed to diagenetic processes by which Cu is solubilized in the anoxic zone or complexed by organic matter and diffused to the surface.

The dominant constituents of sediments controlling uptake and release of Cu appear to be organic matter and free hydrous oxides of Mn and Fe. Between 40 and 60% of Cu present in Sequim Bay sediments is associated with reductant-soluble materials, likely Mn and Fe hydrous oxides, and 8-22% is released upon oxidation of organic matter. Most of the remaining Cu is associated with mineral constituents of the sediment. The development of anoxic conditions will likely enhance the availability of Cu to marine organisms by affecting release of Cu that has coprecipitated with hydrous oxides.

Although as much as 35 µg Cu/l were observed in interstitial waters of Sequim Bay sediments, only about 3 µg/l of ionic Cu could be measured at pH 4.9 using anodic

stripping voltammetry. Most of the Cu appears to be associated with organic ligands. Complexation of Cu by soluble organic materials may enhance diffusion of the metal from the sediment and its entry into the food web by preventing adsorption by hydrous oxides or clays or precipitation as the sulfide.

Transport with suspended particulates is a mechanism by which contaminants from energy facilities may be moved from their source to be dispersed in the far field or perhaps be accumulated in local sediments. Sequim Bay may serve as an illustration of the latter phenomenon. During mid-summer, hourly sampling of suspended matter concentration in the tidal channel was conducted for several diurnal tidal cycles. Analysis of trace metals in the suspended particulates by x-ray fluorescence showed that an estimated mean of 2150 g Cu/day was transported into Sequim Bay by suspended matter during flood tide, and 1650 g Cu/day was removed during ebb tide. The accumulation of Cu appears to exceed the accumulation of suspended material. Transport of suspended particulates was only 10% greater in the flood tides, but introduction of Cu to the bay exceeded the loss by 30%. The flux of Cu in Sequim Bay appears to be little affected by input from freshwater. The exchange of seawater in the bay between mean high and mean low tide is about 35×10^9 l; whereas, surface freshwater flow to the bay is only about $45-60 \times 10^6$ l/day during the summer. Although Cu levels in some stream waters are higher than in water in the bay, the total daily freshwater input of Cu is only about 1% of the amount exchanged during the tidal cycle.

Microbial activity in sediment has an important effect on the transformations of contaminants, but the extent to which this occurs in the marine environment is unknown. The literature indicates that most of the work to date has dealt with identification and isolation of marine bacteria and fungi, but the determination of representative numbers and the role of marine microbes in transformation processes have received little attention. Conventional methods such as pour plating and spread plating are not applicable for marine microbes because a large percentage are motile and psychrophilic. For this reason, preliminary studies to develop plating and incubation techniques have been undertaken prior to initiating investigations of the effects of trace metals on marine microbiota and the role of these organisms in influencing the distribution and form of soluble trace metals in marine sediments.

Biological Availability

The biological availability of an effluent material or materials is the critical factor in determining the consequences to the ecosystem.

However, before we can adequately determine bioavailability, we must understand the processes that are occurring in the natural system, and how these relate to our experimental systems and the biological response. Research related to these two areas is discussed in this section, and under the section, "Effects of Cooling System Effluents on Marine Coastal Organisms and Communities."

Research during the past year has centered on defining the conditions in the test systems being used and the processes by which the contaminants effect the organism. Of particular interest is the work with Cu and shrimp discussed in the earlier "Effects" section. We have found that Cu is toxic at levels of 10 ppb when available in the ionic form in water, while it does not appear to cause any effects when present in food at concentrations of 10,000 ppb in an apparently unavailable form. The entry route of the ionic Cu is via the gills and gut. It is then concentrated in the gill, exoskeleton and hepatopancreas tissue, and causes necrosis in the gills. However, during the moult process, the shrimp is able to eliminate a large portion of the Cu burden via the discarded exoskeleton, and on movement to clean water, all tissues quickly return to background levels except for the hepatopancreas. Thus, the shrimp is a probable route by which Cu at extremely low levels (<20 ppb) can enter into the food web. Transfer rates from the shrimp to prey organisms have not been determined.

With this information and the chemical form data, we are investigating the

availability of Cu from natural and contaminated sediments in closed systems. The studies involve the placement of organisms in aquaria where the concentrations and chemical forms of Cu are closely monitored. This includes the Cu in the water column, sediment, food, and other tank substrates. Once equilibrium is established, the chemical and physical conditions, i.e., temperature, dissolved oxygen and total Cu content, will be controlled to determine their effect on Cu bioavailability.

In addition to the laboratory studies, the work to describe Sequim Bay and use it as a model for the development of an understanding of a Northwest coastal community has continued. Work accomplished during the past year includes the sediment and particulate studies discussed earlier, a zooplankton study by Dr. Thomas Rennie on a Lilly Foundation Fellowship, the initiation of regular finfish sampling, the establishment of a routine water quality monitoring program, and the continued development of a mathematical hydrological model. The results of these studies are being integrated to provide us with an understanding of Sequim Bay as a system, and to provide the background needed to use Sequim Bay for experimental studies such as those discussed under the "Oil and Gas Technology" section. In addition to the work in Sequim Bay, selective sampling in the Strait of Juan de Fuca and northern Puget Sound was conducted, using the R/V Cayuse. This data will be used to relate our Sequim Bay work with the more open waters of the Puget Sound and Strait of Juan de Fuca systems.

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8.0 HANFORD SUPPORT SERVICES

Work reported in this section has been funded through intercontractor agreements in support of the industrial operations actually located on the Hanford site. At the time of this writing, principal effort was directed to establishing, in specific detail, the radioecology of several locations where nuclear fuel reprocessing operations are conducted. The extent of the Hanford site (570 square miles) is such that many different wildlife habitats and landscape subunits are found, each with its preponderant species of organisms and particular food-chains.

In meeting the requests of Atlantic Richfield Hanford Company (ARHCO) to definitively characterize their locations radioecologically and to recommend changes in practice where appropriate, the PNL staff has had to draw on an extensive base of experience and data developed over the past 9 years by DBER. Eight related radioecological projects, of a generic and long-range nature, will be found in Section 4, pp. 4.0-4.172, of this report. Land restoration, also is important in the stabilization of overburden above retired nuclear waste burial sites. These projects are reported here, but background data dealing with climatic, phenological and other botanical considerations will be found in Section 1, pp. 1.24-1.44.

In March 1977, the Hanford site was dedicated as a National Environmental Research Park (NERP), with a broad mandate to further extend ecological investigations at this location. Future development of energy technologies at Hanford can be expected to lead to diversification beyond the nuclear installations already present. At this time much of the site has been systematically characterized, and there is little if any significant environmental impact of the present technology. This provides an excellent background for the development of pilot demonstration units meeting new standards.

• ARHCO STUDIES

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The ARHCO-supported ecological studies are designed to clarify ecosystem structure and function in and near the radioactive waste management areas. To date, emphasis has been placed on characterizing the abiotic and biotic components of these areas.

During the current year, deer, raptors, coots, and mice have been under active study with respect to their potential for dissemination of radioactivity from pond or burial locations via feeding. Both the deer and raptor studies are fairly long-term as regards quantitating their food chains and abundance. Work is also in progress to detect possible changes in habitat that might independently affect populations of these species. Yearly dose commitment also has been calculated for hunters who might eat waterfowl (coots) inhabiting the process waste ponds at Hanford. This would amount to 1.3×10^{-2} man-rem/year. Studies have continued on the rooting depth of vegetation at burial sites. Only 8 of the 14 species studied showed root penetration beyond 5 feet, with bitterbrush the deepest rooted at 10 feet. Preliminary estimates of plant soil concentration ratio for ^{99}Tc ranged from 100 to 400, with as much as 82% of the ^{99}Tc amendment to the soil taken up by tumbleweed and cheatgrass.

Future emphasis will include energy and mineral cycling mechanisms, radionuclide transport by biota and modeling of ecosystem functioning, thereby integrating both fundamental ecological and process studies to application of waste management operations. A pictorial representation showing integration of research segments is included in Figure 8.1. Results from particular studies are included below under separate headings.

Deer Diets

Deer provide an important link to man through food chain transfers. This study was designed to identify the kinds of plants deer consume while foraging in the vicinity of waste management areas.

Three separate study areas were selected. One area was dominated by big sagebrush/cheatgrass and located in the B-C Cribs controlled zone. Two other study areas included Gable Mountain Pond and B-Pond. These areas were dominated by willows, bulrushes, and sweet clover.

FIGURE 8.1. Integration of Research Segments

The experimental design for pellet collection consisted of four transects located near each of the areas. The first 10 pellet groups encountered on a transect were collected. Pellet groups were plucked from the ground by hand and categorized into two age classes: 1) recent pellet groups, less than 1 yr old ranging from gray to a "shiny" brown, and 2) old pellet groups ranging in color from a "dull" brown to gray-brown. Individual pellet groups were dried at 60°C for 48 hr and ground in a Wiley Mill (1 mm screen) to make a homogenous sample. Five slides were prepared for each pellet group. Identification of plant fragments from the slides is based on the structural modification of epidermal cells which are compared to reference plant material. These slides are currently being analyzed. Pellets are also being analyzed for radionuclide content.

Root Distribution Studies

The purpose of this study is to determine maximum rooting depths and rooting structure for plants likely to grow on radioactive waste burial sites.

Eight of the 14 plant species examined to date have had root systems extending below 150 cm (about 5 ft) in depth. The deepest was *Purshia tridentata*, antelope bitterbrush, which penetrated to 300 cm (about 10 ft). Second deepest was *Artemisia tridentata*, big sagebrush, which penetrated to 200 cm. These two shrubs are expected to be important components in the vegetation of the Hanford Reservation for an indefinite period of time. They presently dominate undisturbed areas on the Reservation and might be expected to become the predominant shrub on

disturbed areas of the 200 Area control zone after several decades have elapsed. A total of 90 plant specimens has been examined for root distribution patterns during this study. Additional samples are planned for cheatgrass, tumble mustard, and tansymustard.

Waterfowl Inhabiting Waste Ponds

This study concentrates on analysis of the seasonal body burden and diets of representative waterfowl species inhabiting waste management ponds.

Data collected on coot nests at Gable Mountain Pond are presently being compared to data taken on nests found on control ponds in the Columbia National Wildlife Refuge located near Othello, Washington. Information obtained includes clutch sizes, egg and young weight, nest construction, habitat utilization, nesting phenology and radionuclide content of the eggs. The findings should provide some comparison of nesting performances and facilitate making inferences about potential effects on the coot population. Coot eggs collected at Gable Mountain Pond and the control ponds will be analyzed for various radionuclides.

The "worst possible case" for consuming contaminated wildlife from the Hanford Reservation is probably the coot (*Fulica americana*). The coot inhabits all waste ponds, and because of its dietary habits, it has the highest body burden of any waterfowl on the Reservation. Monthly, or at least bimonthly, collections of coots were made on the waste ponds, concentrating on Gable Mountain Pond (GMP) where the birds have the highest concentration of ^{137}Cs and other fission products. The concentration of ^{137}Cs in the coots' breast muscles averaged over 2 yr was 722 pCi/g dry wt. (approximately 180 pCi/g wet). Assuming a person consumed the muscle from one coot a day (100 g/day), using ICRP#2 values for effective half-time for ^{137}Cs in muscle (138 days) and fraction from GI tract to muscle (0.4), the muscle burden Q_m in the person at equilibrium would be:

$$Q_m(\text{eq}) = (722 \text{ pCi/g dry})(0.25 \text{ dry/wet}) \\ \times \frac{(100 \text{ g wet/day})(0.4)(1-e^{-\infty})}{0.693/138 \text{ day}}$$

$$Q_m(\text{eq}) = 1.44 \text{ } \mu\text{Ci}.$$

Since 79% of ^{137}Cs in the body is in the muscle (ICRP#2), the total body burden (Q_{TB}) would be:

$$Q_{\text{TB}}(\text{eq}) = \frac{1.44 \text{ } \mu\text{Ci muscle}}{0.79 \text{ muscle/TB}} = 1.82 \text{ } \mu\text{Ci TB}.$$

This would be 61% of the maximum ^{137}Cs body burden for nonradiation workers ($3 \mu\text{Ci TB}$) and 1.8 times greater than that for the general public ($1 \mu\text{Ci}$) as recommended by ICRP.

There are approximately 300 coots using the Hanford pond yearly. There are about 150,000 coots in Washington, and approximately 8,000 are shot yearly by hunters. The number of Hanford coots that might be taken annually would be:

$$\frac{300}{150,000} (8,000) = 16 \text{ birds/year.}$$

Then the total amount of ^{137}Cs introduced to food chains of Washingtonians would be:

$$16 \text{ birds/year } (722 \text{ pCi/g dry})$$

$$(0.25 \text{ dry/wet}) (100 \text{ g/bird}) = 0.3 \mu\text{Ci/year.}$$

Assuming the $0.3 \mu\text{Ci}$ would be evenly distributed between the 74,000 hunters in the state each year (4 pCi/individual), annual total dose (D) per individual would be:

$$D = \frac{(51 \frac{\text{rem} \cdot \text{dis} \cdot \text{g}}{\text{mev} \cdot \text{day} \cdot \mu\text{Ci}}) (0.59 \text{ mev/dis})}{(7 \times 10^4 \text{ gTB}) (0.79 \text{ muscle/TB})}$$

$$\int_0^\infty \left[(4 \times 10^{-6} \mu\text{Ci/individual}) \right]$$

$$(0.4)e^{-\frac{0.693}{T_{1/2}} t} dt$$

$$D = 1.73 \times 10^{-7} \text{ rem/individual}$$

The yearly dose commitment to the general public from the Hanford coot population would be:

$$1.73 \times 10^{-7} \text{ rem/individual } (74,000 \text{ individuals/year}) = 1.28 \times 10^{-2} \text{ man} \cdot \text{rem/year}$$

Also, the dose commitment for each coot would be:

$$\frac{1.28 \times 10^{-2} \text{ man} \cdot \text{rem/year}}{16 \text{ coots/year}} =$$

$$8.02 \times 10^{-4} \text{ man} \cdot \text{rem/coot.}$$

Insect Studies

The invertebrate studies are designed to document the taxonomic composition abundance and trophic relationships of major groups inhabiting the vicinity of radioactive waste sites. A study concerning grasshopper populations inhabiting the B-C Cribs and REDOX Pond sites was completed this year (Sheldon and Rogers, 1976).

Grasshoppers are a conspicuous faunal component on the Hanford Reservation. Since they are predominantly herbivores, their presence in large numbers is of special significance in the vicinity of radioactive management areas. The species studied were Agenetettix deorum (Scudder), Conozoa wallula (Scudder), Hesperotettix viridis (Thomas), Melanoplus cinereus (Scudder), Melanoplus yarrowii (Thomas), Oedaleonotus enigma (Scudder), Trimerotropis caeruleipennis Bruner, and the northwestern shield-back katydid, Apote notabilis Scudder.

Insects for diet analysis were collected biweekly between 27 June and 8 August 1974. Adult specimens were collected with a standard aerial insect net and immediately preserved in 95% ETOH. A microscope slide mount was prepared from the crop contents of each grasshopper. Plant species were identified based on structural characteristics of epidermal cells specific to each plant species (comparison was made to a reference collection containing all plant taxa present in the study area).

Diet similarity, as an index of dietary overlap, was evaluated using Kulczynski's formula (Oosting, 1956).

$$S = \frac{2 \sum_{i=1}^s w_i (100)}{\sum_{i=1}^s a_i + b_i}$$

where, a_i represents the mean percentage of food item i in the diet of species a ; b_i represents the mean percentage of food item i in the diet of species b ; and w_i represents a_i if $a_i \leq b_i$ or b_i if $b_i \leq a_i$. A mean was calculated for those indices comparing the diet of each grasshopper species with that of every other species. Analysis of variance was used to determine if the means were statistically different, and Student-Newman-Keuls Multiple-Range Test tested for difference between means.

Crop contents of 576 grasshoppers representing 8 species were examined and a total of 16 plant taxa identified (Table 8.1). In all cases, the "unknown" category constituted 3% or less of the diet. The food niche breadth of the 8 grasshopper species varied from a maximum of 12 vascular plant species recorded from M. yarrowii to a minimum of 2 species from H. viridis. Thirteen of the 24 species of herbaceous vascular plants recorded in Table 8.1 were not encountered in any of the 8 grasshoppers'

diets, although 3 of them were major components of the flora, e.g., six-weeks fescue, winged cryptantha, and microsteris. Three of the common plant species, cheatgrass, Sandberg's bluegrass, and matted cryptantha, were consumed at very low levels. The habitat frequency of four heavily foraged plant species was 1% or less, indicating a positive selection for these species. These species included turpentine cymopterus, Carey's balsamroot, Jim Hill mustard, and green rabbitbrush.

Grasses were not found to constitute a major portion of grasshopper diets (Table 8.1). Needle-and-thread was consumed by *T. caeruleipennis*, *A. doerum*, and *C. wallula* at a higher level than its habitat frequency and was present in the diet of *A. notabilis* (Table 8.1). The small amount of grass consumed by *A. doerum* indicates a somewhat atypical food base for this normally granivorous species. It also contained the highest cryptogam frequency which correlates well with observations reporting a significant level of detritus feeding.

Two species of perennial forbs were widely utilized, although in both cases their habitat frequency was less than 1%. Turpentine

cymopterus was found in the diets of 5 of the 8 species and Carey's balsamroot was utilized by all but two species. Western tansymustard was consumed by 7 of the 8 species, although the overall diet frequency was far below its habitat frequency. Jim Hill mustard was utilized by 4 species.

Both big sagebrush and green rabbitbrush were consumed by 7 of the 8 grasshopper species, and each served as a major food source for 1 species. *Melanoplus cinereus* was usually found on the foliage of big sagebrush and had a diet frequency of 61% for that species. On the other hand, *H. viridis* was found almost exclusively on green rabbitbrush and had a diet frequency for this species of 76%. Cryptogams provided the only other major food source.

Scavenging was observed in only one species, the tettigoniid *A. notabilis*. This species, which is largely nocturnal, in contrast to the diurnal grasshoppers, was often observed feeding on dead insects (including members of its own species) as well as on other detritus. This habit was particularly noticeable along roadways.

The combined results for all grasshopper species showed that the diet frequency of 41%

TABLE 8.1. Relative Frequency of Plant Species in Diets of 8 Species

PLANT SPECIES	GRASSHOPPER SPECIES ⁽¹⁾							
	APNO	TRCA	COWA	MECI	MEYA	OEN	AGDE	HEVI
ANNUAL GRASSES								
<i>Bromus tectorum</i>							3	
PERENNIAL GRASSES								
<i>Poa sandbergii</i>					2			
<i>Stipa comata</i>	< 1	8	4				3	
PERENNIAL FORBS								
<i>Sympterus terebinthinus</i>	34			6	15	2	< 1	
<i>Balsamorhiza careyana</i>	< 1	25	73	< 1	22	3		
<i>Achillea millefolium</i>				2	< 1			
ANNUAL FORBS								
<i>Descurainia pinnata</i>	3	12	3	1	7	4	< 1	
<i>Cryptantha circumsissa</i>	< 1				< 1	< 1		
<i>Sisymbrium altissimum</i>	13			5	3	2		
<i>Salsola kali</i>					< 1		< 1	< 1
<i>Amsinckia lycopsoides</i>		6			< 1	< 1		
SHRUBS								
<i>Artemisia tridentata</i>	18	6	< 1	61	12	47	4	
<i>Synsphythamnus viscidiflorus</i>	15		3	6	6	2	1	76
<i>Synsphythamnus nauseosus</i>				< 1	< 1	< 1	3	
<i>Atriplex spinosa</i>				< 1		< 1		
OTHER								
LICHEN	15	42	16	18	27	36	81	21
UNKNOWN	2	< 1		< 1	< 1	< 1	3	1
NUMBER OF CPOPS EXAMINED	61	17	38	124	137	111	36	52
NUMBER OF FOOD TYPES UTILIZED (EXCLUDING THE UNKNOWN CATEGORY)	9	6	6	10	13	11	9	3

⁽¹⁾ APNO = *Apote notabilis* Scudder; TRCA = *Trimerotropis caeruleipennis* Bruner; COWA = *Conozoa wallula* (Scudder); MECI = *Melanoplus cinereus* Scudder; MEYA = *Melanoplus yarrowii* (Thomas); OEN = *Oedaleonotus enigma* (Scudder); AGDE = *Ageneotettix deorum* (Scudder); HEVI = *Hesperotettix viridis* (Thomas)

for big sagebrush to be more than twice that of any other vascular plant.

Results from this dietary analysis of 8 grasshopper species inhabiting waste management areas at Hanford showed that 15 vascular plant species served as food items. Several plant species occurred at higher frequencies in the diet samples than in the plant community, indicating that they were preferred items. Big sagebrush (*Artemisia tridentata*) was the most frequently selected plant species, followed by turpentine cymopterus (*Cymopterus terebinthinus*), green rabbitbrush (*Chrysothamnus viscidiflorus*), and Carey's balsamroot (*Balsamorhiza careyana*). This report should prove valuable in two ways. If contaminated plants are found at a particular burial site, these data will show if any of the grasshoppers select it as food material--cheatgrass and Russian thistle have low selection values. Alternatively, if a grasshopper "outbreak" should occur, these data will show the preferred food plants for the species. If they tend to select plants occupying burial sites, control measures can be implemented.

Another report documenting darkling beetle populations inhabiting the Hanford Reservation is nearing completion. A total of 18 darkling beetle species has been collected. Some are occasionally very abundant on the Reservation. Their distribution, seasonal occurrence, abundance, and food habits have been determined. A final report is in preparation and will be released this year (Rogers et al., 1977). Both grasshoppers and darkling beetles are important links in the food webs of coyotes, reptiles, and birds.

Small Mammal Study

The purpose of this investigation was to characterize small mammal populations inhabiting areas adjacent to the B-C Cribs waste burial zone, and to compare these populations with the ones observed on a "control" area far removed from a waste management facility.

The study areas are located near the B-C Cribs site on the 200 Area plateau. The Control site is on the Arid Lands Ecology Reserve on the northern slope of Rattlesnake Mountain. The vegetation on both study plots is described as a sagebrush/cheatgrass-Sandberg bluegrass type (Daubenmire, 1970; Cline et al., 1975).

Trapping grids were surveyed in a 10 x 10 pattern at 10 m intervals (100 stations). A Sherman live trap at each station, containing dacron batting and a small amount of mixed seeds, provided nesting material and food. This prevented captured animals from becoming torpid during confinement. Each trap was

placed in a metal can and covered with a sheet-metal tent to shade it from direct sunlight.

During the first afternoon of the trapping sessions, the trap doors were opened and a small quantity of an oatmeal-peanut butter mixture was placed on the door to serve as bait. Early the following morning, the traps were checked and the captured animals examined. All animals were individually marked with a series of toe amputations for future identification. The trap coordinate, species, sex, identification number, reproductive condition, relative age, pelage condition, and weight of all animals were noted.

Reproductive condition was determined by external signs. Males were classified as reproductively active when they had scrotal testes. Females demonstrated reproductive activity by changes in the vulva shape and color during the estrus cycle, palpability of fetuses in the latter stages of pregnancy, and condition of the mammae during lactation. All females demonstrating signs of estrus, observable pregnancy, or lactation were considered reproductively active.

Age categories were limited to adult or subadult. Size and weight were the first criterion for assignment of an age category as newly weaned subadults usually weigh about half of the average adult weight. The appearance of the pelage is also considered since the coloration and texture of hair on subadults is usually different than that of adults.

After the captured animals were released, the traps were reset and were checked in the same manner for the next two mornings.

Data gathered in the field were transcribed to a computer-compatible form for storage and retrieval. Monthly summaries, containing historical information on each individual, were generated at the close of every trapping session. These summaries were used for positive identification in the field during the next trapping session.

From the period of 1 May 1974 through 7 November 1975, nineteen 3-day trapping sessions were conducted on the B-C Cribs and Control plots. The sessions were at approximately monthly intervals and accounted for 5,000 trap nights per plot.

Five species of small mammals were captured on the study plots. These include: Great Basin pocket mouse, *Perognathus parvus*; deer mouse, *Peromyscus maniculatus*; northern grasshopper mouse, *Onychomys leucogaster*; sagebrush vole, *Lagurus curtatus*; and western harvest mouse, *Reithrodontomys megalotis*.

Deer mice were captured 54 times on the B-C Cribs plot and 19 times on the Control plot. The B-C Cribs plot captures comprised only 17 individuals and the Control plot captures comprised only 12 individuals. Northern grasshopper mice were captured 11 times on the B-C Cribs plot (6 individuals) and 8 times on the Control plot (4 individuals). A single sagebrush vole was captured on the B-C Cribs plot and two western harvest mice on the Control plot. The 4 species other than pocket mice represent only 7% of the individual small mammals captured on the combined plots.

The trap catch of Great Basin pocket mice is shown in Figure 8.2. These values are derived from the number of individuals captured during the monthly trapping session rather than from total captures, since recapture frequency varies throughout the year.

Pocket mice tend to develop a trap prone-ness after exposure to the traps and the food contained therein. This is the probable explanation for the extreme differences noted during the initial two trapping sessions since the B-C Cribs plot was recently established and the Control plot had been trapped for several years previous to this study. During 1974, the pocket mouse population on the Control plot remained higher than that found on the B-C Cribs plot. The 1975 data also exhibits a similar trend, although during June and July the number of individuals trapped on the B-C Cribs plot slightly exceeded the number trapped on the Control plot.

Estimates of population size were made using the Jolly-Seber stochastic model (Table 8.2). The estimates for the periods of

TABLE 8.2. Observed and Estimated Population Sizes of *Perognathus parvus* Computed Using Jolly-Seber Stochastic Model

DATE	CONTROL PLOT		B-C CRIBS PLOT	
	NUMBERS OBSERVED	POPULATION ESTIMATE	NUMBERS OBSERVED	POPULATION ESTIMATE
3 MAY 74	37	35	6	13
23 MAY 74	35	35	14	13
21 JUN 74	26	38	16	15
12 JUL 74	35	42	26	26
15 AUG 74	49	54	30	39
13 SEP 74	60	70	43	48
10 OCT 74	72	80	53	57
7 NOV 74	69	*	40	*
12 DEC 74	16	*	11	*
9 JAN 75	5	*	0	*
27 FEB 75	9	27	7	55
20 MAR 75	50	50	34	92
24 APR 75	79	83	59	67
30 MAY 75	52	56	43	44
26 JUN 75	50	53	57	59
1 AUG 75	62	62	64	66
28 AUG 75	69	78	66	72
26 SEP 75	72	72	59	79
7 NOV 75	33	*	31	*

inactivity are not shown since they become unreasonable at this time due to the bias within the model.

The weight of individuals within a population is an indicator of the general health and vigor of that population. The mean weight of the adult pocket mice was calculated monthly for each plot (Table 8.3). There was no significant difference within the sex classes between the Control and B-C Cribs plots. Generally, the males showed a gradual increase in body weight from the time of spring emergence until their disappearance from the trappable population when they began winter torpor. The mean weight of females increased during the periods of reproductivity activity, and decreased after the period when most are pregnant.

FIGURE 8.2. Seasonal Abundance of Pocket Mice Captured on B-C Crib and Control Study Areas

TABLE 8.3. Live Weights of Subadult *Perognathus parvus* Captured on Control and B-C Crib Plots, Categorized by Sex and Age Class (expressed in grams)

DATE	CONTROL PLOT		B-C CRIBS PLOT	
	N	MEAN $\pm$ S.E.*	N	MEAN $\pm$ S.E.*
----- MALE -----				
3 MAY 74	0		0	
23 MAY 74	0		0	
21 JUN 74	5	11.1 $\pm$ 0.9	2	14.3
12 JUL 74	9	14.8 $\pm$ 0.9	7	15.6 $\pm$ 0.8
15 AUG 74	20	15.6 $\pm$ 0.7	12	15.5 $\pm$ 0.7
13 SEP 74	33	17.2 $\pm$ 0.6	19	15.9 $\pm$ 0.7
10 OCT 74	39	17.2 $\pm$ 0.5	25	17.1 $\pm$ 0.6
7 NOV 74	38	17.3 $\pm$ 0.5	20	16.8 $\pm$ 0.5
12 DEC 74	8	17.4 $\pm$ 1.1	8	16.1 $\pm$ 0.8
9 JAN 75	0		0	
27 FEB 75	0		0	
20 MAR 75	0		0	
24 APR 75	0		0	
30 MAY 75	0		0	12.0
26 JUN 75	9	11.2 $\pm$ 0.7	19	13.0 $\pm$ 0.6
31 JUL 75	26	13.6 $\pm$ 0.6	28	15.9 $\pm$ 0.5
28 AUG 75	35	14.7 $\pm$ 0.5	30	17.4 $\pm$ 0.4
26 SEP 75	41	15.0 $\pm$ 0.4	37	15.9 $\pm$ 0.3
7 NOV 75	22	14.9 $\pm$ 0.4	21	15.0 $\pm$ 0.4
----- FEMALE -----				
3 MAY 74	0		0	
23 MAY 74	0		0	
21 JUN 74	2	12.8	1	8.0
12 JUL 74	6	13.0 $\pm$ 1.7	9	13.7 $\pm$ 0.7
15 AUG 74	15	13.8 $\pm$ 1.2	12	15.2 $\pm$ 0.7
13 SEP 74	16	14.2 $\pm$ 0.8	18	14.8 $\pm$ 0.6
10 OCT 74	24	13.8 $\pm$ 0.4	25	14.7 $\pm$ 0.4
7 NOV 74	27	13.5 $\pm$ 0.5	18	13.2 $\pm$ 0.3
12 DEC 74	7	13.0 $\pm$ 0.6	3	14.0 $\pm$ 0.3
9 JAN 75	0		0	
27 FEB 75	0		0	
20 MAR 75	0		0	
24 APR 75	0		0	
30 MAY 75	0		2	12.3
26 JUN 75	9	10.3 $\pm$ 0.6	9	14.3 $\pm$ 0.7
31 JUL 75	16	13.6 $\pm$ 0.7	14	15.8 $\pm$ 0.9
28 AUG 75	27	12.9 $\pm$ 0.5	26	14.0 $\pm$ 0.3
26 SEP 75	26	13.5 $\pm$ 0.2	17	13.5 $\pm$ 0.4
7 NOV 75	11	13.2 $\pm$ 0.4	10	13.1 $\pm$ 0.4

* STANDARD ERROR IS NOT COMPUTED FOR N < 3.

Radiation Doses to Small Mammals at U-Pond

This program was designed to determine radiation doses to small mammals inhabiting radioactive waste pond habitat (U-Pond) located in the 200-West Area of the Hanford Reservation.

Small mammals were trapped along four line transects in slightly different habitat types around the pond. Each line transect consisted of 10 traps. A control transect of 20 traps was set up in a noncontaminated area and away from the pond. A mark and recapture method was employed using Sherman live-traps baited with a peanut butter-oatmeal mixture. Captured animals were toe clipped with a unique identification number permitting later identification of individuals.

Thermoluminescent dosimeters (TLD's) were surgically implanted beneath the skin of the

animals to measure gamma dose to the mice. A general anesthesia was used to quiet them during implantation. When the operation was completed and the animals regained consciousness, they were returned to their trap location and released. Approximately 1 month later, the area was trapped again to recapture mice carrying TLD's. A total of 123 mice have been implanted with dosimeters and 22 TLD's have been recovered for an 18.7% recapture success since the program was initiated in May 1976. The majority of mice captured around the pond were Great Basin pocket mice (*Perognathus parvus*), with deer mice (*Peromyscus maniculatus*), and house mice (*Mus musculus*) next in abundance.

The house mice received the highest doses, deer mice were next and pocket mice generally had lower doses (Table 8.4). The highest doses occurred in a small meadow area located between the two trenches that feed the pond with waste water (Figure 8.3). Animals carrying dosimeters in this area exhibited the highest levels of gamma radiation (Table 8.4).

TABLE 8.4. Radiation Exposure Rates to Mice at U-Pond

Transect	Species	mR/day
Meadow	Mus m	149.80
	Mus m	145.64
	Peromyscus m	38.66
	Peromyscus m	50.72
	Peromyscus m	44.74
	Perognathus p	209.71
Forest	Mus m	60.91
	Mus m	12.60
	Peromyscus m	5.92
	Peromyscus m	6.48
	Peromyscus m	16.35
	Perognathus p	4.83
U-14 Trench	Perognathus p	4.62
	Perognathus p	31.60
	Perognathus p	1.11
	Perognathus p	0.60
Z-19 Trench	Perognathus p	0.62
	Perognathus p	0.42
	Perognathus p	0.32
	Perognathus p	0.32
Control	Peromyscus m	0.18
	Perognathus p	0.24
	Perognathus p	0.23
	Perognathus p	0.21

FIGURE 8.3. U-Pond Small Mammal Transects

Dosimeters were placed in the soil at five locations along each transect to a depth of 5 dm, at 1 dm intervals, to detect stratification of radioactive material at particular depths. In all transects except the control, the top decimeter of soil yielded the highest exposure rates (Figure 8.4).

Raptor Studies

Birds of prey are subjects of great public concern since their populations have drastically declined in the last few decades. Hawks, owls, and eagles are "Birds of the Hour," so to speak. No other raptors seem to arouse such public concern. Government agencies, private groups, and national professional societies are greatly concerned about our bird-of-prey heritage. ARHCO's Raptor Program is one of the first to ascertain the population structure of any raptor species residing on the Hanford Reservation.

Birds of prey as "end of the food chain organisms" can concentrate environmental contaminants which can alter their ability to reproduce. If contaminants are available but in small amounts, effects can be undetected. By comparing raptor productivity on the Hanford Reservation to other regions of the United States, we will be able to assess the relative condition of Hanford birds of prey.

This type of study demands long-term data collection on which to statistically base

FIGURE 8.4. Radiation Dose Rates Measured at Different Soil Depths Near U-Pond

our judgments. To date, only the Bureau of Land Management has a data base on predator and prey populations in western grasslands, which is comparable to that collected on the Hanford Reservation. In the near future, we will need these data in order to accurately assess a variety of impacts, particularly large-scale land disruptions, such as oil shale and coal mining operations.

These raptor studies involve surveying densities and productivity of nesting birds of prey which are known to occur on the Hanford Reservation (Table 8.5).

Dietary data are being collected for many of the nesting and wintering raptors of the Hanford Reservation. Casting analysis, prey item tabulations, and time-lapse photography are all being used to gather data. Casting analysis and time-lapse photographic analysis are tedious, time-consuming operations which yield a relatively accurate description of raptor diets. We are presently working on dietary analysis for Swainson's hawks, great horned owls, and long-eared owls.

Raptor investigations have also involved radiochemical, pesticide and heavy metal

TABLE 8.5. Birds of Prey of the Hanford Reservation

SPECIES	RESIDENCY STATUS
BURROWING OWL (<i>Speotyto cunicularia</i>)	NESTING
GREAT HORNED OWL (<i>Bubo virginianus</i>)	NESTING
LONG-EARED OWL (<i>Asio otus</i>)	NESTING
SHORT-EARED OWL (<i>Asio flammeus</i>)	NESTING
FLAMMULATED OWL (<i>Otus flammeus</i>)	MIGRANT
BARN OWL (<i>Tyto alba</i>)	NESTING
SNOWY OWL (<i>Nyctea scandiaca</i>)	WINTER VISITOR
RED-TAILED HAWK (<i>Buteo jamaicensis</i>)	NESTING
SWAINSON'S HAWK (<i>Buteo swainsoni</i>)	NESTING
MARSH HAWK (<i>Circus cyaneus</i>)	NESTING
AMERICAN ROUGH-LEGGED HAWK (<i>Buteo lagopus</i>)	WINTERING
FERRUGINOUS HAWK (<i>Buteo regalis</i>)	OCCASIONAL VISITOR
GOSHAWK (<i>Accipiter gentilis</i>)	WINTER VISITOR
COOPER'S HAWK (<i>Accipiter cooperii</i>)	WINTER VISITOR
SHARP-SHINNED HAWK (<i>Accipiter striatus</i>)	WINTER VISITOR
GOLDEN EAGLE (<i>Aquila chrysaetos</i>)	WINTER RESIDENT
BALD EAGLE (<i>Haliaeetus leucocephalus</i>)	WINTER RESIDENT
OSPREY (<i>Pandion haliaetus</i>)	WINTER VISITOR
PRAIRIE FALCON (<i>Falco mexicanus</i>)	NESTING
PIGEON HAWK (<i>Falco columbarius</i>)	WINTER VISITOR
AMERICAN KESTREL (<i>Falco sparverius</i>)	NESTING

analyses of bird tissues (eggs and flesh) and their prey. Body burdens of hawks, owls, eagles, and ravens are being determined and will serve as a baseline from which to monitor environmental pollution.

Intensive behavioral investigations have been conducted on the Swainson's hawk (*Buteo swainsoni*). Field observations and time-lapse photography have served for gathering much of the data. During summer 1976, seven adult hawks and nine juveniles were fitted with transmitters and their daily movements were determined. Differences were noted between sex and age groups. We concentrated on the Swainson's hawks which were nesting near the waste management zones. We were able to determine the extent to which each family group used waste management areas for foraging. Birds were also individually marked with colored patagial wing markers. The wing markers are visible on flying and perching birds. Through sightings, we are able to study migration and nest site utilization.

Monthly roadside surveys have been conducted to ascertain seasonal turnover of raptor species. These surveys enable us to document species which migrate through or winter on the Reservation. Bald and golden eagles are both dominant wintering species. There is presently no information as to where these birds come from in the fall or where they depart to in the spring. They may nest far in Canada or in the western United States.

There is an abundance and diversity of raptor species on the Hanford Reservation, not found elsewhere in northwestern grassland habitats. Birds produced on the Reservation serve to supplement populations in other less productive areas. As arid lands undergo

irrigation, the Hanford Reservation may well serve as the major refugium for birds of prey.

Vegetation of Burial Sites

Information concerning the growth habitats of plants commonly found on low-level radioactive waste storage sites is important in preventing uptake of radionuclides by plants and transfers along food webs. The purpose of this study was to characterize the vegetation and backfill with respect to the age of the burial site.

The major vegetation in undisturbed areas is big sagebrush (*Artemisia tridentata*). Other shrubs are rabbitbrush (*Chrysothamnus nauseosus* and *C. viscidiflorus*) and eriogonum (*Eriogonum niveum*). The herbaceous understory is generally sparse and is dominated by cheatgrass (*Bromus tectorum*) and sandberg bluegrass (*Poa sandbergii*).

Five retired burial sites were examined for canopy cover, frequency of occurrence, and biomass of plants. Frequency and cover measurements of plants were collected on replicated 50 m transects located on each burial site. Measurements were collected in reading fifty, 2 x 5 dm plot frames systematically spaced at 1 m intervals on each line transect. Biomass was measured by clipping all plants by species within a 2 x 5 dm plot frame placed at 10 m intervals along the 50 m transect.

Soil samples were collected on each of the burial grounds at 1 dm increments to a depth of 3 dm. All samples of soil were analyzed for texture (Table 8.6).

Average canopy cover by plant species on the burial sites is presented in Table 8.7. Cheatgrass provided for most of the coverage, followed by Russian thistle. Shrubs were sparsely represented and provided little cover.

TABLE 8.6. Textural Analysis of the Upper 0.3 Meters of Soil Profile From Six Sites in the 200 West Area

BURIAL SITE	YEAR COMPLETED	SOIL DEPTH (dm)	% SAND	% CLAY	% SILT	TYPE OF SOIL
216-S-9	1969	0-1	75	1	24	SANDY LOAM
		1-2	73	1	26	SANDY LOAM
		2-3	76	1	23	SANDY LOAM
216-S-22	1967	0-1	70	1	29	SANDY LOAM
		1-2	80	1	19	LOAMY SAND
		2-3	80	1	19	LOAMY SAND
216-U-12	1960	0-1	79	1	20	LOAMY SAND
		1-2	81	1	18	LOAMY SAND
		2-3	77	1	22	SANDY LOAM
218-W-3	1959	0-1	79	1	20	LOAMY SAND
		1-2	80	1	19	LOAMY SAND
		2-3	80	1	19	LOAMY SAND
218-W-4	1964	0-1	77	1	22	SANDY LOAM
		1-2	79	1	20	LOAMY SAND
		2-3	78	1	21	SANDY LOAM
216-Z-7	1967	0-1	80	1	19	LOAMY SAND
		1-2	81	1	18	LOAMY SAND
		2-3	83	1	16	SAND

TABLE 8.7. Percent Cover (%C) and Frequency Percent (%F) by Plant Species on Five Burial Sites, 12 May 1975

SPECIES	BURIAL GROUND DESIGNATION									
	218-W-3		216-U-12		216-Z-7		216-S-9		218-W-9	
	(%C)	(%F)	(%C)	(%F)	(%C)	(%F)	(%C)	(%F)	(%C)	(%F)
DATE BURIAL LAST DISTURBED:	--1961--		--1959--		--1967--		--1969--		--1954--	
ANNUAL GRASSES										
CHEATGRASS (<i>Bromus tectorum</i>)	39.0	100	26.0	100	37.0	100	22.0	100	20.0	100
SIX WEEK FESCUE (<i>Festuca octoflora</i>)	+	1	2.5	9	0.5	13	0.3	11	0.4	11
PERENNIAL GRASSES										
INDIAN RICEGRASS (<i>Oryzopsis hymenoides</i>)	+	1	0.4	1	--	--	0.9	9	5.0	17
SANDBERG BLUEGRASS (<i>Poa sandbergii</i>)	--	--	--	--	--	--	--	--	--	--
FORBS										
RUSSIAN THISTLE (<i>Salsola kali</i>)	6.0	32	2.0	82	3.0	77	2.0	59	3.0	92
BUCKWHEAT (<i>Eriogonum</i> sp.)	--	--	+	8	--	--	--	--	--	--
TANSYMUSTARD (<i>Descurainia pinnata</i>)	1.0	2	0.4	15	0.5	19	0.2	7	0.8	28
JIM HILL MUSTARD (<i>Sisymbrium altissimum</i>)	0.6	4	0.2	7	1.5	56	0.5	2	0.3	6
DRABA (<i>Draba verna</i>)	--	--	--	--	0.1	3	--	--	--	--
EVENING PRIMROSE (<i>Oenothera pallida</i>)	<0.3	1	0.5	3	0.1	3	--	--	0.1	6
CYMOPTERUS (<i>Cymopterus terrestris</i>)	--	--	--	--	--	--	--	--	--	--
MATTED CRYPTANTHA (<i>Cryptantha circumscissa</i>)	9.0	2	0.5	19	0.2	9	0.3	13	0.5	20
WING-NUT CRYPTANTHA (<i>Cryptantha pterocarya</i>)	--	--	0.1	2	--	--	<0.1	1	1.0	4
ASTER (<i>Aster</i> sp.)	0.1	0	<0.1	1	0.1	3	0.2	3	--	--
BURSAGE (<i>Ambrosia acanthioides</i>)	5.0	21	--	--	--	--	1.6	46	0.3	8
LACTUCA (<i>Lactuca</i> sp.)	--	--	0.1	<0.1	--	--	0.2	--	2.0	--
SHRUBS										
RABBITBRUSH (<i>Chrysothamnus viscidiflorus</i>)	6.0	15	--	--	--	--	--	--	--	--

Frequency of occurrence values showed that cheatgrass and Russian thistle were the most common plants on the burial sites examined (Table 8.7). Tansy and Jim Hill mustards are also common on all sites. Bursage has the potential of being very abundant as indicated on the 216-S-9 site, having a 46% frequency of occurrence.

Cheatgrass comprised approximately 94% of the total plant biomass (Table 8.8). Other species of plants encountered are much less abundant. Russian thistle continued to grow until late fall. These estimates obtained for Russian thistle underestimate its true yield which continues growing through the fall season.

TABLE 8.8. Biomass Estimates in gram/m² Dry Weight on Five Burial Sites on May 12, 1975

SPECIES	216-U-12	216-Z-7	216-S-9	218-W-9	AVERAGE
PERENNIAL GRASSES:					
RICEGRASS	0	0	0	15.7	3.1
ANNUAL FORBS:					
BURSAGE	0	0	0.73	0	0.1
CRYPTANTHA	0.04	0	0	0	0
TANSY MUSTARD	0.01	0.15	0	0.46	0.1
DRABA	0	0.15	0	0	0
RUSSIAN THISTLE	0	0.27	0	0	0
TUMBLE MUSTARD	0	4.05	0	0	0
FIREWEED	0	0	0	0	0
ANNUAL GRASS:					
CHEATGRASS	111.7	67.8	102.4	70.5	86.9
TOTAL	111.8	72.4	103.1	86.7	92.2

The soils on the burial sites are either sandy loam or loamy sand with a small amount of clay. Sand comprised an average of 78.2% $\pm$ SE 0.7 on the burial sites with silt averaging 20.8% $\pm$ SE 0.7. Soils of this type have a low water-holding capacity and the cation exchange capacity is also very low.

Plant Uptake of Technetium

Technetium-99 (⁹⁹Tc) is a byproduct of nuclear fission having a fission yield of 6.1%. Until recently, it has received little attention from an environmental standpoint as compared with ¹³⁷Cs and ⁹⁰Sr. However, recent studies have demonstrated that agricultural plants are capable of accumulating ⁹⁹Tc to relatively high tissue concentration, with concentration ratios exceeding 100 (Wildung et al., 1974a; Wildung et al., 1976; Gast, 1975; Cataldo et al., 1975). These observations necessitate additional studies in the area of nuclear waste management and the potential for transfer of Tc in the environs surrounding nuclear processing facilities such as Hanford.

The purpose of the present study was to determine the extent of uptake of ⁹⁹Tc by tumbleweed and cheatgrass using soil characteristics of the Hanford Project. These plant species readily invade disturbed areas such as burial and storage sites and should provide a basis for evaluating the potential for the uptake and transport of ⁹⁹Tc. The objectives of this study were: 1) to evaluate the availability and uptake of ⁹⁹Tc by plants as a function of time at soil concentrations of ⁹⁹Tc at environmental levels, and 2) to

determine the effect of soil properties of the availability of ^{99}Tc to plants.

Cheatgrass and tumbleweed plants were grown in growth chambers for 3 months on five soils found on the Hanford Reservation. Soils were amended with $1.0 \text{ pg } ^{99}\text{TcO}_4^-/\text{g soil}$, traced with $2.2 \times 10^{-4} \text{ } \mu\text{Ci } ^{95\text{m}}\text{TcO}_4^-/\text{g soil}$. In addition, two of the five soils were amended with a 50-fold excess of $^{95\text{m}}\text{Tc}$ ($1.1 \times 10^{-2} \text{ } \mu\text{Ci } ^{95\text{m}}\text{Tc/g soil}$) to assure that $^{95\text{m}}\text{Tc}$ was effectively tracing ^{99}Tc and the plant uptake of ^{99}Tc was not adversely affected by either radiation or toxicity effects. Plants were harvested 30, 60, and 90 days after emergence, oven-dried, analyzed, and concentration ratios determined.

Uptake of ^{99}Tc by tumbleweed and cheatgrass was rapid during the initial 30-day growth period and decreased sharply during the second and third periods. As estimated, 67 to 89% of the uptake occurred during the first 30-day growing period and 82 to 100% of uptake occurred during the first 60 days. Shoot concentration of Tc ranged from 0.06 to 0.33 and from 0.05 to 0.36 $\mu\text{g/g}$ of ^{99}Tc on a dry weight basis for tumbleweed and cheatgrass, respectively. Total uptake ranged from 23 to 82% and 10 to 69% of the ^{99}Tc amended to soils for tumbleweed and cheatgrass, respectively.

Concentration ratios (CR values) were calculated based upon the estimated average soil concentration of ^{99}Tc during each harvest period. These were found to be nearly constant over the three 30-day growth periods for all soils, except Lickskillet, a relatively low pH high organic matter soil, which exhibited a decrease in CR values with time. CR values for tumbleweed and cheatgrass grown on the remaining four soils ranged from 100-400.

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9.0 ENERGY RESEARCH FOR OTHER AGENCIES

- **NUCLEAR REGULATORY COMMISSION**
- **NATIONAL INSTITUTE OF ENVIRONMENTAL AND HEALTH SCIENCES**
- **NATIONAL OCEANOGRAPHIC-ATMOSPHERIC ADMINISTRATION/BUREAU OF LAND MANAGEMENT**
- **BONNEVILLE POWER ADMINISTRATION**
- **ELECTRIC POWER RESEARCH INSTITUTE**

The purpose of this section is to correlate energy research conducted for other sponsors to the ERDA energy programs where interagency and other mutual agreements exist to support work on a non-duplicative basis. The research discussed in this section is complementary to work discussed in other sections of this report. The data bases described provide a unique long-term reference for environmental and energy assessments in the arid West.

NUCLEAR REGULATORY COMMISSION

Evaluation of Nuclear Facility Environmental Impact Prediction, Based on Ecological Monitoring Programs (Principal Investigators: K. L. Gore and D. G. Watson; W. T. Hinds and J. M. Thorp; J. M. Thomas and L. L. Eberhardt)

The ecological monitoring programs from three nuclear power plants were quantitatively evaluated as to their effectiveness in measuring potential environmental impacts. It appears from the data analyzed that more consideration should be given to a statistically oriented sampling design in order to attempt to differentiate between natural variation and plant-induced effects.

The primary objective of this study is to quantitatively evaluate the nonradiological environmental monitoring programs as to their effectiveness in measuring potential environmental impacts. Ten candidate sites qualified for initial screening by meeting the following criteria: 1) at least 1 yr of operating experience, and 2) Battelle-Northwest had assisted the Nuclear Regulatory Commission in the preparation of the Environmental Impact Statement.

During the past year efforts were made to: 1) assemble and review the information available on the nonradiological environmental monitoring programs of the ten operating nuclear power plants, 2) select three of these plants for detailed analyses, and 3) quantitatively evaluate monitoring data from these three plants. The type of information reviewed included Environmental Statements, Environmental Reports, Nonradiological Monitoring Reports, Operating Reports, Unusual Incidence Reports, Nonradiological Environmental Technical Specifications and the open literature. The availability of monitoring information varied widely and played an important role in the plant selection process. In addition to report availability, selection was also based on other factors such as time span covered by the monitoring reports; general quality and quantity of data; consistency of preoperational to operational monitoring; continuity of monitoring programs with respect to methodologies, sampling locations, and sampling program design; habitat type i.e., riverine, lacustrine, estuarine and marine; and time and rate of plant operation.

Out of the ten candidate sites, three were chosen for detailed analyses. The three sites were: 1) Monticello Nuclear Generating Facility, 2) Haddam Neck Atomic Power Plant, and 3) Millstone Nuclear Power Station. Monticello is located in Minnesota and uses the Mississippi River as its cooling water source, thus representing a riverine ecosystem. The Connecticut River is the cooling water source for the Haddam Neck site and is representative of a tidal river ecosystem. The third site, Millstone is also located in Connecticut and is representative of a estuarine-marine ecosystem because the plant uses Niantic Bay (estuarine) as its cooling water source, but discharges into Long Island Sound (marine).

Preoperational as well as operational monitoring data were examined to test the usefulness of baseline information in evaluating impacts. This included an examination of the analytical methods used to measure ecological and physical parameters, and an assessment of sampling periodicity and sensitivity where appropriate data sets were available. From this type of analysis, deficiencies in both preoperational and operational monitoring programs may be identified and provide a basis for suggested improvements.

A considerable amount of time was spent accumulating the raw data and preparing it for analysis; thus this study is largely dependent on applying the proper statistical tests to the appropriate data sets. Both current statistical methodologies and new ones being developed at Battelle Northwest were employed.

The type of data sets examined included at least 2 yr of preoperational and 2 yr of operational monitoring, where the stations, gear and frequency of sampling were consistent throughout the sampling period. Communities such as phytoplankton, periphyton, benthic macroinvertebrates and fish (including ichthyoplankton, juveniles and adults) were evaluated. In addition to community effects, such potential impacts as entrainment, impingement and cold shock were also examined.

Statistical tests employed ranged from an overview of the entire data set via an analysis of variance to a nonparametric test for significant changes due to station or time. We were able to obtain some replicate data for certain data sets, thus enabling us to check the distributional basis for some of the statistical analyses employed. In addition, we have investigated similarity and diversity indices as methods for the analysis of impact data. Considerable effect has been placed on investigating the statistical power for discrimination for several of these measures.

Based on our evaluation of the monitoring programs conducted at these three nuclear power plants where some communities and/or investigations have been extensively monitored, the data recording and synthesis for statistical analyses were very important and at times a cumbersome aspect of this study. However, due to certain inconsistencies (e.g., additions and/or deletions of sampling stations from year to year, changing sampling gear or methodologies, and reporting formats) not all of this extensive data could be quantitatively evaluated. Perhaps, then, more consideration should be given to a sampling design that is statistically oriented in order to try to differentiate between natural variation at a station, between stations or years, and plant-induced effects.

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, as 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 require 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.

NATIONAL OCEANOGRAPHIC-ATMOSPHERIC ADMINISTRATION/
BUREAU OF LAND MANAGEMENT

Accumulation of Organic Constituents and Heavy Metals from Petroleum Impacted Sediments by Marine Detritivores (Principal Investigator: J. W. Anderson)

Three species of benthic organisms have been studied in relation to their capability to take up hydrocarbons and heavy metals from oil-contaminated sediment. Saturate, di- and tri-aromatic compounds were taken up by the deposit-feeders (Macoma and Phascolosoma), but not the filter-feeder (Protothaca) during field experiments of 40 to 60 days. Uptake of C^{14} -labeled hydrocarbons by Macoma exposed to contaminated sediment in the laboratory, showed that most transfer was via the water column and not the sediment. Trace metals, analyzed in tissues and sediment by X-ray fluorescence, were not shown to be significantly taken up by Macoma exposed to clean and oiled sediment.

This research complements that conducted under ERDA funding, since it relates to bioavailability of hydrocarbons from sediment. While the techniques and organisms differ, the findings will aid us in evaluating the fate and effects of petroleum hydrocarbons in marine organisms and the marine environment.

BONNEVILLE POWER ADMINISTRATION

Biological Studies at the Site of an 1100-kV Prototype Transmission Line (Principal Investigator: L. E. Rogers)

A study supported by the Bonneville Power Administration was initiated last year (1976) to evaluate biological effects associated with electrical power transmission over an 1100-KV line. A full scale prototype test facility was constructed near Lyons, Oregon, to test both electrical characteristics and the environmental acceptability of an 1100-kV system.

The biological studies are designed to determine possible changes in the organic productivity and morphology of plants at the site, in behavioral disturbances in cattle and honey bee populations at the site, and in the population dynamics of small mammals. Responses are being measured with respect to altering the voltage gradient to ground and secondary emissions.

These studies complement others funded by the Energy Research and Development Administration and the Electric Power Research Institute both within Battelle and elsewhere.

ELECTRIC POWER RESEARCH INSTITUTE

Synthesis and Analysis of Cooling Pond and Lake Information (Principal Investigators: D. G. Watson and J. A. Strand)

The program entitled, "Synthesis and Analysis of Cooling Impoundment Information", is designed to: 1) synthesize existing ecological data for cooling impoundments with low load ratios (surface acres/plant MWe capacity) less than 10; 2) analyze this data to derive technically sound conclusions concerning plant operational effects on cooling impoundments, and 3) identify what important effects cannot be assessed with existing data. The rationale behind the program is that cooling impoundments represent the simplest aquatic ecosystems for reflecting the impact of electric generating stations using the traditional once-through method of cooling condensers.

This research is compatible with and complementary to several ongoing ERDA supported programs in the Freshwater Sciences Section of the Ecosystems Department. Included in these are studies to determine the effects of coal-fired fossil fuel power plant effluents on the aquatic biota of cooling ponds and natural surface waters; the environmental effects from discharges of a pilot plant concerned with the development of the solvent refined coal process; the effects on aquatic organisms of heat and chemicals typical of the effluents from thermal power plants; and the changes in fish behavior and stamina that result from exposure to combinations of heat and chemicals from thermal power production.

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PUBLICATIONS

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